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CHAPTER 38

Preformulation

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PREFORMULATION CHALLENGES

Bridging Discovery and Development

Preformulation activities range from supporting discovery's identification of new active agents to characterizing physical properties necessary for the design of dosage forms. Critical information provided during preformulation can enhance the rapid and successful introduction of new therapeutic entities for humans. For example, the selection of compounds that have physical properties favorable for oral absorption early in discovery can facilitate the rapid progress of these compounds at all stages of development. Similarly, the adaptation of technologies that permit the rapid selection of a salt that is best suited for development can facilitate the manufacturing of the final market-image dosage form. The broad range of activities in preformulation requires a continuing dialog between scientists in many different disciplines, as shown in Figure 38-1.

Discovery to Development

The introduction of mechanism-based mass screening of small molecules in the late 1980s ushered in a new discovery era. Previously, animal tissue and whole animal screens had been used to find new chemical entities (NCE) that had therapeutic potential. Although the throughput was low, the final candidates for development had proven activity in animals. Today, recombinant enzymes and receptors are used in high-throughput *in vitro* screens that can evaluate quickly the hundreds of thousands of compounds that are found in chemical libraries. Active compounds (mass screen hits) then are evaluated, and some are used as the basis for further synthetic efforts. Because synthesis of new compounds can become rate limiting, combinatorial methods have been developed to synthesize rapidly new compounds using automated technologies. Today, even newer technologies are being used to increase speed and reduce material consumption. This is the attraction for using nanotechnologies in screening, synthesis, purification, and analysis.

All of these innovative changes have had a cascading impact on development. Unprecedented *in vitro* activity and specificity can now be found using recombinant proteins and automated mass screening, but aqueous solubility problems are masked by dimethyl sulfoxide, a universal solvent that is used to dissolve chemical libraries for testing. As a result, although many initially promising NCEs are extremely potent in the *in vitro* enzyme assays, they are inactive *in vivo* because of their unfavorable solubility and dissolution characteristics in the aqueous media of the body. This provides a demanding challenge for the preformulation scientist because, with mechanism-based therapy, testing in humans is often the only means of evaluating the efficacy of a new therapeutic strategy.

Integrating Discovery and Development

If unfavorable physical properties can be minimized before extensive *in vitro* optimization occurs, it may be possible to reduce the time required to discover *active and absorbable* NCEs that are poised for rapid development. Integrating discovery and development, however, will require that preformulation scientists develop a greater understanding of the molecular mechanisms of unfavorable physical properties such as aqueous insolubility. This knowledge then will provide a rational basis for making structural modifications that can enhance physical properties while *in vitro* activity also is being optimized. Figure 38-2 shows the potential time delay in discovering an orally active NCE when only activity is optimized, compared to the potential time savings when both activity and aqueous solubility are balanced for oral absorption.

Assume that a company has a chemical library of thousands of compounds that it wants to screen for a particular therapeutic target. It has isolated the appropriate receptor (protein) and has developed a high-throughput mass screen for its *in vitro* activity. In addition, for every compound that is screened for activity, it can determine aqueous solubility using a high-throughput method. Figure 38-2 shows a plot of activity versus solubility for the screened compounds. For simplicity, an ellipse is used to show regions that are possible for this hypothetical receptor. The inverse relationship shown by the ellipse, with the major axis decreasing from left to right, is based on anecdotal observations that compounds that have high *in vitro* activity often have poor aqueous solubility. A molecular explanation for why such a relationship might exist is given in the section *Aqueous Insolubility*, page 716. The two-phase discovery of an orally active NCE will now be discussed.

During Discovery Phase A, the company used *in vitro* activity as its only criterion for discovering the best compound to develop. Point 0 on the ellipse shows a compound that was chosen for further synthetic optimization on the basis of mass screening. This compound had the highest in vitro activity. During optimization, mass screens were used to provide feedback to direct the synthesis of more active analogs. Compound 1 was the most active NCE the discovery team found. However, this compound is also the most insoluble NCE on the ellipse. Enthusiasm for the compound diminished when *in vivo* animal testing showed inadequate blood levels. A lack of absorption due to poor aqueous solubility was suspected as the cause (other studies had shown that metabolism and permeability did not account for the low blood levels).

During Discovery Phase B, aqueous solubility and *in vitro* activity were optimized simultaneously. The NCE shown at

Figure 38-1. The wheels of product development.

Point 2 was eventually recommended for development. Although this compound is less active than Compound 1, it represented a better compromise between aqueous solubility and *in vitro* activity. Such compromises may be necessary if formulation techniques cannot be used to obtain good *in vivo* activity. The declaration of Compound 1 as a lead in the hope that formulation techniques might solve the absorption issue could slow development. For this reason, it is essential that preformulation provide the discovery process with rapid feedback regarding the feasibility of formulation solutions that may compensate for poor physical properties and subsequent absorption problems.

A strategy in which discovery and development work in concert is also shown in Figure 38-2. In this scenario, both aqueous solubility and *in vitro* activity were used simultaneously in the search to improve Compound 0. By using dual feedback, the company may have been able to progress more directly from Compound 0 to Compound 2. In addition, knowledge of the aqueous solubilities of all the mass screen hits also may have provided alternative starting points. Instead of picking Compound 0 as the only starting point for synthetic optimization, Compound 3, which was not as active but had much better aqueous solubility, could have been chosen. Dual activity and solubility feedback in the co-optimization of both Compounds 0 and 3 would have been used to guide further synthesis.

The potential time saved using the concerted strategy could be considerable. However, for the whole program to be in synchrony, it will require that preformulation scientists develop high-throughput screening methodologies for physical properties, analogous to discovery's biological screens; high-throughput screening methods can predict the feasibility of a formulation solution for poor absorption. Because technological breakthroughs in the discovery process have increased the number of NCEs that will be candidates for development, it is imperative that new candidates have the requisite physical properties that are needed for rapid development. Otherwise, development may become an unacceptable bottleneck.

Preformulation scientists will have to work proactively with discovery scientists to design active NCEs that are active *and* transportable through biological tissues such as the gastrointestinal (GI) tract or the endothelial cells of the blood–brain barrier. New insight into the molecular basis of physical properties and rapid high-throughput physical property screens are needed to accomplish this goal. The section on *Engineering in the Solid State*, page 714, will discuss briefly some of these areas. In the following sections, characteristics of the solid state are discussed. A fundamental understanding of this state of matter is essential for making timely preformulation decisions.

Critical API Decisions

Once a NCE is selected for development, choosing the molecular form that will be the active pharmaceutical ingredient (API) is a critical milestone because all subsequent development will be affected by this decision. For preformulation, physical characterizations should be focused on making decisions that balance solid-state dissolution properties with material consistency under manufacturing and storage conditions. The advantages of having a rapidly dissolving amorphous state have to be balanced against the potential conversion of this state by time, moisture, and heat to a crystalline state that can be less soluble. Similarly, the increased solubility that often can occur with hydrochloride and sodium salts may have to be balanced with a potential for physical or chemical instability due to moisture and heat. These salts are attractive because they are simple to make and are relatively nontoxic. The salt selection process must project its considerations of the "best" properties to encompass dissolution, physical and chemical stability, toxicology, market-image formulations, large scale manufacturing, and product storage.

The following section will outline solid-state changes that might occur with varying moisture content, pH, and temperature. It will be illustrated that water (moisture) is one of the most important environmental factors that influences solid-state stability. The discussion will then focus on identifying the solid-state properties of an NCE that will make it a viable API. Ultimately, the best balance between absorption and material consistency is sought. Later, the discussion of engineering the solid state will explore why these requisite properties should be designed into NCEs from the earliest stages of discovery.

REQUIREMENTS OF THE SOLID STATE

Challenges to the Solid State

Solids are a complex state of matter because intermolecular forces can arrange the molecules in a variety of different ways, each producing a different solid with potentially different physical properties. In this section, a symbolic nomenclature is introduced to specifically address changes that can occur in the solid state (Table 38-1). Application of this notation to the effects of moisture, the major environmental factor influencing the solid state, will then be examined.

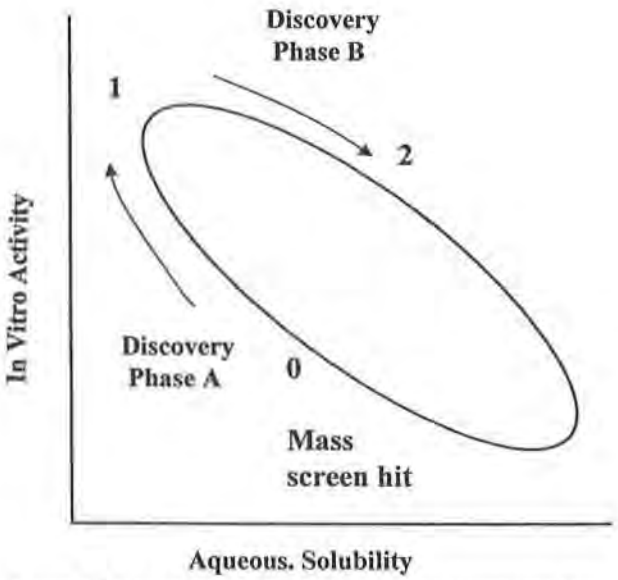


Figure 38-2. Search for an active and orally absorbable NCE.

Table 38-1. List of Symbols

SYMBOL	MEANING
α	Amorphous solid state as left subscript designation
Σ	Surface of solid state as right subscript designation
δ	Defective region of solid state as left subscript designation
ρ	Density
I, II, III	Crystalline polymorphic forms of the solid state as left subscript designation
+	Positively charged, cationic species as superscript designation
−	Negatively charged, anionic species as superscript designation
0	Uncharged, free species as superscript designation
A	Active ingredient in the solid state
a	Dissolved form of the active ingredient
${}_jA^i_\Sigma$	Surface of active ingredient of charge i and solid state j
B	Reactant of A in the solid state
b	Dissolved form of reactant
C_S	Saturation concentration
h	Monohydrate as left subscript designation
$0h$	Anhydrous as left subscript designation
nh	n-Hydrate as left subscript designation
$<h$	Reduced water content as left subscript designation
$>h$	Increased water content as left subscript designation
m	Mass
An^-	Negatively charged anionic counterion
i	Charge on the active ingredient as superscript designation
j	Solid state form of the active ingredient as left subscript designation
k_d	Dissolution rate constant
k_r	Recrystallization rate constant
P	Permeability
Cn^+	Positively charged cationic counterion
S_a	Surface area

SOLID-STATE CHARACTER

In this chapter, ${}_jA^i_\Sigma$ is a notation that will be used to indicate solid-state changes. The A denotes the active drug entity. This may be a weak acid, a weak base, or a nonelectrolyte. When A dissolves, a denotes the presence of this entity in solution; thus, dissolution of the solid A in water to form a will be shown schematically as

$$A \xrightarrow{H_2O} a, \tag{1}$$

The charge of A is denoted by the usual placement of a right superscript, i. The charge of A is assumed to be zero by default. For emphasis, a lack of charge may be shown explicitly as A^0. For a weak acid, A^0 represents the protonated form (in other notations this might be shown as HA). The ionized form of the weak acid, A^-, represents A^0 minus the weak acid proton. For a weak base, A^0 denotes the uncharged base that can be protonated to A^0H^+. Equations with A, shown with arrows, are not stoichiometric. Instead, they only show essential changes, so the focus can be placed on the relevant chemical, ionic, and solid-state alterations in the chemical entity. For example, in Equation 2, in which a chemical reaction changes the parent entity A into a different molecular solid B,

$$A \rightarrow B \tag{2}$$

there is no attempt to show the specific details of the functional groups that were changed to bring about the formation of B. In a similar manner, consider a reversible acid–base reaction

$$A \underset{\leftarrow}{\longrightarrow} A^i \tag{3}$$

where i as a plus sign (+) represents the cationic form, or a minus sign (−) the anionic form, of A. The protonation or deprotonation of a weak basic or acidic group on A will simply be reflected in the charge change that occurs. The scheme is nonstoichiometric because counter ions and charge-balance considerations have not been included.

When a particular molecular organization or emphasis of the solid state is needed, it will be denoted with the left subscript j. A wide variety of different solid states, denoted by ${}_jA$, are possible. For example, amorphous solids that have randomly packed molecules are denoted as ${}_\alpha A$ in this chapter. Crystalline solids, on the other hand, have regular packing arrangements and are denoted in a number of ways. Two types of crystalline phases, polymorphs and solvates, are possible for a given molecule depending on the crystallization conditions.

Polymorphs are crystals that have the same molecule formula but have different crystal structures. The Roman numerals I, II, III, . . . are used to denote polymorphs; the most stable polymorph under ambient conditions is usually designated with Roman numeral I. This solid-state form of A will be denoted as ${}_IA$ in this chapter.

Solvates, on the other hand, are crystals in which a solvent is incorporated into the crystal structure (polymorphs of solvates could exist). The solvent may be highly bound in the crystal or it may be more loosely bound in channels within the crystal. To simplify this discussion, only water of solvation will be considered. Hydrated solids are denoted by ${}_{n\text{h}}A$, where n is a fraction or an integer. For example, ${}_{h/2}A$ denotes a hemihydrate while ${}_{3h}A$ denotes a trihydrate.

In some situations, it will be useful to emphasize that a particular chemical reaction or physical change is occurring on the surface of a particle. For these purposes, the right subscript Σ will be used to emphasize the surface of the solid state. It should be noted that the right superscript i, used for charge designation, and the left subscript j, used for solid-state designation, are only general placeholders for more specific instances that will be detailed below; on the other hand, the right subscript Σ specifically denotes the surface of a solid particle and not a more general entity. For most situations, the full notation will not be used.

In actual APIs, crystal defective regions A_δ are present. These were formed during large-scale synthesis and milling operations that reduced the API's particle size. In Figure 38-3, defective regions as well as crystalline and amorphous regions are shown diagrammatically.

WATER: A MAJOR ENVIRONMENTAL VARIABLE

The presence or absence of moisture is one of the most important environmental factors that can affect solid-state stability. The surface of an API particle can gain or lose water depending on the relative humidity (RH). Figure 38-3 shows how water vapor can form regions of dissolved drug on the surface of the API particle. The amorphous region would be expected to dissolve the fastest, and the crystalline region the slowest; that is, the rank order of dissolution would be $A_\alpha > A_\delta > {}_1A$. In the Figure 38-3 diagram, this is indicated by the font size of the saturated dissolved form of A, a_s, associated with each of these regions. This surface coating results in chemical and physical instability.

Chemical Instability: Water as a Molecular Mobilizer—In general, chemical reactivity is slow in solids because of the spacial separation of different reactive components. For example, if a small amount of an impurity that can act as a catalyst is distributed heterogeneously in an API or a dosage form, the overall rate of reaction is limited because the reaction only occurs in microenvironmental regions. However, in dosage forms, most APIs are usually in contact with moisture-bearing excipients and are stress-tested at elevated temperatures and humidity. The presence of an adsorbed layer of moisture in-

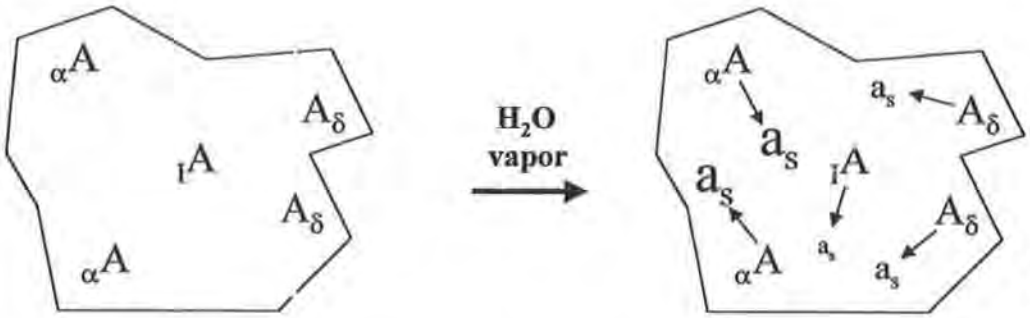


Figure 38-3. Surface of a milled API and dissolution of surface regions due to adsorbed moisture.

creases the catalytic reactivity of the impurity because water, acting as a molecular mobilizer, can transport different chemical species laterally over the surface of the API.[1] Equation 4 shows a chain of reactions from A to a degradant B,:

$$A \xrightarrow{[H_2O]_{low}} a \xrightarrow{[H_2O]_{low},\ catalytic\ impurity} b \xrightarrow{[H^+]_{low}} B \qquad (4)$$

where b is the solubilized form of B. Moisture also induces solid-state changes in A. (Further discussion of moisture-induced chemical instability will be treated in the section *Hydrate Stability: Importance of the Critical Relative Humidity.*)

Microenvironmental pH: Moisture-Induced Sensitivity of Acids/Bases—Acid–base reactivity in the solid-state change will be enhanced by moisture. Equation 5 shows a moisture-induced change of an anionic salt to its free acid on the surface of a drug particle:

$$A_s^- \xrightarrow{[H_2O]_{low}} A_s^0 \qquad (5)$$

Conversely, Equation 6 shows a moisture-induced surface conversion of a cationic salt into its free base,

$$A_s^+ \xrightarrow{[H_2O]_{low}} A_s^0 \qquad (6)$$

where $A^+ = HA^+$. Because the amount of solid drug is large compared to the amount of moisture, Equations 5 and 6 have been diagramed as irreversible reactions. Such solid-state changes can alter the physical properties of the API. For example, if particles of the sodium salt of an insoluble acid form a surface coating of the free acid as in Equation 5, the dissolution rate of the surface will be retarded. Testing methods are needed during the salt selection stage to anticipate this type of solid-state change (see under *Salt Selection*).

Solvent-Mediated Transformations of Polymorphs: Water as a Transporter—If two polymorphic forms can exist at a given temperature, the metastable polymorph will be more soluble (see *Salt Selection*, page 704). When this form is put in contact with water, the following solvent-mediated transformation can be promoted:

$$_{II}A \xrightarrow{H_2O} {}_{I}A \qquad (7)$$

Water, in the vapor phase, has also been shown to be capable of mediating transformations between amorphous and crystalline forms in both directions.[2]

$$_{a}A \xrightarrow[\leftarrow]{[H_2O]_{low}} {}_{I}A \qquad (8)$$

Finally, transformations can occur that incorporate water into the crystal structure. Here, an anhydrous crystalline form is changed into the monohydrate,

$$_{II}A \xrightarrow{H_2O} {}_{h}A \qquad (9)$$

and a salt is transformed into a hemihydrate after passing through the amorphous form:

$$_{II}A^+ \xrightarrow{H_2O} {}_{a}A^+ \xrightarrow{H_2O} {}_{h/2}A^+ \qquad (10)$$

Equations 7 to 10 emphasize solid-state changes. It is likely that most of these transformations may occur only after dissolving and forming a or a species forming a^+.

DECISION-POINTS IN THE DISCOVERY AND DEVELOPMENT OF AN API

The term *active pharmaceutical ingredient* (API), also known as drug substance and bulk pharmaceutical chemical (BPC), highlights both a discovery and a development component. In this section, discovery Steps 1 to 4 will be introduced briefly. The focus will then shift to a detailed discussion of the developmental Steps 5 to 9. Using this background, the section Engineering in the Solid State will outline how early parallel integration of these activities can reduce the time from concept to market.

The term *expansion* is used when choices are being enlarged, and *selection* is used when choices are reduced by decision-making. Ultimately, the expansion and selection phases of discovery lead to a single choice, the best candidate for further development.

1. *Library expansion* refers to additions to a company's chemical library. Established pharmaceutical companies have amassed hundreds of thousands of compounds through previous discovery efforts. These collections are cataloged carefully and are used systematically in mass screens.
2. *Series selection* is a decision-making process in which the most active chemicals in the library are identified using a high-throughput biological assay. Typically, these assays are used to detect the ability of a small molecule to interact with a protein, *in vitro*. In the past, decisions regarding which leads will be pursued further were made based on activity, chemical diversity, patentability, and analog synthetic potential. Today, developmental potential increasingly is part of series selection decision-making.
3. *Analog expansion* is the increase in the number of compounds targeting a specific activity based on synthetic exploitation of the most promising leads.
4. *Analog selection* is the decision-making process in which the best new chemical entity is chosen for further development. In the past, *in vitro* activity alone was the dominating decision-maker; today, a blend of developmental issues are surfacing earlier.

Preformulation, as well as other areas of development such as metabolism, toxicology, and pharmacokinetics, will play an increasingly important role in Steps 1 to 4. Because a fundamental understanding of the solid state is essential for designing appropriate physical property methodologies for Steps 1 to

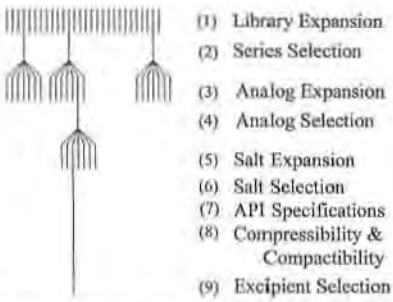


Figure 38-4. Typical API sequential decision-making: selection and expansion cycles.

4, the remainder of this section will deal with how solid-state properties affect absorption and consistency, the two major development issues for an API. Salt selection, which determines the character of $_{f}A^{i}$, is the first critical solid-state decision for preformulation in the developmental arena.

Salt Expansion: Exploring the Molecular Possibilities of A^i

The un-ionized (free) form of weak acids and bases, A^0, may not be the ideal molecular form for development. During the salt expansion Step 5 of Figure 38-4, salts are prepared to explore whether one of them would make a more suitable API. Salts are formed by reacting A^0 with an appropriate counter-acid or counter-base. In this discussion, HA*n* is used to represent a counter-acid that forms an anion An^-. Common counter-acids like HCl and maleic acid are listed in Table 38-2. Similarly, CnOH is used to represent a mineral base of counter cation Cn^+. Common mineral bases like NaOH and KOH are also shown in Table 38-2 along with organic counter-bases.

When A^0 is a weak base, the salt, $(A^0H)^+ An^-$, is composed of the protonated form of the base, $(A^0H)^+$ and the ionized form of the counter-acid HA*n*, An^-. For salt formation, A^0 must be sufficiently basic to remove the proton from HA*n* (see *Salt-Forming Reactivity Potential*, page 705).

Salts have different physical properties than their free forms. Salt selection explores whether a particular salt might have properties that are more appropriate for an API than its parent form. Improving oral absorption by increasing the dissolution rate is often a goal of the salt expansion step. Salts generally dissolve faster in water than their free forms because dissolution is enhanced by the rapid hydration of the ionized salt species with water. Salts of weak bases generally lower the pH of water; salts of weak acids elevate it. For the salt of a weak base in water, the initial dissociation of the salt into the two ions, A^0H^+ and An^- is relatively complete. On the other hand, the deprotonation of A^0H^+ depends on the pK_a of A^0, as shown by these reactions:

$$A^0H^+An^- \longrightarrow A^0H^+ + An^- \quad \text{and} \quad A^0H^+ \underset{\text{high } pK_a}{\overset{\text{low } pK_a}{\rightleftharpoons}} A^0 + H^+ \qquad (11)$$

It is the release of the H^+ in the second reaction by the salt that lowers the pH and increases the solubility (see *pH-Solubility Profiles*, page 717). Hydrochlorides are the most common salts of weak bases.

When A^0 is a weak acid, the salt that forms from a reaction with CnOH is $A^- Cn^+$ (A^- represents A^0 minus a proton). The most common salts for weak acids are the sodium salts.

Even though salts increase aqueous solubility, they only alter the pH of the solution so that more of the ionized form is present in solution. Salts do not change the ionizable character of the free form; this is an intrinsic property of the free acid or free base and their associated pK_a(s). pH-solubility profiles show the solubility relationship between salts and their free forms.

Table 38-2. Molecular Forms Marketed Worldwide Between 1983–1996

SALT FORM	FREQ.	GROUP[a]	pK_a	clogP	MW
No salt form	390	0			
Hydrobromide	1	1	−8	0.45	80.91
Hydrochloride	102	1	−6.1	0.24	36.46
Sulfate	5	1	−3	−1.58	98.08
Nitrate	6	1	−1.44	2.09	63.01
Phosphate	2	1	2.15	−1.95	96.99
Glucuronate	1	1	3.22[b]	−3.74	194.14
Acetate	8	1	4.76	−0.36	59.05
Maleate	3	2	1.92	−0.18	116.07
Fumarate	8	2	3.02	−0.18	116.07
Tartrate	1	2	3.03	−2.21	150.09
Citrate	1	2	3.13	−2.11	189.10
Succinate	2	2	4.21	−0.62	118.09
Mesylate	8	3	−1.20	−1.31	96.11
Acistrate	1	3	4.91[b]	7.98	284.49
Besylate	2	4	−2.80[b]	0.23	157.17
Tosylate	3	4	−1.34	0.88	171.20
Xinafoate	1	4	2.66[b]	3.00	188.18
Potassium	1	1	16		39.10
Sodium	37	1	14.77		23.00
Tromethamine	2	1	8.07[c]	−3.17	121.14
Bismuth	1	1	1.58		208.98
Bromide	6	5			79.90
Chloride	2	5			35.45

[a] Groups: 0 = No salt, 1 = Polar, 2 = Multifunctional, 3 = Flexible aliphatics, 4 = Planar aromatics, 5 = Quartenary.
[b] Calculated pK_a.
[c] *CRC Handbook of Basic Tables for Chemical Analysis*, page 469.
Source: Serajuddin ATM, Sheen P, Augustine MA. To market, to market. In: Bristol J, ed. *Annu Rep Med Chem*. New York: Academic, 1983–1996.

pH-SOLUBILITY PROFILES

For a weak base, a plot of solubility versus pH will show the highest solubility at low pH and the lowest solubility at high pH; for weak acids, the opposite is true. Such plots give a graphic view of the impact of ionization on solubility for an NCE. The pH range of the small intestine, where oral absorption generally occurs, is approximately 6.5 to 8. It is undesirable to have a compound totally charged or uncharged in this region. If it is entirely charged, there are no un-ionized species that can be transported across the GI membrane. If it is totally uncharged, there are no charged species to enhance solubility. For a monoprotic NCE, the pK_a denotes the pH where the number of charged and uncharged species in solution are equal. On the ionized side of the pK_a, the solubility of the salt limits the maximum solubility. The solubility decline at very low pHs is due to activity and solubility-product effects.[3–5] On the un-ionized side, the solubility of A^0 (the intrinsic solubility) marks the lowest solubility. Salts promote a saturated solution to be formed at a pH that is on the ionized side of the pK_a. They cannot alter the pK_a or the intrinsic solubility. Using these parameters, a qualitative pH-solubility profile can be constructed. Figure 38-5 shows pH-solubility profiles for different counter-acid salts.

The synthesis of salts depends on

1. A proton-exchange reactivity between A^0 and the counter-acid/base
2. A long-range order that permits crystal formation.

The discussion that follows will focus on forming salts from weak bases, because they comprise the majority of the new drug candidates. Weak acids would be treated analogously.

SALT-FORMING REACTIVITY POTENTIAL

In order for a salt to form, both the weak base, A^0, and the counter-acid, HAn, must have sufficiently different pK_a values such that a Brönsted-Lowry proton transfer from HAn to A^0 can take place. Table 38-2 gives potential counter-ions and their pK_a values from a listing of all drugs approved worldwide from 1983 to 1996. An acid–base proton transfer should be possible as long as the pK_a of HAn is less than that of the weak base A^0 (recall that the pK_a of A^0 is referenced to its protonated form A^0H^+; see *Solid-State Character*, page 702). If ΔpK_a is defined as

$$\Delta pK_a = pK_a \text{ (weak base)} - pK_a (HAn) \quad (12)$$

a salt-forming reaction should be possible as long as ΔpK_a is positive. For example, a succinate salt (pK_a 4.2) with doxylamine (pK_a 4.4) is possible[6] where the ΔpK_a is 0.2. Nevertheless, the greater the ΔpK_a, the greater the probability that a salt can be formed. Because the pK_a values in Table 38-2 are calculated for an aqueous environment, this rule must be used only as a guide for salt-forming reactivity in organic solvents. In an organic solvent in which the dielectric constant is lower than water, the ionization equilibria would be shifted:

$$HAn \xleftarrow{\text{low dielectric solvents}} H^+ + An \quad (13)$$

$$AH^+ \xrightarrow{\text{low dielectric solvents}} H^+ + A^0 \quad (14)$$

For acridine bases, 50:50 ethanol:water weakens the aqueous pK_a by 1.41 pH units. For the counter-acid, HAn, pK_a weakening is greater than for the protonated base, A^0H^+, because of the greater solubility of HAn in the organic phase and the production of two charges upon ionization. The net effect of organic solvent weakening is to reduce the pK_a difference between the counter-acid and the weak base. This lowers the salt-forming reactive potential. Therefore, in a given organic solvent, if salt formation fails to occur for a particular aqueous ΔpK_a, it is unlikely that salts can be formed in this organic solvent with a smaller aqueous ΔpK_a.

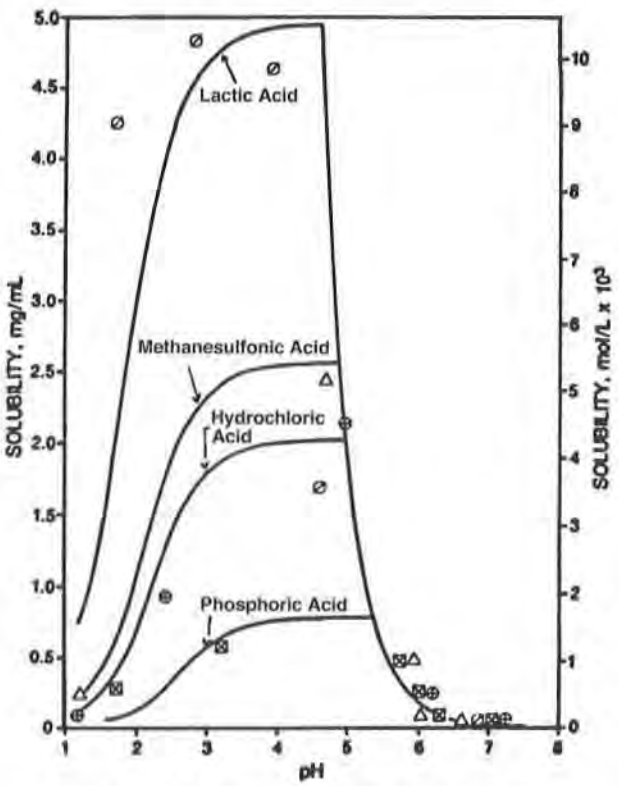


Figure 38-5. pH solubility profile of a weak base.[3]

VARYING SALT PROPERTIES USING COUNTER-ACID GROUPINGS

For weak bases, salt-forming counter-acids can be used to alter an API's solubility, dissolution, hygroscopicity, stability, and processing.[6] Table 38-2 shows counter-acids organized into different functional groups. For each counter-acid, both the pK_a and the log P is given where appropriate. A starting point for salt expansion must begin with the properties of A^0. If, for a weak base, $\Delta pK_a = pK_{a\ A^0} - pK_{a\ \text{counter-acid, } HAn} > 0$, then aqueous salts may be possible. Use of this table and the influence of different counter-acids are covered under *Decision-Tree, Goal-Oriented Approach*, page 712.

CRYSTAL FORMATION REQUIREMENTS

In general, crystalline solids, including salts, make the most promising APIs. The amorphous form of the solid state is usually not as stable as crystals, either physically or chemically. Crystal formation is a special characteristic of a solid in which the molecules self-organize into regular, repeating, molecular patterns. Solvents play at least three roles in crystallization.

1. They provide some solubilizing capacity so that concentrated solutions can be formed.
2. They promote the nucleation process. Nucleation may be from a pure solution (homogeneous nucleation) or from a seed crystal (heterogeneous nucleation). If a solvent binds too strongly to the molecular organizing functionalities of the salt or seed crystal, crystallization will be impeded. Finding appropriate solvents for crystal formation is a very important step in salt expansion. Failure to adequately explore and find solvents that can crystallize salts could mean that very usable salts would not be evaluated in the salt-selection step because they were not synthesized.

3. Solvents, temperature, and cooling rate can impact the crystal-packing pattern of crystals. Stable polymorphic forms usually are desired for APIs. Metastable forms are normally avoided in an API because they are prone to physical and chemical instability. Solvent conditions that promote metastable and stable crystal formations will be explored under *Metastable Polymorph Formation*, page 710.

Salt Selection: Choosing the "Best" API

Salt selection is the first important API decision from the development perspective. Once a salt is chosen, time-consuming and lengthy toxicological studies are initiated that would have to be repeated if the salt form is changed. This decision involves choosing a solid-state phase, $_jA$, which balances potentially conflicting needs: increasing absorption versus maintaining an API that is consistent and can be manufactured in a market-image dosage form (see *Compressibility and Compactibility*, page 712). Figure 38-6 shows some of the factors involved in this decision.

Permeability, solubility (C_S), and pK_a are intrinsic properties of A^0 that have been already determined in the analog selection phase (see Fig 38-4). The major dependent variables, absorption and consistency of the API, can be manipulated and balanced in salt selection. In the following sections, the impact of dissolution and particle size on absorption will be explored. In addition, the consistency of the API solid state under the influence of environmental destabilizing factors—such as exposure time (t), ultraviolet light (UV), pH, moisture (H_2O), temperature (T), and pharmaceutical processing operations like milling, compression, and compaction—will be considered.

ABSORPTION ASSESSMENT

Oral absorption is generally viewed as two-step, sequential process:

$$A_{solid} \xrightarrow{\text{dissolution}} a_{GI\ tract} \xrightarrow{\text{permeation}} a_{blood} \qquad (15)$$

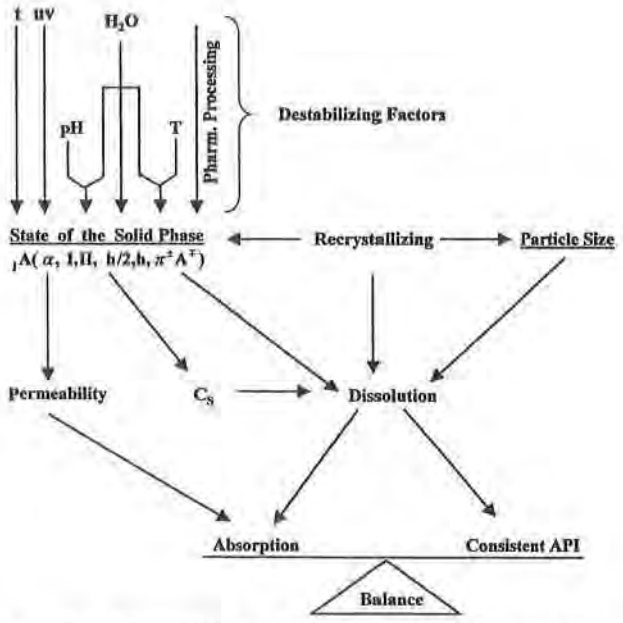


Figure 38-6. API salt selection decision: a balance between absorption and consistency.

Either dissolution of solid drug, A_{solid}, after the dosage form disintegrates in the GI tract, or the permeation of the dissolved drug, $a_{GI\ tract}$, through the GI membrane could be the slowest process. The slower of these two steps determines the overall rate of absorption and is thus rate-limiting.

Dissolution-limited absorption occurs when the rate of appearance in the GI tract by dissolution (a_{GI}) is slower than the rate of appearance in the systemic system (a_{blood}); *permeation-limited* absorption occurs when the a_{blood} appearance is the slowest process. The impact of these two rate processes on *in vitro–in vivo* (IVIV) correlations will be discussed in the section *Biopharmaceutical Classification of API*, page 714. Dissolution-limited absorption will now be considered.

The rate of dissolution of a particle is given by the Noyes–Whitney equation,

$$dA/dt = k_d S_a [C_S - C_{bulk}] \text{ (non-sink conditions)} \qquad (16)$$

where

A is the amount of drug dissolved.
dA/dt is the rate of dissolution (Q sometimes is used for this rate).
k_d is the intrinsic dissolution constant for the drug.
S_a is the total surface area of the dissolving particle.
C_S is the saturation solubility of the drug at the surface of the particle.
C_{bulk} is the concentration of the drug in the bulk solution.

Because the rate of dissolution depends on the concentration difference between C_S and C_{bulk}, the maximum rate of dissolution would occur if $C_{bulk} = 0$ (ie, if drug was removed from solution as fast as it dissolved). This would be analogous to a sink that could drain the water coming out of a water faucet as fast as it comes in so that the water level never built up. This analogy is the basis for referring to Equation 16 as nonsink conditions for dissolution, because drug does build up in the solution and the rate of dissolution is correspondingly reduced.

The expression for the maximum dissolution rate is found by setting C_{bulk} equal to 0:[7]

$$dA/dt = k_d S_a C_S \text{ (sink conditions)} \qquad (17)$$

This initial rate of the Noyes–Whitney equation is termed sink conditions for the dissolution rate.

Particle-Size Effects—For a spherical drug particle of radius r, amount m, and of density ρ, Equation 17 can be rewritten as

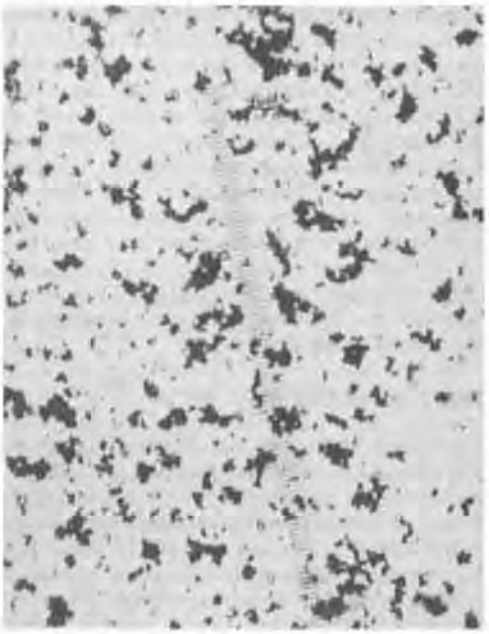
$$dA/dt = (3k_d m/\rho)\,(1/r)\,C_S \qquad (18)$$

This expression emphasizes the inverse relationship between the dissolution rate, dA/dt, and the particle size r, assuming no dissolution rate-reducing factors are present such as adsorbed air bubbles or aggregated particles.

Smaller particles dissolve faster than larger particles. Thus milling, a pharmaceutical unit-operation, increases dissolution because the API particle size is reduced. On the other hand, when drug particles are suspended in an aqueous solution, particles can increase in size due to recrystallization growth[8] (Fig 38-7). Dosing such suspension orally would be expected to reduce absorption because of a reduction in the dissolution rate.

Reactive Media 1: Implications for Salts of Weak Acids and Weak Bases—When a drug reacts with gastric fluids, its dissolution deviates from Equation 17. For dissolution in 0.1 N HCl, acid–base reactivity is most important for salts of weak acids and for free bases. It has been found that the low pH environment of the stomach dissolves a salt of a weak acid 10 to 100 times faster than the weak acid itself.[9] On the other hand, it is the free base, and not its HCl salt, that dissolves faster in this same environment.[10] These deviations from Equation 17 have been shown to be due to differences between bulk-solution pHs and the pH at the surface of the drug particle. Thus, Equation 17 becomes

$$dA/dt = k_d S_a C_{S,h=0} \qquad (19)$$



FORM I

INITIAL SUSPENSION

FORM I

SUSPENSION AFTER 6 HOURS.

Figure 38-7. Photomicrographs showing change in crystal size for a suspension of Form I of an experimental drug.

where $C_{S,h=0}$ is the saturation solubility at the surface of the API.

For weak acid salts, the surface pH has been calculated to be 6.2 to 6.5 for sodium salicylate (pK_a 3.0) and 10.3 for sodium theophylline (pK_a 8.4) in bulk solutions having pHs of 1.10 and 2.1, respectively. On the other hand, the weak base phenazopyridine (pK_a 5.2) sees a surface pH of 3.3 to 3.6, while its HCl salt sees a surface pH of 1.2 for a bulk-solution pH of 1.10. If the solubility due to surface pH and not the pH of the bulk is considered, deviations from Equation 17 become understandable. For the HCl salt, the common-ion effect reduces its solubility from the maximum solubility of the pH-solubility profile at 3.45. Thus, the nonaggregated free base, in this situation, has a surface pH that is optimized to give the highest dissolution rate because it has the highest surface solubility.

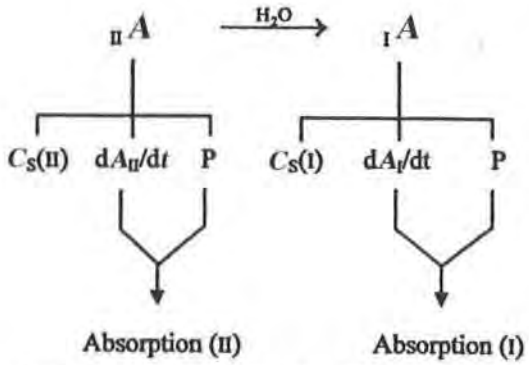


Figure 38-8. Absorption changes due to aqueous-phase transformations.

Reactive Media 2: Implications for Anhydrates and Metastable Polymorphs—Aqueous-phase transformations are solid-state changes in which water acts as a mediator. During the transition from one form to another, dissolution behavior will reflect the switch from the dissolution rate of the initial solid state to that of the more stable state. Two types of aqueous-phase transformations were introduced in Equations 7 and 9: (1) a transformation from Polymorph II to Polymorph I and (2) a transformation from an anhydrous Form II to a hydrated form *h*.[11] In Figure 38-8, the transformation of Equation 7 is shown.

Because the permeability (P) of the dissolved drug is the same for the different crystalline forms, the impact on absorption will be due to differences in their solubilities (C_S) as defined in Equation 17 and thus will be reflected in the dissolution rates, dA_I/dt and dA_{II}/dt, being different.

When a solvent-mediated transformation like that shown in Equation 9 occurs, dissolution profiles become more complex. Figure 38-9 shows the biphasic dissolution characteristics for Equation 9. In this situation, an anhydrous substance, $_{0h}A$,

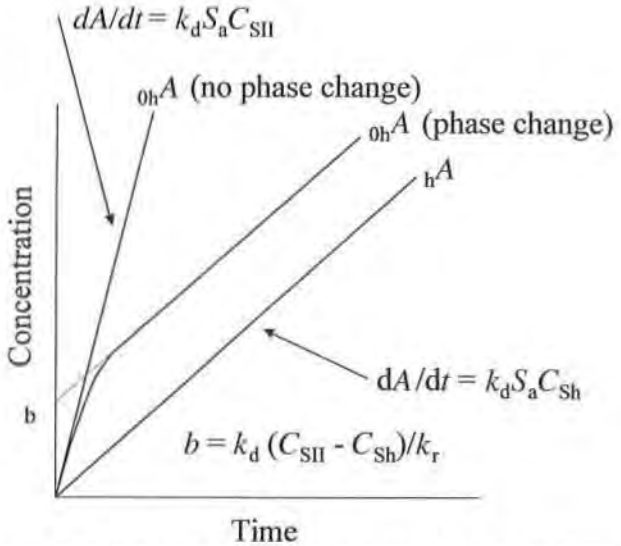


Figure 38-9. Biphasic dissolution of anhydrous to hydrous forms.[11]

becomes hydrated as it dissolves and forms a surface layer of ${}_hA$. It is this latter layer that controls subsequent dissolution. The concentration versus time plot for the net reaction is ${}_{0h}A$ (phase change). Note that initially the slope for ${}_{0h}A$ (phase change) approaches that of the very steep slope ${}_{0h}A$ (no phase change), and that the terminal slope approaches that of ${}_hA$ (no phase change), the hydrated form. Modifications of Equation 17 to take into account surface recrystallization of ${}_hA$ on ${}_{0h}A_\Sigma$ give the biphasic dissolution behavior,

$$dA/dt = k_d S_n\{C_{S_{II}}e^{-k_r t} + C_{S_h}[1 - e^{k_r t}]\} \quad (20)$$

where k_r is the recrystallization rate constant for the second phase, k_d is the intrinsic dissolution constant, C_{SII} is the saturation concentration for the first phase, and C_{Sh} is the saturation concentration for the second hydrate phase.[12]

Enhanced and Retarded Dissolution Due to Sinks and Plugs—The increase in dissolution due to the particle-size reduction of an uncharged API, A^0, can be estimated from Equation 18. Equation 21 shows the resulting surface area increase, $\Sigma\uparrow$, and the corresponding dissolution enhancement.

$$A^0_\Sigma \xrightarrow{\text{milling}} A^0_{\Sigma\uparrow} \xrightarrow{\text{faster}} a^0_S \quad (21)$$

This enhancement, however, is assumed to be under sink conditions and is driven by $C_S = a^0_S$ in Equation 17. If the concentration of drug does build up, dissolution is reduced by and is given by Equation 16. This slower dissolution is diagramed in Equation 22 where $a^0_{\text{bulk}}\uparrow$ indicates the buildup of the drug in the bulk solution.

$$A^0 \xrightarrow{\text{slow}} a^0_{\text{bulk}}\uparrow \quad (22)$$

An ionizable drug, on the other hand, reduces a^0_{bulk}, which is indicated by $\downarrow$ in Equation 23 because it is rapidly converted to a^+_{bulk}, the ionized form. Thus, the ionized form ($a^+_{\text{bulk}} = a^0_{\text{bulk}}H^+$) acts as a sink to remove a^0_{bulk} and promotes the dissolution of A^0 by driving the reaction to the right:

$$A^0 \xrightarrow{\text{fast}} a^0_{\text{bulk}}\downarrow \xrightarrow{\text{very fast}} a^+_{\text{bulk}} \quad \text{(sink)} \quad (23)$$

Reduction of dissolution, on the other hand, can occur for an anhydrous API when the hydrated form recrystallizes on the surface as in Figure 38-9. This effect is the opposite of the sink concept, hence the term plugging. Equation 24 show the species involved in plugging. The subscript Σ emphasizes that this is a surface phenomenon.

$${}_{0h}A_\Sigma \xrightarrow{\text{slow}} a_{\text{bulk}} \xrightarrow{\text{recrystallization}} {}_hA_\Sigma \xrightarrow{\text{slower}} a_{\text{bulk}}\downarrow \quad \text{(plug)} \quad (24)$$

Acceptance Criteria Guidance—A simple model to assess the impact of particle size on dissolution and absorption of a non-ionized drug considers the intestine as a single compartment.[12] If the number of particles of uniform size at time t is

$$N(t) = N_0 e^{-Qt/V} \quad (25)$$

where N_0 is the initial number of particles, Q is the flow rate out of the intestine, and V is the intestinal volume, then the surface area for spherical particles of uniform size, r, as a function of time can be given by

$$S_n = 4\pi r^2(t)N(t) \quad (26)$$

This expression can then be used in the non-sink dissolution expression of Equation 16, with certain assumptions including linear intestinal absorption, to approximate the fraction absorbed as

$$F \propto \frac{k_a X_d \tilde{t}_r}{X_0} \quad (27)$$

where k_a is the absorption rate constant, X_0 is the administered dose, X_d is the amount of drug dissolved in the GI tract at $\tilde{t}_r$, and $\tilde{t}_r$ is the GI transit time. Further refinements to this model include accounting for polydispersed spherical powders and comparing cylindrical with spherical shape factors, with and without time-dependent diffusion layer thickness.

Finally, for poorly soluble drugs, simulated dose absorption studies have been carried out over different ranges of solubility, absorption rate constants, doses, and particle sizes. Table 38-3 shows the percent of drug absorbed for a drug that has a solubility of 10 μg/mL with a k_a of 0.01 min^{-1}. Note that, even though particle-size reduction from 100 to 10 μm increases the percent absorbed, as the dose increases, the impact of this reduction decreases dramatically.

CONSISTENCY ASSESSMENT

Polymorphic Stability: Importance of the Transition Point—Polymorphic systems, in which different crystalline forms of the same molecular composition can exist, vary in their ability to interconvert at different temperatures. The enantiotropic/monotropic classification is based on the observation that some systems can reversibly interconvert and some cannot. In enantiotropic systems, reversible interconversion between the different forms is possible. For monotropic polymorphic systems, interconversion is only possible in one direction, from a metastable form to a more stable form.

For enantiotropic systems, a critical temperature exists, the transition point, T_p, at which the rate of conversion from one form to another is equal. At temperatures below T_p, one form is more stable; at temperatures above T_p, another form is more stable (see the section *Solid-State Character*, page 702; the convention of designating Form I as the most stable polymorph breaks down for such systems because Form I cannot be the most stable form *both* above *and* below T_p).

Figure 38-10 shows a solubility versus temperature diagram for an enantiotropic polymorphic system.[13,14] For the enantiotropic system on the left, at constant pressure, there are three solubility versus temperature curves: Form II is the lowest, Form I is the next higher, and the melting curve is M. The critical temperature, T_p, occurs at the intersection of the Form II and I curves. At this point the solubilities of Form II and Form I are equal and the interconversion rate in any direction is zero.[14] Below the T_p, Form I interconverts to Form II; above the T_p, Form II converts to Form I. The melting point of Form I occurs at the intersection of the Form I curve and the melting curve M.

Because enantiotropic forms show a change in relative physical stability as temperature is changed, it is important to anticipate the impact of temperature on stability. An early warning sign that one is dealing with an enantiotropic system can be found by relating solubilities with thermal parameters. The higher melting Form I has a smaller heat of fusion. Equation 28 gives the relationship between the solubilities,

$$\ln\left[\frac{S_I(T)}{S_{II}(T)}\right] = \left[\frac{\Delta H_{II} - \Delta H_I}{RT}\right]\left[\frac{T_m - T}{T_m}\right] \quad (28)$$

where S_I and S_{II} are the solubilities and ΔH_I and ΔH_{II} are the heats of fusion of Forms I and II, respectively.[15] The more

Table 38-3. Reduced Absorption with Increasing Particle Size for a Poorly Soluble Drug

	PERCENT OF DOSE ABSORBED			
DOSE	10 μm	25 μm	50 μm	100 μm
1	91.3	66.9	38.5	17.5
10	70.0	50.0	30.7	15.4
100	9.0	8.7	8.0	6.3
250	3.6	3.6	3.4	3.1

Source: Johnson KC, Swindell AC. *Pharm Res* 1996; 13: 1795.

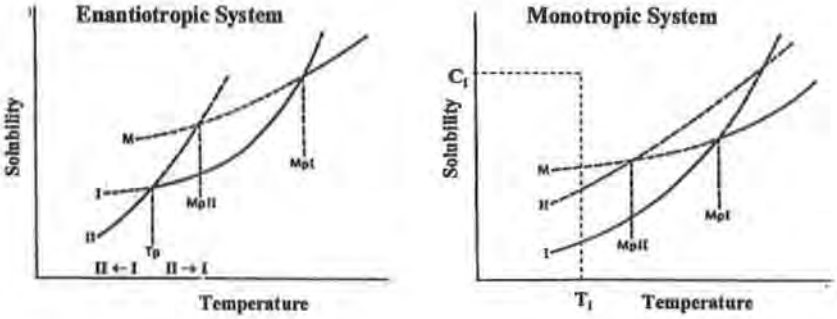


Figure 38-10. Thermal stability of polymorphic systems.[13,14]

stable form at a given temperature will have lower solubility at that temperature.

Enantiotropicity exists only when the transition point is below the melting point of Form I (see Fig 38-10). However, if a transition point is not found below the melting point of Form I, it does not mean that the system is monotropic.[14] The transition point, for example, could be below the lowest temperature studied.

For monotropic systems, interconversion is always from the metastable Form II to Form I. The solubility curve of Form II is always above that of Form I, and a transition point does not exist because a crystal cannot be heated above its melting point (see Fig 38-10). Oswald's Law of Stages dictates that if a system is supersaturated with respect to Form II at concentration C_i and T_i, the metastable Phase II will be the first solid phase that appears.[16] As Form II continues to crystallize, the supersaturation is reduced until it reaches its solubility. At this point, although there is no longer a driving force to crystallize more Form II, the solution continues to be supersaturated with respect to Form I. Thus, crystallization of Form I occurs at the expense of the dissolution of Form II.

Polymorphic Solubility: Difference Between Equilibrium and Dissolution-Based Solubility—Assume Polymorphs I and II are possible for an NCE. Oswald's Law of Stages tells us that a supersaturated solution will first crystallize out as Form II and then ultimately Form I. Thus, the thermodynamic equilibrium solubility will be limited by the solubility of Form I. However, because the rate of nucleation of II and I is a function of a wide variety of variables, equilibrium solubility is not an especially useful parameter in estimating the impact of a polymorph form on the absorption of drug from a dosage form. A dissolution-based solubility definition is more useful in this regard. How might such a solubility be defined?

Because the metastable state Form II has a faster dissolution rate, $dA/dt_{II} > dA/dt_{I}$, where it is assumed that dissolution is carried out under sink conditions of Equation 17. Because $dA/dt = k_d S_a C_S$, we can conclude that $C_S(\text{II}) > C_S(\text{I})$ if we assume that S_a and k_d are the same for both polymorphs. Thus, Equation 17 provides a working definition for the solubility differences between Polymorph II and Polymorph I, and it provides a method for measuring them from dissolution experiments. More precisely, it provides the solubility at the surface of the API, which is the solubility that is most relevant for dissolution (see the section *Reactive Media 1*, page 706).

Polymorph Characterization Techniques—At a given temperature, a fluid-phase transformation can cause a metastable polymorph to change into a more stable, less soluble polymorph. Using a hot-stage microscope, fluid-phase transformations as a function of temperature can be observed.[14] As the temperature is varied, the more soluble polymorph dissolves and the less soluble one grows. If a temperature can be found at which both polymorphs have the same solubility, then the system is enantiotropic, and the temperature is the transition point, T_t. Plots similar to Figure 38-10 can be constructed qualitatively in which the intersection is the measured transition point. These plots are important because they tell which form is most stable at low temperatures, and whether the system is enantiotropic.

Differential scanning calorimetry (DSC) is another characterization tool that is commonly used. It can measure heat changes that occur when a solid undergoes phase transitions. Melting of a solid into a fluid, for example, requires an influx of heat into the crystal. Two techniques are useful for detecting polymorphic systems using DSC: scanning-rate variation and temperature cycling.

Scanning-rate variation has been shown to detect some reversible polymorphic systems. In Figure 38-11, crystallization of the more stable polymorph shows up as exothermic depressions as the scanning-rate increases.[17] Hot-stage microscopy can be used to confirm these thermal changes.

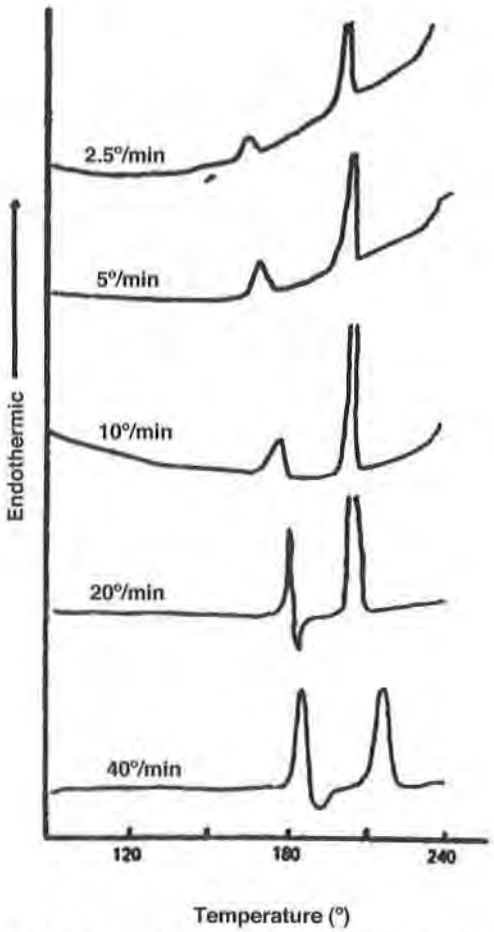


Figure 38-11. Detection of polymorphs by varying the DSC scanning rate.[17]

Temperature cycling using DSC also can be used to study the relative interconvertability of crystalline forms. A loss of the metastable, lower melting point polymorph of metoclopramide base was found after heating, cooling, and then reheating.[18] The more stable polymorph can often be observed as exotherms due to crystallization after heat–cool cycles.[19] In addition, storage of a metastable polymorph below the melting point of either polymorph can result in the formation of the more stable polymorph. For gepirone hydrochloride, this occurred after a heat treatment of 3 hours at 150°.[17]

Powder X-ray diffraction is the most powerful method for detecting polymorphs. Because different polymorphs have different crystal structures, the packing patterns of their atoms are different. Powder X-ray diffraction detects these packing differences as differences in diffraction patterns. Comparisons of diffraction scans between different polymorphs show characteristic differences that can be used for identification (fingerprinting) purposes.

Single-crystal X-ray diffraction is the most definitive characterization tool because the exact relative locations of atoms in the molecular crystal can be determined. However, most often, high-quality crystals for this type of analysis are not available from the bulk API (especially if the material was milled). Recrystallization of suitable crystals from saturated solutions may be possible. If the single-crystal X-ray diffraction problem can be solved, programs are now available that can convert single-crystal diffraction data to a powder X-ray diffraction pattern. This is necessary to ensure that the recrystallization process has not grown a new polymorph.

Solid-state nuclear magnetic resonance (NMR) is also a powerful technique for studying polymorphic systems. In this technique, a powder sample must be rotated at a special angle (the *magic angle*) with respect to the magnetic field so that preferential orientations of the powder particles are averaged. Microcalorimetry also has been used to characterize the thermodynamic properties of different polymorphs. Finally, diffuse reflectance infrared Fourier-transform spectroscopy recently has been used to quantify binary mixtures of polymorphs using the partial least-squares method for spectral analysis.[20]

Metastable Polymorph Formation—Exploring the potential that a given salt has for polymorph formation is a very important aspect of salt selection. It is important that the choice of the final molecular form be based on as much information as possible. Other factors being equal, a molecular entity that forms polymorphs is generally not as desirable as one that does not, because of the potential interconversion of polymorphs and a change in an API's dissolution. This could cause consistency problems both in the API and in the dosage forms. Special techniques are used to attempt to synthesize metastable polymorphs. Preparation of metastable polymorphs requires:

1. Supersaturating conditions for the metastable form, ${}_{II}A$.
2. Crystallization of the metastable state before the stable polymorph forms.
3. Stable conditions for the metastable polymorph so that conversion to the stable ${}_{I}A$ form is prevented.

These steps are shown in Figure 38-12.

For a monotropic system, the metastable state can only change to the stable state; for an enantiotropic system, the transition point is critical for interconversion. Therefore, the formation temperature should be as far above the transition point as practical.

The ideal solution conditions to prevent ${}_{II}A$ from converting to ${}_{I}A$ are such that the solution phase, a, should be highly supersaturated, of a small volume, and in a relatively poor solvent. Rapid cooling is the method of choice for maintaining supersaturation with respect to ${}_{II}A$. To help ensure that the rate of metastable crystallization is much greater than the rate of thermodynamic equilibration, small volumes and poor solvents for ${}_{I}A$ are used. The use of dry ice for rapid

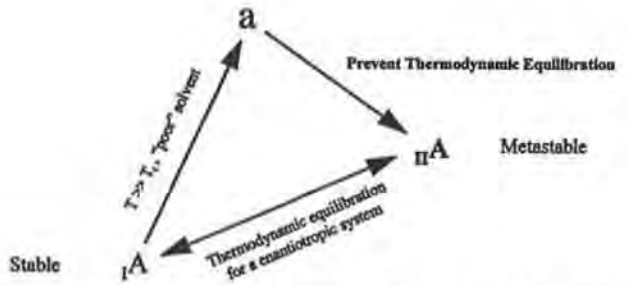
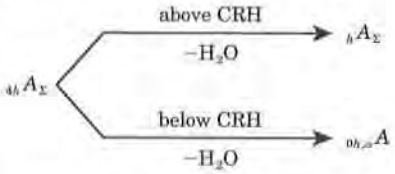


Figure 38-12. Formation of a metastable polymorph in a monotropic system.[14]

cooling with alcohol or acetone is common for these purposes. Once crystallization from the saturated solution phase, a, has occurred, it is important to filter and dry the precipitate as quickly as possible to prevent a fluid-phase transformation to the stable polymorph. Alternatively, if ${}_{I}A$ can be melted without degradation, complete melting and rapid cooling of the melt is an another method of forming metastable forms. This avoids two major problems of solution-phase metastable polymorph formation—filtration and drying, both of which can promote interconversion.

Hydrate Stability: Importance of the Critical Relative Humidity —Relative humidity (RH) is the percentage of the maximum amount of moisture that air can hold. A substance is hygroscopic when it takes up this moisture from air. For a drug substance, the RH that is in equilibrium with a saturated aqueous solution of a solute is termed the critical relative humidity (CRH).[21] It is a key parameter that can influence the physical stability of solid-state hydrates. A number of studies have shown that the gain or loss of water from a hydrate can center on the CRH. Because water in organic crystals is never a passive entity (see *Hydrate Formation*, page 711), solid-state changes in the crystal are very likely to follow.

For the tetrahydrate sodium salt of a tetrazolate derivative, a number of different solid-state forms are possible.[22]



(29)

The conversion of ${}_{4h}A$ to ${}_{h}A$ requires elevated temperature and a RH above the CRH. Water's plasticizing action in reducing the intermolecular H-bonding between adjacent molecules is believed to be the mechanism that facilitates the solid-state transformation to the more stable ${}_{h}A$ crystal form.[23] Similarly, elevation of both temperature and RH were required to convert the ${}_{0h}A$ form of paroxetine HCl to the ${}_{0.5h}A$ form.[24] Water also promoted a solid-state transformation of the ${}_{a}A$ form to the ${}_{0h}A$ form of a disodium leukotriene antagonist. The amorphous form initially picked up a small amount of water (2%) and then slowly released this water as the anhydrous form was formed. Conversely, the humidity-mediated conversion from ${}_{II}A$ to ${}_{\alpha}A$ has been observed for another leukotriene antagonist.[25] Difficult hydrate situations have been dealt with by carefully defining the RH ranges of different species and setting specifications consistent with typical manufacturing environments.[26]

In general, hydrates that are more closely packed tend to be more physically stable with respect to moisture loss. The ideal solid state is one that is stable over a wide range of RH, such as the ${}_{0.5h}A$ form of paroxetine HCl.[24] For the sodium salt of the tetrazole derivative shown in Equations 29 and 30, the denser

$_hA$ structure is physically more stable than the $_{4h}A$ structure. The latter loses four water molecules from crystal channels at a significantly lower temperature than the one water molecule of the $_hA$ form, which is integrated into the crystal structure in a more cohesive manner.[22] In the sections *H-Bonding Networks* (page 717), and *Hydrate Formation* (page 717), hydrate formation is discussed from a molecular point of view. Crystal formation involves two mutually opposing principles: (1) satisfying the molecule's intermolecular H-bonding needs and (2) packing the atoms in the crystal as closely as possible. Hemi- ($h/2$) and monohydrates (h) evidently satisfy both close packing and H-bonding needs more efficiently than hydrates that contain water in channels.

Hysteresis is a general term that is used when a material's response to a second exposure of a stress differs from a prior response. This has been observed in the moisture uptake of an API as a function of RH. A number of instruments are now available that can monitor a sample's weight as RH is cycled from 0% to 95%. The noncoincidence of the weight as the sample is back cycled from 95% to 0% indicates hysteresis. One explanation of this type of behavior is that surface-initiated changes occurred in the solid state below or above the sample's CRH. Dehydration of the surface below the CRH, as in Equation 29, with the formation of an amorphous coat of $_{0h,\alpha}A_\Sigma$ means that any subsequent water vapor will encounter a more hygroscopic surface than $_{4h}A_\Sigma$ and thus a different hydration kinetic behavior. On the other hand, conversion of $_{4h}A$ to $_hA$ above the CRH, as in Equation 30, will produce a different kinetic behavior upon rehydration. Thus, RH hysteresis may result from changes in both the kinetic and equilibrium behavior of the surface of the particle.

Chemical Stability: Common Degradation Sequences—

BELOW CRH

Sorption/Desorption of Surface Water—If an anhydrous form of A is exposed to an RH below the CRH, water molecules will slowly adsorb onto the surface of the drug particle (denoted as $>0h$). Adsorption of up to a monolayer of water has been shown to provide partial protection from oxidation. Dehydrated foods, for example, are more stable when moisture coats reactive sites. For the anhydrous phenylbutazone, the oxidation rate has been shown to be lower below the CRH.[27] For a hydrate, however, the loss of surface water of hydration (denoted as $<h$) at RHs below the CRH has been shown to increase reactivity. Equations 30 and 31 show both of these possibilities.

$$_{0h}A_\Sigma \xrightarrow[+H_2O]{\text{below CRH}} {}_{>0h}A_\Sigma \qquad \text{(partial oxidation protection)} \quad (30)$$

$$A \xrightarrow{\text{below CRH}} A \qquad \text{(increased chemical reactivity)} \quad (31)$$

Formation of an Amorphous (α) Surface—A water enriched/depleted surface, ($>h/<h$), is prone to further solid-state changes shown in Equations 32 and 33. For the water-enriched surface, a chemical reaction is shown in which the crystalline form of A ($j = I$) reacts to form the product $_\alpha B_\Sigma$, which is amorphous. This type of surface hydrolysis at RHs below the CRH was shown to occur for meclofenoxate HCl decomposition[28] and for propantheline bromide hydrolysis.[29] For the latter, a lag time occurred that was attributed to the amount of time that was necessary to form a monolayer. For the water-depleted hydrate ($j = h$), the loss of water initiated the formation of an amorphous surface layer, $_\alpha A_\Sigma$. The consequences of these amorphous surfaces will now be explored.

$$_jA_\Sigma \xrightarrow{-H_2O} {}_{j-h}A_\Sigma \rightarrow {}_\alpha B_\Sigma \qquad (32)$$

$$_hA_\Sigma \xrightarrow{-H_2O} {}_{0h}A_\Sigma \rightarrow {}_\alpha A_\Sigma \qquad (33)$$

Transformation of Amorphous Surfaces—Because amorphous layers are more prone to be hygroscopic than crystalline solids, the chemical transformation of $_jA_\Sigma$ to $_\alpha B_\Sigma$ in Equation 32 is significant because the latter can attract more water to the surface. Dissolution of $_\alpha B_\Sigma$ shown in the first downward reaction of Equation 34 will then form a surface coated with b_Σ, as shown in Figure 38-3. The reaction of meclofenoxate HCl below the CRH to form amorphous dimethylaminoethanol HCl (see Eq 32) is a good example of this.[28] Next, the water adsorbed to the surface due to the dissolved form of B on the surface, b_Σ, promotes the dissolution of the surface of A, A_Σ, to form a surface coated also with a_Σ, the dissolved form of A on the surface, which then undergoes further decomposition to b_Σ. This is shown in the horizontal and final downward reactions of Equation 34.

$$\begin{array}{c} {}_\alpha B_\Sigma \\ \downarrow +H_2O \\ A_\Sigma \xrightarrow{k_1} a_\Sigma + b_\Sigma \\ \downarrow \\ b_\Sigma \end{array} \qquad (34)$$

In Equation 35, two possible solid-state changes for $_\alpha A_\Sigma$ are shown. First, the reactive amorphous surface can undergo a degradation reaction to form C_Σ. Second, the surface can continue to lose water below the CRH so that the subsurface $_hA$ undergoes a solid phase transformation to a crystalline phase, $_IA$. The dehydration changes for cefixime trihydrate are examples of these reactions.[31] The partially dehydrated form of this compound was more unstable than the fully hydrated or the completely dehydrated crystalline forms.

$$\begin{array}{l} \qquad\quad T \qquad C_\Sigma \\ {}_\alpha A_\Sigma \\ -H_2O,\ T \qquad {}_IA_\Sigma \end{array} \qquad (35)$$

ABOVE CRH

When water is adsorbed to the surface of the particle above the CRH, the drug particle becomes coated with a dissolved drug layer, a_Σ, which is assumed to be saturated:[1]

$$A_\Sigma \xrightarrow{\text{excess } H_2O} a_\Sigma \qquad (36)$$

Degradation under these conditions is assumed to occur solely in the dissolved layer. This situation has been extensively discussed.[1] For the Maillard reaction, in which primary amines react with carbohydrates, adsorbed water initially increases the reaction rate to a maximum due to the enhancement of reactant mobility. Greater amounts of water then decrease the reaction rate due to dilution of the reactive species. Similarly, for free-radical auto-oxidation of unsaturated groups, reactivity increases above the CRH because of accelerated reactant mobility. Below the CRH, oxidation decreases due to the immobilization of hydrogen peroxides and trace metal catalysts and the protective effects of a monolayer of water that is insufficient to increase reactant mobility.

Influence of Salt Form on Hygroscopicity—Table 38-2 shows that the non-salt forms, including free bases, free acids, and nonelectrolytes, are the most popular molecular forms on the market. In general, these forms would be expected to be less hygroscopic than salt forms due to their un-ionized character. Although the sodium salt is the most popular weak acid form, this form has a tendency to be hygroscopic. Alternative salts that have proven useful in overcoming hygroscopicity are hydrogen sulfate[32] and tromethamine.[33,34]

Hygroscopic tendencies for weak bases might be overcome by using aromatic counter-ions. Aryl sulfonic acids were shown to provide moisture protection without decreasing dissolution for the sparingly soluble weak base, Xiobam.[35] The free-base form of this drug (pK_a 6.1) was hydrolyzed at 40°C/80% RH. On the other hand, one weak base (pK_a 3.67) was chosen for development because it was less reactive to moisture exposure than the HCl salt. The latter showed chemical instability with moisture and heat and was the only salt that could be formed.[36] Stronger bases like pelrinone (pK_a 4.71) can form stable and nonhygroscopic HCl salts.[30]

Grinding Impact—Processing of solids can have a major impact on dissolution due to solid–solid phase changes. Grinding is one process that has been shown to cause changes in both polymorphs and hydrates. For the ${}_{III}A$ polymorph (Form C) of chloramphenicol palmitate,[37]

$$ {}_{III}A \xrightarrow{\text{grinding}} {}_{II}A \xrightarrow{\text{more grinding}} {}_{I}A \qquad (37) $$

grinding causes a successive change to the ${}_{II}A$ polymorph (Form B) and finally to the ${}_{I}A$ polymorph (Form A).[38] Correspondingly, dissolution from the fastest to the slowest is in the order

$$ {}_{\text{ground II}}A > {}_{\text{ground I}}A > {}_{II}A > {}_{I}A \qquad (38) $$

For hydrates, similar solid-state changes have been observed. When cefixime trihydrate is ground, a solid-phase transformation takes place:

$$ {}_{3H}A \xrightarrow{\text{grinding}} {}_{\alpha,0H}A \qquad (39) $$

Water in this situation plays an essential role in crystal formation. Its removal causes a collapse of the crystal lattice.[39] Other pharmaceutical processing operations and their impact on crystals have been reviewed.[40]

SALT SELECTION DECISION-MAKING

The pressure to increase the productivity of the knowledge worker is readily apparent at the salt-selection stage. Because of increased productivity in discovery, the cascading impact on development to choose rapidly the best molecular form is readily apparent; toxicological and bioavailability studies cannot proceed until the salt is chosen. Once these studies are initiated, it becomes very costly to change the molecular form because many of these biological studies would have to be repeated. More importantly, precious time and a competitive advantage will be lost. However, if an unanticipated, unacceptable property emerges during the development of an API, the sooner the change is made the better. It is for these reasons that efficient paradigms are being sought for this stage of development. Two approaches will be presented that attempt to optimize the probability of success with speed. Previous approaches were criticized for excessive characterization of poor candidates and for a lack of clear go/no-go decision-making.[41] As a practical consideration, it is essential that NCEs have high purity, and that salts be crystallized. In the following discussion, weak bases that are to be absorbed orally are used. Similar approaches can be developed for intravenous NCEs and for weak acids.

Multitiered Selection Approach—One approach in which different critical parameters are used to filter a salt candidate's progression to the next stage has recently been proposed.[41] Crystalline salts are successively sorted by a three-tier system in the following way:

Tier 1. Hygroscopicity
Tier 2. Thermal analysis and X-ray diffraction
Tier 3. Accelerated solid-state stability

Tier 1 eliminates any form with excessive moisture sorption/desorption characteristics. Only the survivors progress to Tier 2. In this second tier, changes in crystal structure are examined under extremes of moisture conditions by using thermal analysis and powder X-ray diffraction to detect desolvation and aqueous-phase transformation problems. In addition, aqueous solubility is determined to address potential dissolution problems. The best candidates for formulation and manufacturing are considered here and survivors proceed onto Tier 3. In this third tier, accelerated thermal and photo-stability testing is carried out. This is considered to be the most time-consuming step so the limiting of candidates saves time and effort. Selected excipient compatibility testing may also occur at this stage. If Tier 2 eliminates all of the candidates, additional salts or free acid/bases are considered before reevaluating any salt that was dropped in an earlier tier.

Several comments can be made regarding this approach.

1. The HCl salt of ranitidine, due to its hygroscopicity,[42] probably would not have been a final candidate in the multi-tiered approach. Yet this is one of the most successful drugs ever marketed. This emphasizes a need for prioritizing the salt selection process so that as wide of a range of development issues are addressed as early as possible and that they all are put in perspective. If a hydrochloride salt has much better absorption properties than the free base but is hygroscopic, it would be very prudent for development to see if it can deal with this problem. Otherwise, bioavailability may be compromised by a single-minded emphasis on API consistency.
2. The free base is not considered in the multi-tiered approach unless all alternatives have failed despite its potentially favorable dissolution in gastric fluids and its sensitivity to particle size reduction with a reactive sink.

The decision-tree, goal-oriented approach discussed below addresses some of these issues.

Decision-Tree, Goal-Oriented Approach—An alternative approach to the multi-tiered go/no-go selection approach is one based on a decision-tree using statistical probabilities and functional grouping of counter-ions to seek prioritized physical properties. In Figure 38-13, prioritized problems are shown, absorption being the highest priority.

The decision-tree considers the free base, the HCl salt, as well as other options. Although this approach uses statistical probabilities for molecular form consideration, ideally, a high-throughput, automated methodology would be available that could determine exhaustively which salts can form crystals and under which conditions. Feasible salts would then be synthesized and placed under accelerated stability and stressing conditions. This would allow for the maximum amount of exposure to the sample before a decision has to be made. Degradant evaluation need not be carried out on these stressed samples immediately; other issues may eliminate a particular candidate and make this unnecessary. However, evaluation for crystallinity should be carried out early to ensure that this does not impact physical or chemical stability. Physical property screens and absorption-dominated prioritization would then force a pharmaceutical evaluation to be made regarding the possibility of overcoming consistency and processing problems.[43] By using functional groupings (see Table 38-2), salt forms would be considered that could address specific problems.[6]

Compressibility and Compactibility

Because tablets remain the preferred oral dosage form due to high-speed manufacturing, information obtained during preformulation studies on the ability of powdered drugs to be compressed and compacted can be a valuable aid to market-image formulators. Compressibility and compactibility relate directly to tableting performance. *Compressibility* can be defined as the ability of a powder to decrease in volume under pressure; *compactibility* can be defined as the ability of a powder to be compressed into a tablet of a certain strength or hardness. Even though powdered drugs usually are formulated with excipients to modify compression and compaction properties, the properties of the powdered drug alone may be the primary determinant of its ability to be manufactured into a tablet. Significant differences in compression and compaction behavior often can be observed in different lots of the same drug. For example, changes in crystallization or milling procedures may produce differences in behavior.

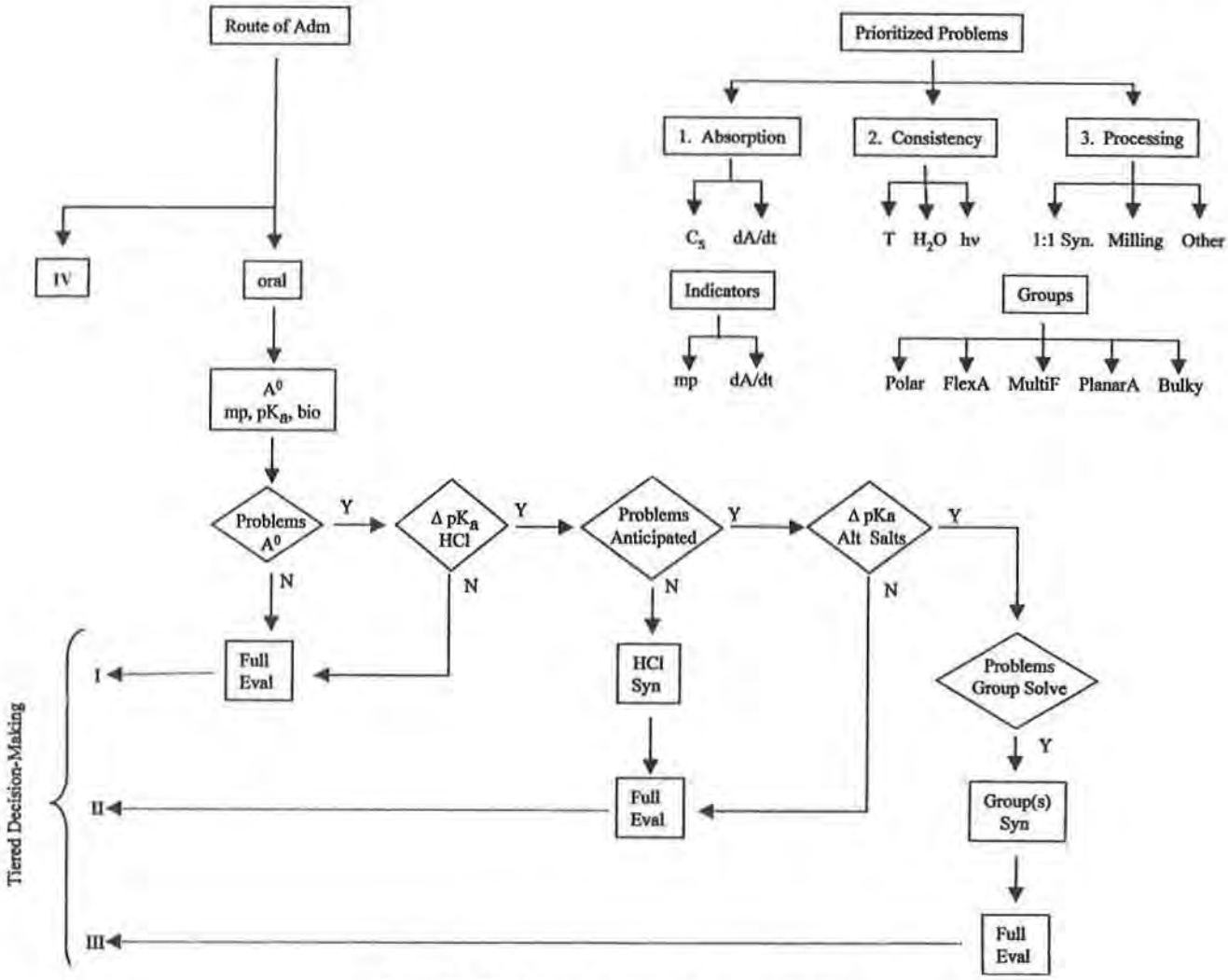


Figure 38-13. Absorption-dominated decision-tree.

Compression and compaction most often are evaluated by measuring the tensile strength and hardness of compacts. Tensile strength commonly is measured by radial compression of round tablets, where the analysis of strength accounts for the dimensions of the tablets. Transverse compression of square compacts between platens narrower than the compact is reported to provide more reproducible results on a wider variety of powders.

Hardness can be defined as the resistance of a solid to local permanent deformation. Static impression or dynamic methods usually measure deformation hardness tests. The static method involves the formation of a permanent indentation on a solid surface by a gradual and regularly increasing stress load. Hardness is determined by the load and size of the indentation and is expressed as force per unit area. In dynamic tests, the solid surface is exposed to an abrupt impact such as a swinging pendulum or an indenter allowed to fall under gravity onto the surface. Hardness then is determined from the rebound height of the pendulum or the volume of the resulting indentation.

Hiestand has used adaptations of a compression test and a hardness test to obtain measurements that are used to formulate three dimensionless parameters or indices.[44] The indices are used to characterize the relative tableting performance of individual components or mixtures. The *Strain Index* is the ratio of dynamic indentation hardness to reduced Young's modulus. The *Bonding Index* is the ratio of tensile strength to indentation hardness. The *Brittle Fracture Index* is obtained by comparing the tensile strengths of square compacts with and without a hole at their center. The indices themselves do not measure intrinsic properties of a chemical compound, but rather the traits that influence the tableting performances of a specific lot of chemical. It is necessary to know the magnitude of all three indices to predict the variety of tableting properties that may be incurred. Such information can act as a guide in selecting excipients to overcome problem properties of a drug ingredient.

Excipient Selection: Formulation Compatibilities

Excipients serve many roles and are the backbone of a formulation. They may be needed to stabilize the API by providing antioxidant, heavy-metal chelating, or light-protection properties. They also may be used to enhance bioavailability and to control the release from dosage forms. For solid dosage forms, they provide suitable properties for dispensing the API in accurate dosage units that have reproducible release properties. Diluents provide a flowable bulk, binders hold powders

together after wet granulation, lubricants provide punch-releasing properties, and disintegrants help to disperse dosage forms in the GI tract. On the other hand, judicious choices must be made to prevent incompatibilities between the API and excipients.

Screens to detect drug-excipient incompatibilities recently have been developed using elevated temperature and added water to accelerate potential interactions in ternary and more complex powder blends.[45] Such methods have been shown to be capable of rapidly detecting chemical incompatibilities and giving good correlations with results using powder blends of drug and excipients at elevated temperatures and humidity.

Processing incompatibilities can be more difficult to troubleshoot than chemical incompatibilities. For example, tablet performance has been shown to vary for ketorolac tromethamine, depending upon the kind of starch that was used. Cornstarch showed a decreased disintegration time and dissolution rate as a function of blending time whereas pregelatinized starch showed no such dependency. The difference between these two excipients was attributed to the formation of drug/cornstarch agglomerates with magnesium stearate.[46] Blending studies have shown the potential benefits of using sodium lauryl sulfate to offset these types of effects.[47]

Finally, manufacturing for a global market has forced a reevaluation of excipients that are used in formulations so that manufacturing can be carried out with internationally acceptable components. The European Economic Community has focused recently the pharmaceutical industry on eliminating excipients that have the potential for transmissible spongiform encephalopathies, replacing ingredients like stearic acid, magnesium stearate, polysorbate 80, and simethicone with vegetable grade sources.

API Specifications: Meeting Product and Regulatory Requirements

POLYMORPHIC FORMS AND HYDRATES DECISION TREES—A major portion of this chapter has been devoted to characterizing the solid state, ${}_jA$. The left side of Figure 38-14[48,49] summarizes some of the potential solid states that can exist for the un-ionized form of A; if a salt form was chosen for the API, the same states also would be possible. Previous sections have discussed the impact on API consistency and dissolution for the different solid states. The critical relative humidity (CRH) and the transition point (T_t) for enantiotropic polymorphic systems are especially important intrinsic physical parameters that control solid-state consistency and potential solid-state interconversion. Moisture and temperature, as we have discussed, are the major environmental variables that can promote these changes. Rapid methods, therefore, are needed to characterize potential solid-state forms and their physical properties. The decision-tree on the right side of Figure 38-14 summarizes when specifications need to be set to maintain API consistency. If the physical properties of the solid states differ, assessments need to determine the impact this will have on a formulated API. Specifications need to be set to ensure a consistent product.

PARTICLE-SIZE ACCEPTANCE CRITERION—Once the solid state, ${}_jA$, has been characterized, the potential impact of particle size on absorption can be assessed. Figure 38-15 shows a decision-tree approach, suggested by the International Committee on Harmonization, for determining whether a particle-size acceptance criterion is needed.[50] Previous sections in this chapter have discussed nearly every aspect of this tree. Although dissolution-limited absorption is a major concern, Figure 38-15 also includes dosage form issues such as content uniformity.

BIOPHARMACEUTICAL CLASSIFICATION OF API—Although it is possible to alter the solid state, ${}_jA$, such that dissolution and absorption can be enhanced, solubility and passive permeability are, in general, intrinsic properties of the NCE. Thus, even though the amorphous state, ${}_\alpha A$, in some situations can be stabilized to enhance dissolution, the equilibrium solubility will be determined by the least soluble solid state. A classification has been proposed to segregate situations when *in vitro* and *in vivo* correlations (IVIV) are expected. Such designations may be used as a guide for determining when bioequivalent studies may need to be carried out. Table 38-4 shows the four major classes based on solubility and passive permeability.

ENGINEERING THE SOLID STATE

Speed is essential for any preformulation innovation if it is to be effective in influencing discovery decision-making. In Figure 38-4, the early discovery stages, Steps 1 to 4 were introduced. The potential focal points of high-throughput physical screening, predictions of physical properties, and artificial intelligence are shown in an expanded version of these early steps in Figure 38-16.

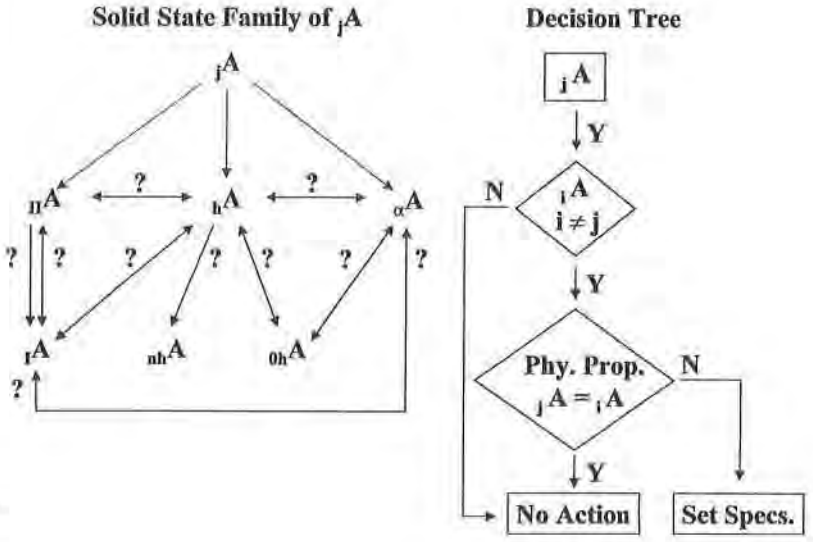


Figure 38-14. Solid-state forms and specification setting.[48,49]

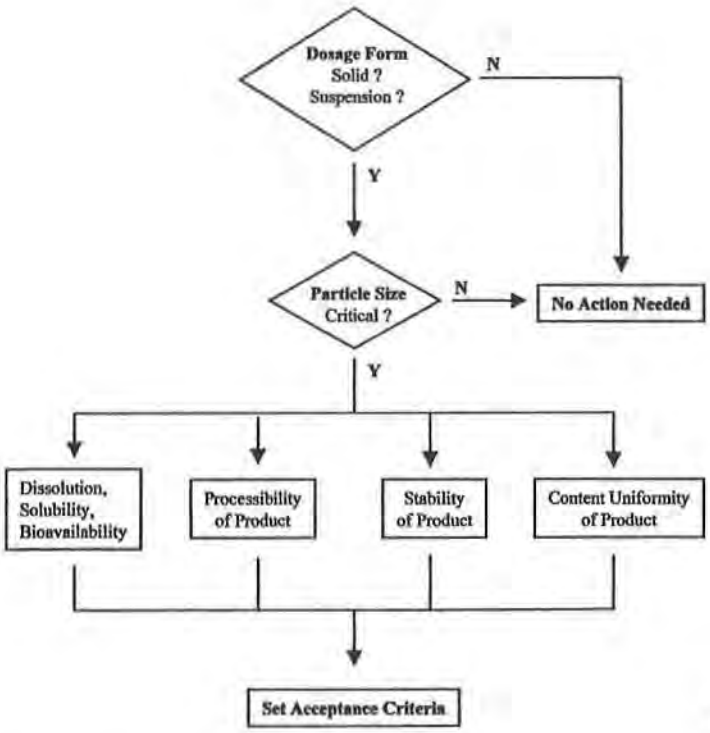


Figure 38-15. Decision-tree for drug substance particle-size distribution.[50]

Library Expansion

The development of mass-screening technologies has spawned a number of technologies that complement a company's in-house library. Besides the massive influx of compounds that can be obtained from combinatorial synthesis, computer-based analyses can be used to assess the diversity of the in-house chemical library and identify areas of weakness. Negotiations with other companies then might take place to fill in deficiencies. In addition, a number of commercial libraries, including natural products, are also available for mass screening. Figure 38-17 shows these aspects of library expansion.

Modern mechanism-based screens, based on recombinant proteins, have vastly increased the number and specificity of *in vitro* screens. However, because the goal of mass screening is to find compounds that have high *in vitro* activity, this exclusive focus tends to produce compounds with poor physical properties. Such compounds, either because of their conformational restriction or their H-bonding with receptors, have much greater activity and selectivity than previous generations of NCEs that were obtained from tissue screens and *in vivo* tests. These attributes have caused modern chemical libraries to expand with compounds that have high melting points and low aqueous solubility. Chemists affectionately call such compounds *brick dust*.

Although brick dust compounds may provide a point of departure for an *in vitro* activity search, most of them are unacceptable for development because of their poor physical properties, especially poor aqueous solubility. It would be undesirable for chemical library expansion to be dominated exclusively by such compounds because of their poor development potential. Selection of a good API, an active chemical with acceptable pharmaceutical properties, could be delayed. For this reason, there is an urgent need to integrate pharmaceutical properties into the chemical library expansion and the mass-screening paradigm.

However, a greater mechanistic understanding is needed of those factors that promote desirable physical properties and good absorption. In lieu of this understanding, computed parameters based on marketed drugs have been used to direct immediate library expansion based on the assumption that these drugs have physical and chemical properties that are desirable.[51] The potential future role pharmaceutics can play in influencing the rational direction of library expansions based on a more fundamental, more molecular-based understanding of physical properties will now be discussed.

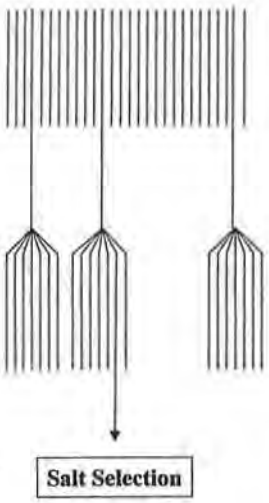
Table 38-4. *In Vitro/In Vivo* Correlation Expectations for Immediate-Release Products Based on Biopharmaceutics Class for Passive Absorption

CLASS	SOLUBILITY	PERMEABILITY	IVIV CORRELATION EXPECTATION
I	High	High	IVIV correlation if dissolution rate is slower than gastric emptying rate. Otherwise limited or no correlation.
II	Low	High	IVIV correlation expected if *in vitro* dissolution rate is similar to *in vivo* dissolution rate (unless dose is very high).
III	High	Low	Absorption (permeability) is rate-determining and limited or no IVIV correlation with dissolution rate.
IV	Low	Low	Limited or no IVIV correlation expected.

Source: Amidon GL, *et al. Pharm Res* 1995; 12: 413.

AQUEOUS INSOLUBILITY: MOLECULAR MECHANISMS

Although aqueous solubility is a major factor that affects drug absorption, better methods of understanding the molecular mechanisms and predicting this parameter are needed. Aqueous insolubility occurs when the attraction between molecules is greater than the ability of water to solvate the molecule and



(1) Library Expansion
- Aqueous Insolubility and Molecular Mechanisms
- Drug Delivery and Molecular Mechanisms

(2) Series Selection
- Physical Property Screens
- pH-Solubility Profiles

(3) Analog Expansion
- Absorption Engineering
- Solid-State Engineering
- Stability Engineering

(4) Analog Selection

Figure 38-16. Proactive pharmaceutical API decision-making: potential opportunities for high-throughput physical innovations.

dislodge it from its solid phase. Generally speaking, most pharmaceutical solids are manufactured in the form of crystals as opposed to amorphous solids because crystals are more stable. Crystals are arrays of molecules that pack in a regular pattern and thus have long-range order, ie, packing patterns that extend in space over large numbers of molecules. Single-crystal X-ray diffraction can be used to visualize the conformation of molecules in the crystal, the interactions between molecules, and exactly how repeating units of molecules are arranged in three-dimensional space. When the forces that form crystals are sufficiently strong, either because the forces are sufficiently strong in themselves or because there are a large number of forces on a given molecule, insolubility results. These forces are termed *intermolecular forces* (between molecules) as opposed to *intramolecular forces* (within a molecule).

In the past, most of the predictive methods for solubility have been either thermodynamically or statistically based. Insight into the molecular basis of insolubility is now possible. By using single crystal X-ray diffraction, correlations between molecular packing motifs and solubility can be carried out. The major intermolecular factors that have been identified to date are

1. Hydrophobicity
2. Conformational restriction
3. H-bonding networks
4. Hydrate formation
5. Zwitterion formation

Hydrophobicity needs no explanation; this brief focus will concentrate on the cohesive aspects of the latter factors.

Conformational Restriction—Using biotechnology, very specific biological targets can be synthesized from genes. For example, pure dopamine receptor subtypes, D_1–D_5, have been used as mechanistic targets for schizophrenia; cycloxygenase$_1$ and cycloxygenase$_2$ similarly are now available for anti-inflammatory screening. Developing a drug specifically for cycloxygenase$_2$ inhibition promises to minimize the side effects of nonsteroidal anti-inflammatory inhibitors like aspirin.

Such molecular specificity is now possible because screening-feedback enables chemists to rigidify drug molecules such that interactions with the target protein are restricted to only a few conformations. However, this rigidity has a physical impact. Rigidified molecules appear to pack better because they can be arranged in fewer ways than flexible molecules. Consequently, such molecules have increased dispersion force interactions (very short ranged). This increased intermolecular interaction leads to a conformationally based insolubility that has been observed especially in molecules that are planar or linear.

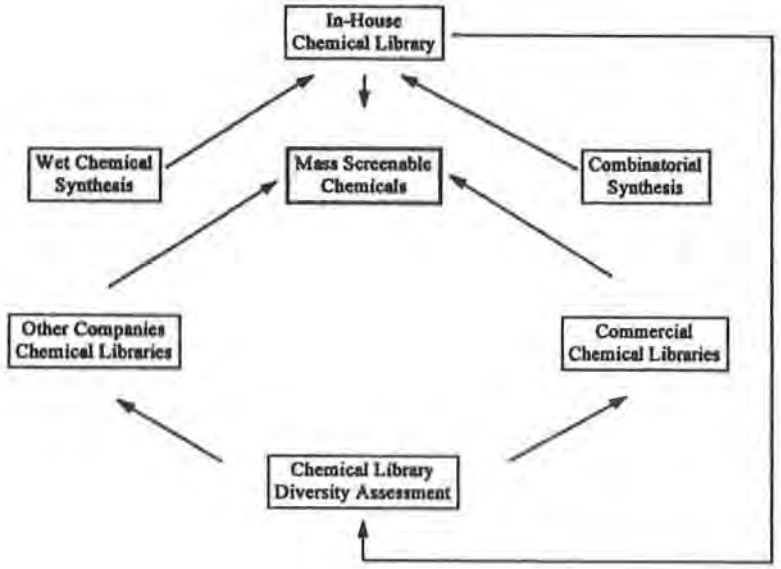


Figure 38-17. Expansion of mass-screenable chemicals.

H-Bonding Networks—Polar groups generally impart water solubility to a flexible molecule. Functional groups that have an H-bond donor and an acceptor group can help the molecule to form a hydration shell around itself and increase solubility. However, in more rigid molecules, these same groups can bind one molecule to another in the crystalline state through intermolecular H-bonding. Single-crystal X-ray diffraction studies of crystals provide a detailed picture of how H-bonds form between such molecules. Insolubility due to H-bonding in conformationally restricted molecules appears to increase as the number of H-bonds per molecule and between molecules increases. Predicting exactly how such molecules arrange in a crystal is difficult because there are two mutually opposing tendencies in crystal formation: (1) packing molecules as closely as possible, and (2) maximizing the number of H-bonding interactions. Crystals often achieve a balance between these opposing tendencies in unexpected ways.

Conformational restriction also seems to increase the efficiency of H-bonding in crystals by increasing molecular rigidity. This may be because rigid molecules can form more uniform, consistent H-bonds that are needed for long-ranged crystal order. Intramolecular H-bonding often adds to this rigidity.

The forces directing initial crystal formation would be expected to be dominated by H-bonds due to their electrostatic nature. These are the longest-ranged intermolecular forces in a nonelectrolyte. Packing and dispersion interactions would then be expected to dominate the final crystal form. For some molecules, the H-bonding of water can be very important in crystal formation. When the number of H-bonding acceptor groups is large compared to the number of donor groups, hydrates are more probable. Water, due to its high H-bonding capacity, often strongly binds molecules together in crystals by making up for molecular deficiencies. This can increase aqueous insolubility. It is generally observed that hemihydrates and monohydrates are more insoluble than the anhydrous forms.

Hydrate Formation—Hydrate formation in organic crystals increases the number of molecular options for satisfying the dual crystal maximization constraints of H-bonding and dense packing. Water, because of its small size and di-donor and di-acceptor capacity for H-bonding, often acts as an interstitial H-bonding cement and spacer.[52] Crystal surveys have found that water is a very weak donor, but the water oxygen is the strongest acceptor. On the other hand, water almost always donates two H-bonds but usually accepts one, not two, H-bonds. Because of its unique characteristics and flexibility, predicting how water will interact with an H-bonding NCE is not possible. The earlier presumption of linear and single acceptor H-bonds has been shown to be wrong. Nevertheless, although the exact structure of water interactions with NCEs cannot be predicted, generalizations can be drawn regarding the type of structure that is most likely to be hydrated. Water with its di-donor/mono-acceptor role tends to reduce proton deficiencies of the parent molecule. Molecules that have donor/acceptor ratios of less that 0.5 are most likely to be hydrate candidates.

Zwitterion Formation—Zwitterions are molecules that at a given pH have both a positive and negative charge. If they are conformationally restricted, they tend to be very insoluble. Evidently the localization of opposite charges at different regions in the same rigid molecule provide scaffolding that enables very efficient salt-bridge dimers to form. Sometimes a zwitterion not only forms dimers, but also has ample H-bonding groups to form H-bonding networks in addition to the dimers. Occasionally, zwitterion insolubility is caused by metabolism, for example by aromatic hydroxylation and subsequent sulfation of a strong basic drug. Such metabolites have the potential to precipitate in the kidneys as urine becomes concentrated in the renal tubules.

DRUG DELIVERY: MOLECULAR MECHANISMS

Membrane-Active Sites—The rich interaction of drugs with membrane receptors is modulated partially by their complex lipid matrix. For drugs that partition into membranes, lateral diffusion provides a rapid surface-dispersion mechanism for transporting drug to any integral membrane receptor. Functional groups on a molecule that help position it within the membrane help explain why some drugs act superficially or more deeply on integral membrane proteins.

Finally, intrinsic membrane curvature, with its concomitant asymmetric distribution of phospholipids, provides a rich potential for specific and allosteric interactions. Drugs, depending on their amphipathic nature, may insert themselves preferentially into the phosphatidylcholine–rich outer leaflet or the phosphatidylserine inner negative leaflet. Protein crystallographic studies have confirmed the relationship between membrane localization and duration of action of a number of drugs.

Membrane Permeability—Computational approaches have been used to develop molecular models for passive membrane permeability. Exploration of a number of models, including homogeneous solubility-diffusion, defect, and free volume, have shown an inability to completely explain the permeability of simple molecules like water and ethanol. Recent progress has been made in this area by dividing the membrane into zones in which the mechanisms of diffusion differ. The preferential impact of different zones on diffusion and the dynamic simulation of spontaneously occurring membrane conformational alterations can then be used to simulate and average diffusion trajectories to estimate permeation rates.

Series Selection

PHYSICAL PROPERTY SCREENS

Until computational methods for predicting physical properties reach an advanced state of reliability, high-throughput screens for physical properties will play a major role in understanding how molecules can be designed for better absorption. New instrumentation makes this task more feasible than it was in the past. Rapid advances in analytical detection sensitivity, especially in powder X-ray diffraction and chromatography–mass spectrometry, have helped reduce material consumption and analytical development time. Robots, and specialized automated dispensers and spectrophotometers that have been developed for mass screens can be used creatively for developmental purposes. In short, the more rapidly and reliably physical properties can be assessed, the more impact these measurements will have on the flow of new leads for development. However, the greatest advantage of automation is not physical evaluations on a grand scale, but rather the ability to customize determinations to solve particular problems rapidly.

pH-SOLUBILITY PROFILES

High-throughput determinations of A^0 and A^I solubilities and pK_a values provide the basis for pH-solubility profiles. Series selection can then focus on the feasibility of modifying the pK_a for a given series as part of an optimization strategy to enhance absorption. Ideally, a pK_a that allows for ionization to enhance solubility while still providing some un-ionized form for absorption is ideal. Consider Equation 40.

$$A^I \text{ (ionizing reservoir)} \overset{\text{rapid equilibration}}{\longleftrightarrow} A^0 \downarrow \xrightarrow{k_a} \text{GI membrane} \rightarrow \text{systemic circulation (sink)} \quad (40)$$

If 99% of the drug in the GI tract is in the ionized form A^I, and 1% in the un-ionized form A^0 (eg, 99%), A^I provides a reservoir of dissolved drug while the systemic circulation provides a sink for A^0. These conditions should allow for good absorption as long as k_m is not rate limiting. Modifying the pH solubility profile to approach this situation is one way to optimize absorption. The impact of this type of optimization cannot be overestimated, because pK_a values and intrinsic solubilities are molecular parameters that neither salt selection nor formulation can alter. Further techniques to enhance absorption will be discussed in the next section.

Analog Expansion

ABSORPTION ENGINEERING

Absorption-enhancement engineering of analogs can be addressed after the rate-limiting mechanism for poor absorption has been identified. Like the pK_a, absorption-enhanced properties must be designed into the NCE before it is passed on to development. For this reason, it is important for preformulation to integrate physical property design into the molecule as early as possible.

Crystal Engineering—If aqueous solubility and dissolution are the problem, crystal engineering might be possible. In this case, it is important to identify the mechanism of insolubility as discussed above. Each mechanism will require a different approach. Hydrophobic problems are usually the simplest and can often be handled using formulation approaches. The more difficult problems require a molecular understanding of the intermolecular forces in the crystal. For H-bonding problems, it may be possible to adjust the mix of H-bond donor and acceptor groups to reduce the number and strength of H-bonds. It has been found that simple changes can alter H-bonding networks and solubility in a dramatic way. The substitution, for example, of a *t*-butyl group for a phenyl group for one insoluble compound increased the intrinsic solubility 4-fold, and the solubility at pH 5 increased it 10,000-fold, despite the fact that the resulting compound had the exact ionization potential of the original compound. These enhancements were due to changes in H-bonding network structure that released a water-solubilizing group for ionization. Other modifications would be directed at minimizing conformational restriction to reduce crystal-packing efficiency, such as by introducing an acyl chain in a compact heterocyclic system. Practical applications of these design suggestions can be difficult because they often reduce activity. However, as preformulation scientists work more closely with synthetic chemists, crystal-packing disruption strategies that are compatible with ease of synthesis and *in vitro* activity will become commonplace. In addition, as computer predictions of crystal-packing structure and H-bonding networks from molecular structure become more practical (see *Engineering The Solid State*, page 714), these types of design considerations will be made as a matter of course as activity is being optimized.

Permeability Enhancement—This is another intrinsic parameter of an API that, in general, is not enhanced in oral formulations. Recently, increased knowledge has helped to design drugs that will passively penetrate membrane barriers more easily. There has been a great emphasis in the past on the partitioning of a solute out of the aqueous phase into a lipophilic membrane and not enough emphasis on the need for a drug molecule to desolvate from the aqueous phase. Molecules have been designed successfully to enhance permeability by reducing the desolvating step. One way this has been accomplished is by reducing a molecule's solvation through the promotion of intramolecular H-bonding in the molecule. In addition, the ability of a membrane-bound drug to flip-flop from the outer leaflet of the bilayer membrane to the inner leaflet appears to be important for efficient membrane permeability. Ultimately, such insight may be possible from molecular modeling studies of membranes.

Intrinsic Dissolution Engineering—Correlating molecular orientation with morphology in crystals has provided insight into molecular mechanisms of dissolution. In one study, it was shown that the relatively strong binding of a solvent at one subset of surface sites and repulsion at others provided a *relay* type of dissolution that favored erosion from particular faces of the crystal. Such a mechanism also perpetuates the natural corrugation of the surface at the molecular level and helps define the factors that may limit dissolution in the bulk phase. In this regard, some progress has been made in predicting the intrinsic dissolution rate of an API from considerations of the surface pH of the API. Modifications of the classic Noyes–Whitney relationship have to be made for weak acids, bases, and their salts. The impact of dissolution in a reactive media was discussed under *Preformulation Challenges*, page 700. Predictions using such considerations are possible for NCEs when the pH of the medium, the solubility of the un-ionized form of the drug in water, and the pK_a of the NCE are known.[53]

SOLID-STATE ENGINEERING

The computational ability to link molecular structure with crystal packing has advanced to the point that polymorphic predictions are becoming more reliable for small molecules. This has a number of implications.

1. Exploring the polymorphic possibilities of a given molecular structure should allow evaluations to be made regarding which structures have more elaborate polymorphic possibilities. In some instances, it may be desirable to avoid such structures; in others, these structures may provide the means for improving physical properties, assuming adequate conditions can be found to ensure physical and chemical stability.
2. As our molecular understanding of the dissolution process increases (see *Intrinsic Dissolution Engineering*, above), it will eventually be possible to predict molecular structures that can enhance dissolution for a particular analog series and to predict the solvents that will be necessary to obtain the most advantageous crystal habit. Hydrate predictions are also within the realm of possibility as the molecular study of existing hydrates yields rules that can be used by expert systems and molecular-modeling programs. Finally, an increased understanding of the molecular conditions necessary for the homogeneous and heterogeneous nucleation process of crystallization will aid in the practical synthesis of industrial APIs.

STABILITY ENGINEERING

The ability to predict the products of chemical reactions means that evaluations of potential NCEs that are being considered at the analog-expansion stage can be considered on the basis of their presumptive chemical stability and degradation pathways before they are even synthesized. Although poor predictions have the potential to inhibit the synthesis of potentially valuable compounds, with future advances in computer-generated molecular diversity such considerations may become less important as predictions become more accurate. The preformulation implications for such predictions are also evident. Anticipation of potential degradants and their characterization can be used to identify proactively unknown chromatography peaks and predict pharmaceutical excipient incompatibilities.

Analog Selection

Physical properties that are oriented toward *in vivo* conditions are most useful at this stage. Solubility and dissolution determinations in media and pHs that mimic physiological pHs can be used as an early indicator of how well an *in vitro/in vivo* correlation can be drawn. At this stage, a number of other

studies from different divisions will be carried out. *In vitro* and *in vivo* metabolism studies, bioavailability studies in different animals, as well as possible selective toxicological studies can be used to determine the best analog. Degradant predictions of the different analogs at this stage may also help to differentiate and minimize problems that can occur later in development. In addition, high-throughput methods to determine the best salt form for a particular analog would mean that therapeutic testing could be carried out on the salt form that will be eventually used in development.

Conclusion: Application of Knowledge

"The actual product of the pharmaceutical industry is knowledge; pills and prescriptions ointments are no more than packaging for knowledge."[54] The introduction of methods to probe and exploit human and animal genomics has had a cascading impact on the industry. These new concepts had a number of qualities that ensured adaptation.[55] The systematic use of mechanism-based reagents was a tangibly better solution for finding new therapeutic entities than the more serendipitous methods of the past. Such high-throughput screens were compatible with increasing use of robotics whose advantages could easily be understood by all in the pharmaceutical industry. Each company was able to hold trial runs to test the utility of such screens and in the end obtain observable results. Today, the recombinant DNA innovations of the 1980s still provide the driving force for other innovations in the pharmaceutical industry: miniaturization, customizing, and artificial intelligence.

Miniaturization began in earnest with the micronization of the transistor concept onto silicone chips. In the pharmaceutical industry, mass screening, the demand for higher and higher throughput, and the need to conserve chemical libraries have accelerated analytical and synthetic nanotechnology. This latter need is extremely important because chemical libraries are expendable resources that are not easily replaced. Old library entries were synthesized in gram quantities, and newer entries in milligrams. Conservation of this resource will require a combination of nanotechnology along with a host of regeneration technologies including combinatorial synthesis, high-throughput purification, and promotion of an increasingly diverse molecular library for mass screening. In addition, chromatographic columns, HPLCs, and electrophoresis on the nanoscale hold promise for extremely high resolution with extremely low material consumption. On this scale, area can efficiently be converted to a linear dimension. Thus a chip 10×10 mm can be converted easily to an electrophoretic path of 9.5 cm. The potential for massive parallel processing is evident when one contemplates the possibilities of 100 nanolaboratories on a single chip.

Customization at low cost also will be possible with new technology. DNA probes located on biochips will permit the individualization of a treatment course depending on a person's ability to metabolize a given drug. Such innovations likely will cause a cascading demand on development to individualize dosage forms. Finally, the rapid and parallel demands placed on preformulation will force more decisions to be made using artificial intelligence. High-throughput determinations of physical properties will result in high quality databases, which can in turn be systematically exploited by expert systems. Highly accurate predictions of solubility, permeability, and dissolution will be possible in the 21st century.

Although artificial intelligence is still in its infancy, the benefits of its applications can be appreciated from a consideration of the differences between knowledge and information. A chemical reaction database, for example, stores information on particular reactions. However, it cannot apply this information to new molecules. Expert systems, on the other hand, so codify knowledge that they can be applied to entirely new situations. Knowledge differs from information in that information is random and miscellaneous, and it tends to expand too rapidly and overwhelm us.[56] Knowledge, on the other hand, requires that the structure of a subject be understood in a way that permits other things to be related to it in a meaningful way; it permits intuitive heuristic procedures to be developed to solve problems when no algorithms are available.[57] Such applications of artificial intelligence, however, are still in the early-stage knowledge revolution, in which knowledge is applied to produce results. In the postcapitalist society, knowledge will be applied toward systematic innovation: "It will be applied systematically and purposefully to define what new knowledge is needed, whether it is feasible, and what has to be done to make knowledge more effective."[54]

Knowledge and the productive application of knowledge are anticipated to be the sole factors that will drive the postcapitalist society into the 21st century. In the pharmaceutical industry, massive diffusion of innovations from discovery into development will pose an accelerating challenge for preformulation. To meet this challenge, preformulation, through a better understanding of the solid state, must seek to design improved characteristics into APIs at the earliest stages of discovery. This will be the edge that any company will need to facilitate the rapid movement of new therapeutics entries to marketplace. The patient is waiting!

REFERENCES

1. Shalaev EY, Zografi G. *J Pharm Sci* 1996; 85: 1137.
2. Sokoloski TD, *et al. Pharm Res* 1994; 11: S152. Duddu SP, *et al. Pharm Res* 1994; 11: S153. Vadas EB, et al. *Pharm Res* 1994; 8: 148.
3. Streng WH, *et al. J Pharm Sci* 1984; 73: 1679.
4. Serajuddin ATM, Mufson D. *Pharm Res* 1985; 2: 65.
5. Serajuddin ATM, Sheen P, Augustine MA. *J Pharm Pharmacol* 1986; 39: 587.
6. Wells JI. *Pharmaceutical Preformulation: The Physicochemical Properties of Drug Substances.* New York: Wiley, 1988, p 38.
7. Hussain A. *J Pharm Sci* 1972; 61: 811.
8. Nielsen AE. *Croatica Chemica Acta* 1987; 60: 531.
9. Serajuddin ATM, Jarowski CI. *J Pharm Sci* 1985; 74: 148.
10. Serajuddin ATM, Jarowski CI. *J Pharm Sci* 1985; 74: 142.
11. Nogami H, Nagai T, Yotsuyanagi T. *Chem Pharm Bull* 1969; 17: 499.
12. Dressman JB, Fleisher D. *J Pharm Sci* 1986; 75: 109.
13. Kuhnert-Bradnstatter M. *Thermomieroscopy in the Analysis of Pharmaceuticals.* New York: Pergamon, 1971, pp 35–36.
14. Heleblian J, McCrone W. *J Pharm Sci* 1969; 58: 911.
15. Rocco WL, Swanson JR. *Int J Pharm* 1995; 117: 231.
16. Cardew PT, Davey R. *Proc Roy Soc Lond A* 1985; 398: 415.
17. Behme RJ, *et al. J Pharm Sci* 1985; 74: 1041.
18. Mitchell AG. *J Pharm Pharmacol* 1985; 37: 601.
19. Shah AC, Britten NJ. *J Pharm Pharmacol* 1987; 39: 736.
20. Hartauer KJ, Miller ES, Guillory JK. *Int J Pharm* 1992; 85: 163.
21. Admirat P, Grenier JC. *J Rech Atmos* 1975; 9: 97.
22. Kitamura S, *et al. Pharm Res* 1992; 9: 138.
23. Tada T, *et al. J Pharm Sci* 1987; 76: S302.
24. Buxton PC, Lynch IR, Roe JM. *Int J Pharm* 1988; 42: 135.
25. Vadas EB, Toma P, Zografi G. *Pharm Res* 1991; 8: 148.
26. Morris KR, *et al. Int J Pharm* 1994; 108: 195.
27. Yoshioka S, Shibazaki T, Uchiyama M. *J Pharmacobiodyn* 1986; 9: S6.
28. Yoshioka S, Shibazaki T, Ejima A. *Chem Pharm Bull* 1982; 30: 3734.
29. Yoshioka S, Uchiyama M. *J Pharm Sci* 1986; 75: 92.
30. Hajdu J, Adams G, Lee H. *J Pharm Sci* 1988; 77: 921.
31. Kitamura S, *et al. Int J Pharm* 1990; 59: 217.
32. Gu L, *et al. Drug Devel Ind Pharm* 1987; 13: 437.
33. Gu L, Strickley RG. *Pharm Res* 1987; 4: 255.
34. Roseman TJ, Yalkowsky SH. *J Pharm Sci* 1973; 62: 1680.
35. Walkling WD, *et al. Drug Dev Ind Pharm* 1983; 9: 809.
36. Serajuddin ATM, *et al. J Pharm Sci* 1986; 75: 492.
37. Aguiar AJ. *J Pharm Sci* 1969; 58: 963.
38. Kaneniwa N, Otsuka M. *Chem Pharm Bull* 1985; 33: 1660.
39. Kitamura S, *et al. Int J Pharm* 1989; 56: 125.
40. Grant DJW, York P. *Int J Pharm* 1986; 30: 161.
41. Morris KR, *et al. Int J Pharm* 1994; 105: 209.

42. Teraoka R, Otsuka M, Matsuda Y. *J Pharm Sci* 1993; 82: 601.
43. Gould PL. *Int J Pharm* 1986; 33: 201.
44. Hiestand EH, Smith D. *Powder Tech* 1984; 38: 145.
45. Serajuddin ATM, et al. *Pharm Res* 1991; 8(suppl): S103.
46. Chowhan ZT, Chi LH. *Pharm Technol* 1985; 9: 84.
47. Wand LH, Chowhan ZT. *Int J Pharm* 1990; 60: 61.
48. Byrn S, *et al. Pharm Res* 1995; 9: 84.
49. Byrn S, *et al.* Bulk drug guidance: quality control reports. *Gold Sheet* 1996; 30(6): 1.
50. Byrn S, *et al.* Specifications for new drug substances and products: chemical substances, ICH4. Fourth International Conference on Harmonization, Brussels, 16 July 1997.
51. Lipinsky CA. *Adv Drug Del Rev* 1997; 23: 3.
52. Desiraju GR. *J Chem Soc, Chem Commun* 1991; 6: 426.
53. Al-Janabi II. *Drug Dev Ind Pharm* 1990; 16: 347.
54. Drucker P. Post-capitalist society. New York: Harper Business, 1993, p182.
55. Rogers EM. *Diffusion of Innovations.* Glencoe, NY: The Free Press, 1962, Chap 4.
56. Boorstin DJ. *Readers Digest* Sept 54, 1982.
57. Bruner JS. *The Process of Education.* Cambridge: Harvard University Press, 1960. Source: Serajuddin ATM, Sheen P, Augustine MA. To market, to market. In: Bristol J, ed. *Annu Rep Med Chem.* New York: Academic, 1983–1996.

CHAPTER 45

Oral Solid Dosage Forms

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Drug substances most frequently are administered orally by means of solid dosage forms such as tablets and capsules. Large-scale production methods used for their preparation, as described later in the chapter, require the presence of other materials in addition to the active ingredients. Additives also may be included in the formulations to facilitate handling, enhance the physical appearance, improve stability, and aid in the delivery of the drug to the bloodstream after administration. These supposedly inert ingredients, as well as the production methods employed, have been shown in some cases to influence the absorption or bioavailability of the drug substances.[1] Therefore, care must be taken in the selection and evaluation of additives and preparation methods to ensure that the drug-delivery goals and therapeutic efficacy of the active ingredient will not be diminished.

In a limited number of cases it has been shown that the drug substance's solubility and other physicochemical characteristics have influenced its physiological availability from a solid dosage form. These characteristics include its particle size, whether it is amorphous or crystalline, whether it is solvated or nonsolvated, and its polymorphic form. After clinically effective formulations are obtained, such variations among dosage units of a given batch, as well as batch-to-batch differences, should be reduced to a minimum through proper in-process controls and good manufacturing practices. The recognition of the importance of validation for both equipment and processes has enhanced assurance in the reproducibility of formulations greatly. It is in these areas that significant progress has been made with the realization that large-scale production of a satisfactory tablet or capsule depends not only on the availability of a clinically effective formulation but also on the raw materials, facilities, personnel, documentation, validated processes and equipment, packaging, and the controls used during and after preparation (Fig 45-1).

TABLETS

Tablets may be defined as solid pharmaceutical dosage forms containing drug substances with or without suitable diluents and prepared by either compression or molding methods. They have been in widespread use since the latter part of the 19th century, and their popularity continues. The term *compressed tablet* is believed to have been used first by John Wyeth and Brother of Philadelphia. During this same period, molded tablets were introduced to be used as *hypodermic* tablets for the extemporaneous preparation of solutions for injection. Tablets remain popular as a dosage form because of the advantages afforded both to the manufacturer (eg, simplicity and economy of preparation, stability, and convenience in packaging, shipping, and dispensing) and the patient (eg, accuracy of dosage, compactness, portability, blandness of taste, and ease of administration).

Although the basic mechanical approach for their manufacture has remained the same, tablet technology has undergone great improvement. Efforts are being made continually to understand more clearly the physical characteristics of powder compaction and the factors affecting the availability of the drug substance from the dosage form after oral administration. Tableting equipment continues to improve in both production speed and the uniformity of tablets compressed. Recent advances in tablet technology have been reviewed.[2–13]

Although tablets frequently are discoid in shape, they also may be round, oval, oblong, cylindrical, or triangular. They may differ greatly in size and weight depending on the amount of drug substance present and the intended method of administration. They are divided into two general classes by whether they are made by compression or molding. Compressed tablets usually are prepared by large-scale production methods, while molded tablets generally involve small-scale operations. The various tablet types and abbreviations used in referring to them are listed below.

COMPRESSED TABLETS (CT)

These tablets are formed by compression and contain no special coating. They are made from powdered, crystalline, or granular materials, alone or in combination with binders, disintegrants, controlled-release polymers, lubricants, diluents, and in many cases colorants.

Sugar-Coated Tablets (SCT)—These are compressed tablets containing a sugar coating. Such coatings may be colored and are beneficial in covering up drug substances possessing objectionable tastes or odors and in protecting materials sensitive to oxidation.

Film-Coated Tablets (FCT)—These are compressed tablets that are covered with a thin layer or film of a water-soluble material. A number of polymeric substances with film-forming properties may be used. Film coating imparts the same general characteristics as sugar coating, with the added advantage of a greatly reduced time period required for the coating operation.

Enteric-Coated Tablets (ECT)—These are compressed tablets coated with substances that resist solution in gastric fluid but disintegrate in the intestine. Enteric coatings can be used for tablets containing drug substances that are inactivated or destroyed in the stomach, for those that irritate the mucosa, or as a means of delayed release of the medication.

Multiple Compressed Tablets (MCT)—These are compressed tablets made by more than one compression cycle.

Layered Tablets—Such tablets are prepared by compressing additional tablet granulation on a previously compressed granulation. The operation may be repeated to produce multilayered tablets of two or

Figure 45-1. Tablet press operators checking batch record in conformance with Current Good Manufacturing Practices (courtesy, Lilly).

three layers. Special tablet presses are required to make layered tablets such as the Versa press *(Stokes/Pennwalt)*.

Press-Coated Tablets—Such tablets, also referred to as dry-coated, are prepared by feeding previously compressed tablets into a special tableting machine and compressing another granulation layer around the preformed tablets. They have all the advantages of compressed tablets, ie, slotting, monogramming, speed of disintegration, etc, while retaining the attributes of sugar-coated tablets in masking the taste of the drug substance in the core tablets. An example of a press-coated tablet press is the *Manesty* Drycota. Press-coated tablets also can be used to separate incompatible drug substances; in addition, they can provide a means of giving an enteric coating to the core tablets. Both types of multiple-compressed tablets have been used widely in the design of prolonged-action dosage forms.

Controlled-Release Tablets—Compressed tablets can be formulated to release the drug slowly over a prolonged period of time. Hence, these dosage forms have been referred to as *prolonged-release* or *sustained-release* dosage forms as well. These tablets (as well as capsule versions) can be categorized into three types: (1) those that respond to some physiological condition to release the drug, such as enteric coatings; (2) those that release the drug in a relatively steady, controlled manner; and (3) those that combine combinations of mechanisms to release *pulses* of drug, such as repeat-action tablets. The performance of these systems is described in more detail in Chapter 47.

Tablets for Solution—Compressed tablets to be used for preparing solutions or imparting given characteristics to solutions must be labeled to indicate that they are not to be swallowed. Examples of these tablets are Halazone Tablets for Solution and Potassium Permanganate Tablets for Solution.

Effervescent Tablets—In addition to the drug substance, these contain sodium bicarbonate and an organic acid such as tartaric or citric. In the presence of water, these additives react, liberating carbon dioxide that acts as a distintegrator and produces effervescence. Except for small quantities of lubricants present, effervescent tablets are soluble.

Compressed Suppositories or Inserts—Occasionally, vaginal suppositories, such as Metronidazole Tablets, are prepared by compression. Tablets for this use usually contain lactose as the diluent. In this case, as well as for any tablet intended for administration other than by swallowing, the label must indicate the manner in which it is to be used.

Buccal and Sublingual Tablets—These are small, flat, oval tablets. Tablets intended for buccal administration by inserting into the buccal pouch may dissolve or erode slowly; therefore, they are formulated and compressed with sufficient pressure to give a hard tablet. Progesterone Tablets may be administered in this way.

Some newer approaches use tablets that melt at body temperatures. The matrix of the tablet is solidified while the drug is in solution. After melting, the drug is automatically in solution and available for absorption, thus eliminating dissolution as a rate-limiting step in the absorption of poorly soluble compounds. Sublingual tablets, such as those containing nitroglycerin, isoproterenol hydrochloride, or erythrityl tetranitrate, are placed under the tongue. Sublingual tablets dissolve rapidly, and the drug substances are absorbed readily by this form of administration.

MOLDED TABLETS OR TABLET TRITURATES (TT)

Tablet triturates usually are made from moist material, using a triturate mold that gives them the shape of cut sections of a cylinder. Such tablets must be completely and rapidly soluble. The problem arising from compression of these tablets is the failure to find a lubricant that is completely water-soluble.

Dispensing Tablets (DT)—These tablets provide a convenient quantity of potent drug that can be incorporated readily into powders and liquids, thus circumventing the necessity to weigh small quantities. These tablets are supplied primarily as a convenience for extemporaneous compounding and should never be dispensed as a dosage form.

Hypodermic Tablets (HT)—Hypodermic tablets are soft, readily soluble tablets and originally were used for the preparation of solutions to be injected. Since stable parenteral solutions are now available for most drug substances, there is no justification for the use of hypodermic tablets for injection. Their use in this manner should be discouraged, since the resulting solutions are not sterile. Large quantities of these tablets continue to be made, but for oral administration. No hypodermic tablets ever have been recognized by the official compendia.

Compressed Tablets (CT)

For medicinal substances, with or without diluents, to be made into solid dosage forms with pressure, using available equipment, it is necessary that the material, either in crystalline or powdered form, possess a number of physical characteristics. These characteristics include the ability to flow freely, cohesiveness, and lubrication. The ingredients such as disintegrants designed to break the tablet up in gastrointestinal (GI) fluids and controlled-release polymers designed to slow drug release ideally should possess these characteristics or not interfere with the desirable performance traits of the other excipients. Since most materials have none or only some of these properties, methods of tablet formulation and preparation have been developed to impart these desirable characteristics to the material that is to be compressed into tablets.

The basic mechanical unit in all tablet-compression equipment includes a lower punch that fits into a die from the bottom and an upper punch, with a head of the same shape and dimensions, which enters the die cavity from the top after the tableting material fills the die cavity (see Fig 45-2). The tablet is formed by pressure applied on the punches and subsequently is ejected from the die. The weight of the tablet is determined by the volume of the material that fills the die cavity. Therefore, the ability of the granulation to flow freely into the die is important in ensuring a uniform fill, as well as the continuous movement of the granulation from the source of supply or feed hopper. If the tablet granulation does not possess cohesive properties, the tablet after compression will crumble and fall apart on handling. As the punches must move freely within the die and the tablet must be ejected readily from the punch faces, the material must have a degree of lubrication to minimize friction and allow the removal of the compressed tablets.

There are three general methods of tablet preparation: the wet-granulation method, the dry-granulation method, and direct compression. The method of preparation and the added ingredients are selected to give the tablet formulation the desirable physical characteristics allowing the rapid compression of tablets. After compression, the tab-

Figure 45-2. Basic mechanical unit for tablet compression: lower punch, die, and upper punch (courtesy, Vector/Colton).

lets must have a number of additional attributes such as appearance, hardness, disintegration ability, appropriate dissolution characteristics, and uniformity, which also are influenced both by the method of preparation and by the added materials present in the formulation. In the preparation of compressed tablets, the formulator also must be cognizant of the effect that the ingredients and methods of preparation may have on the availability of the active ingredients and, hence, the therapeutic efficacy of the dosage form. In response to a request by physicians to change a dicumarol tablet so that it might be broken more easily, a Canadian company reformulated to make a large tablet with a score. Subsequent use of the tablet, containing the same amount of drug substance as the previous tablet, resulted in complaints that larger-than-usual doses were needed to produce the same therapeutic response. On the other hand, literature reports indicate that the reformulation of a commercial digoxin tablet resulted in a tablet that, although containing the same quantity of drug substance, gave the desired clinical response at half its original dose. Methods and principles that can be used to assess the effects of excipients and additives on drug absorption have been reviewed.[2,14,15] See Chapters 38, 53, and 58.

TABLET INGREDIENTS

In addition to the active or therapeutic ingredient, tablets contain a number of inert materials. The latter are known as additives or *excipients*. They may be classified according to the part they play in the finished tablet. The first group contains those that help to impart satisfactory processing and compression characteristics to the formulation. These include diluents, binders, glidants, and lubricants. The second group of added substances helps to give additional desirable physical characteristics to the finished tablet. Included in this group are disintegrants, colors, and, in the case of chewable tablets, flavors and sweetening agents, and in the case of controlled-release tablets, polymers or waxes or other solubility-retarding materials.

Although the term *inert* has been applied to these added materials, it is becoming increasingly apparent that there is an important relationship between the properties of the excipients and the dosage forms containing them. Preformulation studies demonstrate their influence on stability, bioavailability, and the processes by which the dosage forms are prepared. The need for acquiring more information and use standards for excipients has been recognized in a joint venture of the Academy of Pharmaceutical Sciences and the Council of the Pharmaceutical Society of Great Britain. The result is called the *Handbook of Pharmaceutical Excipients*. This reference now is distributed widely throughout the world.[16]

Diluents

Frequently, the single dose of the active ingredient is small, and an inert substance is added to increase the bulk to make the tablet a practical size for compression. Compressed tablets of dexamethasone contain 0.75 mg steroid per tablet; hence, it is obvious that another material must be added to make tableting possible. Diluents used for this purpose include dicalcium phosphate, calcium sulfate, lactose, cellulose, kaolin, mannitol, sodium chloride, dry starch, and powdered sugar. Certain diluents, such as mannitol, lactose, sorbitol, sucrose, and inositol, when present in sufficient quantity, can impart properties to some compressed tablets that permit disintegration in the mouth by chewing. Such tablets commonly are called *chewable tablets*. Upon chewing, properly prepared tablets will disintegrate smoothly at a satisfactory rate, have a pleasant taste and feel, and leave no unpleasant aftertaste in the mouth. Diluents used as excipients for direct compression formulas have been subjected to prior processing to give them flowability and compressibility. These are discussed under *Direct Compression*, page 869.

Most formulators of immediate-release tablets tend to use consistently only one or two diluents selected from the above group in their tablet formulations. Usually, these have been selected on the basis of experience and cost factors. However, in the formulation of new therapeutic agents, the compatibility of the diluents with the drug must be considered; eg, calcium salts used as diluents for the broad-spectrum antibiotic tetracycline have been shown to interfere with the drug's absorption from the GI tract. When drug substances have low water solubility, it is recommended that water-soluble diluents be used to avoid possible bioavailability problems. Highly adsorbent substances, eg, bentonite and kaolin, are to be avoided in making tablets of drugs used clinically in small dosage, such as the cardiac glycosides, alkaloids, and the synthetic estrogens. These drug substances may be adsorbed after administration. The combination of amine bases with lactose, or amine salts with lactose in the presence of an alkaline lubricant results in tablets that discolor on aging.

Microcrystalline cellulose (Avicel) usually is used as an excipient in direct-compression formulas. However, its presence in 5 to 15% concentrations in wet granulations has been shown to be beneficial in the granulation and drying processes in minimizing case-hardening of the tablets and in reducing tablet mottling.

Many ingredients are used for several different purposes, even within the same formulation; eg, corn starch can be used in paste form as a binder. When added in drug or suspension form, it is a good disintegrant. Even though these two uses are to achieve opposite goals, some tablet formulas use corn starch in both ways. In some controlled-release formulas, the polymer hydroxypropylmethylcellulose (HPMC) is used both as an aid to prolong the release from the tablet as well as a film-former in the tablet coating. Therefore, most excipients used in formulating tablets and capsules have many uses, and a thorough understanding of their properties and limitations is necessary to use them rationally.

Binders

Agents used to impart cohesive qualities to the powdered material are referred to as binders or granulators. They impart a cohesiveness to the tablet formulation that ensures the tablet remaining intact after compression, as well as improving the free-flowing qualities by the formulation of granules of desired hardness and size. Materials commonly used as binders include starch, gelatin, and sugars such as sucrose, glucose, dextrose, molasses, and lactose. Natural and synthetic gums that have been used include acacia, sodium alginate, extract of Irish moss, panwar gum, ghatti gum, mucilage of isapol husks, carboxymethylcellulose, methylcellulose, polyvinylpyrrolidone, Veegum, and larch arabogalactan. Other agents that may be considered binders under certain circumstances are polyethylene glycol, ethylcellulose, waxes, water, and alcohol.

The quantity of binder used has considerable influence on the characteristics of the compressed tablets. The use of too much binder or too strong a binder will make a hard tablet that will not disintegrate easily and will cause excessive wear of punches and dies. Differences in binders used for CT Tolbutamide resulted in differences in hypoglycemic effects observed clinically. Materials that have no cohesive qualities of their own will require a stronger binder than those with these qualities. Alcohol and water are not binders in the true sense of the word, but because of their solvent action on some ingredients such as lactose, starch, and celluloses, they change the powdered material to granules, and the residual moisture retained enables the materials to adhere together when compressed.

Binders are used both as a solution and in a dry form, depending on the other ingredients in the formulation and the method of preparation. However, several *pregelatinized* starches available are intended to be added in the dry form so

that water alone can be used as the granulating solution. The same amount of binder in solution will be more effective than if it were dispersed in a dry form and moistened with the solvent. By the latter procedure, the binding agent is not as effective in reaching and wetting each of the particles within the mass of powders. Each of the particles in a powder blend has a coating of adsorbed air on its surface, and it is this film that must be penetrated before the powders can be wetted by the binder solution. After wetting, a certain period of time is necessary to dissolve the binder completely and make it completely available for use. Since powders differ with respect to the ease with which they can be wetted and their rate of solubilization, it is preferable to incorporate the binding agent in solution. By this technique it often is possible to gain effective binding with a lower concentration of binder.

The direct-compression method for preparing tablets (see page 869) requires a material that is not only free-flowing but also sufficiently cohesive to act as a binder. This use has been described for a number of materials including microcrystalline cellulose, microcrystalline dextrose, amylose, and polyvinylpyrrolidone. It has been postulated that microcrystalline cellulose is a special form of cellulose fibril in which the individual crystallites are held together largely by hydrogen bonding. The disintegration of tablets containing the cellulose occurs by breaking the intercrystallite bonds by the disintegrating medium.

STARCH PASTE—Corn starch is used widely as a binder. The concentration may vary from 10 to 20%. It usually is prepared as it is to be used, by dispersing corn starch in sufficient cold purified water to make a 5 to 10% *w/w* suspension and warming in a water bath with continuous stirring until a translucent paste forms. It has been observed that during paste formation, not all of the starch is hydrolyzed. Starch paste then is not only useful as a binder, but also as a method to incorporate some disintegrant inside the granules.

GELATIN SOLUTION—Gelatin generally is used as a 10 to 20% solution; gelatin solutions should be prepared freshly as needed and used while warm or they will solidify. The gelatin is added to cold purified water and allowed to stand until it is hydrated. It then is warmed in a water bath to dissolve the gelatin, and the solution is made up to the final volume on a weight basis to give the concentration desired.

CELLULOSIC SOLUTIONS—Various cellulosics have been used as binders in solution form. Hydroxypropylmethylcellulose (HPMC) has been used widely in this regard. Typical of a number of cellulosics, HPMC is more soluble in cold water than hot. It also is more dispersable in hot water than cold. Hence, to obtain a good, smooth gel that is free from lumps or *fisheyes*, it is necessary to add the HPMC in hot, almost boiling water and, under agitation, cool the mixture down as quickly as possible, as low as possible. Other water-soluble cellulosics such as hydroxyethylcellulose (HEC) and hydroxypropylcellulose (HPC) have been used successfully in solution as binders.

Not all cellulosics are soluble in water. Ethylcellulose can be used effectively when dissolved in alcohol or as a dry binder that then is wetted with alcohol. It is used as a binder for materials that are moisture-sensitive.

POLYVINYLPYRROLIDONE—PVP can be used as an aqueous or alcoholic solution, and this versatility has increased its popularity. Concentrations range from 2% and vary considerably.

It will be noted that binder solutions usually are made up to weight rather than volume. This is to enable the formulator to determine the weight of the solids that have been added to the tablet granulation in the binding solution. This becomes part of the total weight of the granulation and must be taken into consideration in determining the weight of the compressed tablet, which will contain the stated amount of the therapeutic agent.

As can be seen by the list of binders in this chapter, most modern binders used in solution are polymeric. Because of this, the flow or spreadability of these solutions becomes important when selecting the appropriate granulating equipment. The rheology of polymeric solutions is a fascinating subject in and of itself and should be considered for these materials.

Lubricants

Lubricants have a number of functions in tablet manufacture. They prevent adhesion of the tablet material to the surface of the dies and punches, reduce interparticle friction, facilitate the ejection of the tablets from the die cavity, and may improve the rate of flow of the tablet granulation. Commonly used lubricants include talc, magnesium stearate, calcium stearate, stearic acid, hydrogenated vegetable oils, and polyethylene glycol (PEG). Most lubricants, with the exception of talc, are used in concentrations below 1%. When used alone, talc may require concentrations as high as 5%. Lubricants are in most cases hydrophobic materials. Poor selection or excessive amounts can result in *waterproofing* the tablets, resulting in poor tablet disintegration and/or delayed dissolution of the drug substance.

The addition of the proper lubricant is highly desirable if the material to be tableted tends to stick to the punches and dies. Immediately after compression, most tablets have the tendency to expand and will bind and stick to the side of the die. The choice of the proper lubricant will overcome this effectively.

The method of adding a lubricant to a granulation is important if the material is to perform its function satisfactorily. The lubricant should be divided finely by passing it through a 60- to 100-mesh nylon cloth onto the granulation. In production this is called *bolting* the lubricant. After adding the lubricant, the granulation is tumbled or mixed gently to distribute the lubricant without coating the particles too well or breaking them down to finer particles. Some research has concluded that the order of mixing of lubricants and other excipients can have a profound effect on the performance of the final dosage form. Thus, attention to the mixing process itself is just as important as the selection of lubricant materials.

These process variables can be seen in the prolonged blending of a lubricant in a granulation. Overblending materially can affect the hardness, disintegration time, and dissolution performance of the resultant tablets.

The quantity of lubricant varies, being as low as 0.1% and, in some cases, as high as 5%. Lubricants have been added to the granulating agents in the form of suspensions or emulsions. This technique serves to reduce the number of operational procedures and thus reduce the processing time.

In selecting a lubricant, proper attention must be given to its compatibility with the drug agent. Perhaps the most widely investigated drug is acetylsalicylic acid. Different talcs varied significantly the stability of aspirin. Talc with a high calcium content and a high loss on ignition was associated with increased aspirin decomposition. From a stability standpoint, the relative acceptability of tablet lubricants for combination with aspirin was found to decrease in the following order: hydrogenated vegetable oil, stearic acid, talc, and aluminum stearate.

The primary problem in the preparation of a water-soluble tablet is the selection of a satisfactory lubricant. Soluble lubricants reported to be effective include sodium benzoate, a mixture of sodium benzoate and sodium acetate, sodium chloride, leucine, and Carbowax 4000. However, it has been suggested that formulations used to prepare water-soluble tablets may represent a number of compromises between compression efficiency and water solubility. While magnesium stearate is one of the most widely used lubricants, its hydrophobic properties can retard disintegration and dissolution. To overcome these waterproofing characteristics, sodium lauryl sulfate sometimes is included. One compound found to have the lubricating properties of magnesium stearate without its disadvantages is magnesium lauryl sulfate. Its safety for use in pharmaceuticals has not been established.

Glidants

A glidant is a substance that improves the flow characteristics of a powder mixture. These materials always are added in the dry state just prior to compression (ie, during the lubrication step). Colloidal silicon dioxide Cab-o-sil *(Cabot)* is the most commonly used glidant and generally is used in low concentrations of 1% or less. Talc (asbestos-free) also is used and may serve the dual purpose of lubricant/glidant.

It is especially important to optimize the order of addition and the mixing process for these materials, to maximize their effect and to make sure that their influence on the lubricant(s) is minimized.

Disintegrants

A disintegrant is a substance or a mixture of substances added to a tablet to facilitate its breakup or disintegration after administration. The active ingredient must be released from the tablet matrix as efficiently as possible to allow rapid dissolution. Materials serving as disintegrants have been classified chemically as starches, clays, celluloses, algins, gums, and cross-linked polymers.

The oldest and still the most popular disintegrants are corn and potato starch that have been well dried and powdered. Starch has a great affinity for water and swells when moistened, thus facilitating the rupture of the tablet matrix. However, others have suggested that its disintegrating action in tablets is due to capillary action rather than swelling; the spherical shape of the starch grains increases the porosity of the tablet, thus promoting capillary action. Starch, 5%, is suggested, but if more rapid disintegration is desired, this amount may be increased to 10 or 15%. Although it might be expected that disintegration time would decrease as the percentage of starch in the tablet increased, this does not appear to be the case for tolbutamide tablets. In this instance, there appears to be a critical starch concentration for different granulations of the chemical. When their disintegration effect is desired, starches are added to the powder blends in the dry state.

A group of materials known as *super disintegrants* have gained in popularity as disintegrating agents. The name comes from the low levels (2 to 4%) at which they are completely effective. Croscarmelose, crospovidone, and sodium starch glycolate represent examples of a cross-linked cellulose, a cross-linked polymer, and a cross-linked starch, respectively.

The development of these disintegrants fostered new theories about the various mechanisms by which disintegrants work. Sodium starch glycolate swells 7- to 12-fold in less than 30 sec. Croscarmelose swells 4- to 8-fold in less than 10 sec. The starch swells equally in all three dimensions, while the cellulose swells only in two dimensions, leaving fiber length essentially the same. Since croscarmelose is the more efficient disintegrating agent, it is postulated that the rate, force, and extent of swelling play an important role in those disintegrants that work by swelling. Cross-linked PVP swells little but returns to its original boundaries quickly after compression. Wicking, or capillary action, also is postulated to be a major factor in the ability of cross-linked PVP to function.[17–19]

In addition to the starches, a large variety of materials have been used and are reported to be effective as disintegrants. This group includes Veegum HV, methylcellulose, agar, bentonite, cellulose and wood products, natural sponge, cation-exchange resins, alginic acid, guar gum, citrus pulp, and carboxymethylcellulose.[20] Sodium lauryl sulfate in combination with starch also has been demonstrated to be an effective disintegrant. In some cases the apparent effectiveness of surfactants in improving tablet disintegration is postulated as due to an increase in the rate of wetting.

The disintegrating agent usually is mixed with the active ingredients and diluents prior to granulation. In some cases it may be advantageous to divide the starch into two portions: one part is added to the powdered formula prior to granulation, and the remainder is mixed with the lubricant and added prior to compression. Incorporated in this manner, the starch serves a double purpose; the portion added to the lubricant rapidly breaks down the tablet to granules, and the starch mixed with the active ingredients disintegrates the granules into smaller particles. Veegum has been shown to be more effective as a disintegrator in sulfathiazole tablets when most of the quantity is added after granulation and only a small amount before granulation. Likewise, the montmorillonite clays were found to be good tablet disintegrants when added to prepared granulations as powder. They are much less effective as disintegrants when incorporated within the granules.

Factors other than the presence of disintegrants can affect the disintegration time of compressed tablets significantly. The binder, tablet hardness, and the lubricant have been shown to influence the disintegration time. Thus, when the formulator is faced with a problem concerning the disintegration of a compressed tablet, the answer may not lie in the selection and quantity of the disintegrating agent alone.

The evolution of carbon dioxide is also an effective way to cause the disintegration of compressed tablets. Tablets containing a mixture of sodium bicarbonate and an acidulant such as tartaric or citric acid will effervesce when added to water. Sufficient acid is added to produce a neutral or slightly acidic reaction when disintegration in water is rapid and complete. One drawback to the use of the effervescent type of disintegrator is that such tablets must be kept in a dry atmosphere at all times during manufacture, storage, and packaging. Soluble, effervescent tablets provide a popular form for dispensing aspirin and noncaloric sweetening agents.

Coloring Agents

Colors in compressed tablets serve functions other than making the dosage form more esthetic in appearance. Color helps the manufacturer to control the product during its preparation, as well as serving as a means of identification to the user. The wide diversity in the use of colors in solid dosage forms makes it possible to use color as an important category in the identification code developed by the AMA to establish the identity of an unknown compressed tablet in situations arising from poisoning.

All colorants used in pharmaceuticals must be approved and certified by the FDA. For several decades colorants have been subjected to rigid toxicity standards, and as a result, a number of colorants have been removed from an approved list of Food, Drug and Cosmetic Act (FD&C) colors, or *delisted*. Several have been listed as well. The colorants currently approved in the US are listed in Table 45-1. Each country has its own list of approved colorants, and formulators must consider this in designing products for the international market.[21]

Any of the approved, certified, water-soluble FD&C dyes, mixtures of the same, or their corresponding lakes may be used to color tablets. A color lake is the combination by adsorption of a water-soluble dye to a hydrous oxide of a heavy metal resulting in an insoluble form of the dye. In some instances multiple dyes are used to give a purposefully heterogeneous coloring in the form of speckling to compressed tablets. The dyes available do not meet all the criteria required for the ideal pharmaceutical colorants. The photosensitivity of several of the commonly used colorants and their lakes has been investigated, as well as the protection afforded by a number of glasses used in packaging tablets.

Another approach for improving the photostability of dyes has been in the use of ultraviolet-absorbing chemicals in the tablet formulations with the dyes. The Di-Pac line *(Amstar)* is a series of commercially available colored, direct-compression sugars.

Table 45-1. Colors Approved for Use in the US in Oral Dosage Forms[a,b]

COLOR	OTHER NAMES	COLOR INDEX (CI 1971)	USE RESTRICTION (US)
FD&C Red 40	Allura red	16035	FDA certification on each lot of dye
D&C Red 33	Acid fuchsin D Naphtalone red B	17200	ADI 0–0.76 mg
D&C Red 36			ADI 0–1.0 mg
Canthaxanthinin	Food orange 8	40850	None
D&C Red 22	Eosin Y	45380	FDA certification on each lot of dye
D&C Red 28	Phloxine B	45410	FDA certification on each lot of dye
D&C Red 3	Erythrosine	45430	FDA certification on each lot of dye
Cochineal extract	Natural red 4 Carmine	75470	None
Iron oxide—red	—	77491	ADI 0–5 mg elemental iron
FD&C Yellow 6	Sunset yellow FCF Yellow orange 5	15985	None
FD&C Yellow 5	Tartrazine	19140	Label declaration and FDA certification on each lot of dye
D&C Yellow 10	Quinoline yellow WS	47005	FDA certification on each lot of dye
Beta-carotene	—	40800	
Iron oxide—yellow	—	77492	ADI 0–5 mg elemental iron
FD&C Blue 1	Brilliant blue FCF	42090	FDA certification on each lot of dye
FD&C Blue 2	Indigotine Indigo carmine	73015	None
FD&C Green 3	Fast green FCF	42035	FDA certification on each lot of dye
Iron oxide—black	—	77499	ADI 0–5 mg elemental iron
Caramel	Burnt sugar	—	None
Titanium dioxide	—	77891	None

[a] Abbreviations: ADI, acceptable daily intake (per kg body weight); CI, color index numbers of 1971 (US); D&C, Drug and Cosmetic Dyes (US); FD&C, Food, Drug and Cosmetic Dyes (US); FDA, Food and Drug Administration (US).
[b] As of February, 1988 and subject to revision.

The most common method of adding color to a tablet formulation is to dissolve the dye in the binding solution prior to the granulating process. Another approach is to adsorb the dye on starch or calcium sulfate from its aqueous solution; the resultant powder is dried and blended with the other ingredients. If the insoluble lakes are used, they may be blended with the other dry ingredients. Frequently during drying, colors in wet granulations migrate, resulting in an uneven distribution of the color in the granulation. After compression, the tablets will have a mottled appearance due to the uneven distribution of the color. Migration of colors may be reduced by drying the granulation slowly at low temperatures and stirring the granulation while it is drying. The affinity of several water-soluble, anionic, certified dyes for natural starches has been demonstrated; in these cases this affinity should aid in preventing color migration.

Other additives have been shown to act as dye-migration inhibitors. Tragacanth (1%), acacia (3%), attapulgite (5%), and talc (7%) were effective in inhibiting the migration of FD&C Blue No 1 in lactose. In using dye lakes, the problem of color migration is avoided since the lakes are insoluble. Prevention of mottling can be helped also by the use of lubricants and other additives that have been colored similarly to the granulation prior to their use. The problem of mottling becomes more pronounced as the concentration of colorants increases. Color mottling is an undesirable characteristic common to many commercial tablets.

Flavoring Agents

In addition to the sweetness that may be afforded by the diluent of the chewable tablet, eg, mannitol or lactose, artificial sweetening agents may be included. Formerly, the cyclamates, either alone or in combination with saccharin, were used widely. With the banning of the cyclamates and the indefinite status of saccharin, new natural sweeteners are being sought. Aspartame *(Searle)*, has found applications in pharmaceutical formulations. Sweeteners other than the sugars have the advantage of reducing the bulk volume, considering the quantity of sucrose required to produce the same degree of sweetness. Being present in small quantities, they do not affect markedly the physical characteristics of the tablet granulation.

POWDER COMPACTION

Compressed tablets became a commercially viable and efficient dosage form with the invention of tablet machines. In 1843 William Brockendon, a British inventor, author, artist, and watchmaker, received British Patent #9977 for *Shaping Pills, Lozenges, and Black Lead by Pressure in Dies.*[32] In over 150 years of tablet manufacture, the basic process has not changed. Surprisingly, improvements have been made only with regards to speed of manufacture and quality control.

The process of compaction has several identifiable phases. As can be seen in Figure 45-3, when powders undergo compression (a reduction in volume), the first process to occur is a consolidation of the powders. During this consolidation phase, the powder particles adopt a more efficient packing order. The

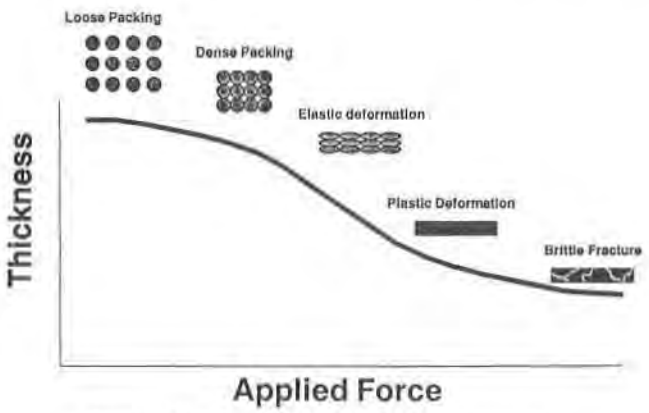


Figure 45-3. The stages of powder compaction.

second phase of the compaction process is elastic, or reversible deformation. If the force were to be removed during this phase, the powder would recover completely to the efficiently packed state. For most pharmaceutical powders, this phase is very short in duration and very difficult to identify on most instrumented tablet presses. The third phase of compaction is plastic, or irreversible, deformation of the powder bed. It is this phase of the compaction process that is the most critical in tablet formation. If too much force is applied to the powder, brittle fracture occurs. If the force was applied too quickly, fracture and debonding during stress relaxation can occur.

In 1950, Stewart reported on the importance of plastic flow and suggested that if a material has significant plastic flow under compression, it will be more likely to form a compact.[23] David and Augsburger evaluated stress-relaxation data, using the Maxwell model of viscoelastic behavior in an attempt to quantify the rate of plastic deformation of some direct compression excipients.[24] Jones has used the term *contact time* to describe the total time for which a moving punch applies a detectable force to the die contents during the compression and decompression event, excluding ejection.[25]

Rees and Rue evaluated three parameters: stress relation during compaction, effect of contact time on tablet density, and rate of application of diametrical compression on tablet deformation.[26]

Jones[25] outlined numerous techniques to evaluate the compactability of powders. Because of the completeness of his review, these parameters are discussed below.

Tablet Strength—Compression Pressure Profile

Most formulators use tablet *hardness*, or tensile strength, as a measure of the cohesiveness of a tablet. With even the simplest of instrumented tablet presses, it is possible to plot tensile strength versus the force applied to the tablet. Figure 45-4 illustrates such a plot. These plots can be useful in identifying forces that can cause fracture and can lead to a quick, tangible assessment of the compatibility of the formulation. However, there are many limitations to this method, as these plots cannot predict *lamination* or *capping*. In addition, the cohesiveness of a tablet can change upon storage, in either a positive or negative direction.

Tablet Friability

This test is discussed later in the chapter, and there have been many suggestions about how they should be performed. Many formulators believe this is an important indicator of cohesiveness but is of limited value in predicting failure in the field.

Changes in Bed Density during Compression

As applied stress (force) increases, elastic and plastic deformation of the particles occurs, which results in plastic flow and a reduction in inter- and intraparticulate void spaces. This lowers the overall compact density.

For highly cohesive systems, the reduction in void space may yield a compact of sufficient strength for insertion into a capsules shell. However, the inherent cohesiveness for most drugs and excipients is not suitable alone for tablet manufacture.

The Heckel equation is given below; K can be considered equal to the reciprocal of the mean yield pressure, and A is a function of the original compact volume and is related to the densification and particle rearrangement prior to bonding.

$$\text{Log } [1/(1 - D)] = KP + A$$

where D is the relative density at pressure P, and K and A are constants.

Hersey and Rees[28] have classified Heckel plots into two categories. Figure 45-5 shows both types of Heckel plots. Type 2 differs from Type 1 in that above a certain pressure a single linear relationship occurs irrespective of the initial bed density. This is independent of particle size and is probable due to fragmentation of particles and their subsequent compaction by plastic deformation. For Type 1 materials, no such fracture occurs, but adjacent particles simply deform plastically.

The pressure at which the plots transition to a linear portion is approximately equal to the minimum pressure required to form a coherent compact.

Changes in Surface Area during Compression

Bulk powders change their state of packing during compaction, and individual particles fracture and/or plastically deform. During this process, the surface area of the powders and the compact in whole, changes. Conventional nitrogen absorption techniques can estimate these changes. Although this can be tedious, these measurements can give a means of examining lamination tendency.

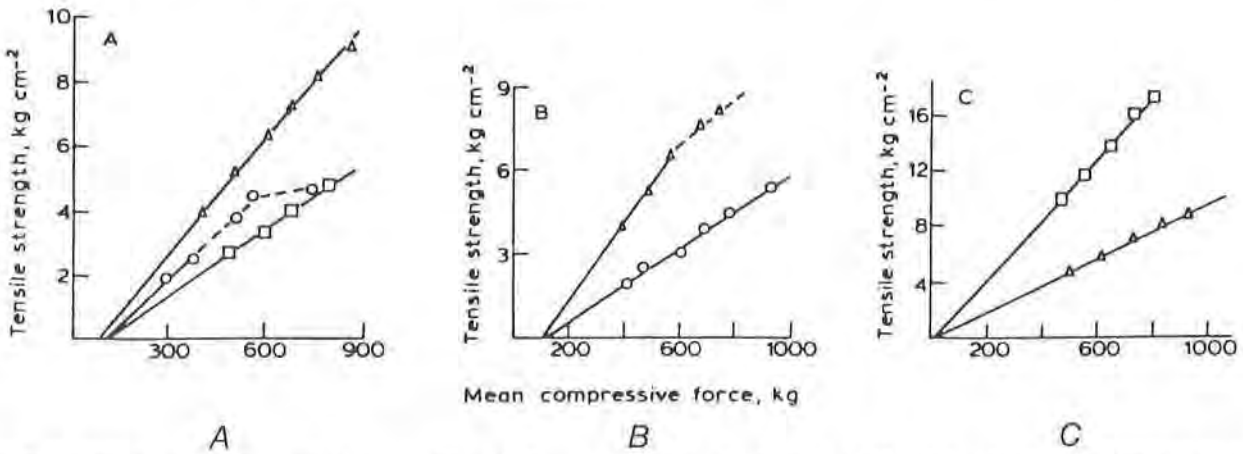
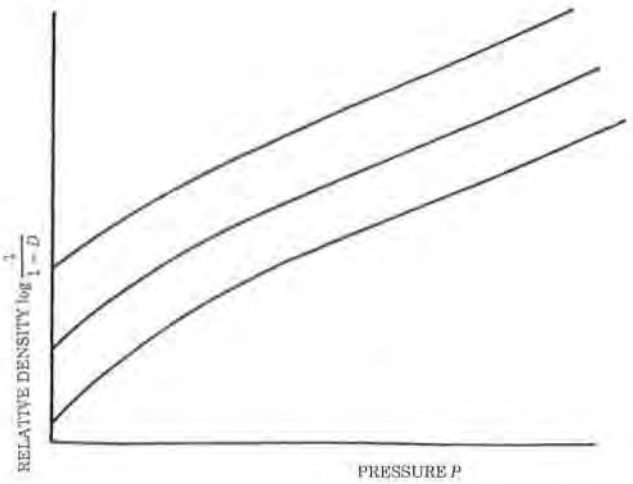
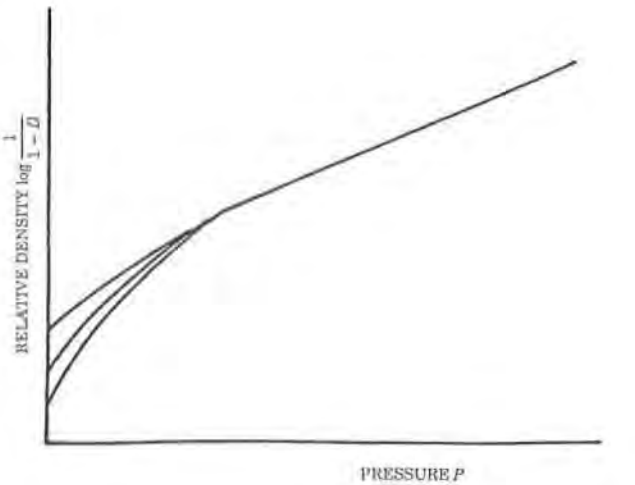


Figure 45-4. Tensile strength of compacts prepared from different crystal forms. *A:* Barbitone (104–152 μm)—○, Form I; □, Form II; △, Form III. *B:* Sulfathiazole (104–152 μm)—○, Form I; △, Form II. *C:* Aspirin (250–353 μm)—△, Form I; □, Form IV (courtesy, Summers *et al*[27]).



A



B

Figure 45-5. Heckel plots. *A:* Type I. *B:* Type II (courtesy, Jones[29]).

Stress Relaxation

The experimental technique consists of holding the compression process at a point of maximum compression and observing the compression force over various periods of time. By increasing the duration of this period (dwell time), plastic flow is maximized, and tablet strength increases.

Stress Transmissions during Compression

If the stresses in the upper punch, lower punch, and die wall are monitored, as in Figure 45-6, a general plot can be constructed showing the relationship between these forces. The elastic limit is reached at point *A*. At point *B*, the applied force is released, and the transmitted force on the wall of the die falls rapidly. The upper punch ceases to contact the powder/compact at point *C*, where the transmitted force falls rapidly to a residual force, point *D*. The force needed to eject the tablet from the die must be greater than the residual force holding it to the sides of the die. Therefore, residual forces tend to be proportional to ejection forces. In addition, these plots can give a good assessment of the elastic component of the compaction process of a powder.

Work and Compaction

Force-displacement (*F-D*) curves are useful in determining the *work* involved in forming a compact. Curves, such as shown in Figure 45-7,[29] represent the work of the compression process, but all compacts expand somewhat during decompression, and this force is transferred back to the punch. Therefore, by performing a second compression of the compact, the second result can be subtracted from the first for a *corrected F-D curve*. The corrected curve represents the work associated with plastic deformation during powder compaction, as well as a determination of the work of friction of the die wall and the work of elastic deformation.

GRANULATION METHODS

Wet Granulation

The most widely used and most general method of tablet preparation is the wet-granulation method. Its popularity is due to the greater probability that the granulation will meet all the physical requirements for the compression of good tablets. Its chief disadvantages are the number of separate steps involved, as well as the time and labor necessary to carry out the procedure, especially on a large scale. The steps in the wet method are weighing, mixing, granulation, screening the damp mass, drying, dry screening, lubrication, and compression. The equipment involved depends on the quantity or size of the batch. The active ingredient, diluent, and disintegrant are mixed or blended well. For small batches the ingredients may be mixed in stainless steel bowls or mortars. Small-scale blending also can be carried out on a large piece of paper by holding the opposite edges and tumbling the material back and forth. The powder blend may be sifted through a screen of suitable fineness to remove or break up lumps. This screening also affords additional mixing. The screen selected always should be of the same type of wire or cloth that will not affect the potency of the ingredients through interaction. For example, the stability of ascorbic acid is affected deleteriously by even small amounts of copper, thus care must be taken to avoid contact with copper or copper-containing alloys.

For larger quantities of powder, the Patterson-Kelley twin-shell blender and the double-cone blender offer a means of precision blending and mixing in short periods of time (Fig 45-8). Twin-shell blenders are available in many sizes from laboratory models to large production models. Planetary mixers, eg, the Glen mixer and the Hobart mixer, have served this

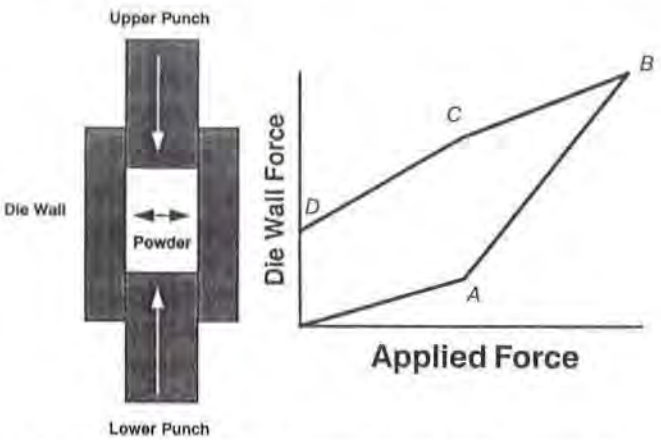


Figure 45-6. Transmitted stresses during tablet compaction.

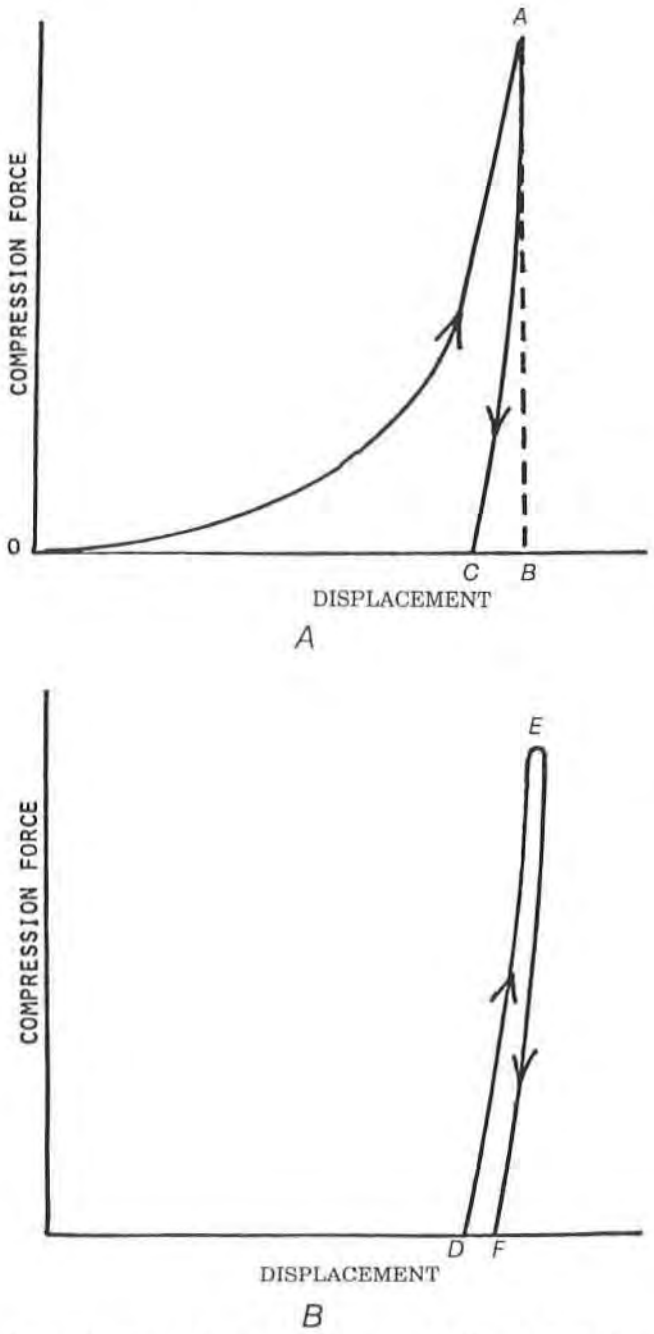


Figure 45-7. Typical forces. *A:* Displacement (F–D) curve; *B:* displacement (F–D), second compression (courtesy, Jones[29]).

function in the pharmaceutical industry for many years (Fig 45-9). On a large scale, ribbon blenders also are employed frequently and may be adapted for continuous-production procedures. Mass mixers of the sigma-blade type have been used widely in the pharmaceutical industry.

Rapidly increasing in popularity are the high-speed, high-shear mixers such as the Lodige/Littleford, Diosna, Fielder, and Baker-Perkins. For these mixers a full range of sizes are available. The processing of granulations in these machines is generally faster than in conventional granulators. However, control over the process is critical, and scale-up issues may become extremely important.[30] Fluid-bed granulation (discussed below) also is gaining wide acceptance in the industry. For both of these types of processing, slight modifications to the following procedures are required.

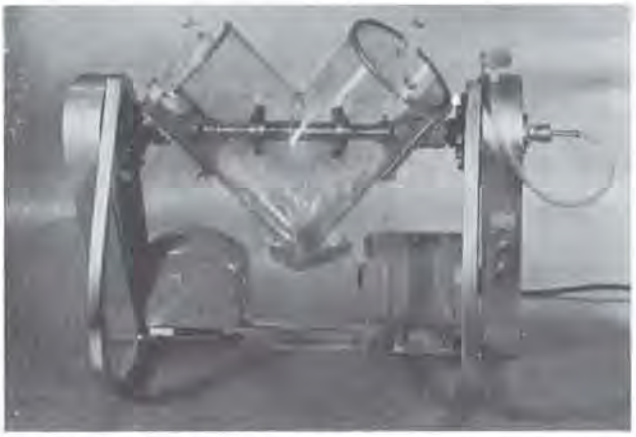


Figure 45-8. Twin-shell blender for solids or liquid-solids blending (courtesy, Patterson-Kelley).

Solutions of the binding agent are added to the mixed powders with stirring. The powder mass is wetted with the binding solution until the mass has the consistency of damp snow or brown sugar. If the granulation is overwetted, the granules will be hard, requiring considerable pressure to form the tablets, and the resultant tablets may have a mottled appearance. If the powder mixture is not wetted sufficiently, the resulting granules will be too soft, breaking down during lubrication and causing difficulty during compression.

The wet granulation is forced through a 6- or 8-mesh screen. Small batches can be forced through by hand using a manual screen. For larger quantities, one of several comminuting mills suitable for wet screening can be used. These include the Stokes oscillator, Colton rotary granulator, Fitzpatrick comminuting mill, or Stokes tornado mill. See Figure 45-10. In comminuting mills the granulation is forced through the sieving device by rotating hammers, knives, or oscillating bars. Most

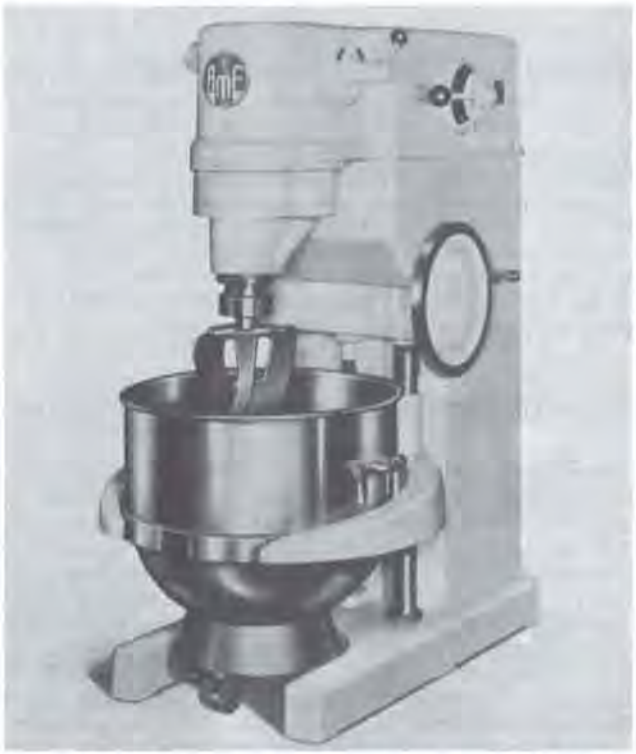


Figure 45-9. The Glen powder mixer (courtesy, Am Machine).

Figure 45-10. Rotary granulator and sifter (courtesy, Vector/Colton).

high-speed mixers are equipped with a chopper blade that operates independently of the main mixing blades and can replace the wet milling step, ie, can obviate the need for a separate operation.

For tablet formulations in which continuous production is justified, extruders such as the Reitz extructor have been adapted for the wet-granulation process. The extruder consists of a screw mixer with a chamber where the powder is mixed with the binding agent, and the wet mass gradually is forced through a perforated screen, forming threads of the wet granulation. The granulation then is dried by conventional methods. A semiautomatic, continuous process using the Reitz extructor has been described for the preparation of the antacid tablet Gelusil *(Warner-Lambert)*.

Moist material from the wet milling step is placed on large sheets of paper on shallow wire trays and placed in drying cabinets with a circulating air current and thermostatic heat control. See Figure 45-11. While tray drying was the most widely used method of drying tablet granulations in the past, fluid-bed drying is now equally popular. Notable among the newer methods being introduced are the fluid-bed dryers. In drying tablet granulation by fluidization, the material is suspended and agitated in a warm air stream while the granulation is maintained in motion. Drying tests comparing the fluidized bed and a tray dryer for a number of tablet granulations indicated that the former was 15 times faster than the conventional method of tray drying. In addition to the decreased drying time, the fluidization method is claimed to have other advantages such as better control of drying temperatures, decreased handling costs, and the opportunity to blend lubricants and other materials into the dry granulation directly in the fluidized bed. See Figure 45-12.[31]

The application of radio-frequency drying and infrared drying to tablet granulations has been reported as successful for most granulations tried. These methods readily lend themselves to continuous granulation operations. The study of drying methods for tablet granulations led to the development of the Rovac dryer system by Ciba pharmacists and engineers. The dryer is similar in appearance to the cone blender except for the heating jacket and vacuum connections. By excluding oxygen and using the lower drying temperatures made possible by drying in a vacuum, opportunities for degradation of the ingredients during the drying cycle are minimized. A greater uniformity of residual moisture content is achieved because of the moving bed, controlled temperature, and controlled time period of the drying cycle. Particle-size distribution can be controlled by varying the speed of rotation and drying temperature as well as by comminuting the granulation to the desired granule size after drying.

In drying granulations it is desirable to maintain a residual amount of moisture in the granulation. This is necessary to maintain the various granulation ingredients, such as gums, in a hydrated state. Also, the residual moisture contributes to the reduction of the static electric charges on the particles. In the selection of any drying process, an effort is made to obtain a uniform moisture content. In addition to the importance of moisture content of the granulation in its handling during the manufacturing steps, the stability of the products containing moisture-sensitive active ingredients may be related to the moisture content of the products.

Previously it was indicated that water-soluble colorants can migrate toward the surface of the granulation during the drying process, resulting in mottled tablets after compression. This is also true for water-soluble drug substances, resulting in tablets unsatisfactory as to content uniformity. Migration can be reduced by drying the granulation slowly at low temperatures or using a granulation in which the major diluent is present as granules of large particle size. The presence of microcrystalline cellulose in wet granulations also reduces migration tendencies.

After drying, the granulation is reduced in particle size by passing it through a smaller-mesh screen. Following dry screening, the granule size tends to be more uniform. For dry granulations the screen size to be selected depends on the diameter of the punch. The following sizes are suggested:

Tablets up to 3/16 inch diameter, use 20-mesh
Tablets 7/32 to 5/16 inch, use 16-mesh
Tablets 11/32 to 13/32 inch, use 14-mesh
Tablets 7/16 inch and larger, use 12-mesh

For small amounts of granulation, hand screens may be used and the material passed through with the aid of a stainless steel spatula. With larger quantities, any of the comminuting mills with screens corresponding to those just mentioned may be used. Note that the smaller the tablet, the finer the dry granulation to enable more uniform filling of the die cavity; large granules give an irregular fill to a comparatively small die cavity. With compressed tablets of sodium bicarbonate, lactose, and magnesium trisilicate, a relationship has been demonstrated between the particle size of the granulated material and the disintegration time and capping of the resultant tablets. For a sulfathiazole granulation, however, the particle-size distribution did not appear to influence hardness or disintegration.

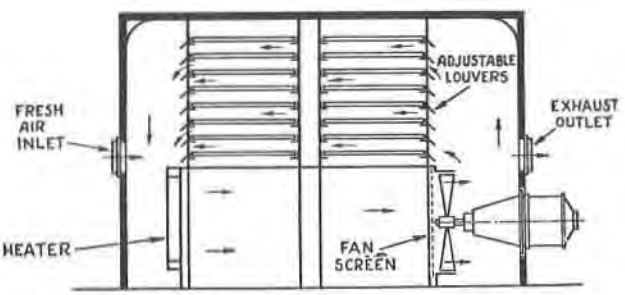


Figure 45-11. Cross-section of tray dryer.

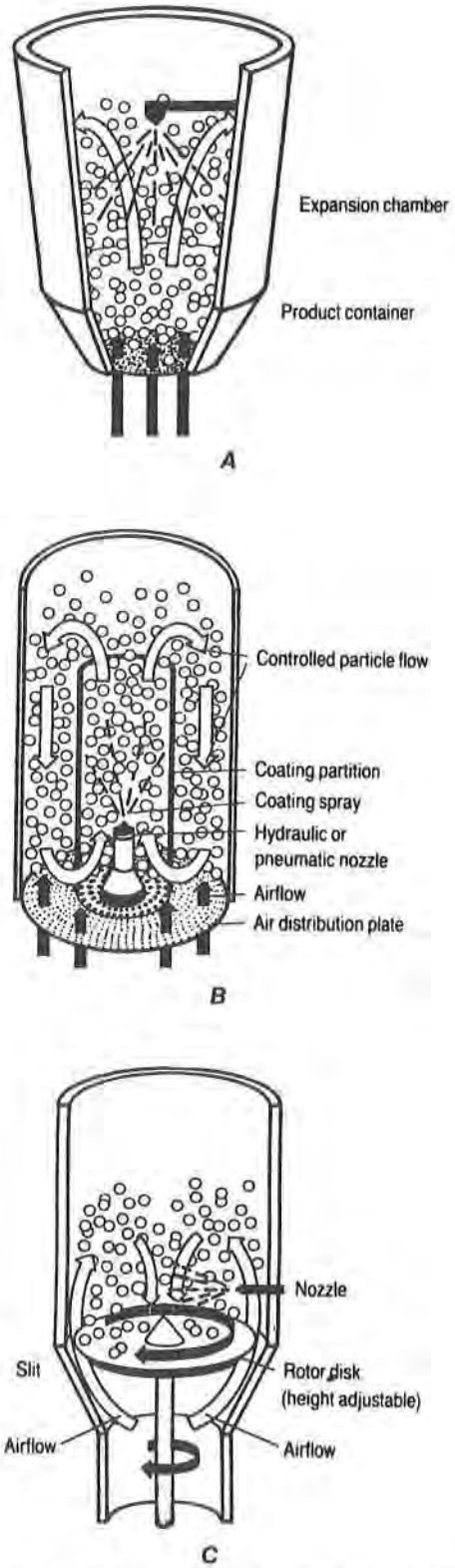


Figure 45-12. Three versions of fluidized-bed granulation and drying. *A:* Top-spray method used in conventional fluid-bed granulation coaters; *B:* bottom-spray method used in Wurster air-suspension columns; *C:* tangential-spray method used in rotary fluid-bed coaters/granulators (courtesy, Aster Publ, adapted from Reference 31).

After dry granulation, the lubricant is added as a fine powder. It usually is screened onto the granulation through 60- or 100-mesh nylon cloth to eliminate small lumps as well as to increase the covering power of the lubricant. As it is desirable for each granule to be covered with the lubricant, the lubricant is blended with the granulation very gently, preferably in a blender using a tumbling action. Gentle action is desired to maintain the uniform granule size resulting from the granulation step. It has been claimed that too much fine powder is not desirable because fine powder may not feed into the die evenly; consequently, variations in weight and density result. Fine powders, commonly designated as *fines,* also blow out around the upper punch and down past the lower punch, making it necessary to clean the machine frequently. Fines, however, at a level of 10 to 20%, traditionally are sought by the tablet formulator. The presence of some fines is necessary for the proper filling of the die cavity. Now, even higher concentrations of fines are used successfully in tablet manufacture. Most investigators agree that no general limits exist for the amount of fines that can be present in a granulation; it must be determined for each specific formula.

Many formulators once believed (and some still believe) that overblending resulted in an increased amount of fines and, hence, caused air entrapment in the formula. The capping and laminating of tablets associated with overblending lubricants was thought to be caused by these air pockets. Most scientists now recognize that a more plausible explanation has to do with the function of the lubricants themselves. Since the very nature of a lubricant tends to make surfaces less susceptible to adhesion, overblending prevents the intergranular bonding that takes place during compaction.

Fluid-Bed Granulation

A new method for granulating evolved from the fluid-bed drying technology described earlier. The concept was to spray a granulating solution onto the suspended particles, which then would be dried rapidly in the suspending air. The main benefit from this system is the rapid granulation and drying of a batch. The two main firms that developed this technology are *Glatt* and *Aeromatic.* The design of these systems is basically the same with both companies (see Fig 45-12). In this method, particles of an inert material or the active drug are suspended in a vertical column with a rising air stream; while the particles are suspended, the common granulating materials in solution are sprayed into the column. There is a gradual particle buildup under a controlled set of conditions resulting in a tablet granulation that is ready for compression after the addition of the lubricant. An obvious advantage exists, since granulating and drying can take place in a single piece of equipment. It should be noted, however, that many of the mixers discussed previously can be supplied with a steam jacket and vacuum and can provide the same advantage.

In these systems a granulating solution or solvent is sprayed into or onto the bed of suspended particles. The rate of addition of the binder, temperature in the bed of particles, temperature of the air, volume, and moisture of the air all play an important role in the quality and performance of the final product. Many scientists feel that this method is an extension of the wet-granulation method, as it incorporates many of its concepts. However anyone who has developed a formulation in a fluid-bed system knows that the many operating parameters involved make it somewhat more complex.[31] In addition to its use for the preparation of tablet granulations, this technique also has been proposed for the coating of solid particles as a means of improving the flow properties of small particles. Researchers have observed that, in general, fluid-bed granulation yields a less dense particle than conventional methods, and this can affect subsequent compression behavior. A large-scale fluid-bed granulation process has been described for Tylenol *(McNeil).* Methods for the preparation of compressed tablets have been reviewed in the literature.[32]

In the *Merck* facility at Elkton, VA, the entire tablet-manufacturing process based on a wet-granulation method is

computer-controlled. By means of a computer, the system weighs the ingredients, blends, granulates, dries, and lubricates to prepare a uniform granulation of specified particle size and particle-size distribution. The computer directs the compression of the material into tablets with exacting specifications for thickness, weight, and hardness. After compression, the tablets are coated with a water-based film coating. The computer controls and monitors all flow of material. The plant represents the first totally automated pharmaceutical manufacturing facility. See Figure 45-13.

Although the Merck facility represents the most fully automated production operation, there are many others throughout the industry that have parts of the operation (such as a coating, compressing, or fluid-bed granulation process) operating under a high degree of sophistication and automation. This is the trend for the future. Equipment suppliers work closely with individual pharmaceutical companies in designing specialized and unique systems.

Dry Granulation

When tablet ingredients are sensitive to moisture or are unable to withstand elevated temperatures during drying, and when the tablet ingredients have sufficient inherent binding or cohesive properties, slugging may be used to form granules. This method is referred to as dry granulation, precompression, or double-compression. It eliminates a number of steps but still includes weighing, mixing, slugging, dry screening, lubrication, and compression. The active ingredient, diluent (if required), and part of the lubricant are blended. One of the constituents, either the active ingredient or the diluent, must have cohesive properties. Powdered material contains a considerable amount of air; under pressure this air is expelled, and a fairly dense piece is formed. The more time allowed for this air to escape, the better the tablet or slug.

When slugging is used, large tablets are made as slugs because fine powders flow better into large cavities. Also, producing large slugs decreases production time; ⅞ to 1 in are the most practical sizes for slugs. Sometimes, to obtain the pressure that is desired the slug sizes are reduced to ¾ in. The punches should be flat-faced. The compressed slugs are comminuted through the desirable mesh screen either by hand or, for larger quantities, through the Fitzpatrick or similar comminuting mill. The lubricant remaining is added to the granulation and blended gently, and the material is compressed into tablets. Aspirin is a good example of where slugging is satisfactory. Other materials such as aspirin combinations, acetophenetidin, thiamine hydrochloride, ascorbic acid, magnesium hydroxide, and other antacid compounds may be treated similarly.

Results comparable to those accomplished by the slugging process also are obtained with compacting mills. In the compaction method the powder to be densified passes between high-pressure rollers that compress the powder and remove the air. The densified material is reduced to a uniform granule size and compressed into tablets after the addition of a lubricant. Excessive pressures that may be required to obtain cohesion of certain materials may result in a prolonged dissolution rate. Compaction mills available include the Chilsonator *(Fitzpatrick)*, Roller Compactor *(Vector)*, and the Compactor Mill *(Allis-Chalmers)*.

Direct Compression

As its name implies, direct compression consists of compressing tablets directly from powdered material without modifying the

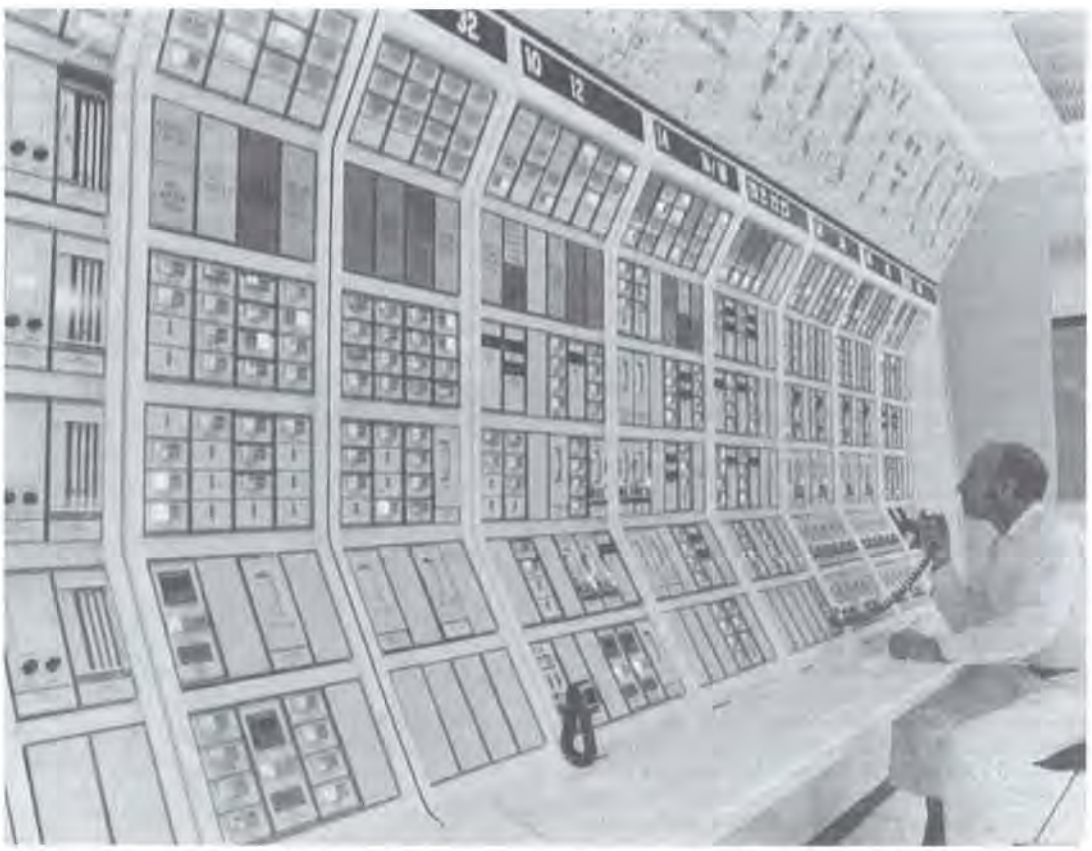


Figure 45-13. Computer control room for the first large-scale computer-controlled tablet manufacturing facility (courtesy, Merck).

physical nature of the material itself. Formerly, direct compression as a method of tablet manufacture was reserved for a small group of crystalline chemicals having all the physical characteristics required for the formation of a good tablet. This group includes chemicals such as potassium salts (chlorate, chloride, bromide, iodide, nitrate, permanganate), ammonium chloride, and methenamine. These materials possess cohesive and flow properties that make direct compression possible.

Since the pharmaceutical industry constantly is making efforts to increase the efficiency of tableting operations and reduce costs by using the smallest amount of floor space and labor as possible for a given operation, increasing attention is being given to this method of tablet preparation. Approaches being used to make this method more universally applicable include the introduction of formulation additives capable of imparting the characteristics required for compression and the use of force-feeding devices to improve the flow of powder blends.

For tablets in which the drug itself constitutes a major portion of the total tablet weight, it is necessary that the drug possess those physical characteristics required for the formulation to be compressed directly. Direct compression for tablets containing 25% or less of drug substances frequently can be used by formulating with a suitable diluent that acts as a carrier or vehicle for the drug.[32–34]

Direct-compression vehicles or carriers must have good flow and compressible characteristics. These properties are imparted to them by a preprocessing step such as wet granulation, slugging, spray drying, spheronization, or crystallization. These vehicles include processed forms of most of the common diluents including dicalcium phosphate dihydrate, tricalcium phosphate, calcium sulfate, anhydrous lactose, spray-dried lactose, pregelatinized starch, compressible sugar, mannitol, and microcrystalline cellulose. These commercially available direct-compression vehicles may contain small quantities of other ingredients (eg, starch) as processing aids. Dicalcium phosphate dihydrate (Di-Tab, *Stauffer*) in its unmilled form has good flow properties and compressibility. It is a white, crystalline agglomerate insoluble in water and alcohol. The chemical is odorless, tasteless, and nonhygroscopic. Since it has no inherent lubricating or disintegrating properties, other additives must be present to prepare a satisfactory formulation.

Compressible sugar consists mainly of sucrose that is processed to have properties suitable for direct compression. It also may contain small quantities of dextrin, starch, or invert sugar. It is a white crystalline powder with a sweet taste and complete water solubility. It requires the incorporation of a suitable lubricant at normal levels for lubricity. The sugar is used widely for chewable vitamin tablets because of its natural sweetness. One commercial source is Di-Pac *(Amstar)* prepared by the cocrystallization of 97% sucrose and 3% dextrins. Some forms of lactose meet the requirements for a direct-compression vehicle. Hydrous lactose does not flow, and its use is limited to tablet formulations prepared by the wet-granulation method. Both anhydrous lactose and spray-dried lactose have good flowability and compressibility and can be used in direct compression provided a suitable disintegrant and lubricant are present. Mannitol is a popular diluent for chewable tablets because of its pleasant taste and mouth feel resulting from its negative heat of solution. In its granular form *(ICI Americas)* it has good flow and compressible qualities. It has a low moisture content and is not hygroscopic.

The excipient that has been studied extensively as a direct compression vehicle is microcrystalline cellulose (Avicel, *FMC*). This nonfibrous form of cellulose is obtained by spray-drying washed, acid-treated cellulose and is available in several grades that range in average particle size from 20 to 100 μm. It is water-insoluble, but the material has the ability to draw fluid into a tablet by capillary action; it swells on contact and thus acts as a disintegrating agent. The material flows well and has a degree of self-lubricating qualities, thus requiring a lower level of lubricant than other excipients.

Forced-flow feeders are mechanical devices, available from pharmaceutical equipment manufacturers, designed to deaerate light and bulky material. Mechanically, they maintain a steady flow of powder moving into the die cavities under moderate pressure. By increasing the density of the powder, higher uniformity in tablet weights is obtained. See Figure 45-14.

Recently, many companies have reversed their optimism for some direct-compression systems. Some formulations made by direct compression were not as *forgiving* as the older wet-granulated products were. As raw material variations occurred, especially with the drug, many companies found themselves with poorly compactable formulations. Interest in direct compression also is stimulating basic research on the flowability of powders with and without additives. Direct compression formulas are included in the formula section found on pages 878 to 881.

Related Granulation Processes

SPHERONIZATION—Spheronization, a form of pelletization, refers to the formation of spherical particles from wet granulations. Since the particles are round, they have good flow properties when dried. They can be formulated to contain sufficient binder to impart cohesiveness for tableting. Spheronization equipment such as the Marumerizer *(Luwa)* and the CF-Granulator *(Vector)* is commercially available. A wet granulation containing the drug substance, diluent (if required), and binder, is passed first through an extruding machine to form rod-shaped cylindrical segments ranging in diameter from 0.5 to 12 mm. The segment diameter and the size of the final spherical particle depend on the extruder screen size. After extrusion the segments are placed into the Marumerizer where they are shaped into spheres by centrifugal and frictional forces on a rotating plate (see Fig 45-15). The pellets then are dried by conventional methods, mixed with suitable lubricants, and compressed into tablets or used as capsule-fill material. Microcrystalline cellulose has been shown to be an effective diluent and binder in granulations to be spheronized.[35–38] The advantages of the process include the production of granules, regular in shape, size, and surface characteristics; low friability resulting in fewer fines and less dust; and the ability to regulate the size of the spheres within a narrow particle-size distribution.

Spheres also can be produced by fluid-bed granulation techniques and by other specialized equipment such as the

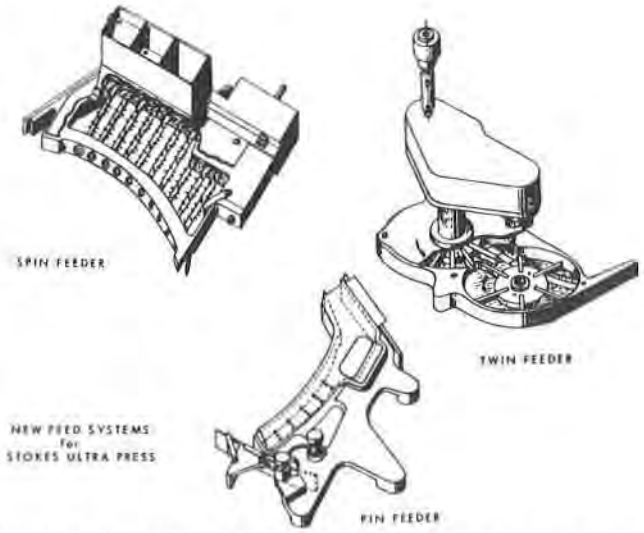


Figure 45-14. Feeding devices designed to promote flow of granulations for high-speed machines (courtesy, Stokes/Pennwalt).

Figure 45-15. The inside of a QJ-400 Marumerizer (courtesy, Luwa).

CF-Granulator *(Vector)*. These processes, however, must begin with crystals or nonpareil seeds followed by buildup. Exact results, such as sphere density, are different for the various methods and could be important in product performance. These processes can be run as batches or continuously.

SPRAY-DRYING—A number of tableting additives suitable for direct compression have been prepared by the drying process known as spray-drying. The method consists of bringing together a highly dispersed liquid and a sufficient volume of hot air to produce evaporation and drying of the liquid droplets. The feed liquid may be a solution, slurry, emulsion, gel, or paste, provided it is pumpable and capable of being atomized. As shown in Figure 45-16, the feed is sprayed into a current of warm filtered air. The air supplies the heat for evaporation and conveys the dried product to the collector; the air is then exhausted with the moisture. As the liquid droplets present a large surface area to the warm air, local heat and transfer coefficients are high.

The spray-dried powder particles are homogeneous, approximately spherical in shape, nearly uniform in size, and frequently hollow. The latter characteristic results in low bulk density with a rapid rate of solution. Being uniform in size and spherical, the particles possess good flowability. The design and operation of the spray-dryer can vary many characteristics of the final product, such as particle size and size distribution, bulk and particle densities, porosity, moisture content, flowability, and friability. Among the spray-dried materials available for direct compression formulas are lactose, mannitol, and flour. Another application of the process in tableting is spray-drying the combination of tablet additives as the diluent, disintegrant, and binder. The spray-dried material then is blended with the active ingredient or drug, lubricated, and compressed directly into tablets.

Since atomization of the feed results in a high surface area, the moisture evaporates rapidly. The evaporation keeps the product cool and as a result the method is applicable for drying heat-sensitive materials. Among heat-sensitive pharmaceuticals successfully spray-dried are the amino acids; antibiotics as aureomycin, bacitracin, penicillin, and streptomycin; ascorbic acid; cascara extracts; liver extracts; pepsin and similar enzymes; protein hydrolysates; and thiamine.[39]

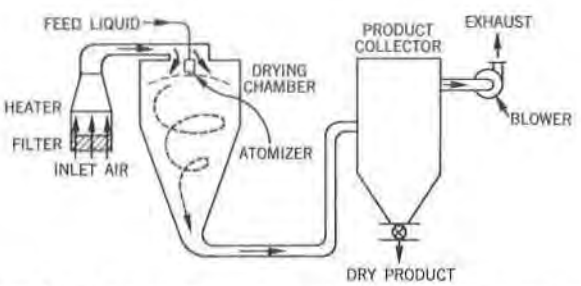


Figure 45-16. Typical spray-drying system (courtesy, Bowen Eng).

Frequently, spray-drying is more economical than other processes, since it produces a dry powder directly from a liquid and eliminates other processing steps as crystallization, precipitation, filtering or drying, particle-size reduction, and particle classifying. By the elimination of these steps, labor, equipment costs, space requirements and possible contamination of the product are reduced. Intrinsic factor concentrate obtained from hog mucosa previously was prepared by *Lederle*, using a salt-precipitation process followed by a freeze-drying. By using spray-drying it was possible to manufacture a high-grade material by a continuous process. The spherical particles of the product facilitated its subsequent blending with vitamin B_{12}. Similar efficiencies have been found in processes producing magnesium trisilicate and dihydroxyaluminum sodium carbonate; both chemicals are used widely in antacid preparations.

Encapsulation of chemicals also can be achieved using spray-drying equipment. The process is useful in coating one material on another to protect the interior substance or to control the rate of its release. The substance to be coated can be either liquid or solid but must be insoluble in a solution of the coating material. The oil-soluble vitamins, A and D, can be coated with a variety of materials such as acacia gum to prevent their deterioration. Flavoring oils and synthetic flavors are coated to give the so-called dry flavors.

SPRAY-CONGEALING—Also called spray-chilling, spray-congealing is a technique similar to spray-drying. It consists of melting solids and reducing them to beads or powder by spraying the molten feed into a stream of air or other gas. The same basic equipment is used as with spray-drying, although no source of heat is required. Either ambient or cooled air is used, depending on the freezing point of the product. For example, monoglycerides and similar materials are spray-congealed with air at 50°F. A closed-loop system with refrigeration cools and recycles the air. Using this process, drugs can be dissolved or suspended in a molten wax and spray-congealed; the resultant material then can be adapted for a prolonged-release form of the drug.

Among the carbohydrates used in compressed tablets, mannitol is the only one that possesses high heat stability. Mannitol melts at 167° and, either alone or in combination with other carbohydrates, can be fused and spray-congealed. Selected drugs have been shown to be soluble in these fused mixtures, and the resultant spray-congealed material possesses excellent flow and compression characteristics.

TABLET MACHINES

As mentioned previously, the basic mechanical unit in tablet compression involves the operation of two steel punches within a steel die cavity. The tablet is formed by the pressure exerted on the granulation by the punches within the die cavity, or cell. The tablet assumes the size and shape of the punches and die used. See Figures 45-17 and 45-18. While round tablets are used more generally, oval, capsule-form, square, triangular, or other irregular shapes may be used. Likewise, the curvature of

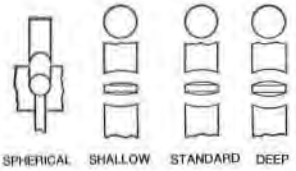


Figure 45-17. Concave punches.

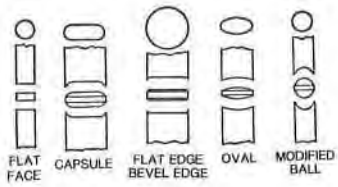


Figure 45-18. Specially shaped punches.

the faces of the punches determines the curvature of the tablets. The diameters generally found to be satisfactory and frequently referred to as standard are as follows: 3/16, 7/32, 1/4, 9/32, 5/16, 11/32, 7/16, 1/2, 9/16, 5/8, 11/16 and 3/4 in. Punch faces with ridges are used for compressed tablets scored for breaking into halves or fourths, although it has been indicated that variation among tablet halves is significantly greater than among intact tablets. However, a patented formulation[40] for a tablet scored to form a groove that is one-third to two-thirds the depth of the total tablet thickness is claimed to give equal parts containing substantially equal amounts of the drug substance. Tablets, engraved or embossed with symbols or initials, require punches with faces embossed or engraved with the corresponding designs. See Figures 45-19 and 45-20. The use of the tablet sometimes determines its shape; effervescent tablets are usually large, round, and flat, while vitamin tablets frequently are prepared in capsule-shaped forms. Tablets prepared using deep-cup punches appear to be round and when coated take on the appearance of pills. Veterinary tablets often have a bolus shape and are much larger than those used in medical practice.

The quality-control program for punches and dies, frequently referred to as tooling, instituted by large pharmaceutical companies, emphasizes the importance of their care in modern pharmaceutical production. To produce physically perfect compressed tablets, an efficient punch-and-die program must be set up. Provisions for inspection of tooling, parameters for cost-per-product determination, product identification, and tooling specifications must all be considered. A committee of the Industrial and Pharmaceutical Technology Section of the APhA Academy of Pharmaceutical Sciences has established a set of dimensional specifications and tolerances for standard punches and dies.[41]

Figure 45-19. Collection of punches (courtesy, Stokes/Pennwalt).

Figure 45-20. Collection of dies (courtesy, Stokes/Pennwalt).

Regardless of the size of the tableting operation, the attention that must be given to the proper care of punches and dies should be noted. They must be highly polished and kept free from rust and imperfections. In cases in which the material pits or abrades the dies, chromium-plated dies have been used. Dropping the punches on hard surfaces will chip their fine edges. When the punches are in the machine, the upper and lower punches should not be allowed to contact each other; otherwise, a curling or flattening of the edges will result that is one of the causes of capping. This is especially necessary to observe in the case of deep-cup punches.

When the punches are removed from the machine, they should be washed thoroughly in warm soapy water and dried well with a clean cloth. A coating of grease or oil should be rubbed over all parts of the dies and punches to protect them from the atmosphere. They should be stored carefully in boxes or paper tubes.

Single-Punch Machines

The simplest tableting machines available are those having the single-punch design. A number of models are available as outlined in Table 45-2. While most of these are power-driven, several hand-operated models are available. Compression is accomplished on a single-punch machine as shown in Figure 45-21. The feed shoe filled with the granulation is positioned over the die cavity, which then fills. The feed shoe retracts and scrapes all excess granulation away from the die cavity. The upper punch lowers to compress the granulation within the die cavity. The upper punch retracts, and the lower punch rises to eject the tablet. As the feed shoe returns to fill the die cavity, it pushes the compressed tablet from the die platform. The weight of the tablet is determined by the volume of the die cavity; the lower punch is adjustable to increase or decrease the volume of granulation, thus increasing or decreasing the weight of the tablet.

Table 45-2. Single-Punch Tablet Machines

MACHINE MODEL	MAXIMUM TABLET DIAMETER (INCHES)	PRESS SPEED (TABLETS/MIN)	DEPTH OF FILL (INCHES)
Stokes-Pennwalt equipment[a]			
511-5	½	40–75	7/16
206-4	1¾	10–40	1 1/16
530-1	2	12–48	1⅝
525-2	3	16–48	2
Manesty equipment (Thomas Eng)			
Hand machine	½	100	7/16
Model F3	⅞	85	1 1/16
Model 35T[a]	3	36	2¼

[a] Widely used for veterinary boluses.

For tablets having diameters larger than ½ inch, sturdier models are required. This is also true for tablets requiring a high degree of hardness, as in the case of compressed lozenges. The heavier models are capable of much higher pressures and are suitable for slugging.

OPERATION OF SINGLE-PUNCH MACHINES

In installing punches and dies in a single-punch machine, insert the lower punch first by lining up the notched groove on the punch with the lower punch setscrew and slipping it into the smaller bore in the die table; the setscrew is not tightened yet. The lower punch is differentiated from the upper punch in that it has a collar around the punch head. Slip the die over the punch head so that the notched groove (with the widest area at the top) lines up with the die setscrew. Tighten the lower punch setscrew after seating the lower punch by pressing on the punch with the thumb. Tighten the die setscrew, making certain that the surface of the die is flush with the die table. Insert the upper punch, again lining up the grooved notch with the upper punch setscrew. To be certain that the upper punch is seated securely, turn the machine over by hand with a block of soft wood or wad of cloth between the upper and lower punches. When the punch is seated, tighten the upper punch setscrew. Adjust the pressure so that the upper and lower punches will not come in contact with each other when the machine is turned over. Adjust the lower punch so that it is flush with the die table at the ejection point. Install the feed shoe and hopper.

After adding a small amount of granulation to the hopper, turn the machine over by hand and adjust the pressure until a tablet is formed. Adjust the tablet weight until the desired weight is obtained. The pressure will have to be altered concurrently with the weight adjustments. It should be remembered that as the fill is increased the lower punch moves farther away from the upper punch, and more pressure will have to be applied to obtain comparable hardness. Conversely, when the fill is decreased, the pressure will have to be decreased. When all the adjustments have been made, fill the hopper with granulation and turn on the motor. Hardness and weight should be checked immediately, and suitable adjustments made if necessary. Periodic checks should be made on the tablet hardness and weight during the running of the batch, at 15- to 30-min intervals.

When the batch has been run off, turn off the power and remove loose dust and granulation with the vacuum cleaner. Release the pressure from the punches. Remove the feed hopper and the feed shoe. Remove the upper punch, the lower punch, and the die. Clean all surfaces of the tablet machine, and dry well with clean cloth. Cover surfaces with thin coating of grease or oil prior to storage.

As tablets are ejected from the machine after compression, they usually are accompanied by powder and uncompressed granulation. To remove this loose dust, the tablets are passed over a screen, which may be vibrating, and cleaned with a vacuum line.

Rotary Tablet Machines

For increased production, rotary machines offer great advantages. A head carrying a number of sets of punches and dies revolves continuously while the tablet granulation runs from the hopper, through a feed frame and into the dies placed in a large, steel plate revolving under it. This method promotes a uniform fill of the die and therefore an accurate weight for the tablet. Compression takes place as the upper and lower punches pass between a pair of rollers, as can be seen in Figure 45-21. This action produces a slow squeezing effect on the material in the die cavity from the top and bottom and so gives a chance for the entrapped air to escape. The lower punch lifts up and ejects the tablet. Adjustments for tablet weight and hardness can be made without the use of tools while the machine is in operation. Figure 45-22 shows a high speed press. Figure 45-23 shows the tooling in a 16-station rotary press in the positions of a complete cycle to produce 1 tablet per set of tooling. One of the factors that contributes to the variation in tablet weight and hardness during compression is the internal flow of the granulation within the feed hopper.

On most rotary machine models there is an excess pressure release that cushions each compression and relieves the machine of all shocks and undue strain. The punches and dies can be removed readily for inspection, cleaning, and inserting different sets to produce a great variety of sizes and shapes. Many older presses have been modernized with protective shields to prevent physical injury and to comply with OSHA standards (see Fig 45-24). It is possible to equip the machine with as few punches and dies as the job requires and thus economize on installation costs. For types of rotary machines available, see Table 45-3.

OPERATION OF ROTARY MACHINES

Before inserting punches and dies, make certain that the pressure has been released from the pressure wheel. The die holes should be cleaned

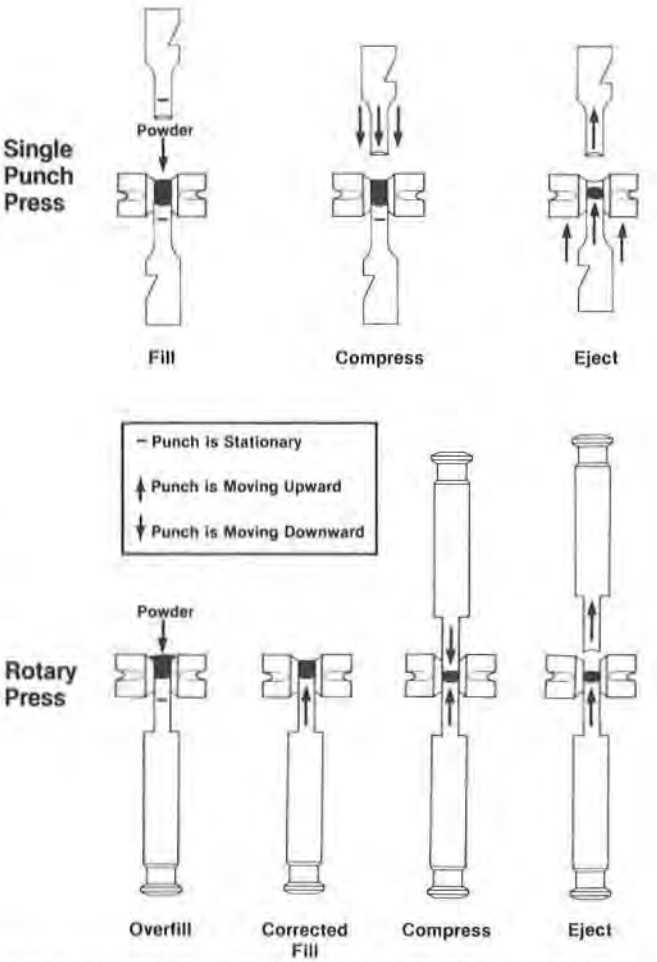


Figure 45-21. The steps associated with single-punch and rotary tablet machines.

Figure 45-22. Model 747 High Speed Press, double-sided rotary compacting press designed to produce at speeds over 10,000/min (courtesy, Stokes/Pennwalt).

thoroughly, making certain that the die seat is completely free of any foreign materials. Back off all die locks, and loosely insert dies into the die holes, then tap each die securely into place with a fiber of soft metal rod through the upper punch holes. After all the dies have been tapped into place, tighten each die lockscrew progressively and securely. As each screw is tightened the die is checked to see that it does not project above the die table. Insert the lower punches through the hole made available by removing the punch head. Turn the machine by hand until the punch bore coincides with the plug hole. Insert each lower punch in its place progressively. Insert the upper punches by dropping them into place in the head. Each punch (upper and lower) should be coated with a thin film of mineral oil before insertion into the machine. Adjust the ejection cam so that the lower punch is flush with the die table at the ejection point.

After insertion of the punches and dies, adjust the machine for the tablet weight and hardness. The feed frame should be attached to the

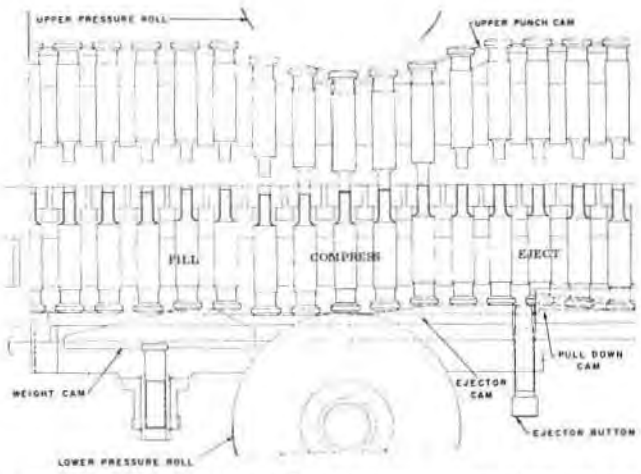


Figure 45-23. Tooling for a 16-station rotary press showing positions of the cycle required to produce one tablet per set of tooling (courtesy, Vector/Colton).

Figure 45-24. Research technicians use an instrumented tablet press to develop processes at Schering-Plough.

machine along with the feed hopper. Add a small amount of the granulation through the hopper and turn over the machine by hand. Increase the pressure by rotating the pressure wheel until a tablet is formed. Check the weight of the tablet and adjust the fill to provide the desired tablet weight. Most likely more than one adjustment of the fill will be necessary before obtaining the acceptable weight. When the fill is decreased, the pressure must be decreased to provide the same hardness in the tablet. Conversely, when the fill is increased, the pressure must be increased to obtain comparable hardness.

Fill the hopper with the granulation and turn on the power. Check tablet weight and hardness immediately after the mechanical operation begins, and make suitable adjustments, if necessary. Check these properties routinely and regularly at 15- to 30-min intervals while the machine is in operation. When the batch has been run, turn off the power. Remove the hopper and feed frame from the machine. Remove loose granulation and dust with a vacuum line. Remove all pressure from the wheel. Remove the punches and dies in the reverse order of that used in setting up the machine. First, remove the upper punches individually, then the lower punches, and finally the dies. Wash each punch and die in alcohol and brush with a soft brush to remove adhering material. Dry them with a clean cloth, and cover them with a thin coating of grease or oil before storing.

High-Speed Rotary Tablet Machines

The rotary tablet machine has evolved gradually into models capable of compressing tablets at high production rates. See Figures 45-22, 45-25, and 45-26. This has been accomplished by increasing the number of stations, ie, sets of punches and dies, in each revolution of the machine head, improving feeding devices, and on some models installing dual compression points. In Figure 45-26, the drawing shows a rotary machine with dual compression points. Rotary machines with dual compression points are referred to as double rotary machines, and those with one compression point, single rotary. In the diagram, half of the tablets are produced 180° from the tablet chute. They travel outside the perimeter and discharge with the second tablet production. While these models are mechanically capable of operating at the production rates shown in Table 45-3, the actual speed still depends on the physical characteristics of the tablet granulation and the rate that is consistent with compressed tablets having satisfactory physical characteristics. The main difficulty in rapid machine operation is ensuring adequate filling of the dies. With rapid filling, dwell time of the die cavity beneath the feed frame is insufficient to ensure the requirements of uniform flow and packing of the dies. Various methods of force-feeding the granulation into the dies have been devised to refill the dies in the very short

Table 45-3. High-Speed Rotary Tablet Machines

MACHINE MODEL	TOOL SETS	MAXIMUM TABLET DIAMETER (INCHES)	PRESS SPEED (TABLETS/MIN)	DEPTH OF FILL (INCHES)
Vector-Colton equipment				
2216	16	5/8	1180	3/4
240	16	7/8	640	13/16
250	12	1 1/4	480	1 1/8
260	25	1 3/16	1450	1 3/8
	31	1	1800	1 3/8
	33	15/16	1910	1 3/8
	43	5/8	2500	1 3/8
270	25	1 3/8	450	2 3/4
Stokes/Pennwalt equipment				
Manesty equipment (Thomas Eng)				
B3B	16	5/8	350–700	11/16
	23	7/16	500–1000	11/16
BB3B	27	5/8	760–1520	11/16
	33	7/16	924–1848	11/16
	35	5/8	1490–2980	11/16
	45	7/16	1913–3826	11/16
D3B	16	1	260–520	13/16
Key equipment				
DC-16	16	15/16	210–510	13/16
BBC	27	5/8	1025–2100	11/16
	35	5/8	1325–2725	11/16
	45	7/16	1700–3500	11/16
Cadpress	37	15/16	850–3500	13/16
	45	5/8	2000–6000	11/16
	55	7/16	2500–7500	11/16
Fette equipment (Raymond Auto)		(mm)		(mm)
Perfecta 1000	28	16	2100	18
	33	13	2475	18
Perfecta 2000	29	25	2175	22
	36	16	3600	18
	43	13	4300	18
Courtoy equipment (AC Compact)				
R-100	24	25	285–2260	20
	30	19	356–2850	20
	36	13	550–440	16
Kikusui equipment				
Hercules	18	37	180–540	16
	21	26	210–630	16
	29	25	290–870	16
Virgo	19	16	418–1330	16
	24	11	528–1680	16
Killian equipment				
TX21	21	28	231–1386	20
TX25	25	22	275–2166	20
TX30	30	16	330–3150	20
TX21D	21	25	231–1826	20
TX30A	30	16	330–3150	16
TX40A	40	13	440–4200	16
Korsch equipment				
PH 250/20	20	25	240–1640	22
PH 250/25	25	16	270–2700	18
PH 250/30	30	13	315–3233	18
Elizabeth-Hata equipment				
AP-15-SSU	15	17	300–1050	8–18
AP-18-SSU	18	13	360–1260	8–18
AP-22-SSU	22	11	440–1540	8–18
AP-32-MSU	32	17	640–2240	8–18
AP-38-MSU	38	13	760–2660	8–18
AP-45-MSU	32	11	900–3150	8–18
Vector-Colton equipment				
2247	33	5/8	3480	3/4
	41	7/16	4300	3/4
	49	7/16	5150	3/4
Magna	66	22/32	10,560	3/4
	74	1/2	11,840	3/4
	90	7/16	14,400	3/4
Stokes/Pennwalt equation				
552-2	35	5/8	800–3200	11/16
328-4	45	3/4	1600–4500	1 3/8
610	65	7/16	3500–10,000	11/16
747	65	7/16	3000–10,000	11/16
	53	5/8	2900–8100	11/16
	41	15/16	2150–6150	11/16
Direct Triple Compression Type				
580-1	45	7/16	525–2100	11/16
580-2	35	5/8	400–1600	11/16
610	65	7/16	3500–10,000	11/16
	53	5/8	2900–8100	11/16
Manesty equipment (Thomas Eng)				
Betapress	16	5/8	600–1500	11/16
	23	7/16	860–2160	11/16
Express	20	1	800–2000	13/16
	25	5/8	1000–2500	11/16
	30	7/16	1200–3000	11/16
Unipress	20	1	970–2420	13/16
	27	5/8	1300–3270	11/16
	34	7/16	1640–4120	11/16
Novapress	37	1	760–3700	13/16
	45	5/8	900–4500	11/16
	61	7/16	1220–6100	11/16
BB3B	35	5/8	1490–2980	11/16
BB4	27	5/8	900–2700	11/16
	35	5/8	1167–3500	11/16
	45	7/16	1500–4500	11/16
Rotapress				
Mark IIA	37	1	710–3550	13/16
	45	5/8	1640–8200	11/16
	61	7/16	2200–11,100	11/16
Mark IV	45	1	2090–6000	13/16
	55	5/8	2550–7330	11/16
	75	7/16	3500–10,000	11/16
Fette tool systems		(mm)		(mm)
PT 2080	29	25	435–2900	18
	36	16	540–4100	18
	43	16	645–4900	18
PT 2090IC	22	34	1760	18
	29	25	2900	18
	36	16	4140	18
	43	13	5160	18
	47	11	6110	18
PT 3090IC	37	34	5920	18
	49	25	7840	18
	61	16	9760	18
	73	13	16,748	18
P 3100	37	25	5618	22
	45	16	8100	18
	55	13	9900	18
Courtoy equipment (AC Compact)				
R-200	43	25	750–5833	20
	55	19	916–8500	20
	65	13	1083–10,000	16
Kikusui equipment				
Libra	36	16	900–2520	16
	45	11	1125–3150	16
	49	8	1225–3430	16

Table 45-3. High-Speed Rotary Tablet Machines (continued)

MACHINE MODEL	TOOL SETS	MAXIMUM TABLET DIAMETER (INCHES)	PRESS SPEED (TABLETS/MIN)	DEPTH OF FILL (INCHES)
Gemini	55	16	2200–7700	16
	67	11	2680–9380	16
	73	8	2920–10,200	16
Elizabeth-Hata equipment				
AP-45-LDU	45	17	1800–6300	8–18
AP-55-LDU	55	13	2200–7700	8–18
AP-65-LDU	65	11	2600–9100	8–18
AP-71-LDU	71	11	2840–9940	8–18
51-XLDU	51	17	2040–7140	8–18
65-XLDU	61	13	2440–8540	8–18

dwell time permitted on the high-speed machine. These devices are illustrated in Figure 45-14. Presses with triple compression points (see Table 45-3) permit the partial compaction of material before final compaction. This provides for partial deaeration and particle orientation of material before final compression. This helps in the direct compacting of materials and reduces laminating and capping due to entrapped air.

Multilayer Rotary Tablet Machines

The rotary tablet machines also have been developed into models capable of producing multiple-layer tablets; the machines are able to make 1, 2 or 3-layer tablets (Versa Press, *Stokes/Pennwalt*). Stratified tablets offer a number of advantages. Incompatible drugs can be formed into a single tablet by separating the layers containing them with a layer of inert material. It has permitted the formulation of time-delay medication and offers a wide variety of possibilities in developing color combinations that give the products identity.

Figure 45-25. Rotapress Mark IIA. Designed for improvements in sound reduction, operator safety, cleanliness, and operational convenience; note the control panel on front of machine (courtesy, Thomas/Manesty).

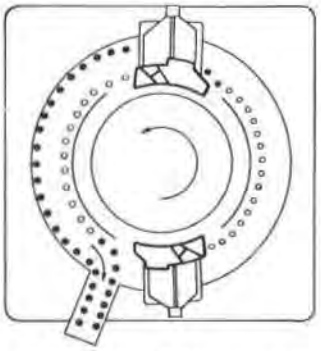


Figure 45-26. The movement of tablets on die table of a double rotary press (courtesy, Vector/Colton).

Originally, the tablets were prepared by a single-compression method. The dies were filled with the different granulations in successive layers, and the tablet was formed by a single compression stroke. The separation lines of the tablets prepared by this method tended to be irregular. In the machines now available for multilayer production the granulation receives a precompression stroke after the first and second fill, which lightly compacts the granulation and maintains a well-defined surface of separation between each layer. The operator is able to eject either precompressed layer with the machine running at any desired speed for periodic weight and analysis checks.

Other multiple-compression presses can receive previously compressed tablets and compress another granulation around the preformed tablet. An example of a press with this capability is the Manesty Drycota *(Thomas/Manesty)*. Pressure-coated tablets can be used to separate incompatible drug substances and also to give an enteric coating to the core tablets.

Capping and Splitting of Tablets

The splitting or capping of tablets is one of great concern and annoyance in tablet making. It is quite difficult to detect while the tablets are being processed but can be detected easily by vigorously shaking a few in the cupped hands. A slightly chipped tablet does not necessarily mean that the tablet will cap or split.

There are many factors that may cause a tablet to cap or split:

Excess *fines* or powder, which traps air in the tablet mixture.

Deep markings on tablet punches. Many designs or *scores* on punches are too broad and deep. Hairline markings are just as appropriate as deep, heavy markings.

Worn and imperfect punches. Punches should be smooth and buffed. Nicked punches often cause capping. The development of fine feather edges on tablets indicates wear on punches.

Worn dies. Dies should be replaced or reversed. Dies that are chrome-plated or have tungsten carbide inserts wear longer and give better results than ordinary steel dies.

Too much pressure. By reducing the pressure on the machines the condition may be corrected.

Unsuitable formula. It may be necessary to change the formula.

Moist and soft granulation. This type of granulation will not flow freely into the dies, thus giving uneven weights and soft or capped tablets.

Poorly machined punches. Uneven punches are detrimental to the tablet machine itself and will not produce tablets of accurate weight. One punch out of alignment may cause one tablet to split or cap on every revolution.

Figure 45-27. Courtoy R-100 with computer-controlled operation.

Instrumented Tablet Presses

Compressional and ejectional forces involved in tablet compression can be studied by attaching strain gauges to the punches and other press components involved in compression. The electrical output of the gauges has been monitored by telemetry or use of a dual-beam oscilloscope equipped with camera.[42,43] Instrumentation permits a study of the compaction characteristics of granulations, their flowabilities, and the effect of formulation additives, such as lubricants, as well as differences in tablet press design, as shown in Figures 45-27 to 45-30. Physical characteristics of tablets, such as hardness, friability, disintegration time, and dissolution rate, are influenced not only by the nature of the formulation but by the compressional force as well.

Figure 45-28. Direct weighing of tablets produced gives actual weight feedback for the controller of the Courtoy R-100 (seen in the bottom left of Fig 45-27).

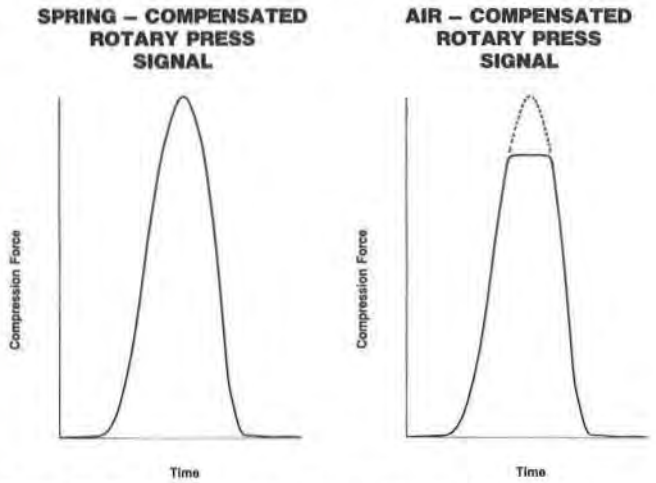


Figure 45-29. Force-time curves for two types of tablet press.

As can be seen in Figures 45-29 and 45-30, the rate and duration of compaction forces can be quantified. The rate of force application has a profound effect on powder consolidation within the die and, hence, efficiency of packing and powder compaction. The rate of release of force, or *decompression* has a direct effect on the ability of the tablet to withstand relaxation. A prominent hypothesis, fostered by Hiestand[44,45] and later Luenberger[46], suggested that capping and laminating of tablets is caused by too-rapid stress relaxation or decompression. This explains why slowing a tablet press and using tapered dies is useful in such situations. Most prominent pharmaceutical scientists have embraced this theory and largely have discounted air entrapment as a cause of capping and laminating.

Figure 45-30 presents an interesting set of plots. Walter and Augsburger reported that as compaction force rises, the steel tooling actually compresses in accommodation to the forces applied. The forces used to produce a tablet are considerable and should be monitored and understood.[47] Therefore, definition of the compressional force and duration of force (dwell time) giving a satisfactory tablet for a formulation provides an in-process control for obtaining both tablet-to-tablet and lot-to-lot uniformity (see Figs 45-24 and 45-31).

Instrumentation has led to the development of on-line, automatic, electromechanical tablet weight-control systems capa-

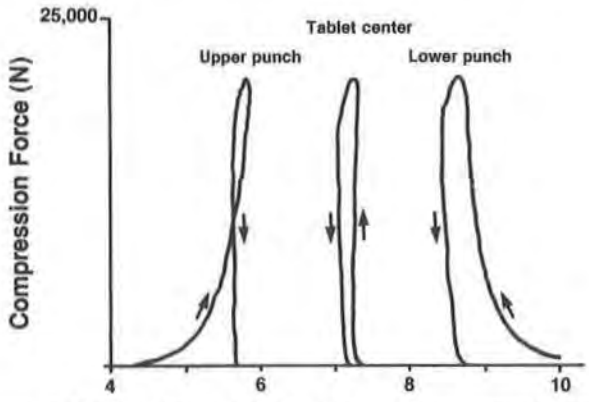


Figure 45-30. Plot showing the upper and lower punch forces as functions of the position of the punch face within the die. A biaxial force/displacement curve also shown is a plot of the position of the tablet center as a function of the compression force.

ble of continuously monitoring the weights of tablets as they are produced. Units are available commercially (Thomas Tablet Sentinel *(Thomas Eng);* Fette Compression Force Monitor *(Raymond Auto);* Vali-Tab *(Stokes/Pennwalt)*) and are applicable to single or rotary tablet machines. Most commercial presses today can be delivered with some sort of instrumentation attached. When tablet weights vary from preset limits, the monitor automatically will adjust the weight control mechanism to reestablish weights within acceptable limits. If the difficulty continues, the unit will activate an audible warning signal or an optional shut-down relay on the press (see Figs 45-27 and 45-28). Most production-model tablet presses come equipped with complete instrumentation (optional) and with options for statistical analysis and print out of compression/ejection signals. The techniques and applications of press instrumentation have been reviewed.[48,49]

Contamination Control

While good manufacturing practices used by the pharmaceutical industry for many years have stressed the importance of cleanliness of equipment and facilities for the manufacture of drug products, the penicillin contamination problem resulted in renewed emphasis on this aspect of manufacturing. Penicillin, as either an airborne dust or residual quantities remaining in equipment, is believed to have contaminated unrelated products in sufficient concentrations to cause allergic reactions in individuals hypersensitive to penicillin who received these products. This resulted in the industry spending millions of dollars to change or modify buildings, manufacturing processes, equipment, and standard operating procedures to eliminate penicillin contamination.

With this problem has come renewed emphasis on the dust problem, material handling, and equipment cleaning in dealing with drugs, especially potent chemicals. Any process using chemicals in powder form can be a dusty operation; the preparation of compressed tablets and encapsulation fall in this category. In the design of tablet presses attention is being given to the control and elimination of dust generated in the tableting process. In the Perfecta press shown in Figure 45-32, the pressing compartment is completely sealed off from the outside environment, making cross-contamination nearly impossible. The pressing compartment can be kept dust-free by the air supply and vacuum equipment developed for the machine. It removes airborne dust and granular particles that have not been compressed, thus keeping the circular pressing compartment and the upper and lower punch guides free of dust.

Drug manufacturers have the responsibility to make certain that microorganisms present in finished products are unlikely to cause harm to the patient and will not be deleterious to the product. An outbreak of *Salmonella* infections in Scandinavian countries was traced to thyroid tablets that had been prepared from contaminated thyroid powder. This concern eventually led to the establishment of microbial limits for raw materials of animal or botanical origin, especially those that readily support microbial growth and are not rendered sterile during subsequent processing. Harmful microorganisms when present in oral products include *Salmonella* spp, *Escherichia coli,* certain *Pseudomonas* spp such as *P aeruginosa,* and *Staphylococcus aureus*. The compendia have microbial limits on raw materials such as aluminum hydroxide gel, corn starch, thyroid, acacia, and gelatin.

These represent examples of the industry's efforts to conform with the intent of current good manufacturing practice as defined by the FDA.

Figure 45-32. Fette Perfecta 3000 high-speed tablet press with pressing compartment completely sealed off from outside environment, making cross-contamination impossible (courtesy, Raymond Auto).

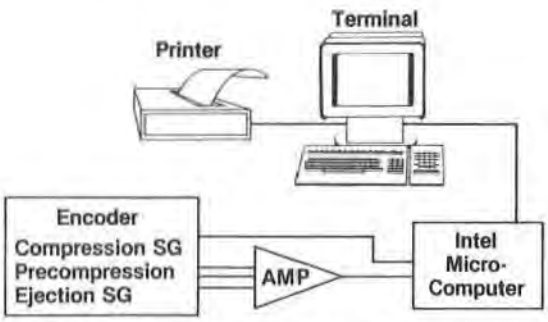


Figure 45-31. Schematic of an instrumentation system using a microcomputer as developed by Schering-Plough.

Tablet Formulations

WET GRANULATION

CT Acetaminophen, 300 mg

INGREDIENTS	IN EACH	IN 10,000
Acetaminophen	300 mg	3000 g
Polyvinylpyrrolidone	22.5 mg	225 g
Lactose	61.75 mg	617.5 g
Alcohol SD3A—200 proof	4.5 mL	45 L
Stearic acid	9 mg	90 g
Talc	13.5 mg	135 g
Corn starch	43.25 mg	432.5 g

Blend acetaminophen, polyvinylpyrrolidone, and lactose together; pass through a 40-mesh screen. Add the alcohol slowly, and knead well. Screen the wet mass through a 4-mesh screen. Dry the granulation at 50° overnight. Screen the dried granulation through a 20-mesh screen. Bolt the stearic acid, talc, and cornstarch through a 60-mesh screen prior to mixing by tumbling with the granulation. Compress, using 7/16-inch standard concave punch. Ten tablets should weigh 4.5 g (courtesy, Abbott).

CT Ascorbic Acid USP, 50 mg

INGREDIENTS	IN EACH	IN 7000
Ascorbic acid USP (powder No. 80)[a]	55 mg	385 g
Lactose	21 mg	147 g
Starch (potato)	13 mg	91 g
Ethylcellulose N 100 (80–105 cps)	16 mg	112 g
Starch (potato)	7 mg	49 g
Talc	6.5 mg	45.5 g
Calcium stearate (impalpable powder)	1 mg	7 g
Weight of granulation		836.5 g

[a] Includes 10% in excess of label claim.

Granulate the first three ingredients with ethylcellulose (5%) dissolved in anhydrous ethyl alcohol, adding additional anhydrous alcohol to obtain good, wet granules. Wet-screen through a #8 stainless steel screen and dry at room temperature in an air-conditioned area. Dry-screen through a #20 stainless steel screen and incorporate the remaining three ingredients. Mix thoroughly and compress. Use a flat, beveled, ¼-inch punch. Twenty tablets should weigh 2.39 g.

Chewable Antacid Tablets

INGREDIENTS	IN EACH	IN 10,000
Magnesium trisilicate	500 mg	5000 g
Aluminum hydroxide, dried gel	250 mg	2500 g
Mannitol	300 mg	3000 g
Sodium saccharin	2 mg	20 g
Starch paste, 5%	qs	qs
Oil of peppermint	1 mg	10 g
Magnesium stearate	10 mg	100 g
Corn starch	10 mg	100 g

Mix the magnesium trisilicate and aluminum hydroxide with the mannitol. Dissolve the sodium saccharin in a small quantity of purified water, then combine this with the starch paste. Granulate the powder blend with the starch paste. Dry at 140°F and screen through 16-mesh screen. Add the flavoring oil, magnesium stearate, and corn starch; mix well. Age the granulation for at least 24 hr and compress, using a ⅝-inch, flat-face, bevel-edge punch (courtesy, *Atlas*).

CT Hexavitamin

INGREDIENTS	IN EACH	IN 7000
Ascorbic acid USP (powder)[a]	82.5 mg	577.5 g
Thiamine mononitrate USP (powder)[a]	2.4 mg	16.8 g
Riboflavin[a]	3.3 mg	23.1 g
Nicotinamide USP (powder)[a]	22 mg	154 g
Starch	13.9 mg	97.4 g
Lactose	5.9 mg	41.2 g
Zein	6.4 mg	45 g
Vitamin A acetate	6250 U	
Vitamin D_2[a] (use Pfizer crystalets medium granules containing 500,000 U vitamin A acetate and 50,000 U vitamin D_2/g)	625 U	87.5 g
Magnesium stearate		7.5 g
Weight of granulation		1050 g

[a] Includes the following in excess of label claim: ascorbic acid 10%, thiamine mononitrate 20%, riboflavin 10%, nicotinamide 10%, and vitamin A acetate–vitamin D_2 crystalets 25%.

Thoroughly mix the first six ingredients and granulate with zein (10% in ethyl alcohol, adding additional alcohol if necessary to obtain good, wet granules). Wet-screen through a #8 stainless steel screen and dry at 110 to 120°F. Dry-screen through a #20 stainless steel screen and add the vitamin crystalets. Mix thoroughly, lubricate, and compress. Ten tablets should weigh 1.50 g. Coat with syrup.

CT Theobromine-Phenobarbital

INGREDIENTS	IN EACH	IN 7000
Theobromine	325 mg	2275 g
Phenobarbital	33 mg	231 g
Starch	39 mg	273 g
Talc	8 mg	56 g
Acacia (powder)	8 mg	56 g
Stearic acid	0.7 mg	4.9 g
Weight of granulation		2895.9 g

Prepare a paste with the acacia and an equal weight of starch. Use this paste for granulating the theobromine and phenobarbital. Dry and put through a 12-mesh screen, add the remainder of the material, mix thoroughly, and compress into tablets, using a 1 3/32-inch concave punch. Ten tablets should weigh 4.13 g.

FLUID-BED GRANULATION

CT Ascorbic Acid USP, 50 mg

INGREDIENTS	IN EACH	IN 10,000
Ascorbic acid USP (powder no 80)[a]	55 mg	550 g
Lactose	21 mg	210 g
Starch (potato)	13 mg	130 g
Ethylcellulose N100 (80–105 cps)	16 mg	160 g
Starch (potato)	7 mg	70 g
Talc	6.5 mg	65 g
Calcium stearate	1 mg	10 g
Weight of granulation		1195.0 g

[a] Includes 10% in excess of claim.
Add the first three ingredients to the granulator. Mix for 5 to 15 min or until well mixed. Dissolve the ethylcellulose in anhydrous ethanol and spray this solution and any additional ethanol into the fluidized mixture. Cease spraying when good granules are produced. Dry to approximately 3% moisture. Remove the granules and place them in a suitable blender. Sequentially add the remaining three ingredients with mixing steps in between each addition. Compress, using a flat, beveled, ⅛-inch punch. Twenty tablets should weigh 2.39 g.

Sustained-Release (SR) Procainamide Tablets

INGREDIENTS	IN EACH	IN 10,000
Procainamide	500 mg	5000 g
HPMC 2208, USP	300 mg	3000 g
Carnauba wax	60 mg	600 g
HPMC 2910, USP	30 mg	300 g
Magnesium stearate	4 mg	40 g
Stearic acid	11 mg	110 g
Talc	5 mg	50 g
Weight of granulation		9100 g

Place the first three ingredients in the granulator and mix for 5 to 15 min. Dissolve the HPMC in water (mix in hot water, then cool down) and spray into the fluidized mixture. Dry to approximately 5% moisture. Sequentially add the last three ingredients, with mixing steps in between each addition. Compress, using capsule-shaped tooling. Ten tablets should weigh 9.1 g.

DRY GRANULATION

CT Acetylsalicylic Acid

INGREDIENTS	IN EACH	IN 7000
Acetylsalicylic Acid (crystals 20-mesh)	0.325 g	2275 g
Starch		226.8 g
Weight of granulation		2501.8 g

Dry the starch to a moisture content of 10%.Thoroughly mix this with the acetylsalicylic acid. Compress into slugs. Grind the slugs to 14- to 16-mesh size. Recompress into tablets, using a 1 3/32-inch punch. Ten tablets should weigh 3.575 g.

CT Sodium Phenobarbital

INGREDIENTS	IN EACH	IN 7000
Phenobarbital sodium	65 mg	455 g
Lactose (granular, 12-mesh)	26 mg	182 g
Starch	20 mg	140 g
Talc	20 mg	140 g
Magnesium stearate	0.3 mg	2.1 g
Weight of granulation		919.1 g

Mix all the ingredients thoroughly. Compress into slugs. Grind and screen to 14- to 16-mesh granules. Recompress into tablets, using a 3/32-inch concave punch. Ten tablets should weigh 1.3 g.

CT Vitamin B Complex

INGREDIENTS	IN EACH	IN 10,000
Thiamine mononitrate[a]	0.733 mg	7.33 g
Riboflavin[a]	0.733 mg	7.33 g
Pyridoxine hydrochloride	0.333 mg	3.33 g
Calcium pantothenate[a]	0.4 mg	4 g
Nicotinamide	5 mg	50 g
Lactose (powder)	75.2 mg	752 g
Starch	21.9 mg	219 g
Talc	20 mg	200 g
Stearic acid (powder)	0.701 mg	7.01 g
Weight of granulation		1250 g

[a] Includes 10% in excess of label claim.
Mix all the ingredients thoroughly. Compress into slugs. Grind and screen to 14- to 16-mesh granules. Recompress into tablets, using a ¼-inch concave punch. Ten tablets should weigh 1.25 g.
Sufficient tartaric acid should be used in these tablets to adjust the pH to 4.5.

DIRECT COMPRESSION

APC Tablets

INGREDIENTS	IN EACH	IN 10,000
Aspirin (40-mesh crystal)	224 mg	2240 g
Phenacetin	160 mg	1600 g
Caffeine (anhyd USP gran)	32 mg	320 g
Compressible sugar (Di-Pac[a])	93.4 mg	934 g
Sterotex	7.8 mg	78 g
Silica gel (Syloid 244[b])	2.8 mg	28 g

[a] *Amstar.*
[b] *Davison Chem.*
Blend ingredients in a twin-shell blender for 15 min and compress on a 13/32-inch standard concave punch (courtesy, *Amstar*).

CT Ascorbic Acid USP, 250 mg

INGREDIENTS	IN EACH	IN 10,000
Ascorbic Acid USP (Merck, fine crystals)	255 mg	2550 g
Microcrystalline cellulose[a]	159 mg	1590 g
Stearic acid	9 mg	90 g
Colloidal silica[b]	2 mg	20 g
Weight of granulation		4250 g

[a] Avicel-PH-101.
[b] Cab-O-Sil.
Blend all ingredients in a suitable blender. Compress, using 7/16-inch standard concave punch. Ten tablets should weigh 4.25 g (courtesy, *FMC*).

Breath Freshener Tablets

INGREDIENTS	IN EACH	IN 10,000
Wintergreen oil	0.6 mg	6 g
Menthol	0.85 mg	8.5 g
Peppermint oil	0.3 mg	3 g
Silica gel (Syloid 244[a])	1 mg	10 g
Sodium saccharin	0.3 mg	3 g
Sodium bicarbonate	14 mg	140 g
Mannitol USP (granular)	180.95 mg	1809.5 g
Calcium stearate	2 mg	20 g

[a] *Davison* Chem.
Mix the flavor oils and menthol until liquid. Adsorb onto the silica gel. Add the remaining ingredients. Blend and compress on 5/16-inch, flat-face bevel-edge punch to a thickness of 3.1 mm (courtesy, *Atlas*).

Chewable Antacid Tablets

INGREDIENTS	IN EACH	IN 10,000
Aluminum hydroxide and magnesium carbonate, codried gel[a]	325 mg	3250 g
Mannitol USP (granular)	675 mg	6750 g
Microcrystalline cellulose[b]	75 mg	750 g
Corn starch	30 mg	300 g
Calcium stearate	22 mg	220 g
Flavor	qs	qs

[a] *Reheis F-MA-11.*
[b] *Avicel*
Blend all ingredients in a suitable blender. Compress, using a 5/8-inch, flat-face, bevel-edge punch (courtesy, *Atlas*).

Chewable Multivitamin Tablets

INGREDIENTS	IN EACH	IN 10,000
Vitamin A USP (dry, stabilized form)	5000 USP units	50 million units
Vitamin D dry, stabilized form)	400 USP units	4 million units
Ascorbic Acid USP	60.0 mg	600 g
Thiamine Hydrochloride USP	1 mg	10 g
Riboflavin USP	1.5 mg	15 g
Pyridoxine Hydrochloride USP	1 mg	10 g
Cyanocobalamin USP	2 μg	20 mg
Calcium Pantothenate USP	3 mg	30 g
Niacinamide USP	10 mg	100 g
Mannitol USP (granular)	236.2 mg	2362 g
Corn starch	16.6 mg	166 g
Sodium saccharin	1.1 mg	11 g
Magnesium stearate	6.6 mg	66 g
Talc USP	10 mg	100 g
Flavor	qs	qs

Blend all ingredients in a suitable blender. Compress, using a 5/8-inch, flat-face, bevel-edge punch (courtesy, *Atlas*).

CT Ferrous Sulfate

INGREDIENTS	IN EACH	IN 7000
Ferrous Sulfate USP (crystalline)	0.325 g	2275 g
Talc		0.975 g
Sterotex		1.95 g
Weight of granulation		2277.93 g

Grind to 12- to 14-mesh, lubricate, and compress. Coat immediately to avoid oxidation to the ferric state with 0.410 gr of tolu balsam (dissolved in alcohol) and 0.060 gr of salol and chalk. Use a deep, concave, 13/32-inch punch. Ten tablets should weigh 3.25 g.

CT Methenamine

INGREDIENTS	IN EACH	IN 7000
Methenamine (12- to 14-mesh crystals)	0.325 g	2275 g
Weight of granulation		2275 g

Compress directly, using a 7/16-inch punch. Ten tablets should weigh 3.25 g.

CT Phenobarbital USP, 30 mg

INGREDIENTS	IN EACH	IN 10,000
Phenobarbital	30.59 mg	305.9 g
Microcrystalline cellulose[a]	30.59 mg	305.9 g
Spray-dried lactose	69.16 mg	691.6 g
Colloidal silica[b]	1.33 mg	13.3 g
Stearic acid	1.33 mg	13.3 g
Weight of granulation		1330 g

[a] Avicel-PH-101.
[b] QUSO F-22.
Screen the phenobarbital to break up lumps and blend with the microcrystalline cellulose. Add spray-dried lactose and blend. Finally, add the stearic acid and colloidal silica; blend to obtain a homogeneous mixture. Compress, using a 3/32-inch, shallow, concave punch. Ten tablets should weigh 1.33 g (courtesy, *FMC*).

Molded Tablets or Tablet Triturates (TT)

Tablet triturates are small, discoid masses of molded powders weighing 30 to 250 mg each. The base consists of lactose, β-lactose, mannitol, dextrose, or other rapidly soluble materials. It is desirable in making tablet triturates to prepare a solid dosage form that is rapidly soluble; as a result they are generally softer than compressed tablets.

This type of dosage form is selected for a number of drugs because of its rapidly dissolving characteristic. Nitroglycerin in many concentrations is prepared in tablet triturate form since the molded tablet rapidly dissolves when administered by placing under the tongue. Potent alkaloids and highly toxic drugs used in small doses are prepared as tablet triturates that can serve as dispensing tablets to be used as the source of the drug in compounding other formulations or solutions. Narcotics in the form of hypodermic tablets originally were made as tablet triturates because they rapidly dissolve in sterile water for injection prior to administration. Today with stable injections of narcotics available, there is no longer any justification for their use in this manner. Although many hypodermic tablets currently are made, they are used primarily for oral administration.

Tablet triturates are made by forcing a moistened blend of the drug and diluent into a mold, extruding the formed mass, which is allowed to dry. This method is essentially the same as it was when introduced by Fuller in 1878. Hand molds may vary in size, but the method of operation is essentially the same. Molds consist of two plates made from polystyrene plastic, hard rubber, nickel-plated brass, or stainless steel. The mold plate contains 50 to 500 carefully polished perforations. The other plate is fitted with a corresponding number of projecting pegs or punches that fit the perforations in the mold plate. The mold plate is placed on a flat surface, the moistened mass is forced into the perforations, and the excess is scraped from the top surface. The mold plate is placed over the plate with the corresponding pegs and lowered. As the plates come together, the pegs force the tablet triturates from the molds. They remain on the tops of the pegs until dry, and they can be handled (see Fig 45-33). In some hand molds, as shown in Figure 45-34, the pegs are forced down onto the plate holding the moist trituration.

FORMULATION

In developing a formula it is essential to know the blank weight of the mold that is to be used. To determine this, the weight of the diluent that exactly fills all the openings in the mold is determined by experiment. This amount of diluent is weighed and placed aside. The total amount of the drug required is determined by multiplying the number of perforations in the plate used in the previous experiment by the amount of drug desired in each tablet. The comparative bulk of this medication is compared with that of an equal volume of diluent and that quantity of diluent is removed and weighed. The drug and the remaining diluent are mixed by trituration, and the resulting triturate is moistened and forced into the openings of the mold. If the perforations are not filled completely, more diluent is added, its weight noted, and the formula written from the results of the experiments.

It is also permissible in the development of the formula to weigh the quantity of medication needed for the number of tablets represented by the number of perforations in the mold, triturate with a weighed portion (more than ½) of the diluent, moisten the mixture, and press it into the perforations of the mold. An additional quantity of the diluent is moistened immediately and also forced into the perforations in the plate until they are filled completely. All excess diluent is removed, the trial tablets are forced from the mold, then triturated until uniform, moistened again, if necessary, and remolded. When these tablets are dried thoroughly and weighed, the difference between their total weight and the weight of medication taken will indicate the amount of diluent required and accordingly supply the formula for future use for that particular tablet triturate.

For proper mixing procedures of the medication with the diluent see Chapter 37.

Figure 45-33. Hand-molding tablet triturates (courtesy, Merck).

Figure 45-34. Tablet triturate mold (courtesy, Vector/Colton).

PREPARATION

The mixed powders are moistened with a proper mixture of alcohol and water, although other solvents or moistening agents such as acetone, petroleum benzin, and various combinations of these may be used in specific cases; the agent of choice depends on the solvent action that it will exert on the powder mixture. Often the moistening agent is 50% alcohol, but this concentration may be increased or decreased depending on the constituents of the formula. Care must be used in adding the solvent mixture to the powder. If too much is used, the mass will be soggy and will require a long time to dry, and the finished tablet will be hard and slowly soluble; if the mass is too wet, shrinkage will occur in the molded tablets; finally, a condition known as creeping will be noticed. Creeping is the concentration of the medication on the surface of the tablet caused by capillarity and rapid evaporation of the solvent from the surface. Because molded tablets by their very nature are quite friable, an inaccurate strength in each tablet may result from creeping if powder is lost from the tablet's surface. On the other hand, if an insufficient amount of moistening agent is used, the mass will not have the proper cohesion to make a firm tablet. The correct amount of moistening agent can be determined initially only by experiment.

HAND-MOLDING TABLET TRITURATES

In preparing hand-molded tablets place the mold plate on a glass plate. The properly moistened material is pressed into the perforations of the mold with a broad spatula, exerting uniform pressure over each opening. The excess material is removed by passing the spatula at an oblique angle, with strong hand pressure, over the mold to give a clean, flat surface. The material thus removed should be placed with the remainder of the unmolded material.

The mold with the filled perforations should be reversed and moved to another clean part of the plate where the pressing operation with the spatula is repeated. It may be necessary to add more material to fill the perforations completely and uniformly. The mold should be allowed to stand in a position so that part of the moistening agent will evaporate equally from both faces. While the first plate is drying, another mold can be prepared. As soon as the second mold has been completed, the first mold should be sufficiently surface-dried so that the pegs will press the tablets from the mold with a minimum of sticking.

To remove the tablets from the mold, place the mold over the peg plate so that the pegs and the perforations are in juxtaposition. The tablets are released from the mold by hand pressure, which forces the pegs through the perforations. The ejected tablets are spread evenly in single layers on silk trays and dried in a clean, dust-free chamber with warm, circulating air. If only a small quantity of tablet triturates is made and no warm-air oven is available, the tablet triturates may be dried to constant weight at room temperature.

MACHINE-MOLDING TABLET TRITURATES

Tablet triturates also can be made using mechanical equipment. The automatic tablet triturate machine illustrated in Figure 45-35 makes tablet triturates at a rate of 2500/min. For machine-molding, the powder mass need not be as moist as for plate-molding, since the time interval between forming the tablets and pressing them is considerably shorter. The moistened mass passes through the funnel of the hopper to the feed plates below. In this feed plate are four holes having the same diameter as the mouth of the funnel. The material fills one hole at a time and, when filled, revolves to a position just over the mold plate. When in position the weighted pressure foot lowers and imprisons the powder. At the same time a spreader in the sole of the pressure foot rubs it into the mold cavities and evens it off so that the triturates are smooth on the surface and are of uniform density. When this operation is completed, the mold passes to the next position, where it registers with a nest of punches or pegs that eject the tablets from the mold plate onto a conveyor belt. The conveyor belt sometimes is extended to a length of 8 or 10 ft. under a battery of infrared drying lamps to hasten the setting of the tablets for more rapid handling. This method of drying can be used only if the drug is chemically stable to these drying conditions.

Figure 45-35. Automatic tablet triturate machine (courtesy, Vector-Colton).

COMPRESSED TABLET TRITURATES

Frequently, tablet triturates are prepared on compression tablet machines using flat-face punches. When solubility and a clear solution are required, water-soluble lubricants must be used to prevent sticking to the punches. The granulations are prepared as directed for ordinary compressed tablets; lactose generally is used as the diluent. Generally, tablet triturates prepared by this method are not as satisfactory as the molded type regarding their solubility and solution characteristics.

TABLET CHARACTERISTICS

Compressed tablets may be characterized or described by a number of specifications. These include the diameter size, shape, thickness, weight, hardness, disintegration time, and dissolution characteristics. The diameter and shape depend on the die and the punches selected for the compression of the tablet. Generally, tablets are discoid in shape, although they may be oval, oblong, round, cylindrical, or triangular. Their upper and lower surfaces may be flat, round, concave, or convex to various degrees. The concave punches (used to prepare convex tablets) are referred to as shallow, standard, and deep cup, depending on the degree of concavity (see Figs 45-17 to 45-20). The tablets may be scored in halves or quadrants to facilitate breaking if a smaller dose is desired. The top or lower surface may be embossed or engraved with a symbol or letters that serve as an additional means of identifying the source of the tablets. These characteristics along with the color of the tablets tend to make them distinctive and identifiable with the active ingredient that they contain.

The remaining specifications assure the manufacturer that the tablets do not vary from one production lot to another. In the case of new tablet formulations their therapeutic efficacy is demonstrated through clinical trials, and it is the manufacturer's aim to reproduce the same tablet with the exact characteristics of the tablets that were used in the clinical evaluation of the dosage form. Therefore, from the control viewpoint these specifications are important for reasons other than physical appearance.

Tablet Hardness

The resistance of the tablet to chipping, abrasion, or breakage under conditions of storage, transportation, and handling before usage depends on its hardness. In the past, a rule of thumb described a tablet to be of proper hardness if it was firm enough to break with a sharp snap when it was held between the 2nd and 3rd fingers and using the thumb as the fulcrum, yet didn't break when it fell on the floor. For obvious reasons and control purposes a number of attempts have been made to quantitate the degree of hardness.

A small and portable hardness tester was manufactured and introduced in the mid-1930s by *Monsanto*. It now is distributed by the Stokes Div *(Pennwalt)* and may be designated as either the Monsanto or Stokes hardness tester. The instrument measures the force required to break the tablet when the force generated by a coil spring is applied diametrically to the tablet. The force is measured in kilograms and when used in production, a hardness of 4 kg is considered to be minimum for a satisfactory tablet.

The Strong-Cobb hardness tester introduced in 1950 also measures the diametrically applied force required to break the tablet. In this instrument the force is produced by a manually operated air pump. As the pressure is increased, a plunger is forced against the tablet placed on anvil. The final breaking point is indicated on a dial calibrated into 30 arbitrary units. The hardness values of the Stokes and Strong-Cobb instruments are not equivalent. Values obtained with the Strong-Cobb tester have been found to be 1.6 times those of the Stokes tester.

Another instrument is the Pfizer hardness tester, which operates on the same mechanical principle as ordinary pliers. The force required to break the tablet is recorded on a dial and may be expressed in either kilograms or pounds of force. In an experimental comparison of testers the Pfizer and the Stokes testers were found to check each other fairly well. Again the Strong-Cobb tester was found to give values 1.4 to 1.7 times the absolute values on the other instruments.

The most widely used apparatus to measure tablet hardness or crushing strength is the Schleuniger apparatus, also known as the Heberlein, distributed by *Vector*. This and other, newer, electrically operated test equipment eliminate the operator variability inherent in the measurements described above. Newer equipment is also available with printers to provide a record of test results. See Figure 45-36.

Manufacturers, such as *Key, Van Kel, Erweka,* and others, make similar hardness testers.

Hardness (or more appropriately, crushing strength) determinations are made throughout the tablet runs to determine the need for pressure adjustments on the tableting machine. If the tablet is too hard, it may not disintegrate in the required period of time or meet the dissolution specification; if it is too soft, it will not withstand the handling during subsequent processing such as coating or packaging and shipping operations.

A tablet property related to hardness is *friability,* and the measurement is made by use of the Roche friabilator. Rather than a measure of the force required to crush a tablet, the instrument is designed to evaluate the ability of the tablet to withstand abrasion in packaging, handling, and shipping. A number of tablets are weighed and placed in the tumbling

Figure 45-36. The Schleuniger or Heberlein tablet hardness tester shown with calibration blocks (courtesy, Vector).

Figure 45-37. The Roche friabilator (courtesy, Hoffmann-LaRoche).

apparatus where they are exposed to rolling and repeated shocks resulting from freefalls within the apparatus. After a given number of rotations the tablets are weighed, and the loss in weight indicates the ability of the tablets to withstand this type of wear (Fig 45-37).

Recent research has proposed that there are at least three measurable hardness parameters that can give a clue to the compatibility and intrinsic strength of powdered materials. These include bonding strength, internal strain, and brittleness. Hiestand proposed indices to quantify these parameters, and they are listed in Table 45-4 for a number of materials.

The higher the bonding index, the stronger a tablet is likely to be. The higher the strain index, the weaker the tablet. Since the two parameters are opposite in their effect on the tablet, it is possible for a material (such as Avicel) to have a relatively high strain index, but yet have superior compaction properties because of an extraordinary bonding potential. The higher the brittleness index, the more friable the tablet is likely to be. For a more detailed discussion of this subject, the reader is directed to References 22, 37, 38.

A similar approach is taken by many manufacturers when they evaluate a new product in the new market package by sending the package to distant points and back using various methods of transportation. This is called a *shipping test*. The condition of the product on its return indicates its ability to withstand transportation handling.

Tablet Thickness

The thickness of the tablet from production-run to production-run is controlled carefully. Thickness can vary with no change in weight because of difference in the density of the granulation and the pressure applied to the tablets, as well as the speed of tablet compression. Not only is the tablet thickness important in reproducing tablets identical in appearance but also to en-

Table 45-4. Hiestand Compaction Indices for a Number of Materials

MATERIAL	BONDING INDEX	STRAIN INDEX	BRITTLENESS INDEX
Aspirin	1.5	1.11	0.16
Dicalcium phosphate	1.3	1.13	0.15
Lactose anhydrous	0.8	1.40	0.27
Avicel pH 102	4.3	2.20	0.04
Corn starch	0.4	2.48	0.26
Sucrose NF	1.0	1.45	0.35
Erythromycin dihydrate	1.9	2.13	0.98

sure that every production lot will be usable with selected packaging components. If the tablets are thicker than specified, a given number no longer may be contained in the volume of a given size bottle. Tablet thickness also becomes an important characteristic in counting tablets using filling equipment. Some filling equipment uses the uniform thickness of the tablets as a counting mechanism. A column containing a known number of tablets is measured for height; filling is accomplished by continually dropping columns of tablets of the same height into bottles. If thickness varies throughout the lot, the result will be variation in count. Other pieces of filling equipment can malfunction because of variation in tablet thickness, since tablets above specified thickness may cause wedging of tablets in previously adjusted depths of the counting slots. Tablet thickness is determined with a caliper or thickness gauge that measures the thickness in millimeters. Plus or minus 5% may be allowed, depending on the size of the tablet.

Uniformity of Dosage Forms

TABLET WEIGHT—The volumetric fill of the die cavity determines the weight of the compressed tablet. In setting up the tablet machine the fill is adjusted to give the desired tablet weight. The weight of the tablet is the quantity of the granulation that contains the labeled amount of the therapeutic ingredient. After the tablet machine is in operation the weights of the tablets are checked routinely, either manually or electronically, to ensure that proper-weight tablets are being made. This has become rather routine in most manufacturing operations with newer, electronically controlled tablet presses. The USP has provided tolerances for the average weight of uncoated compressed tablets. These are applicable when the tablet contains 50 mg or more of the drug substance or when the latter comprises 50% or more, by weight, of the dosage form. Twenty tablets are weighed individually, and the average weight is calculated. The variation from the average weight in the weights of not more than two of the tablets must not differ by more than the percentage listed below; no tablet differs by more than double that percentage. Tablets that are coated are exempt from these requirements but must conform to the test for content uniformity if it is applicable.

AVERAGE WEIGHT	PERCENT DIFFERENCE
130 mg or less	10
More than 130 mg through 324 mg	7.5
More than 324 mg	5

CONTENT UNIFORMITY—To ensure that every tablet contains the amount of drug substance intended, with little variation among tablets within a batch, the USP includes the content uniformity test for certain tablets. Due to the increased awareness of physiological availability, the content uniformity test has been extended to monographs on all coated and uncoated tablets and all capsules intended for oral administration where the range of sizes of the dosage form available includes a 50 mg or smaller size, in which case the test is applicable to all sizes (50 mg and larger and smaller) of that tablet or capsule. The official compendia can be consulted for the details of the test. Tablet monographs with a content uniformity requirement do not have a weight variation requirement.

Tablet Disintegration

It is recognized generally that the *in vitro* tablet disintegration test does not necessarily bear a relationship to the *in vivo* action of a solid dosage form. To be absorbed, a drug substance must be in solution, and the disintegration test is a measure only of the time required under a given set of conditions for a group of tablets to disintegrate into particles. Generally, this test is useful as a quality-assurance tool for conventional (non-sustained-release) dosage forms. In the present disintegration test the particles are those that will pass through a 10-mesh screen. In a comparison of disintegration times and dissolution rates or initial absorption rates of several brands of aspirin tablets, it was found that the faster-absorbed tablets had the longer disintegration time. Regardless of the lack of significance as to *in vivo* action of the tablets, the test provides a means of control in ensuring that a given tablet formula is the same as regards disintegration from one production batch to another. The disintegration test is used as a control for tablets intended to be administered by mouth, except for tablets intended to be chewed before being swallowed or tablets designed to release the drug substance over a period of time.

Exact specifications are given for the test apparatus, inasmuch as a change in the apparatus can cause a change in the results of the test. The apparatus consists of a basket rack holding six plastic tubes, open at the top and bottom; the bottom of the tubes is covered with 10-mesh screen. See Figure 45-38. The basket rack is immersed in a bath of suitable liquid, held at 37°, preferably in a 1-L beaker. The rack moves up and down in the fluid at a specified rate. The volume of the fluid is such that on the upward stroke the wire mesh remains at least 2.5 cm below the surface of the fluid and descends to not less than 2.5 cm from the bottom on the downward stroke. Tablets are placed in each of the six cylinders along with a plastic disc over the tablet unless otherwise directed in the monograph. The endpoint of the test is indicated when any residue remaining is a soft mass with no palpably soft core. The plastic discs help to force any soft mass that forms through the screen.

For compressed, uncoated tablets the testing fluid is usually water at 37°, but in some cases the monographs direct that Simulated Gastric Fluid TS be used. If one or two tablets fail to disintegrate, the test is to be repeated using 12 tablets. Of the 18 tablets then tested, 16 must have disintegrated within the given period of time. The conditions of the test are varied somewhat for coated tablets, buccal tablets, and sublingual

Figure 45-38. Vanderkamp tablet disintegration tester (courtesy, VanKel).

tablets. Disintegration times are included in the individual tablet monograph. For most uncoated tablets the period is 30 min, although the time for some uncoated tablets varies greatly from this. For coated tablets up to 2 hr may be required, while for sublingual tablets, such as CT Isoproterenol Hydrochloride, the disintegration time is 3 min. For the exact conditions of the test, consult the USP.

Dissolution Test

For certain tablets the monographs direct compliance with limits on dissolution rather than disintegration. Since drug absorption and physiological availability depend on having the drug substance in the dissolved state, suitable dissolution characteristics are an important property of a satisfactory tablet. Like the disintegration test, the dissolution test for measuring the amount of time required for a given percentage of the drug substance in a tablet to go into solution under a specified set of conditions is an *in vitro* test. It is intended to provide a step toward the evaluation of the physiological availability of the drug substance, but as described currently, it is not designed to measure the safety or efficacy of the tablet being tested. Both the safety and effectiveness of a specific dosage form must be demonstrated initially by means of appropriate *in vivo* studies and clinical evaluation. Like the disintegration test, the dissolution test does provide a means of control in ensuring that a given tablet formulation is the same as regards dissolution as the batch of tablets shown initially to be clinically effective. It also provides an *in vitro* control procedure to eliminate variations among production batches. Refer to Chapter 35 for a complete discussion of dissolution testing.

Validation

In this era of increasing regulatory control of the pharmaceutical industry, manufacturing procedures cannot be discussed without the mention of some process-validation activity. By way of documentation, product testing, and perhaps in-process testing as well, manufacturers can demonstrate that their formulas and processes perform in the manner expected and that they do so reproducibly.

Although the justification for requiring validation is found in the regulations relating to *Current Good Manufacturing Practices for Finished Pharmaceuticals* as well as other sources, there is still much room for interpretation, and the process varies from one company to another. General areas of agreement appear to be that

The validation activity must begin in R&D and continue through product introduction.
Documentation is the key.
In general, three batches represent an adequate sample for validation.

The FDA has rejected historical data or *retrospective validation*. They require that new products be validated from beginning to end, a process called *prospective validation*.

CAPSULES

Capsules are solid dosage forms in which the drug substance is enclosed in either a hard or soft, soluble container or shell of a suitable form of gelatin. The soft gelatin capsule was invented by Mothes, a French pharmacist, in 1833. During the following year DuBlanc obtained a patent for his soft gelatin capsules. In 1848 Murdock patented the two-piece hard gelatin capsule. Although development work has been done on the preparation of capsules from methylcellulose and calcium alginate, gelatin, because of its unique properties, remains the primary composition material for the manufacture of capsules. The gelatin used in the manufacture of capsules is obtained from collagenous material by hydrolysis. There are two types of gelatin, Type A, derived mainly from pork skins by acid processing, and Type B, obtained from bones and animal skins by alkaline processing. Blends are used to obtain gelatin solutions with the viscosity and bloom strength characteristics desirable for capsule manufacture.[50]

The encapsulation of medicinal agents remains a popular method for administering drugs. Capsules are tasteless, easily administered, and easily filled either extemporaneously or in large quantities commercially. In prescription practice the use of hard gelatin capsules permits a choice in prescribing a single drug or a combination of drugs at the exact dosage level considered best for the individual patient. This flexibility is an advantage over tablets. Some patients find it easier to swallow capsules than tablets, therefore preferring to take this form when possible. This preference has prompted pharmaceutical manufacturers to market the product in capsule form, even though the product already has been produced in tablet form. While the industry prepares approximately 75% of its solid dosage forms as compressed tablets, 23% as hard gelatin capsules, and 2% as soft elastic capsules, market surveys have indicated a consumer preference of 44.2% for soft elastic capsules, 39.6% for tablets, and 19.4% for hard gelatin capsules.[51]

HARD GELATIN CAPSULES

The hard gelatin capsule, also referred to as the dry-filled capsule (DFC), consists of two sections, one slipping over the other, thus completely surrounding the drug formulation. The classic capsule shape is illustrated in Figure 45-39. These capsules are filled by introducing the powdered material into the longer end or body of the capsule and then slipping on the cap. Hard gelatin capsules are made largely from gelatin, FD&C colorants, and sometimes an opacifying agent such as titanium dioxide; the USP permits the gelatin for this purpose to contain 0.15% sulfur dioxide to prevent decomposition during manufacture. Hard gelatin capsules contain 12 to 16% water, but the water content can vary depending on the storage conditions. When the humidity is low, the capsules become brittle; if stored at high humidities, the capsules become flaccid and lose their shape. Storage in high-temperature areas also can affect the quality of hard gelatin capsules. Gelatin capsules do not protect hygroscopic materials from atmospheric water vapor, as moisture can diffuse through the gelatin wall.

Companies having equipment for preparing empty hard gelatin capsules include *Lilly, Parke-Davis, Scherer,* and *SmithKline*. The latter's production is mainly for its own use; the others are suppliers to the industry. With this equip-

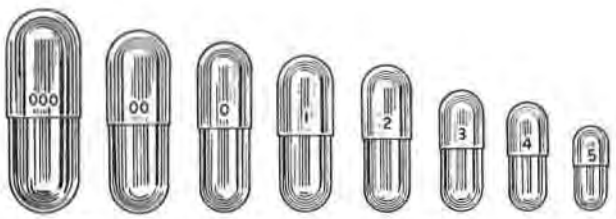


Figure 45-39. Hard gelatin capsules showing relative sizes (courtesy, Parke-Davis).

ment, stainless steel pins, set in plates, are dipped into the gelatin solution, which must be maintained at a uniform temperature and an exact degree of fluidity. If the gelatin solution varies in viscosity, it correspondingly will decrease or increase the thickness of the capsule wall. This is important since a slight variation is sufficient to make either a loose or a tight joint. When the pins have been withdrawn from the gelatin solution, they are rotated while being dried in kilns through which a strong blast of filtered air with controlled humidity is forced. Each capsule is stripped, trimmed to uniform length and joined, the entire process being mechanical. Capsule-making equipment is illustrated in Figures 45-40 and 45-41. These show the stainless steel pins being dipped into the gelatin solutions and then being rotated through the drying kiln.

Capsules are supplied in a variety of sizes. The hard, empty capsules (Fig 45-39) are numbered from 000, the largest size that can be swallowed, to 5, which is the smallest. Larger sizes are available for use in veterinary medicine. The approximate capacity for capsules from 000 to 5 ranges from 600 to 30 mg, although this will vary because of the different densities of powdered drug materials.

Commercially filled capsules have the conventional oblong shape illustrated, with the exception of capsule products by *Lilly* and *SmithKline*, which are of distinctive shape. For Lilly products, capsules are used in which the end of the base is tapered to give the capsule a bullet-like shape; products encapsulated in this form are called *Pulvules*. The *SmithKline* capsules differ in that both ends of the cap and body are angular, rather than round.

After hard gelatin capsules are filled and the cap applied, there are a number of methods used to ensure that the capsules will not come apart if subjected to vibration or rough handling, as in high-speed counting and packaging equipment. The capsules can be spot-welded by means of a heated metal pin pressed against the cap, fusing it to the body, or they may be banded with molten gelatin laid around the joint in a strip and dried. Colored gelatin bands around capsules have been used for many years as a trademark by *Parke-Davis* for their line of capsule products, *Kapseals*. Another approach is used in the *Snap-Fit* and *Coni-Snap* capsules. A pair of matched locking rings are formed into the cap and body portions of the capsule. Prior to filling, these capsules are slightly longer than regular capsules of the same size. When the locking rings are engaged after filling, their length is equivalent to that of the conventional capsule.

Following several tampering incidents, many pharmaceutical companies now use any number of locking and sealing technologies to manufacture and distribute these very useful dosage forms safely. Unfortunately, tamper-resistant packaging has become standard for capsule products.

Figure 45-40. Manufacture of hard gelatin capsules by dipping stainless steel pins into gelatin solutions (courtesy, Lilly).

Figure 45-41. Formed capsules being dried by rotating through a drying kiln (courtesy, Lilly).

It is usually necessary for the pharmacist to determine the size of the capsule needed for a given prescription through experimentation. The experienced pharmacist, having calculated the weight of material to be held by a single capsule, often will select the correct size immediately. If the material is powdered, the base of the capsule is filled and the top is replaced. If the material in the capsule proves to be too heavy after weighing, a smaller size must be taken and the test repeated. If the filled capsule is light, it is possible that more can be forced into it by increasing the pressure or, if necessary, some of the material may be placed in the cap. This is not desirable as it tends to decrease the accuracy of subdivision and it is much better to select another size, whose base will hold exactly the correct quantity. In prescription filling it is wise to check the weight of each filled capsule.

In addition to the transparent, colorless, hard gelatin capsule, capsules are also available in various transparent colors such as pink, green, reddish brown, blue, yellow, and black. If they are used, it is important to note the color as well as the capsule size on the prescription so that in the case of renewal the refilled prescription will duplicate the original. Colored capsules have been used chiefly by manufacturers to give a specialty product a distinctive appearance. Titanium dioxide is added to the gelatin to form white capsules or to make an opaque, colored capsule. In addition to color contrasts, many commercial products in capsules are given further identification by markings, which may be the company's name, a symbol on the outer shell of the capsule, or banding. Some manufacturers mark capsules with special numbers based on a coded system to permit exact identification by the pharmacist or physician.

Extemporaneous Filling Methods

When filling capsules on prescription, the usual procedure is to mix the ingredients by trituration, reducing them to a fine and uniform powder. The principles and methods for the uniform distribution of an active medicinal agent in a powder mixture are discussed in Chapter 37. Granular powders do not pack readily in capsules, and crystalline materials, especially those that consist of a mass of filament-like crystals such as the quinine salts, are not fitted easily into capsules unless powdered. Eutectic mixtures that tend to liquefy may be dispensed in capsules if a suitable absorbent such as magnesium carbonate is used. Potent drugs given in

small doses usually are mixed with an inert diluent such as lactose before filling into capsules. When incompatible materials are prescribed together, it is sometimes possible to place one in a smaller capsule and then enclose it with the second drug in a larger capsule.

Usually, the powder is placed on paper and flattened with a spatula so that the layer of powder is not greater than about ⅓ the length of the capsule that is being filled. This helps to keep both the hands and capsules clean. The cap is removed from the selected capsule and held in the left hand; the body is pressed repeatedly into the powder until it is filled. The cap is replaced and the capsule is weighed. In filling the capsule the spatula is helpful in pushing the last quantity of the material into the capsule. If each capsule has not been weighed, there is likely to be an excess or a shortage of material when the specified number of capsules have been packed. This condition is adjusted before dispensing the prescription.

A number of manual filling machines and automatic capsule machines are available for increasing the speed of the capsule-filling operation. Figure 45-42 illustrates a capsule-filling machine that was known formerly as the Sharp & Dohme machine. This equipment is now available through *ChemiPharm*. Many community pharmacists find this a useful piece of apparatus, and some pharmaceutical manufacturers use it for small-scale production of specialty items. The machine fills 24 capsules at a time with the possible production of 2000 per day. Entire capsules are placed in the machine by hand; the lower plate carries a clamp that holds the capsule bases and makes it possible to remove and replace the caps mechanically. The plate holding the capsule bases is perforated for three sizes of capsules. The powder is packed in the bases; the degree of accuracy depends on the selection of capsule size and the amount of pressure applied in packing. The hand-operated machine (Model 300, *ChemiPharm*) illustrated in Figure 45-43 has a production capacity of 2000 capsules per hour. The machine is made for a single capsule size and cannot be changed over for other sizes. A different machine is required for any additional capsule size. Its principle of operation is similar to that of the Sharp & Dohme machine.

Machine Filling Methods

Large-scale filling equipment for capsules operates on the same principle as the manual machines described above, namely the filling of the base of the capsule. Compared with tablets, powders for filling into hard gelatin capsules require a minimum of formulation efforts. The powders usually contain diluents such as lactose, mannitol, calcium carbonate, or magnesium carbonate. Since the flow of material is of great importance in the rapid and accurate filling of the capsule bodies, lubricants such as the stearates also are used frequently.

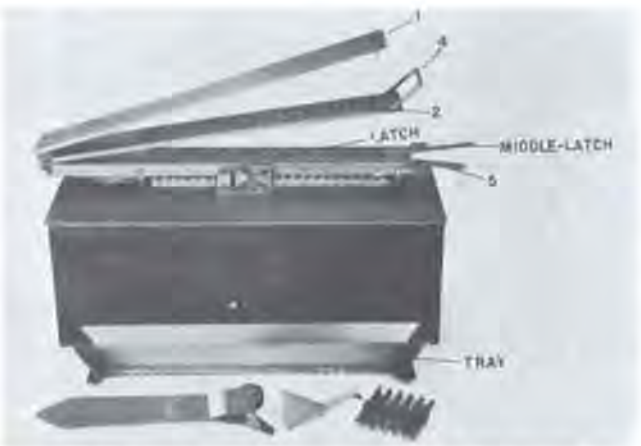


Figure 45-42. Hand-operated capsule machine (courtesy, ChemiPharm).

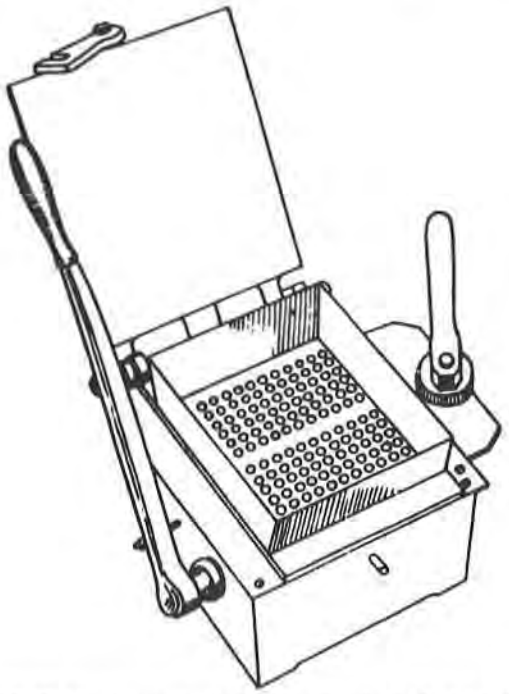


Figure 45-43. Hand-operated capsule machine, Model 300 (courtesy, ChemiPharm).

Because of the absence of numerous additives and manufacturing processing, the capsule form is used frequently to administer new drug substances for evaluation in initial clinical trials. However, it is now realized that the additives present in the capsule formulation, like the compressed tablet, can influence the release of the drug substance from the capsule. Tablets and capsules of a combination product containing triamterene and hydrochlorothiazide in a 2:1 ratio were compared clinically. The tablet caused approximately twice as much excretion of hydrochlorothiazide and three times as much triamterene as the capsule.[52]

Most equipment operates on the principle by which the base of the capsule is filled and the excess is scraped off. Therefore, the active ingredient is mixed with sufficient volume of a diluent, usually lactose or mannitol, to give the desired amount of the drug in the capsule when the base is filled with the powder mixture. The manner of operation of the machine can influence the volume of powder that will be filled into the base of the capsule; therefore, the weights of the capsules must be checked routinely as they are filled. See Table 45-5.

Semiautomatic capsule-filling machines manufactured by *Parke-Davis* and *Lilly* are illustrated in Figures 45-44 and 45-45. The Type 8 capsule-filling machine performs mechanically under the same principle as the hand filling of capsules. This includes separation of the cap from the body, filling the body half, and rejoining the cap and body halves.

Empty capsules are taken from the bottom of the capsule hopper into the magazine. The magazine gauge releases one capsule from each tube at the bottom of each stroke of the machine. Leaving the magazine, the capsules drop onto the tracks of the raceway and are pushed forward to the rectifying area with a push blade. The rectifier block descends, turning the capsules in each track, cap up, and drops them into each row of holes in the capsule-holding ring assembly.

As the capsules fall into the holding ring, the cap half has a seat on the counter bore in each hole for the top ring. The body

Table 45-5. Capsule Fill Chart
Capsule Fill Weights (mg) Based on Size and Density

	CAPSULE VOLUME (mL)									
	0.95	0.78	0.68	0.54	0.5	0.37	0.3	0.25	0.21	0.13
POWDER DENSITY (g/mL)	CAPSULE SIZE									
	OO	Oel	O	1el	1	2	3	4el	4	5
0.3	285	234	204	162	150	111	90	75	63	39
0.4	380	312	272	216	200	148	120	100	84	52
0.5	475	390	340	270	250	185	150	125	105	65
0.6	570	468	408	324	300	222	180	150	126	78
0.7	665	546	476	378	350	259	210	175	147	91
0.8	760	624	544	432	400	296	240	200	168	104
0.9	855	702	612	486	450	333	270	225	189	117
1.0	950	780	680	540	500	370	300	250	210	130
1.1	1045	858	748	594	550	407	330	275	231	143
1.2	1140	936	816	648	600	444	360	300	252	156
1.3	1235	1014	884	702	650	481	390	325	273	169
1.4	1330	1092	952	756	700	518	420	350	294	182
1.5	1425	1170	1020	810	750	555	450	375	315	195

half is pulled by vacuum down into the bottom ring. When all rows in the ring assembly are full, the top ring, filled with caps only, is removed and set aside for later assembly. The body halves now are located in the bottom ring, ready for filling.

The ring holding the body halves is rotated at one of eight speeds on the rotary table. The drug hopper is swung over the rotating ring, and the auger forces drug powder into the open body cavities. When the ring has made a complete revolution and the body halves have been filled, the hopper is swung aside. The cap-holding ring is placed over the body-holding ring and the assembly is ready for joining. The capsule-holding ring assembly is placed on the joiner and the joiner plate is swung down into position to hold the capsules in the ring. The peg ring pins are entered in the holes of the body holding ring and tapped in place by the air cylinder pushing the body halves back into the cap halves.

The holding-ring assembly is now pushed by hand back onto the peg ring away from the joiner plate, thus pushing the capsules out of the holding-ring assembly. The joined capsules then fall through the joiner chute into the capsule receiver box. The capsule receiver box screens the excess powder from the capsules and delivers them to any convenient container.

Many companies use the Type 8 capsule-filling equipment for small-scale manufacture and clinical supplies for investigational use because of its ease of operation, low cost, and extreme flexibility. A Type 8 capsule filling machine will produce approximately 200,000 capsules per day. This, of course, depends upon the operator and the type of material being filled. For this machine, a mathematical model has been developed that describes the effect of selected physical powder properties as well as mechanical operating conditions on the capsule-filling operation. While the Type 8 capsule-filling machine has been in existence for many years, recent modifications have been made to this machine to improve the capsule-filling operations.

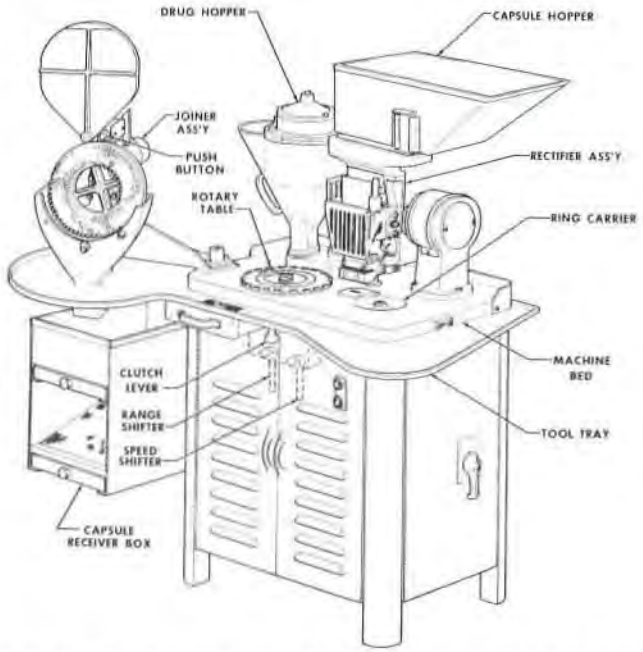


Figure 45-44. Schematic of Type 8 capsule-filling machine (courtesy, Parke-Davis).

Figure 45-45. Type 8 capsule-filling machine (courtesy, Lilly).

There are several pieces of equipment available that are classified as automatic capsule-filling machines. These are automatic in the sense that one operator can handle more than one machine. In this category are the Italian-made Zanasi *(United Machinery)* and MG-2 *(Supermatic)* models, plus the West German–made Hoefliger & Karg models *(Bosch)*.

Automatic capsule machines are capable of filling either powder or granulated products into hard gelatin capsules. With accessory equipment these machines also can fill pellets or place a tablet into the capsule with the powder or pellets. The capsules are fed at random into a large hopper. They are oriented as required and transferred into holders where the two halves are separated by suction. The top-half and bottom-half of the capsules are in separate holders, which at this stage take diverting directions.

A set of filling heads collects the product from the hopper, compresses it into a soft slug, and inserts this into the bottom half of the capsule. After filling, each top-half is returned to the corresponding bottom-half. The filled capsules are ejected, and an air blast at this point separates possible empty capsules from the filled. The machines can be equipped to handle all sizes of capsules. Depending upon the make and model, speeds from 9000 to 150,000 units per hour can be obtained (see Figs 45-46 to 45-48).

All capsules, whether they have been filled by hand or by machine, will require cleaning. Small quantities of capsules may be wiped individually with cloth. Larger quantities are rotated or shaken with crystalline sodium chloride. The capsules then are rolled on a cloth-covered surface.

Figure 45-47. Zanasi automatic filling machine, Model AZ-60. The set of filling heads shown at the left collects the powder from the hopper, compresses it into a soft slug, and inserts it into the bottom half of the capsule (courtesy, United Machinery).

Uniformity of Dosage Units

The uniformity of dosage forms can be demonstrated by either of two methods, weight variation or content uniformity. Weight variation may be applied when the product is a liquid-filled, soft, elastic capsule or when the hard gelatin capsule contains 50 mg or more of a single active ingredient comprising 50% or more, by weight, of the dosage form. See the official compendia for details.

Disintegration tests usually are not required for capsules unless they have been treated to resist solution in gastric fluid (enteric-coated). In this case they must meet the requirements for disintegration of enteric-coated tablets. For certain capsule dosage forms a dissolution requirement is part of the monograph. Procedures used are similar to those employed in the case of compressed tablets. See Chapter 35.

SOFT ELASTIC CAPSULES

The soft elastic capsule (SEC) is a soft, globular, gelatin shell somewhat thicker than that of hard gelatin capsules. The gelation is plasticized by the addition of glycerin, sorbitol, or a similar polyol. The soft gelatin shells may contain a preservative to prevent the growth of fungi. Commonly used preservatives are methyl- and propylparabens and sorbic acid. When the suspending vehicle or solvent can be an oil, soft gelatin capsules provide a convenient and highly acceptable dosage form. Large-scale production methods generally are required for the preparation and filling of soft gelatin capsules.

Formerly, empty soft gelatin capsules were available to the pharmacist for the extemporaneous compounding of solutions

Figure 45-46. MG-2, automatic capsule-filling machine (courtesy, Supermatic).

Figure 45-48. Hoefliger & Karg automatic capsule-filling machine, Model GFK 1200 (courtesy, Amaco).

or suspensions in oils. Commercially filled soft gelatin capsules come in a wide choice of sizes and shapes; they may be round, oval, oblong, tubular, or suppository-shaped. Some sugar-coated tablets are quite similar in appearance to soft gelatin capsules. The essential differences are that the soft gelatin capsule has a seam at the point of closure of the two halves, and the contents can be liquid, paste, or powder. The sugar-coated tablet will not have a seam but will have a compressed core.

Oral SEC dosage forms generally are made so that the heat seam of the gelatin shell opens to release its liquid medication into the stomach less than 5 min after ingestion. Its use is being studied for those drugs poorly soluble in water having bioavailability problems. When used as suppositories, it is the moisture present in the body cavity that causes the capsule to come apart at its heat-sealed seam and to release its contents.

Plate Process

In this method a set of molds is used. A warm sheet of prepared gelatin is laid over the lower plate, and the liquid is poured on it. A second sheet of gelatin is carefully put in place, and this is followed by the top plate of the mold. The set is placed under the press where pressure is applied to form the capsules, which are washed off with a volatile solvent to remove any traces of oil from the exterior. This process has been adapted and is used for encapsulation by *Upjohn*. The sheets of gelatin may have the same color or different colors.

Rotary-Die Process

In 1933 the rotary-die process for elastic capsules was perfected by Robert P Scherer.[53] This process made it possible to improve the standards of accuracy and uniformity of elastic gelatin capsules and globules.

The rotary-die machine is a self-contained unit capable of continuously and automatically producing finished capsules from a supply of gelatin mass and filling material, which may be any liquid, semiliquid, or paste that will not dissolve gelatin. Two continuous gelatin ribbons, which the machine forms, are brought into convergence between a pair of revolving dies and an injection wedge. Accurate filling under pressure and sealing of the capsule wall occur as dual and coincident operations; each is delicately timed against the other. Sealing also severs the completed capsule from the net. The principle of operation is shown in Figure 45-49. See also Figure 45-50.

Figure 45-49. Rotary-die elastic capsule filler.

Figure 45-50. Scherer soft elastic capsule machine (courtesy, Scherer).

By this process the content of each capsule is measured individually by a single stroke of a pump so accurately constructed that plunger travel of 0.025 inch will deliver 1 ℥ (apoth). The Scherer machine contains banks of pumps so arranged that many capsules may be formed and filled simultaneously. All pumps are engineered to extremely small mechanical tolerances and to an extremely high degree of precision and similarity. All operations are controlled on a weight basis by actual periodic checks with a group of analytical balances. Individual net-fill weights of capsules resulting from large-scale production vary no more than ±1 to 3% from theory, depending upon the materials used.

The rotary-die process makes it possible to encapsulate heavy materials such as ointments and pastes. In this manner solids can be milled with a vehicle and filled into capsules. When it is desirable to have a high degree of accuracy and a hermetically sealed product, this form of enclosure is suited ideally.

The modern and well-equipped capsule plant is completely air conditioned, a practical necessity for fine capsule production. Its facilities and operations include the availability of carbon dioxide at every exposed point of operation for the protection of oxidizable substances before encapsulation. Special ingredients also have been used in the capsule shell to exclude light wavelengths that are destructive to certain drugs.

Norton Capsule Machine

This machine produces capsules completely automatically by leading two films of gelatin between a set of vertical dies. These dies as they close, open, and close are in effect a continual vertical plate forming row after row of pockets across the gelatin film. These are filled with medicament and, as they progress through the dies, are sealed, shaped, and cut out of the film as capsules, which drop into a cooled solvent bath.

Accogel Capsule Machine

Another means of soft gelatin encapsulation uses the Accogel machine and process which were developed at *Lederle*. The Accogel, or Stern machine, uses a system of rotary dies but is unique in that it is the only machine that successfully can fill dry powder into a soft gelatin capsule. The machine is available to the entire pharmaceutical industry by a lease arrangement and is used in many countries of the world. It is extremely

versatile, not only producing capsules with dry powder but also encapsulating liquids and combinations of liquids and powders. By means of an attachment, slugs or compressed tablets may be enclosed in a gelatin film. The capsules can be made in a variety of colors, shapes, and sizes.

Microencapsulation

As a technology, microencapsulation is placed in the section on capsules only because of the relationship in terminology to mechanical encapsulation described above. The topic also could have been included in a discussion of coating procedures. Essentially, microencapsulation is a process or technique by which thin coatings can be applied reproducibly to small particles of solids, droplets of liquids, or dispersions, thus forming microcapsules. It can be differentiated readily from other coating methods in the size of the particles involved; these range from several tenths of a micrometer to 5000 μm in size.

A number of microencapsulation processes have been disclosed in the literature.[54] Some are based on chemical processes and involve a chemical or phase change; others are mechanical and require special equipment to produce the physical change in the systems required.

A number of coating materials have been used successfully; examples of these include gelatin, polyvinyl alcohol, ethylcellulose, cellulose acetate phthalate, and styrene maleic anhydride. The film thickness can be varied considerably, depending on the surface area of the material to be coated and other physical characteristics of the system. The microcapsules may consist of a single particle or clusters of particles. After isolation from the liquid manufacturing vehicle and drying, the material appears as a free-flowing powder. The powder is suitable for formulation as compressed tablets, hard gelatin capsules, suspensions, and other dosage forms.

The process provides answers for problems such as masking the taste of bitter drugs, a means of formulating prolonged-action dosage forms, a means of separating incompatible materials, a method of protecting chemicals against moisture or oxidation, and a means of modifying a material's physical characteristics for ease of handling in formulation and manufacture.

Among the processes applied to pharmaceutical problems is that developed by the National Cash Register Co (NCR). The NCR process is a chemical operation based on phase separation or coacervation techniques. In colloidal chemistry, coacervation refers to the separation of a liquid precipitate, or phase, when solutions of two hydrophilic colloids are mixed under suitable conditions.

The NCR process, using phase separation or coacervation techniques, consists of three steps:

1. Formation of three immiscible phases: a liquid manufacturing phase, a core material phase, and a coating material phase.
2. Deposition of the liquid polymer coating on the core material.
3. Rigidizing the coating, usually by thermal, cross-linking, or desolvation techniques, to form a microcapsule.

In Step 2, the deposition of the liquid polymer around the core material occurs only if the polymer is absorbed at the interface formed between the core material and the liquid vehicle phase. In many cases physical or chemical changes in the coating polymer solution can be induced so that phase separation (coacervation) of the polymer will occur. Droplets of concentrated polymer solution will form and coalesce to yield a two-phase, liquid-liquid system. In cases in which the coating material is an immiscible polymer or insoluble liquid polymer, it may be added directly. Also monomers can be dissolved in the liquid vehicle phase and, subsequently, polymerized at the interface.

Equipment required for microencapsulation by this method is relatively simple; it consists mainly of jacketed tanks with variable-speed agitators. Figure 45-51 shows a typical flow diagram of a production installation.

Other Oral Solid Dosage Forms

PILLS

Pills are small, round, solid, dosage forms containing a medicinal agent and are intended for oral administration. Pills were formerly the most extensively used oral dosage form, but they have been replaced largely by compressed tablets and capsules. Substances that are bitter or unpleasant to the taste, if not corrosive or deliquescent, can be administered in this form if the dose is not too large.

Formerly, pills were made extemporaneously by the community pharmacist whose skill at pill-making became an art. However, the few pills that are now used in pharmacy are prepared on a large scale with mechanical equipment. The pill formulas of the NF were introduced largely for the purpose of establishing standards of strength for the well-known and currently used pills. Hexylresorcinol Pills consist of hexylresorcinol crystals covered with a rupture-resistant coating that is dispersible in the digestive tract. It should be noted that the official hexylresorcinol pills are prepared not by traditional methods but by a patented process, the gelatin coating being sufficiently tough that it cannot be broken readily, even when chewed. Therefore, the general method for the preparation of pills does not apply to hexylresorcinol pills.

Previous editions of this text should be consulted for methods of pill preparation.

TROCHES

These forms of oral medication, also known as *lozenges* or *pastilles,* are discoid-shaped solids containing the medicinal agent in a suitably flavored base. The base may be a hard sugar

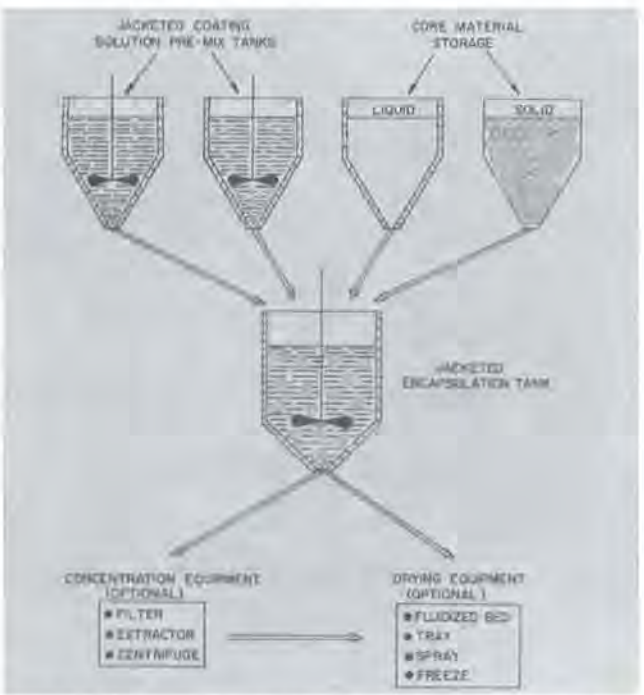


Figure 45-51. Production installation for the microencapsulation process (courtesy, NCR).

candy, glycerinated gelatin, or the combination of sugar with sufficient mucilage to give it form. Troches are placed in the mouth, where they slowly dissolve, liberating the active ingredient. The drug involved can be an antiseptic, local anesthetic, antibiotic, antihistaminic, antitussive, analgesic, or a decongestant.

Formerly, troches were prepared extemporaneously by the pharmacist. The mass is formed by adding water slowly to a mixture of the powdered drug, powdered sugar, and a gum until a pliable mass is formed. Powdered acacia in 7% concentration gives sufficient adhesiveness to the mass. The mass is rolled out and the troche pieces cut out using a cutter, or else the mass is rolled into a cylinder and divided. Each piece is shaped and allowed to dry before dispensing.

If the active ingredient is heat-stable, it may be prepared in a hard candy base. Syrup is concentrated to the point at which it becomes a pliable mass, the active ingredient is added, and the mixture is kneaded while warm to form a homogeneous mass. The mass is worked gradually into a pipe form having the diameter desired for the candy piece, and the lozenges are cut from the pipe and allowed to cool. This is an entirely mechanical operation with equipment designed for this purpose.

If the active ingredient is heat-labile, it may be made into a lozenge preparation by compression. The granulation is prepared in a manner similar to that used for any compressed tablet. The lozenge is made using heavy compression equipment to give a tablet that is harder than usual, as it is desirable for the troche to dissolve or disintegrate slowly in the mouth. In the formulation of the lozenge the ingredients are chosen that will promote its slow-dissolving characteristics. Compression is gaining in popularity as a means of making troches and candy pieces because of the increased speeds of compression equipment. In cases in which holes are to be placed in troches or candy pieces, core-rod tooling is used (see Fig 45-52). Core-rod tooling includes a rod centered on the lower punch around which the troche is compressed in the die cavity. The upper punch has an opening in its center for the core rod to enter during compression. It is evident that maximum accuracy is needed to provide alignment as the narrow punches are inserted into the die.

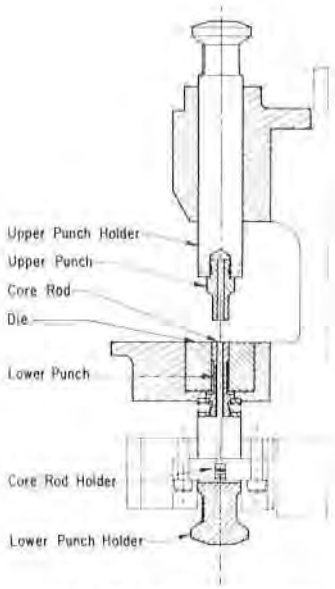


Figure 45-52. Core-rod tooling for compressing troches or candy pieces with hole in center (courtesy, Vector/Colton).

CACHETS

Related to capsules, inasmuch as they provide an edible container for the oral administration of solid drugs, cachets formerly were used in pharmacy. They varied in size from 3/4 to 1/8 inch in diameter and consisted of two concave pieces of wafer made of flour and water. After one section was filled with the prescribed quantity of the medicinal agent, they were sealed tightly by moistening the margins and pressing them firmly together. When moistened with water, their character was changed entirely; they became soft, elastic, and slippery. Hence, they could be swallowed easily by floating them on water.

PELLETS

The term pellet is now applied to small, sterile cylinders about 3.2 mm in diameter by 8 mm in length, which are formed by compression from medicated masses.[55] Whenever prolonged and continuous absorption of testosterone, estradiol, or desoxycorticosterone is desired, pellets of these potent hormones may be used by implantation.

REFERENCES

1. Rowland M, Tozer TN. *Clinical Pharmacokinetics: Concepts & Applications*. Baltimore: Lippincott Williams & Wilkins, 1995.
2. Benet LZ, Levy G, Ferraiolo BL. *Pharmacokinetics—A Modern View*. New York: Plenum, 1984.
3. Manninen V, Ojala K, Reisell P. *Lancet* 1972; 2: 922.
4. Katchen B, Symchowisz S. *J Pharm Sci* 1967; 56: 1108.
5. Katchen B. *Acta Pharmacol Toxicol* 1971; 29: 88.
6. Hansch C, Dunn WJ III. *J Pharm Sci* 1972; 61: 1.
7. Flynn GL, Yalkowsky SH, Roseman T. *Ibid* 1974; 63: 479.
8. Lieberman HA, Lachman L, eds. *Pharmaceutical Dosage Forms: Tablets*, vols I, II, and III. New York: Dekker, 1980, 1981, 1982, 2nd rev 1989.
9. Evans AJ, Train D. *A Bibliography of the Tableting of Medicinal Substances*. London: Pharmaceutical Press, 1963.
10. Evans AJ. *A Bibliography of the Tableting of Medicinal Substances*. London: Pharmaceutical Press, 1964.
11. Lachman L, *et al. The Theory and Practice of Industrial Pharmacy*, 3rd ed, Philadelphia: Lea & Febiger, 1988.
12. Banker G, Rhodes CT. *Modern Pharmaceutics*, New York: Dekker, 1995.
13. Ansel HC. *Introduction to Pharmaceutical Dosage Forms*, 3rd ed, Philadelphia: Lea & Febiger, 1981.
14. Monkhouse DC, Lach JL. *Can J Pharm Sci* 1972; 7: 29.
15. Blanchard J. *Am J Pharm* 1978; 150: 132.
16. *Handbook of Pharmaceutical Excipients*. Washington, DC: APhA/Pharm Soc Great Britian, APhA, 1986.
17. Rudnic EM, Kanig JL, Rhodes CT. *J Pharm Sci* 1985; 74: 647.
18. Rudnic EM, *et al. Drug Dev Ind Pharm* 1982; 8: 87.
19. Kanig JL, Rudnic EM. *Pharm Technol* 1984; 8: 50.
20. Rudnic EM, *et al. Drug Dev Ind Pharm* 1981; 7: 347.
21. *Capsugel List of Colorants for Oral Drugs*. Basel: Capsugel AG, 1988.
22. Foley VL, Belcastro PF. *Pharm Technol* 1987; 9: 110.
23. Stewart A. *Engineering* 1950; 169: 203.
24. David ST, Augsburger LL. *J Pharm Sci* 1977; 66: 155.
25. Jones TM. In *Formulation and Preparation of Dosage Forms*, Poldermand J, ed. North Holland: Elsevier, 1977, p 29.
26. Rees JE, Rue PJ. *J Pharm Pharmacol* 1987; 30: 601.
27. Summers MP, Enever RP, Carless JE. *J Pharm Sci* 1977; 66: 1172.
28. Hersey JA, Rees JE. In *Particle Size Analysis*. Groves MJ, Wyatt-Sargent JL, eds. London: Soc Anal Chem, 1970.
29. Jones TM. *Acta Pharm Tech* 1978.
30. Chowhan ZT. *Pharm Technol* 1988; 12: 46.
31. Meht AM. *Ibid* 1988; 12: 46(Dec):
32. Mendes RW, Roy SB. *Ibid* 1978; 2(3): 35.
33. Wurster DE. *J APhA Sci Ed* 1960; 49: 82.
34. Mendes RW, Roy SB. *Pharm Technol* 1978; 2(9): 61.
35. Malinowski HJ, Smith WE. *J Pharm Sci* 1974; 63: 285.

36. Woodruff CW, Nuessle NO. *Ibid* 1972; 61: 787.
37. O'Connor RE, Holinej J, Schwartz JB. *Am J Pharm* 1984; 156: 80.
38. O'Connor RE, Schwartz JB. *Drug Dev Ind Pharm* 1985; II: 1837.
39. Newton JM. *Mfg Chem Aerosol News* 1966; 37(Apr): 33.
40. US Pat 3,883647, May 13, 1975.
41. *Tableting Specification Manual*. Washington DC: APhA, 1981.
42. Knoechel EL *et al. J Pharm Sci* 1967; 56: 116.
43. Wray PE. *Drug Cosmet Ind* 1969; 105(3): 53.
44. Hiestand EN, Smith DP. *Powder Tech* 1984; 38: 145.
45- Hiestand EN, *et al. J Pharm Sci* 1977; 66: 510.
46. Luenberger H. *Int J Pharm* 1982; 12: 41.
47. Walter JT, Augsburger, LL. *Pharm Technol* 1986; 10: 26.
48. Schwartz JB. *Ibid* 1981; 5(9): 102.
49. Marshall K. *Ibid* 1983; 7(3): 68.
50. Jones BE. *Mfg Chem Aerosol News* 1969; 40(Feb): 25.
51. Delaney R. *Pharm Exec* 1982; 2(3): 34.
52. Tannenbaum PJ *et al. Clin Pharmacol Ther* 1968; 9: 598.
53. Ebert WR. *Pharm Technol* 1977; 1(10): 44.
54. Madan PL. *Ibid* 1978; 2(9): 68.
55. Cox PH, Spanjers F. *Pharm Weekbl* 1970; 105: 681.

CHAPTER 106

Vitamins and Other Nutrients

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Man consumes food to provide him with energy for growth, maintenance of normal body functions, and work. Energy is made available through conversion of carbohydrate, fat, and protein, which yield 4, 9, and 4 kilocalories per gram of the nutrient, respectively, when completely metabolized. The proportion or each of these nutrient sources in the human diet varies with environment, food availability, culture, and personal food behavior of the individual.

In the US the percentage of total calories provided by carbohydrate, fat, and protein in most diets is approximately 50, 38, and 12%, respectively. There is growing evidence that the amount and form of carbohydrates and fats have a profound effect on the development of degenerative diseases.

Metabolism, growth, and tissue repair require adequate ingestion of protein, minerals, vitamins, water, and oxygen. The latter two generally are not classed as nutrients in the usual sense but are substances that must be supplied on a continuing basis and in sufficient amount to sustain life. Current recommendations are that diets should not contain more than 30% of total calories from fat, of which not more than ⅓ should be from saturated fat and not more than ⅓ from polyunsaturated fat. The remainder of the fat will be monounsaturated. Olive oil is a good source of monounsaturated fatty acids.

Mineral elements present in organic compounds serve structural, catalytic, and modulator roles in the metabolic processes. Minerals are present as free ions in body fluids, where they act osmotically as electrolytes. The solid structure of the body, which is primarily bone tissue, contains mineral compounds. Vitamins are a heterogeneous group of organic compounds that participate in metabolic processes in minute amounts compared with other nutrients. The combination of complex processes through which living animal organisms obtain and utilize these materials is nutrition. The various disciplines of study aimed at elucidating those processes are collectively termed nutritional science.

Understanding of the significance of nutrients in human physiology has evolved largely from research studies on lower forms of life, mainly bacteria and animals such as the chicken, rat, guinea pig, mouse, dog, pig, and monkey. These studies have been substantiated and enlarged by clinical observations on human populations in healthy states and various conditions of disease, in malnutrition, and by some experimental studies conducted with human subjects.

MISINFORMATION ABOUT FOOD—A vast amount of confusion and nonscientific information surrounds the relationship of foods, as specially formulated food products, to health and prevention or cure of various disease conditions. The consumption of appropriate amounts of food selected from a variety of plant and animal sources will, over a period of time, furnish adequate to abundant amounts of all known essential nutrients for virtually the entire population.

A very few individuals with inborn errors of metabolism or who have injury or diseases of the gastrointestinal (GI) tract or are exposed to substances that decrease absorption or alter metabolism of certain nutrients may need supplements or special foods to meet their needs. However, food behaviors of increasing numbers of people are influenced by misrepresentations and false claims made for *health* foods, fad diets, and miracle cures by individuals and groups who profit from sale of such foods or ideas. Often these purveyors are convincing in their approach, claiming to have experienced a *cure* or presenting evidence of their product's success in curing people of a variety of real or imagined illnesses. When the unwary consumer uncritically accepts the advice of the purveyors of falsely labeled products in place of needed medical treatment, serious consequences can result. Risks incurred through following bizarre diet schemes for weight loss can be equally serious. Deaths have occurred from causes directly or indirectly associated with fad diets and other forms of self-diagnosis and treatment.

The Food and Drug Administration (FDA) has the responsibility and authority to control interstate traffic of products that are promoted falsely. This includes authority to regulate nutritional supplements for safety; ensure that labeling is informative and accurate and does not contain false or misleading statements; and ensure that supplements for infants, children under 12 years, pregnant women, and lactating women have appropriate potency. Compliance with regulations that pertain to safety, label requirements, and promotion is accomplished through nationwide monitoring of labeling and composition of the enormous number and variety of packaged foods in the market. Pharmacists and others who are informed in the sciences that make up nutritional science should report instances of false and misleading claims in labeling to the FDA.

Particularly in the field of nutrition, where misinformation may endanger the health of individuals, consumers must be provided opportunity to learn to make sound decisions regarding their health and nutritional status. Effective nutrition education programs are perceived to be essential if consumers are to make decisions in their own best interest when faced with the complexities of the modern marketplace.

Pharmacists, because of their day-to-day contact with the public most directly concerned, have a responsibility to be well-informed to allay the fears that are created by pseudoscientific writings of sensationalists and to protect the health as well as the pocketbooks of patrons.

NUTRIENT REQUIREMENTS AND DIETARY STANDARDS—The determination of quantitative human requirements for nutrients could be made if it were possible to correlate known nutrient intake with specific biological responses in precisely controlled studies. Although that is not possible, there are three kinds of studies that do yield information that can be used for close estimation of requirements.

Table 106-1. Summary Table: Estimated Safe and Adequate Daily Dietary Intakes of Selected Vitamins and Minerals[a,b]

	VITAMINS			TRACE ELEMENTS[c]				
CATEGORY	AGE (yr)	BIOTIN (μg)	PANTOTHENIC ACID (mg)	AGE (yr)	COPPER (mg)	MANGANESE (mg)	FLUORIDE (mg)	CHROMIUM (μg)	MOLYBDENUM (μg)
Infants	0–0.5	10	2	0–0.5	0.4–0.6	0.3–0.6	0.1–0.5	10–40	15–30
	0.5–1	15	3	0.5–1	0.6–0.7	0.6–1.0	0.2–1.0	20–60	20–40
Children and adolescents	1–3	20	3	1–3	0.7–1.0	1.0–1.5	0.5–1.5	20–80	25–50
	4–6	25	3–4	4–6	1.0–1.5	1.5–2.0	1.0–2.5	30–120	30–75
	7–10	30	4–5	7–10	1.0–2.0	2.0–3.0	1.5–2.5	50–200	50–150
	11+	30–100	4–7	11+	1.5–2.5	2.0–5.0	1.5–2.5	50–200	75–250
Adults		30–100	4–7		1.5–3.0	2.0–5.0	1.5–4.0	50–200	75–250

[a] Reproduced from Recommended Dietary Allowances, ed 10. Washington, DC: NAS, 1989.
[b] Because there is less information on which to base allowances, these figures are provided as ranges of recommended intakes.
[c] Since the toxic levels for many trace elements may be only several times usual intakes, the upper levels for the trace elements given in this table should not be exceeded habitually.

Balance studies, which employ a method of comparing nutrient intake and output and therefore measure body gain or loss of a stable component.

Biochemical measurements of a nutrient, nutrient metabolites, or related functional and structural components in a body fluid, compartment, tissue, or excreta.

Clinical evaluation and performance tests on subjects maintained on carefully controlled nutrient intakes to determine dietary levels that maintain health and will prevent deterioration of physiological and cognitive functions.

Ideally, data from all three enable the investigator to determine the smallest amount of a nutrient that will prevent deficiency symptoms or support a well-defined physiological or biochemical response, eg, the maintenance of serum ferritin levels in women of childbearing ages. An *average* requirement, however, is derived most often from such data to denote the amount of a nutrient that will support health in most persons of a given population group. It implies that the *true* requirement for *individuals* may be either above or below the average for the group. Obviously, neither the perfect tool for determining human requirements nor the perfect criterion of physiological and cognitive responses have yet been devised or ascertained.

To use the knowledge about nutrient requirements in a practical way, ie, to develop dietary standards as goals for food selection, it is necessary to add amounts above estimated requirements as *safety factors* to cover both variation among individuals and the lack of precision inherent in the estimated requirement. The resulting values are called *allowances*, and the dietary standards used in the US are the Recommended Dietary Allowances (RDAs) developed by the Food and Nutrition Board of the National Academy of the Sciences–National Research Council (NAS-NRC) (see Chapter 100). The Food and Nutrition Board also published Estimated Safe and Adequate Daily Dietary Intakes for 12 nutrients for which less information existed than was necessary to establish allowances (Table 106-1).

In 1940 the FDA independently established a set of dietary standards called Minimum Daily Requirements (MDRs), which were used in labeling to help consumers relate the nutrient content claimed for certain foods to their own nutrient needs. In 1974 these were replaced by the FDA with a new set of labeling standards, the US Recommended Daily Allowances (US RDAs), which include values for more nutrients and which were adapted and condensed from the Food and Nutrition Board's RDAs. To reduce confusion the FDA has renamed these labeling standards Reference Daily Intakes (RDIs). See Table 106-2. Federal regulations require that manufacturers who make nutritional claims on the label of foods, including dietary

Table 106-2. Reference Daily Intakes (RDIs) for Labeling Purposes

	UNITS	INFANTS	CHILDREN UNDER 4 YR OF AGE	ADULTS AND CHILDREN 4 OR MORE YR OF AGE	PREGNANT OR LACTATING WOMEN
Vitamin A	IU	1500	2500	5000	8000
Vitamin D	IU	400	400	400	400
Vitamin E	IU	5	10	30	30
Vitamin C	mg	35	40	60	60
Folacin	mg	0.1	0.2	0.4	0.8
Thiamin	mg	0.5	0.7	1.5	1.7
Riboflavin	mg	0.6	0.8	1.7	2.0
Niacin	mg	8	9	20	20
Vitamin B_6	mg	0.4	0.7	2	2.5
Vitamin B_{12}	μg	2	3	6	8
Biotin	mg	0.05	0.15	0.3	0.3
Pantothenic Acid	mg	3	5	10	10
Calcium	g	0.6	0.8	1.0	1.3
Phosphorus	g	0.5	0.8	1.0	1.3
Iodine	μg	45	70	150	150
Iron	mg	15	10	18	18
Magnesium	mg	70	200	400	450
Copper	mg	0.6	1.0	2.0	2.0
Zinc	mg	5	8	15	15
Protein	g	14[a]	16[a]	50[b]	60, 65[b]

[a] Quality measured by Protein Efficiency Ratio (PER).
[b] Value for protein in adults and children 4 or more years of age is referred as a *daily reference value* and quality is measured by a scoring process based on amino acid content.

supplements, must include a statement of the percentages of the RDIs of the vitamins, minerals, and protein supplied by an amount of the food usually consumed or recommended for consumption in 1 day.

Dietary standards are necessary and useful tools and are a means through which the findings in nutritional science can be applied for the improvement and maintenance of human health. They are assessed periodically and revised as new data become available.

THERAPEUTIC NUTRITION—Any interference with the body's ability to use the nutrients present in available food or, for that matter, its inability to obtain enough nutrients from the available food calls for the intervention by professionals who are able to diagnose and treat the condition. The treatment or therapy, may involve a range of actions from simple adjustment of nutrient intake to the intravenous feeding of special nutrient formulas. Diet therapy is practiced when a change in nutritional status of a patient can be effected gradually. This would be in cases in which it is important to maintain optimal nutritional status during prolonged periods of physical stress, to bring about changes in body weight, and to adjust food intake (both qualitatively and quantitatively) when the body is functioning abnormally or when surgery or trauma have depleted the body's reserves. Specific kinds of diet therapy also are needed for long periods, often for life, to compensate for inborn errors of metabolism.

Radical means of therapy in both the management and treatment of certain conditions is often necessary. For example, in correcting a nutritional deficiency such as pernicious anemia and preventing its recurrence in a susceptible individual, large doses of the missing nutrient are administratered parenterally. Feeding by nasogastric tube or by gastrostomy or jejunostomy is instituted when it is not possible for a patient to take food by mouth. Patients with extensive burns present nutritional problems much more far-reaching than those who have undergone major surgery or sustained severe hemorrhage. The first need for those individuals is for fluid and electrolyte replacement, followed as quickly as possible by a diet or intravenous solution markedly increased in protein, calories, and vitamins. The focus in all these cases is on restoration of nutrient supply commensurate with the specific need as soon as possible.

An understanding of the necessity for nutrient therapy is aided by recognizing the following factors that can affect nutrient needs:

Interference with food consumption (eg, impaired appetite, GI disease, traumatic neurological disorders interfering with self-feeding, neuropsychiatric disorders, disease of soft or hard oral tissue, alcoholism, pregnancy anorexia and vomiting, food allergy, and disease requiring a restricted diet).

Interference with absorption (eg, absence of normal digestive secretions, intestinal hypermotility, reduction of effective absorbing surface, impairment of intrinsic mechanism of absorption, and drugs preventing absorption.)

Interference with utilization or storage (eg, impaired liver function, hypothyroidism, neoplasm of GI tract, and drug therapy or radiation).

Increased destruction of tissues and/or function (eg, severe trauma, achlorhydria in the GI tract, heavy metals, and other metabolic antagonists).

Increased excretion or loss of nutrients (eg, lactation, burns, glycosuria and albuminuria and acute chronic blood loss).

Increased nutrient requirements (eg, increased physical activity, periods of rapid growth, pregnancy and lactation, fever, hyperthyroidism, and drug therapy).

VITAMINS

Vitamins are organic compounds required for normal growth and maintenance of life by animals, including man. As a rule, animals are unable to synthesize these compounds by anabolic processes that are independent of environment other than air. These compounds are effective in small amounts, do not furnish energy, and are not used as building units for the structure of the organism, but are essential for transformation of energy and for regulation of the metabolism of structural units. They or their precursors are found in plants and, so far as is known, have specific metabolic functions to perform in plant cells. Plant tissues are sources for the animal kingdom of these protective nutritional factors. In addition to carbohydrates, fats, proteins, mineral salts, and water, it is essential that the food of man and animals contain small amounts of these organic substances called vitamins. If any one of at least 13 of these compounds is lacking in the diet, this breakdown results in a reduced rate or complete lack of growth in children and in symptoms of malnutrition that are known as deficiency diseases.

Vitamins are unlike each other in chemical composition and function. They are alike only in that they cannot be synthesized at all or at least not at an adequate rate in the tissues of animals or humans. The functions they serve fall into two categories, the maintenance of normal structure and of normal metabolic functions. For example, vitamin A is essential for the maintenance of normal epithelial tissue; vitamin D functions in the absorption of normal bone salts for the formation and growth of bone and other tissue. Certain vitamins of the water-soluble group, among them thiamine, riboflavin, pantothenic acid, and niacin, are known to be essential constituents of the respiratory enzymes that are required in the use of energy from oxidative catabolism of sugars and fats.

It is convenient in a discussion of this subject to divide these nutritional substances into two groups, the *fat-soluble* and the *water-soluble factors*. Vitamins A, D, E and K fall into the fat-soluble group, since they can be extracted with fat solvents and are found in the fat fractions of animal tissues. The water-soluble vitamins include ascorbic acid and the B group of vitamins, which consists of some 10 or more well-defined compounds. Additional vitamin nomenclature can be found in Table 106-3. The characterization of vitamins as essential metabolic factors with discrete chemical structures required their isolation in pure form from natural sources and subsequent laboratory synthesis. Commercial chemical or microbiological syntheses, some from relatively simple compounds, are the source of most of the vitamins now used in pharmaceutical preparations, dietary supplements, and fortified foods.

STANDARDIZATION—Vitamin activity or potency is measured by three principal types of methods:

Biological, in which rats, mice, guinea pigs, and chickens serve as the assay animals.

Microbiological, which employ bacteria that require certain of the water-soluble vitamins, are rapid, specific, and precise. Such methods are used for manufacturing and laboratory control of the production of some vitamins.

Chemical, using a characteristic color or a sensitive reaction specific for the compounds, are available for most vitamins in uncomplicated mixtures. Chromatographic separations followed by a variety of detection techniques provide alternative means of quantification.

The status of vitamin methods of assay is now such that manufacturers of vitamin preparations find it possible to state with precision the potency of their products, and tables of vitamin content of foods are, for most vitamins, quite complete. Methods of assay are described briefly in the individual vitamin sections.

In the interest of improvement and uniformity of expressing the results of such assays, the World Health Organization (WHO) of the United Nations has sponsored the preparation and distribution of Standards. As a rule, an International Standard is no longer provided once the substance responsible for its characteristic activity has been isolated, identified, and

Table 106-3. Vitamin Nomenclature

VITAMIN	SYNONYM OR DESCRIPTIVE TERMS
A group	Antixerophthalmic vitamin
A_1	Retinol
A_2	Dehydroretinol
A acid	Retinoic acid (tretinoin)
Provitamin A carotenoids	Carotene (α & β, cryptoxanthin (hydroxy β-carotene)
B group	Formerly vitamin B complex
Thiamin	Vitamin B_1, aneurin, antiberiberi vitamin
Riboflavin	Vitamin B_2, lactoflavin
Niacin	Nicotinic acid and nicotinamide, pellagra-preventive factor
Pantothenic acid	Formerly vitamin B_3
B_6	Pyridoxine, pyridoxal, pyridoxamine
Biotin	Coenzyme R
Folacin	Folic acid (pteroylmonoglutamic acid, PGA) and folic acid polyglutamates, tetrahydrofolic acid, formyl tetrahydrofolic acid (formerly citrovorum factor, folinic acid)
B_{12}	Antipernicious anemia vitamin, cyanocobalamin, hydroxocobalamin (formerly vitamin B_{12b}), nitritocobalamin (formerly vitamin B_{12c})
C	L-Ascorbic acid, antiscorbutic vitamin
D group	Antirachitic vitamin
D_2	Ergocalciferol (formerly calciferol), activated ergosterol
D_3	Cholecalciferol, activated 7-dehydrocholesterol
E group	Possess vitamin E activity in varying degrees; occur as fatty acid esters
alpha-, beta-, gamma-, delta-	tocopherols & tocotrienols
K group	Antihemorrhagic vitamin
K_1	Phylloquinone (naturally occurring)
K_2	Farnoquinone (naturally occurring)
K_3	Menadione, menaquinone
K_{4-7}	Biologically active analogs of menadione (synthetic)

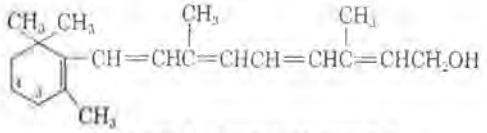
made readily available. The USP has set up comparable Reference Standards in this country, and the biological potency of vitamins A and D is expressed in USP Units that are equal to International Units (IUs). However, availability of the vitamins in pure form encourages transition from the use of units to the use of weight in expressing amounts present in vitamin products.

THE FAT-SOLUBLE VITAMINS

VITAMIN A AND CAROTENE

Vitamin A was the first fat-soluble vitamin discovered. Animal nutritionists observed growth failures in calves born of cows maintained on wheat or oats alone, whereas whole corn plants supported growth and development of the animals. The vitamin was found to be related to chlorophyll and carotenoid-containing plants. Later study revealed that the vitamin is essential for the maintenance of normal tissue structure and for other important physiological functions such as vision and reproduction.

Chemistry and Assay—Vitamin A is represented primarily by the cyclic polyene alcohol vitamin A_1 (retinol) with an empirical formula of $C_{20}H_{30}O$ and whose four conjugated double bonds in the side chain are in the *trans* arrangement.

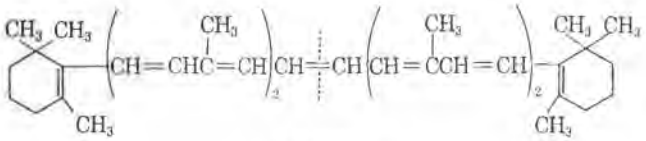


Vitamin A (Retinol) (Vitamin A_1)

Another representative of vitamin A occurring in nature is vitamin A_2, which has an additional double bond in the ring at the 3–4 position. It has only about ¼ to ½ the biological activity of vitamin A_1 for the rat and has no commerical significance. A third such representative is neovitamin A-a, in which the terminal double bond in the side chain of vitamin A_1 is *cis*. It has low biological activity.

Vitamin A_1 is a pale yellow crystalline compound, is soluble in lipid solvents, and has a UV absorption maximum at 328 nm. The vitamin is not readily destroyed by heat but is oxidized easily and is less stable in acid than in alkaline solution. The esters of vitamin A_1 with the fatty acids acetic and palmitic are commercially important, since they are considerably more stable than the alcohol.

The source of most of the vitamin A in animals, birds, and fish is the carotenoid pigments, the yellow-colored compounds in all chlorophyll-containing plants. At least 10 different carotenoids exhibit provitamin A activity, but only α- and β-carotene and cryptoxanthin (founded in yellow corn) are important in animal nutrition, β-carotene being the most important.



β-Carotene

Theoretically one molecule of β-carotene should yield two molecules of vitamin A_1; however, the availability of carotene in foods as sources of vitamin A for humans is low and extremely variable. Often, factors of ½, ⅓, ¼, or less are used arbitrarily to compensate for this. This utilization efficiency of carotene is generally considered to be ⅙ for humans; that is, 1 μg of β-carotene would have the same biological activity as 0.167 μg of retinol. This conservatively takes into account the decremental effects on carotene utilization of absorption, transport, and tissue conversion to the active vitamin. The conversion of the provitamin to vitamin A occurs primarily in the walls of the small intestine and perhaps to a lesser degree in the liver; conversion is linked to body stores of vitamin A. Like vitamin A_1, the carotenes are soluble in fat solvents, in crystalline form appear deep orange or copper-colored, and have characteristic absorption spectra.

Total synthesis of vitamin A_1 and β-carotene is achieved commercially, vitamin A usually being prepared as the acetate. Concentration of vitamin A from animal fats and fish liver oil is still important. The principal steps in the process are molecular distillation, saponification and crystallization of the distillate, and acylation to the desired ester.

The USP Unit for vitamin A is identical to the International Unit. The USP Reference Standard for vitamin A is a solution of crystalline vitamin A acetate in cottonseed oil such that there is contained 1 USP Unit (0.344 μg)/0.1 mg of solution. Although there is no USP Unit for carotene, there is an International Unit (IU); the relation between carotene and vitamin A is 6 to 3.44 by weight of the respective pure compounds.

Vitamin A can be assayed by direct measurement of its ultraviolet absorption by photometric evaluation of the color reaction with antimony trichloride in chloroform (the Carr-Price reaction), by high-pressure liquid chromatographic separation and ultraviolet and visible spectrometry, or by a biological method based on the resumption of growth of rats when the vitamin activity is added to a vitamin A–deficient diet. The chemical or physicochemical determination of β-carotene depends on measurement of the yellow color of its solutions in organic solvents. Chromatographic separation of associated carotenoids is usually necessary before an accurate analysis of the biologically active compounds can be made.

Carotenoids are photodegradable, and deficiency resulting from excessive exposure of the human to UV light has been reported.

Metabolic Functions—Of the known functions of vitamin A in the body, its role in the visual process is established best. The retina of man contains two distinct photoreceptor systems. The rods, which are the structural components of one system, are especially sensitive to light of low intensity. A specific vitamin A aldehyde is essential for the formation of rhodopsin (the high-molecular-weight glycoprotein part of the

visual pigment within the rods) and the normal functioning of the retina. By virtue of this relation to the visual process, vitamin A alcohol has been named retinol, and the aldehyde form named retinal. A vitamin A–deficient person has impaired dark adaptation *(night-blindness)*.

Vitamin A also participates in the maintenance of the integrity of the epithelial membranes such that normal structures may be substituted by stratified keratinizing epithelium in the eyes, paraocular glands, and respiratory, alimentary, and genitourinary tracts under the stresses of a deficiency. The basal cells do not lose their function under such conditions, however, and are able to be restored to normal when sufficient vitamin A is absorbed. Abnormalities of nerve and connective tissue and of bones are further consequences of a dietary deficiency of the vitamin. In severe deficiency the affected epithelial and connective tissue may become the site of infections because of the cells' reduced resistance to bacterial invasion. This gave rise to the notion that administration of vitamin A was useful in the treatment of skin infections. Both topical and oral vitamin A, and especially vitamin A acid (*trans*-retinoic acid, tretinoin), are prescribed by some physicians to treat acne vulgaris; however, *trans*-retinoic acid has been shown to be equally effective, with less harmful side effects than oral isotretinoin (*cis*-retinoic acid).

There is a growing body of epidemiological data that suggest that foods that are a good source of vitamin A and carotenoids are protective against a variety of epithelial cancers. This association simply may be a result of a chronic vitamin A deficiency, since vitamin A is required for normal cell differentiation of stem cells in epithelial tissue. Also, there is the possibility that the observed protective effect could have been due to other undetected carotenoids, other vitamins, indoles, or unknown compounds present in these foods. Some, but not all, animal studies show a positive effect for vitamin A and synthetic retinoids against epithelial cancers of the skin, lung, bladder, and breast.

The common severe deficiency symptoms are increased susceptibility to microbial infections, xerophthalmia and other eye disorders, loss of appetite and weight, and sterility, conditions that require a long time for their development. Although the recommended dietary allowance is no more than 6000 IU/day, in a deficiency much greater amounts are indicated. For example, the usual therapeutic oral dose range is from 10,000 to 20,000 IU daily for 7 to 10 days for infants and growing children and 25,000 to 100,000 IU daily for 7 to 10 days for older children and adults.

If large doses of vitamin A are ingested for long periods of time, manifestations of toxicity develop. In the absence of a deficiency, chronic administration of 25,000 to 50,000 IU of vitamin A daily induces pathological changes in bone and periosteal tissues, skin and mucous membranes, and liver and changes in behavior. Doses as low as 18,500 IU of a water-dispersed vitamin A preparation daily for 1 to 3 months are reported to be toxic for infants 3 to 6 months of age. Vitamin A toxicity has occurred in infants who were given liver daily for a period of 3 months. Animal studies show that levels as low as four times the requirements increase the incidence of birth defects. Epidemiological studies in humans have indicated that levels as low as 15,000 IU during the first trimester of pregnancy may increase the risk of birth defects.

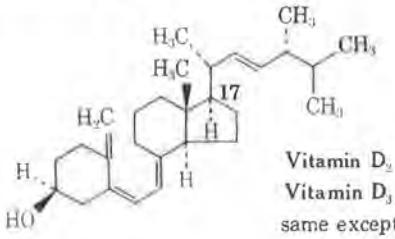
Dietary Requirement and Food Sources—According to the NRC's *Recommended Dietary Allowances*, the requirement for vitamin A appears to be proportional to body weight. The recommended allowances for the maintenance of good nutrition of healthy adults in the United States is 1000 Retinol equivalents (RE) for males and 800 RE for females per day (1000 RE is equivalent to 5000 IU), although the adult requirement for maintenance of normalcy in important vitamin A functions is about ½ this amount. Somewhat more vitamin A than the allowance should be provided during the latter ⅔ of pregnancy and even more during lactation. These increments would ensure the nutritional well-being of the rapidly growing fetus and nursing infant, who depend on the mother's vitamin A intake.

About ½ of the vitamin A activity in the average American diet comes from β-carotene and related compounds. The other ½ is provided by the vitamin itself present in foods of animal origin. Not all of the carotene present in the food eaten is converted into vitamin A. Some passes through the digestive tract and is excreted as such. Of that absorbed, only the amounts necessary to meet requirements are converted to vitamin A. The rest is stored in the body or excreted. Intake of large amounts of carotene frequently causes a yellow-orange color to the skin, which is considered to be harmless. The richest sources of carotene are yellow and green (leafy) vegetables and yellow fruits. Preformed vitamin A_1 is supplied primarily from the fat of dairy products and egg yolk, but other important sources in some diets are liver, kidney, and fish. Federal regulations provide for the optional addition of 15,000 IU of vitamin A per pound of margarine. Almost all margarine is so fortified. There are also provisions for marketing vitamins A and D–fortified nonfat dry milk containing 500 IU vitamin A and 100 IU vitamin D/8 fl oz reconstituted.

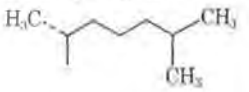
VITAMIN D

Vitamin D is the antirachitic vitamin effective in promoting calcification of the bony structures of man and animals. It sometimes is known popularly as the *sunshine* vitamin because it is formed by the action of the sun's ultraviolet rays on precursor sterols in the skin. Exposure to sunlight, therefore, has a powerful antirachitic effect. The term *rachitic* denotes the condition of a person or animal affected with the deficiency disease rickets, in which bone is poorly mineralized and unable to support the weight of the body.

Chemistry and Assay—The two immediate biological precursors (provitamins) of the vitamins D are the steroid alcohols ergosterol (egosta-5,7,22*E*-trien-3β-ol) and 7-dehydrocholesterol (cholesta-5,7-dien-3β-ol). Under the influence of UV light, each undergoes scission of the 9(10) bond of the steroid nucleus with the simultaneous creation of a 10(19) double bond yielding, respectively, vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol).



Vitamin D_2 (Ergocalciferol)
Vitamin D_3 (Cholecalciferol):
same except C_{17} side chain is



Pure vitamins D_2 and D_3 are white, odorless crystals that are soluble in fat solvents such as either, alcohol, or chloroform but insoluble in water. The compounds have characteristic absorption spectra, which property is useful in their identification. Both forms of the vitamin are stable to oxidation by air and to moderate heat in neutral and alkaline solutions. Upon alkaline saponification of fats, the vitamin appears in the nonsaponifiable fraction. It withstands autoclaving temperatures of 120° in the absence of air but at this temperature is subject to oxidation, and it is destroyed completely by heating at 170°. Vitamin D is stable over long periods of storage in oil solution but is quite unstable in the presence of mineral salts, such as tricalcium phosphate, when compounded in tablet form. It may be stabilized by dispersion in gelatin or a similar protective coating.

The international standard for vitamin D is a crystalline preparation of pure vitamin D_3 assigned a potency of 40 million units/g. The USP adopted an equivalent standard of vitamin D_3 with the same assigned potency, distributed in the form of a cottonseed oil solution. The USP unit for vitamin D, therefore, is equivalent to the IU.

The provitamins D are found in both plant and animal tissue; 7-dehydrocholesterol is found principally in animal skin and ergosterol in relatively large amounts in yeasts, although it was first isolated from ergot. The vitamin D that is absorbed through the intestinal wall from dietary sources or that is formed in the skin from 7-dehydrocholesterol enters the circulatory system, and excesses are stored. Like vitamin A, vitamin D is stored in animal body fats, principally in the liver. The liver oils, particularly of fish, are the most potent natural sources of the vitamin. The vitamin D of commerce now is synthesized principally from readily available, structurally related compounds, such as cholesterol, which often are obtained as packing house by-products.

There are three methods for quantitative physicochemical assay of vitamin D. For years, the biological assay based on the curative effects of the vitamin on experimental rickets in young rats has been used to measure the total biological activity of the vitamin in complex materials of low potency. Minimal amounts of the vitamin are needed by the rat; therefore, the rachitic condition is produced by using an extremely low-calcium, low-phosphorus diet. Now the preferred method for minimal amounts is high-pressure liquid chromatography for separation and UV spectrometry. For relatively concentrated solutions of vitamin D in alcohol (but not in oil), UV spectrometric determination is made at the wavelength of maximum absorption. Antimony trichloride reacts with various vitamins D in a Carr-Price reaction to yield a yellow color whose intensity is proportional to the vitamin D present. The reaction is satisfactory only for concentrated preparations; cholesterol and vitamin A interfere only when present in amounts in excess of certain limits.

Metabolic Functions—Both vitamin D_2 and vitamin D_3 are biologically inactive molecules. After absorption, they are converted, primarily in the liver, to 25-hydrocholecalciferol D_2 and D_3 (25-HCCD$_2$ and 25-HCCD$_3$ calciferol), respectively, and are the most predominant forms found in the blood. Both of these compounds appear to facilitate phosphate resorption in the renal tubule; however, their most important function is as a precursor of 1,25-dihydroxycholecalciferol (1,25-DHCC calciferol), which is formed in the kidney. This compound is a true hormone and is excreted in response to specific stimuli from an organ distal to its target organ. Calcitriol is transported in the blood bound to a protein. There is a rapid turnover of 1,25-DHCC, which depends on vitamin D status (greater turnover if body stores and plasma levels are low). Normal plasma values range from 18 to 60 pg/mL in children and 15 to 45 pg/mL in adults. Vitamin D, therefore, is a precursor of a true hormone, 1,25-DHCC, which is secreted by an organ and performs a vital function. It is likely that some forms of vitamin D–resistant rickets can be explained by possible genetic inability of the body to produce adequate amounts of either 25-HCC or 1,25-DHCC. Conversely, some children may have an enhanced capacity to convert vitamin D to the more active metabolites and, thereby, manifest a hyperreactivity to amounts of the ingested vitamin very slightly in excess of recommended dietary allowances.

Vitamin D, through the action of these active metabolites, aids in the absorption of calcium from the intestinal tract and the resorption of phosphate in the renal tubule. Vitamin D is necessary for normal growth in children, probably having a direct effect on the osteoblast cells that influence calcification of cartilage in the growing areas of bone. 1,25-DHCC also plays an essential management role in the regulation of various genes important to cell proliferation and lymphokine expression in systems not involved in mineral homeostasis.

A deficiency of vitamin D leads to inadequate absorption of calcium and phosphorus from the intestinal tract and retention of these minerals in the kidney and thence to faulty mineralization of bone structures. The inability of the soft bones to withstand the stress of weight results in skeletal malformations. Early rickets is difficult to diagnose, but fully developed cases in infants and children present characteristic signs. These include delayed closure of the fontanelles and softening of the skull; soft fragile bones with bowing of the legs and spinal curvature; enlargement of wrist, knee, and ankle joints; poorly developed muscles; and restlessness and nervous irritability. A form of *adult rickets* called osteomalacia similarly may occur. It, too, represents a failure of the process of calcification caused by simple vitamin D lack and calcium or phosphorus inadequacy.

With adequate calcium-phosphorus intake, adult osteomalacia and uncomplicated rickets can be cured by the ordinary daily intake of 400 IU of vitamin D. Larger doses (about 1600 IU or more daily) are more rapidly effective, the first evidence of improvement—a rise in serum phosphorus—occurring in about 10 days.

Vitamin D has a serious toxic potential. There is a wide range of susceptibility to the toxic effects of vitamin D. Most adults will require more than 50,000 units of vitamin D/day to produce intoxication. However, levels as low as 15,000 IU/kg for 2 weeks have produced acute toxicity in adults. Long-term consumption of as little as 1000 IU/kg may lead to hypercalcemia and attendant complications, such as metastatic calcification and renal calculi in adults, provided there are high levels of calcium in the diet. As little as 2000 IU can inhibit linear growth of normal children. In advanced stages, demineralization of bones occurs, and multiple fractions may result from very slight trauma. Chronic excessive intake will result in liver accumulation, and detoxification will take several months. Classic features of vitamin D intoxication are hypercalcemea, hyperphosphatemia, and impaired renal function. Painful joints and muscle weakness also may occur, which impair mobility.

Dietary Requirement and Food Sources—Requirements for vitamin D vary with the amount of exposure to UV light. Some individuals can obtain their entire requirements by skin irradiation, but age, skin pigment, and other conditions can effect the need for dietary supplies.

There are few reliable data concerning minimum vitamin D requirements, except for infants. For most healthy individuals 400 IU/day is sufficient to meet requirements without exposure to sun. However, studies on older individuals indicate that higher levels may be desirable. In normal full-term infants, intakes of as little as 100 IU/day have prevented rickets. There is no evidence that diets need supply more than 400 IU/day for normal growth of infants and children.

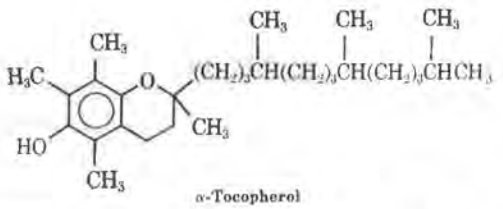
Vitamin D is not found naturally in many food sources. Egg yolks, which are the best food source, vary in content from winter to summer depending most upon the content of the vitamin in the hen's diet. Unfortified dairy products contain some vitamin D, but again the potency varies with the season. Varieties of fish, whose muscle tissues contain substantial quantities of oil and fat, may supply an appreciable part of the dietary requirement. The livers of a number of fish, or the oils extracted from the livers, are extremely rich in vitamin D. Addition of vitamin D to appropriate foods has been an important factor in the prevention of any significant incidence of rickets in this country.

The major sources of vitamin D in the diets of most Americans are those foods that have been fortified. Vitamin D–fortified whole milk, nonfat dry milk, and evaporated milk containing 400 IU/qt (or reconstituted quart in the case of nonfat dry milk and evaporated milk) are particularly effective because of their use in infant feeding during the stage of growth most susceptible to rachitic changes. Fortification is accomplished by addition of vitamin D concentrates, mainly in the form of vitamin D_3. Fortification of other foods, such as processed cereals and margarine, is practiced to a limited degree.

VITAMIN E

Vitamin E designates the group of compounds (tocol and tocotrienol derivatives) that exhibit qualitatively the biological activity of α-tocopherol. Studies that led to its discovery as an essential factor in animal metabolism showed that it was, among other things, necessary for reproduction in rats. It often is called the antisterility vitamin, an inappropriate term, since it is not known to specifically function in this capacity in humans.

Chemistry and Assay—As with several of the other vitamins, there are a series of closely related compounds, tocopherols, known to occur in nature. Biological activity associated with the vitamin nature of the group is exhibited by four major compounds: α-, β-, γ-, and δ-tocopherol, each of which can exist in various stereoisomeric forms. These are all methyl-substituted tocols; α-tocopherol, the most important member of the series because of its activity and occurrence, is 5,7,8-trimethyltocol, ie, 2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)-6-chromanol.



α-Tocopherol

The tocopherols are oily liquids at room temperature. High temperatures and acids do not affect the stability of vitamin E, but oxidation does take place readily in the presence of iron salts or in rancid fats. The tocopherols themselves act as antioxidants, δ-tocopherol having the greatest antioxidant power. Decomposition also occurs in UV light. Tocopherols are isolated on a commercial scale from vegetable oils, usually by molecular distillation, extraction with organic solvents or by absorption chromatography, α-tocopherol is usually the most important homolog isolated from these sources; it also can be prepared synthetically and made available as the acetate and acid succinate esters.

The international standard for vitamin E used as a reference in all assays for this vitamin is a solution of *dl*-α-tocopheryl acetate in coconut oil. Each 0.1 g of this solution contains 1 mg of the acetate. Results of an assay are expressed in terms of milligrams of the vitamin. The following relationship exists between IUs (or the equivalent USP Units) of the vitamin and the respective weights of the common forms: 1 USP or IU = 1 mg *dl*-α-tocopheryl acetate = 0.91 mg *dl*-α-tocopherol = 0.735 mg *d*-α-tocopheryl acetate (the ester of the natural form) = 0.671 mg *d*-α-tocopherol (the natural form). The IU represents biological activity as determined by the rat antisterility test.

The usual methods for quantitative assay of vitamin E depend either directly or indirectly upon the ease with which free α-tocopherol is oxidized. The esters, which are almost exclusively used in pharmaceuticals, must first be hydrolyzed. The free alcohol, then, because of its instability, must be handled with care in all other analytical operations. The physicochemical methods generally applied employ either of two oxidation-reduction reactions: (1) the formation of a red orthoquinone by treatment of the tocopherol with concentrated nitric acid or (2) the reduction of ferric chloride in the presence of α,α′-dipyridyl, which forms a red-colored complex with ferrous ions. Both methods are relatively nonspecific and are suitable only when combined with adequate separation procedures. A gas-liquid chromatographic procedure coupled with a visible-light detector and a high-pressure liquid chromatographic procedure using a UV detector provides highly specific determinations.

The classic biological method is the rat assay in which female rats are depleted of vitamin E and mated with normal males. The dose of the material to be tested and of the standard is administered over a period

of several days after conception. On the 20th day of pregnancy the female rats are killed, and the numbers of living and dead fetuses and resorption sites are recorded. Another, simpler bioassay is based on the dialuric acid hemolysis test in which the red-blood-cell fragility is measured as a criterion of vitamin E status in the rat.

Metabolic Functions, Dietary Requirement, and Food Sources—The exact biochemical mechanism whereby vitamin E functions in the body is still unknown; however, its most critical function occurs in the membranous parts of cells. Here, it interdigitates with phospholipids, cholesterol, and triglycerides, the three main structural elements of membranes. Since vitamin E is an antioxidant, a favored reaction at this site is with very reactive and usually destructive compounds called free radicals. These are products of oxidative deterioration of such substances as polyunsaturated fat. Vitamin E converts the free radical into a less reactive, nonharmful form. In its role as a protector against oxidation, vitamin E shows nutritional interactions with a wide variety of nutrients: vitamin A, the trace element selenium, the sulfur amino acids methionine and cysteine/cystine, polyunsaturated fatty acids, and, to a lesser extent, vitamin C. Interestingly enough, the order of antioxidant power among the tocopherols, as measured by their effect on the rate of peroxide formation in fats, is the reverse of the order of biological potencies. Other physiological functions probably include participation in nucleic acid metabolism, and it appears also that the tocopherols may be a component of the cytochrome reductase segment of the terminal respiratory chain in intermediary metabolism. In general, it appears that vitamin E plays an important role in ensuring the stability and integrity of cellular membranes; thus far in man, the only such demonstrated effect is on the red blood cell. The effect also is modified by the level of polyunsaturated fatty acids in the diet.

The therapeutic effectiveness of vitamin E in the prevention of abortion, in certain menstrual disorders, in the improvement of lactation, in muscular dystrophy, or in cardiovascular diseases has not been substantiated, and the promotion of vitamin E for such purposes is fraudulent. One use that is established and sound is in hemolytic anemia in premature infants. Vitamin E also generally is considered to provide protection against pulmonary oxygen poisoning. Essentially all other examples of clinical indications of need for vitamin E at nutritional levels are related to malnourishment or malabsorption problems. The latter are found in humans with cystic fibrosis, liver cirrhosis, postgastrectomy, obstructive jaundice, pancreatic insufficiency, and sprue.

There are some data that suggest that vitamin E may be useful in protecting the epithelial tissue of the lungs from free radical damage associated with air pollution, but more research is needed to achieve a consensus of medical opinion. Similarly, more data are required to substantiate claims that vitamin E promotes rapid healing of tissue damaged by severe burns or other skin injuries. Studies that suggest that vitamin E is useful for preventing some forms of cancer and preventing and treating coronary heart disease have been supported by epidemiological data, but replication of the finding is still needed using human subjects.

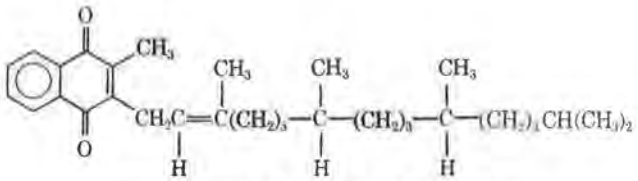
A clearly defined uncomplicated vitamin E–deficiency disease has not been recognized as a public health problem. A deficiency state with respect to vitamin E has been demonstrated in human subjects, especially in premature and newborn infants and in infants with steatorrhea. The evidence rests mainly on determination of *in vitro* hemolysis and blood tocopherol level. However, peripheral neuropathy and vitamin E–deficient nerves in deficient patients have been reported. Vitamin E requirement apparently is not related to body weight directly or to a caloric intake, but seems to be related to body weight in kilograms to the ¾ power, sometimes designated as physiological or metabolic size. Requirements for vitamin E are known to increase with high intakes of polyunsaturated fatty acids and in selenium deficiency.

Vitamin E is ubiquitous in its distribution and is found particularly in vegetable fats and oils, dairy products and meat, eggs, cereals, nuts, and leafy green and yellow vegetables. *Vitamin E is distributed so widely in nature that it is difficult to prepare a diet that does not meet NAS RDAs for all sex and age groups. However, attainment of levels expected to be needed for lowering the risk of cancer and heart disease likely will require supplementation.* In direct contrast to the more rapid turnover of some of the water-soluble vitamins, vitamin E is stored in fatty tissue and is removed from it only when the fat is mobilized. This means that many months of deprivation would have to pass to deplete the body stores.

VITAMIN K

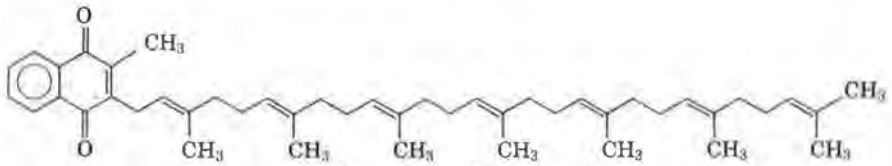
Vitamin K refers to a group of substances, widespread in nature, with similar biological activity; one form was isolated first from alfalfa and the other from putrefied fish meal. The primary activity that makes the vitamin essential in humans is its function in posttranslation of γ-carboxylation of glutamate in a number of proteins associated with blood clotting.

Chemistry and Assay—The parent structure of the K family of vitamins is 2-methyl-1,4-naphthoquinone. The various forms of vitamin K differ in the hydrophobic substituent at 3-position. This fat-soluble compound and several water-soluble derivatives such as the sodium bisulfite and diphosphoric acid ester are the common commercial forms used in medical practice. Vitamin K_1 (isolated from plants) is 2-methyl-3-phytyl-1,4-naphthoquinone.



Vitamin K_1—phylloquinone; phytonadione

Vitamin K_2 exists as a chemical series that, instead of the phytyl side chain in the 3-position, has side chains of varying numbers of unhydrogenated isoprene units, depending on the bacterial source. The vitamin K_2 with a 35-carbon side-chain and originally isolated from the putrefied fish meal is 2-methyl-3-all-*trans*-farnesylgeranylgeranyl-1,4-naphthoquinone. The synthetic compound, menadione, lacks a hydrophobic group at position 3 but can be alkylated in mammalian liver. The synthetic form is used as a source of vitamin K in most commercial animal feeds.



Vitamin $K_{2(35)}$ (farnoquinone)

The naturally occurring substances in pure form are light-yellow solids or oils, insoluble in water but soluble in fat solvents. Transparent colloidal solutions of vitamin K_1 can be prepared by means of nonionic surfactants. Although menadione, too, is fat-soluble, it is easily soluble in boiling water, and it is also slightly volatile at room temperature. Vitamins K_1 and K_2 as well as menadione are redox substances stable in the quinone form. In this respect there is a structural analogy between the vitamins K and E and a series of naturally occurring quinones called *ubiquinones*. The latter do not possess any demonstrable vitamin activity. Vitamins K have characteristic absorption spectra in the UV range and are sensitive to alkali, light, and ionizing radiation.

There is neither an international nor a USP standard (or Unit) for vitamin K. There is, however, a USP Reference Standard of menadione. The activity of test materials is generally measured in terms of biological equivalency to milligrams or micrograms of menadione in a chick-feeding test.

After extraction and separation from interfering substances, the vitamins K can be determined by their UV spectra or by color reactions. They react with sodium ethylate to give a blue color, which changes to brown. A more sensitive reaction occurs with sodium diethyldithiocarbamate to give a transient blue color. A method for assay of menadione in injections is the photometric assay of Menotti, in which 2,4-dinitrophenylhydrazine in ethanol is heated with menadione in the

presence of HCl. The vitamin thus is converted to the hydrazone, which when treated with ammonia yields a blue-green color. Vitamin K also can be assayed by the use of high-performance liquid chromatography (HPLC) coupled with UV detection. Vitamin K_1 content of food homogenates and plasma is analyzed using reverse-phase HPLC with post-column solid-phase reduction of vitamin K_1 to its hydroquinone form, followed by fluorometric detection.

The chick is suited particularly for the biological assay of vitamin K because of the ease in producing a dietary vitamin deficiency and the high requirement, and the criterion of activity (blood *prothrombin time*) is readily measurable, but species differences in biological activity are known to occur.

Metabolic Functions, Dietary Requirement, and Food Sources—Vitamin K is necessary for the formation of prothrombinogen and other blood-clotting factors in the liver. During clotting, circulating prothrombin is required for the production of thrombin; in turn, the thrombin converts fibrinogen to fibrin, the network of which constitutes the clot. It is obvious from this description that interference with formation of prothrombin will reduce the clotting tendency of the blood. In a severe deficiency of the vitamin, a condition of hypoprothrombinemia occurs, and blood-clotting time may be prolonged greatly or even indefinitely. Internal or external hemorrhages may ensue, either spontaneously or following injury or surgery. Other vitamin K–dependent proteins, including osteocalcin and matrix gla protein, have been identified in bone.

A group of substances termed vitamin K antagonists are characterized by their property to decrease plasma prothrombin levels and their usefulness in medicine as anticoagulants (see page 1252). Representative of this group is dicumarol, originally isolated from spoiled sweet clover hay, in which it is formed by bacterial action on coumarin. An important use of vitamin K is in the treatment of hypoprothrombinemia consequent to prothrombopenic anticoagulant therapy. Vitamin K_1 is the preferred form. Large doses of salicylates also antagonize vitamin K.

A few chemically related derivatives of dicumarol are commercially used as rodenticides. Another compound with similar antagonist activity is sulfaquinoxaline, a sulfonamide drug used in veterinary medicine for treatment of various infectious intestinal diseases. It increases the animal's requirement for vitamin K in some undetermined manner, probably by eliminating vitamin K–synthesizing enteric bacteria, upon which the animal depends, in part, for a source of the vitamin. Extended treatment with antibacterial drugs that alter the enteric flora also increases the dietary vitamin K requirement in man.

Optimal absorption of vitamins K requires the presence of bile or bile salts in the intestine. Menadione, the synthetic water-soluble analog, is absorbed easily in the absence of bile. The average diet apparently contains adequate amounts of vitamin K_1, since few if any malnourished humans have presented findings of dietary lack of vitamin K uncomplicated by intestinal disease, which prevents absorption. In 1989 the NAS established RDAs for vitamin K_1 at 80 μg/day for men and 65 μg/day for women.

The premature infant appears to be particularly sensitive to a lack of the vitamin and to an excess in the case of menadione. Because of this potential toxicity, the inclusion of menadione in OTC dietary supplements for the pregnant women is prohibited. Vitamin K_1 does not exhibit this toxicity and is the preferred form. For newborn infants and especially those born prematurely (and anoxic), a single dose of 1 mg of vitamin K_1, immediately after birth, is often a routine measure to prevent hemorrhagic disease. Vitamin K_1 may be administered to the mother 12 to 24 hr prior to the expected delivery or at the first sign of labor, especially if the mother has been receiving prothrombopenic anticoagulants. Requirements normally decrease after the neonatal period; however, it is important to ensure that adequate amounts of vitamin K_1 are present in infant formulas, since these are likely to be the sole nutriment during this period. Milk-substitute formulas containing less than 4 μg/100 kcal are required to have vitamin K_1 added to attain the level of 4 μg/100 kcal required by infant formula regulations.

Although extensive measurements of dietary intakes and food content of the vitamins K_1 have not been made, primarily because suitable analytical methods have not been developed, most diets contain sufficient amounts as evident by adequate body stores for a very high proportion of the population. The green, leafy vegetables, tomatoes, cauliflower, egg yolk, soybean oil, and liver of all kinds are good sources. Since it is insoluble in water, there is no loss in ordinary cooking. The human also uses vitamin K synthesized by certain enteric bacteria.

FAT-SOLUBLE VITAMIN PREPARATIONS

CHOLECALCIFEROL

(3β)-9,10-Secocholesta-5,7,10(19)-trien-3-ol, Vitamin D_3; Activated 7-Dehydrocholesterol

9,10-Secocholesta-5,7,10(19)-trien-3β-ol [67-97-0] $C_{27}H_{44}O$ (384.64); an antirachitic vitamin obtained from natural sources or prepared synthetically. See page 1800.

Description—White, odorless crystals; affected by air and light; melts between 84 and 88°.

Solubility—Insoluble in water; soluble in alcohol, chloroform, or fatty oils.

Comments—The only valid therapeutic (as opposed to dietary) uses are in the *treatment* of vitamin D *deficiency* or in the *prophylaxis* of deficiency in persons with a known deficiency, a high requirement or an absorption defect. However, the substance may be employed to treat *hypocalcemic tetany* and *hypoparathyroidism*. Also, there is a growing medical opinion that it facilitates the prophylaxis of osteoporosis by calcium in postmenopausal women. It should not be employed in the presence of renal insufficiency or hyperphosphatemia.

COD LIVER OIL

Oleum Morrhuae; Oleum Jecoris Aselli; Oleum Gadi

The partially destearinated fixed oil obtained from fresh livers of *Gadus morrhua* Linné and other species of the Family *Gadidae;* contains in each gram not less than 255 μg (850 USP Units) of vitamin A and not less than 2.125 μg (85 USP Units) of vitamin D.

It may be flavored by the addition of not more than 1% of a suitable flavoring substance or a mixture of such substances.

Preparation—The highest grade of this medicinal oil is manufactured from fresh cod livers from healthy fish, removed from the fish within a few hours after they are caught. The oil is separated from the livers by heating with low-pressure steam. When livers of high quality are used and the manufacturing procedure is carried out under carefully controlled sanitary conditions the resulting crude oil is a light yellow color and has good flavor and odor. Such an oil requires no purification or chemical refining.

Due, however, to long-established trade demands, it is necessary to remove the cod liver stearin so that the oil will remain clear at temperatures above freezing. To accomplish this, the oil is chilled to precipitate the stearin, which is removed by pressure filtration. To preserve the natural vitamin content of the oil it should be stored out of contact with air and light, preferably in a cold place.

Constituents—Consists chiefly of unsaturated glycerides but contains *palmitin* and *stearin*, as well as traces of *chlorine, bromine, phosphorus,* and *sulfur*. American cod liver oils may contain as much as 3 ppm of arsenic, but there is little evidence as to how completely it may be assimilated. American cod liver oils are rich in *iodine*—one sample was found to contain nearly 15,000 parts of iodine/billion parts of oil.

The vitamins of this oil occur in the unsaponifiable fraction. Since some persons object to taking oils, tablets and capsules containing the unsaponifiable fraction of the oil are manufactured. In general the procedure consists of saponifying the oil, separating the unsaponifiable portion, and extracting it with suitable solvents. The extract is diluted with corn oil and filled with capsules or mixed with solid materials and manufactured into tablets. The vitamin potency of these preparations can be adjusted to the patient's requirements, but obviously they do not supply the constituents present in the saponifiable portion of the oil from which they were prepared.

Description—Thin, oily liquid, with a characteristic, slightly fishy, but not rancid, odor and a fishy taste; specific gravity, 0.918 to 0.927.

Solubility—Slightly soluble in alcohol; freely soluble in either, chloroform, carbon disulfide, or ethyl acetate.

Comments—A source of vitamins A and D. The vitamins are present in such proportion that an oral dose of 5 mL provides the daily requirements for children or adults of both of these dietary essentials. However, it may not provide 100% of a US RDA. It has been employed in the prophylaxis of rickets in infants.

DIHYDROTACHYSTEROL—page 1377.

ERGOCALCIFEROL

(3β,5*Z*,7*E*,22*E*)-9,10-Secoergosta-5,7,10(19),22-tetraen-3-ol, Calciferol; Vitamin D_2

See page 1800 for the structure.

[50-14-6] $C_{28}H_{44}O$ (396.65). It is obtained by exposing ergosterol to UV light for the proper length of time. Insufficient irradiation results in

the production of products with little or no antirachitic activity, and prolonged exposure causes the production of toxic products. See page 1800.

Note—In stating the potency and dosage of vitamin D (cholecalciferol, ergocalciferol) dosage forms it is customary to use either the International Unit (IU) or the equivalent USP Unit. One USP Unit (or International Unit) of vitamin D (cholecalciferol or ergocalciferol) is defined as the specific biological activity of 0.025 μg of the crystalline international standard or pure vitamin D_3.

Description—White, odorless crystals; affected by light and air; melting range, 115 to 118°.

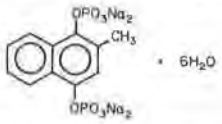
Solubility—Insoluble in water; soluble in alcohol, chloroform, ether, or fatty oils.

Comments—Like other forms of vitamin D, it exhibits both antirachitic and calcemic effects. It has a relatively high potency and is thus especially useful for the treatment of severe or refractory *rickets*. It also may be used in the management of *hypocalcemia* and *hypoparathyroidism*.

Care must be exercised to prevent overdosage. It should not be employed when renal insufficiency or hyperphosphatemia prevails. The serious toxic effects that may be caused by vitamin D are summarized in the general statement on *Vitamin D* under *Metabolic Functions*.

MENADIOL SODIUM DIPHOSPHATE

1,4-Naphthalenediol, 2-methyl-, bis(dihydrogen phosphate), tetrasodium salt, hexahydrate; Vitamin K_4; Kappadione; Synkavite



2-Methyl-1,4-naphthalenediol bis(dihydrogen phosphate) tetrasodium salt, hexahydrate [6700-42-1] $C_{11}H_8Na_4O_8P_2 \cdot 6H_2O$ (530.18); *anhydrous* [131-13-5] (422.09).

Preparation—Reduction of menadione to the diol compound by treatment with zinc in the presence of acid, followed by double esterification with HI, metathesis of the resulting 1,4-diiodo compound with AgH_2PO_4, and neutralization of the bis(dihydrogen phosphate) ester thus formed with NaOH.

Description—White to pink powder, with a characteristic odor; hygroscopic; solutions are neutral or slightly alkaline to litmus, pH about 8.

Solubility—Very soluble in water; insoluble in alcohol.

Comments—See *Menadione* and *Phytonadione*. In the body it is converted to menadione, and consequently, it has the same uses and limitations, except that it is water-soluble and does not require the presence of bile salts for its absorption; therefore, it is especially useful in the presence of biliary obstruction.

PHYTONADIONE

***R*-[*R**,*R**(*E*)]]-1,4-Naphthalenedione, 2-methyl-3-(3,7,11,15-tetramethyl-2-hexadecenyl)-, 2-Methyl-3-phytyl-1,4-naphthoquinone; Vitamin K_1; Mephyton**

Phylloquinone [84-80-0] $C_{31}H_{46}O_2$ (450.70). It is a mixture of *cis*- and *trans*-isomers; it contains not more than 20.0% of the *cis*-isomer. See page 1802.

Description—Clear, yellow to amber, very viscous, odorless or nearly odorless liquid; specific gravity about 0.967; stable in air but decomposes on exposure to sunlight; solution (1 in 20) in alcohol is neutral to litmus; refractive index, 1.523 to 1.526 at 25°.

Solubility—Insoluble in water; soluble in dehydrated alcohol, benzene, chloroform, ether, or vegetable oils.

Comments—The natural product, vitamin K_1. For the metabolic functions of vitamin K, see the general statement.

It has a more prompt and prolonged action than menadiol and other synthetic analogs of vitamin K, and it is more reliable in restoring prothrombin to the blood in conditions of *hypoprothrombinemia*. *Hypoprothrombinemia in the newborn* may be prevented or treated by the administration of phytonadione to the mother shortly before parturition or by giving the infant a single dose shortly after birth. In *hypoprothrombinemia consequent to prothrombopenic anticoagulant therapy*, an adequate intravenous injection usually will stop hemorrhage within 3 to 4 hr and restore the plasma prothrombin level to normal in 12 to 24 hr. In hypoprothrombinemia resulting from liver disease it may have limited value, especially if the disease is hepatocellular; in *biliary obstruction or fistula*, in which only the absorption of vitamin K is impaired, hypoprothrombinemia responds promptly to parenteral phytonadione. In other enteric diseases in which absorption is defective—as in *sprue, regional enteritis, enterocolitis, ulcerative colitis, dysentery,* and *extensive bowel resection*—it will correct hypoprothrombinemia if given parenterally.

It must be emphasized that it cannot be used to check bleeding irrespective of its origin. It is of no benefit in diseases of the blood-forming organs, thrombocytopenic purpura, hemophilia, etc.

Excessive doses occasionally may cause hyperprothrombinemia and a tendency toward thrombosis.

TRETINOIN—page 879.

VITAMIN A

Contains a suitable form of retinol ($C_{20}H_{30}O$; vitamin A alcohol). It may consist of retinol or esters of retinol formed from edible fatty acids, principally acetic and palmitic acids. It may be diluted with edible oils, or it may be incorporated in solid, edible carriers or excipients, and it may contain suitable antimicrobial agents, dispersants, and antioxidants. See page 1799.

Note—In stating the potency and dosage of vitamin A dosage forms it is customary to use either the International Unit (IU) or the equivalent USP Unit. One USP Unit (or International Unit) of vitamin A is defined as the specific biological activity of 0.3 μg of the all-*trans* isomer of retinol.

Description—Yellow to red, oily liquid that may solidify upon refrigeration; in solid form, it has the appearance of any diluent that has been added; may be nearly odorless or may have a fish odor but has no rancid odor or taste; unstable to air and light.

Solubility—In liquid form, insoluble in water or glycerin; soluble in absolute alcohol or vegetable oils; very soluble in ether or chloroform. In solid form, may be dispersible in water.

Comments—The only valid therapeutic uses are in the treatment of vitamin A *deficiency* or in the *prophylaxis* of deficiency in persons with a known dietary deficiency, a high requirement, or an absorption defect. Large doses produce toxicity (see the general statement), symptoms of which may not be evident for 6 months or longer. Daily doses larger than 25,000 USP Units should not be prescribed unless severe deficiency exists.

VITAMIN E

A form of alpha-tocopherol [$C_{29}H_{50}O_2$ = 430.71]. See page 1801. It includes the following: *d*- or *dl*-alpha-tocopherol ($C_{29}H_{50}O_2$); *d*- or *dl*-alpha-tocopheryl acetate [$C_{31}H_{52}O_3$ = 472.75]; *d*- or *dl*-alpha-tocopheryl acid succinate [$C_{33}H_{54}O_5$ = 530.79].

The generic title *Vitamin E Preparation* is officially recognized for any single form of the vitamin with one or more inert substances. The product may be in liquid or solid form, and it must contain not less than 95.0% and not more than 120.0% of the labeled amount of the vitamin. For a preparation labeled to contain a *dl*-form of the vitamin allowance is made for it to contain a small amount of a *d*-form occurring as a minor constituent of an added substance.

Alpha-tocopherol (also written α-tocopherol) is a trivial generic name that embraces all stereoisomeric forms of 2,5,7,8-tetra-methyl-2-(4,8,12-trimethyltridecyl)-6-chromanol. The term *d*-alpha-tocopherol is employed in the pharmaceutical field to designate that form of the compound that (1) occurs naturally and (2) is dextrorotatory. The term *dl*-alpha-tocopherol designates the mixture of stereoisomers prepared synthetically, commonly from racemic isophytol.

The phenolic hydroxyl is readily susceptible to acylation, and the resulting esters, eg, the acetate and acid succinate, are much more resistant to oxidation and discoloration on exposure to air and light than the phenolic form.

Description—Little or no odor or taste. *The alpha-tocopherols and alpha-tocopheryl acetates:* clear, yellow, viscous oils. *d-Alpha-tocopheryl acetate:* may solidify in the cold. *Alpha-tocopheryl acid succinate:* white powder; the *d*-isomer melts at about 75°, and the *dl*-form melts at about 70°. *The esters:* stable to air and to light but unstable to alkali; *the acid succinate:* also unstable when held molten.

Solubility—*Alpha-tocopheryl acid succinate:* insoluble in water; slightly soluble in alkaline solutions; soluble in alcohol, ether, acetone, or vegetable oils; very soluble in chloroform. *Other forms of vitamin E:* insoluble in water; soluble in alcohol; miscible with ether, acetone, vegetable oils, or chloroform.

Comments—The only valid therapeutic use is as a supplement to the diet of the newborn infant, especially if premature, or in the treatment of the infant with steatorrhea, in which the GI absorption of it is impaired. No need for administration to children or adults has been demonstrated. For additional information see the general statement.

OTHER FAT-SOLUBLE VITAMINS

Calcifediol (3β,5*Z*,7*Z*)-9,10-Secocholesta-5,7,10(19)-triene-3,25-diol monohydrate $C_{27}H_{44}O_2 \cdot H_2O$ (418.66) Calderol—The form of vitamin D_3 found in the circulation; differs from calcitriol (below) in that hy-

droxylation in the liver occurs only at C-25. Produced synthetically; see *Am J Clin Nutr* 1969; 22:412. A white powder practically insoluble in water. *Comments:* In the treatment and management of metabolic bone disease or hypocalcemia associated with chronic renal failure. It should not be given to patients with hypercalcemia or evidencing toxicity to vitamin D.

Calcitriol—For the full monograph, see page 1800. *Comments:* The form of vitamin D_3 that stimulates intestinal calcium transport. Based on the observation that in acutely uremic rats it stimulates intestinal calcium absorption, it has been suggested that a vitamin D–resistant state exists in uremic patients because of failure of the kidney to convert precursors to calcitriol; hence the indication for use of the latter compound in the management of hypocalciuria in patients undergoing chronic renal dialysis. Its efficacy in not only reversing the calcium metabolic disorder but also reducing elevated parathyroid hormone levels in some patients has been demonstrated.

Vitamin A Acetate [Retinol Acetate; $C_{22}H_{32}O_2$]—Light-yellow to red oil with a slight fishy odor; light and oxygen cause deterioration; tasteless. Soluble in lipid solvents; insoluble in water. *Comments:* A form of vitamin A; 0.344 μg is equivalent to 1 USP Unit or to 0.6 μg of β-carotene.

Vitamin A Palmitate [Retinol Palmitate; $C_{36}H_{60}O_2$]—Light-yellow to red oil; odorless in the pure state but otherwise has a slight fishy odor; unstable in light and air. Soluble in oils and lipid solvents; insoluble in water. *Comments:* A form of vitamin A.

THE WATER-SOLUBLE VITAMINS

Except for ascorbic acid, all the vitamins in this water-soluble category belong to the B-group of vitamins. Some still retain their original individual designations, such as B_1, B_6, and B_{12}, whereas comparable names for other vitamins have become obsolete.

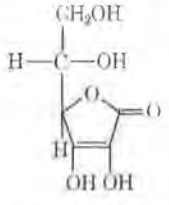
In 1930, when it was clear that vitamin B was of multiple nature, the term vitamin B complex was coined to refer to the group of water-soluble animal growth factors found in relatively high concentrations in such products as liver, yeast, and rice bran. This was a convenient term to use in the early scientific literature, but it was not intended to be a specific name for pharmaceutical preparations that contain varying proportions of the B vitamins. The term was intended to apply to a group of vitamins whose identity was being sought, rather than to a group of compounds whose identity had been established. Since the nature of the *complex* has been characterized, the term vitamin B complex is no longer appropriate.

ASCORBIC ACID (VITAMIN C)

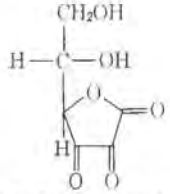
Vitamin C, or ascorbic acid (antiscorbutic vitamin), is necessary for the prevention and cure of the deficiency disease scurvy.

Scurvy has been recognized since the Middle Ages and was found widespread in northern Europe and among the crews of sailing ships. During the 18th century it was learned that when fresh fruit was made available aboard sailing vessels, scurvy was avoided. In 1907 Holst and Frolich observed a scurvy-like syndrome in guinea pigs that was similar to human scurvy and cured it by feeding citrus juices. This gave an experimental means for the rapid development of our knowledge of vitamin C, to which many workers have contributed.

Chemistry and Assay—Ascorbic acid is a white, crystalline compound structurally related to the monosaccharides. It exists in nature in both a reduced and the oxidized form, dehydroascorbic acid. These substances are in a state of reversible equilibrium in biological systems, and both have the same biological activity.



L-Ascorbic Acid



Dehydroascorbic Acid

Ascorbic acid is stable in the dry state but is easily oxidized in aqueous solution in the presence of air. Oxidation is accelerated by heat, light, alkalies, oxidative enzymes, and traces of copper and iron. Because of its relative instability, ascorbic acid is readily lost during cooking if simple precautions to avoid aeration are not taken. Also, because of its high aqueous solubility, the vitamin is lost to a considerable extent when large amounts of cooking water are discarded. Progressive loss of vitamin C in fresh fruits and vegetables occurs during storage.

Solutions of ascorbic acid are strongly reducing, and the vitamin is oxidized easily. In animal tissues the greater part of the vitamin is in the reduced form, but as scurvy develops, the ratio of oxidized to reduced form rises. This property of reversible oxidation-reduction is the most likely basis for the role of the vitamin in biochemical reactions.

The article of commerce is produced exclusively by synthesis. Sorbitol, a hexose occurring in several fruits but commercially obtained by hydrogenating dextrose, is the raw material for production of ascorbic acid. Amounts of ascorbic acid are expressed in terms of weight, as milligrams. The USP provides a Reference Standard of L-ascorbic acid for assay purposes. The practical methods of ascorbic acid assay are based on its powerful reducing properties, which enable determination by oxidimetric titration. The three most-used reagents for this titration are chloramine-T, 2,6-dichlorophenolindophenol, and iodine. Another practical assay is based on the conversion of ascorbic acid to oxalic acid 2-nitrophenylhydrazide by treatment with diazotized 2-nitroaniline. This yields a colored compound that is measured photometrically. Still another is the photometric assay of total ascorbic acid (ascorbic acid plus dehydroascorbic acid) by conversion of the vitamin to its 2,4-dinitrophenylhydrazone.

Metabolic Function, Dietary Requirement, and Food Sources—Vitamin C is known to be essential for the formation of intercellular collagen. In scorbutic tissues the amorphous ground substance and the fibroblasts in the area between the cells appear normal but without the matrix of collagen fibers. These bundles of collagenous material appear within a few hours after the administration of ascorbic acid. This points to the relationship of the vitamin in maintenance of tooth structures, matrix of bone, and the walls of capillaries. In scurvy, these are the tissues found to be faulty.

The picture of clinical scurvy in humans is one that can be related to the general breakdown of intercellular collagen substance. Bleeding is common, particularly at sites of pressure. The occurrence of petechiae, pinpoint hemorrhages that occur in the skin under reduced pressure, has been used as a diagnosis of scurvy. This is an indication of weakness or fragility of the walls of capillaries. Bones become brittle and cease to grow, and normal structures are replaced by connective tissue that contains calcified cartilage. Anemia is a common occurrence in scurvy, caused by an impairment of hematopoiesis. Also, vitamin C has been shown to change iron absorption. Tooth enamel, cementum, and particularly dentin change in structure, and the gums about the teeth become spongy and bleed easily. Keratoconjunctivitis sicca, xerostomia, salivary gland enlargement, xerosis, hyperpigmentation, ichthyosis, neuropathies, and mental depression may occur, even when the full-blown picture of scurvy is absent.

Vitamin C is essential for the healing of bone fractures. Such fractures heal slowly in a patient deficient in vitamin C. Wound-healing also is impaired.

There is evidence to indicate that the vitamin functions in the metabolism of tyrosine. There is an abnormal excretion of homogentisic, *p*-hydroxyphenylpyruvic, and *p*-hydroxyphenyllactic acids in scorbutic guinea pigs following administration of tyrosine, which, of course, is corrected with ascorbic acid. The excretion of *tyrosyl* derivatives in humans on a low–vitamin C diet given 20 g of tyrosine daily also is affected by ascorbic acid administration. In some newborns, the occurrence of tyrosinemia possibly accruing to high protein intakes suggests that this relationship be taken into consideration in evaluating the ascorbic acid requirement for the infant.

An intake of 10 to 20 mg a day of ascorbic acid is sufficient to protect an adult from classical scurvy, and 45 mg a day will maintain an adequate body pool of 1500 mg. Except for pregnant and lactating women, 60 mg is the recommended dietary allowance (Table 106-1) for both males and females over 11 years. For infants, 35 mg of ascorbic acid provides about the same amount as supplied daily by 850 mL of milk from mothers living in the US. The vitamin C requirements are increased following trauma, during infections, and during periods of vigorous physical activity; in such circumstances the requirement may be 100 to 200 mg a day.

The regular ingestion of 1 to 4 g of ascorbic acid a day has been suggested as a means of shortening the illness period and alleviating the symptoms of the *common cold*. A few clinical studies offer some support for this hypothesis, but other studies have failed to replicate these results. Definitive long-term studies with large populations, which might confirm the practice as a reliable public-health measure, have not been done.

A number of epidemiological studies show a protective association between the consumption of foods that contain vitamin C and cancers of the esophagus, stomach, and cervix. Animal studies testing precursors of known carcinogens showed a reduced number of tumors when the animals were given vitamin C. Biochemical studies suggest that vitamin C blocks the formation of active carcinogenics from precursors. There is also the hypothesis that vitamin C has an effect as a free radial scavenger. Although vitamin C in large amounts may have some pharmacological effects, these are not related to the normal functioning of the vitamin at nutritional levels. There is no evidence that levels exceeding the recommended RDA amount have any additional benefit, and contrary to those who advocate the use of megadose quantities (gram quantities), such practices can be harmful to some individuals.

The prolonged ingestion of supplements of ascorbic acid in excess of about 3 g a day is not without potential danger. GI disturbances (nausea followed by diarrhea), kidney or bladder stone formation (resulting from an increased excretion of oxalate, urate, and calcium), prenatal conditioning of the fetus to deficiency symptoms, interference with simple tests for glycosuria, and interference with the anticoagulant effect of heparin are clinical problems that may occur.

For therapeutic purposes in treatment of adult scurvy, 1000 mg of ascorbic acid a day, in divided doses, for 1 week is recommended, then 500 mg until all signs disappear. It also is used in the treatment of idiopathic methemoglobinemia to reduce the ferric iron in heme to the ferrous state.

Ascorbic acid facilitates the absorption of iron by keeping the iron in the reduced form. A few microcytic anemias respond to ascorbic acid treatment, which may be in part due to improved absorption of iron.

Vitamin C is found in all living plant cells, is synthesized during the germination of seeds, and is concentrated relatively in the rapidly growing parts of the plant. It is present in all animal tissues as well, but only guinea pigs, primates, a few exotic animal species and human are unable to meet body needs by synthesis and must rely upon a dietary source.

Although vitamin C appears to be present in all living tissues, our best sources of supply are fresh fruits such as citrus fruits, strawberries, and melons and green vegetables such as lettuce and cabbage. An average serving of potatoes contains enough vitamin C when first harvested to meet the adult male RDA, but contains only half that amount by the following spring. It is a common practice, and a sound one, to rely to a large extent on citrus fruits and juices as important vitamin C carriers, particularly in infant feeding. An ounce of orange or lemon juice a day is sufficient to prevent scurvy in humans on an otherwise low–vitamin C diet.

It is fairly common practice to add ascorbic acid to foods for technical purposes; eg, as an antioxidant to protect natural flavors and colors.

THE B VITAMINS

The *water-soluble B* of McCollum, or the *antiberiberi vita mine* of Funk, has now been differentiated into at least 11 separate and distinct chemical entities. It has been established that 8 of these are required in human nutrition. They are thiamine, riboflavin, niacin, folic acid, pyridoxine, biotin, pantothenic acid, and vitamin B_{12}. *p*-Aminobenzoic acid, choline, and inositol have an essential part in cellular metabolism in plants and animals, but this alone does not constitute presumptive evidence of their importance in human nutrition. When the dietary intake of methionine is adequate, choline can be synthesized endogenously; therefore, the human requirement is relative to the methionine intake, similar to the relationship between niacin and tryptophan. It can be stated categorically that the human does not require either an exogenous or endogenous source of *p*-aminobenzoic acid. Although inositol deficiency has not been demonstrated in humans, it may be an important nutrient in infant nutrition. Mammalian milk contains inositol, and since milk is the sole item of the diet of infants during this critical growth period, it is appropriate to include it in non-milk-based formulas, a practice that has existed since the early 1960s.

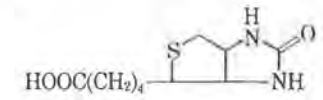
There is no one natural source of the B vitamins as a group that is necessarily superior to another source. No natural source contains all the water-soluble factors in the proportions that are needed in human nutrition, and the therapeutic value of any vitamin-containing material depends on the needs of the individual to whom it is being administered. Nevertheless, multiple deficiencies of B vitamins often coexist. Furthermore, the repair of one B-vitamin deficiency may increase the need for another; thus, the administration of thiamine in clinical or subclinical beriberi increases the need for riboflavin. Consequently, there is some justification for multivitamin therapy with those five B vitamins for which clinical deficiencies occur (thiamine, niacin, riboflavin, folic acid, and vitamin B_{12}). Human deficiencies in biotin and pantothenic acid have only been produced experimentally, and pyridoxine deficiency has occurred in infants fed an unfortified formula.

BIOTIN

***cis*-Hexahydro-2-oxothieno[3,4-*d*]imidazole-4-valeric acid**

Before this nutritional factor was identified as a discrete chemical substance, it variously was called vitamin H, anti-egg-white injury factor, coenzyme R, Bios II, and others. Its discovery was an outgrowth of studies on the *toxicity* of large amounts of unheated egg white as the sole source of protein for rats.

Chemistry and Assay—Biotin is a colorless, crystalline, monocarboxylic acid, only slightly soluble in water or alcohol (its salts are quite soluble). Water solutions are stable at 100°, and the dry substance is both thermostable and photostable. Biotin is unstable, however, in strong acids and alkaline solutions and in oxidizing agents. The vitamin is optically active, and the natural isomer, which alone possesses biological activity, is the D-form (rings are *cis*-fused and the isomer is designated (+)-biotin).



Biotin

Although biotin with the above structure is the compound present in food sources, the sulfur atom can be replaced with an oxygen atom without reduction in its metabolic activity. Biotin occurs in animal and plant tissues primarily in combined forms that are liberated by enzymatic hydrolysis during digestion. One of the simplest such complexes is biocytin, ε-*N*-biotinyl-L-lysine. The amount of the vitamin in a product is expressed solely in terms of the weight of the chemically pure substance, the free monocarboxylic acid.

Only microbiological methods are feasible for the quantitative assay of biotin because of their sensitivity to the low concentrations usually encountered. After simple aqueous or acid extraction combined with heating, a microbiological assay using growth of the test organisms *Allescheria boydii* or *Lactobacillus arabinosus* as the criterion is carried out.

Metabolic Functions, Dietary Requirement, and Food Sources—Attempts to induce deficiency in man by inclusion of large amounts (200 g) of dried unheated egg white for several days in the diet have resulted in the appearance of vague symptoms such as change in skin color and dermatoses, slight change in lingual papillae of the tongue, muscle pains, loss of appetite, sleeplessness, and extreme lassitude. Raw egg white contains a protein, avidin, which combines with biotin and prevents absorption of the vitamin from the intestine. Rapid relief from such symptoms was observed with administration of biotin. This condition is difficult to produce in human subjects, and since a frank and specific deficiency disease is not discernible, there is uncertainty as to the exact nature of the deficiency syndrome as well as the need for a dietary source of biotin in human nutrition. Intestinal synthesis is undoubtedly the important factor in the supply of biotin to the body.

Biotin functions in carbon dioxide fixation reactions in intermediary metabolism, transferring the carboxyl group to acceptor molecules. It similarly acts also in decarboxylation reactions. For its part in these vital enzymatic steps, in catalyzing deamination of amino acids and in oleic acid synthesis, biotin is essential in human metabolism and presumed to be a dietary essential in the absence of adequate microbial synthesis in the intestine.

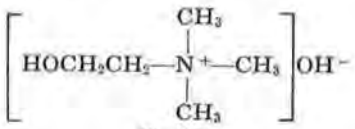
Diets providing a daily intake of 150 to 300 μg of biotin are considered adequate. These amounts are readily met and exceeded when milk, meat, and eggs are frequent items of the diet.

CHOLINE

The propriety of classifying choline as a vitamin and a member of the B group is questionable because it is synthesized in the human body, and there is no evidence that a lack of choline has a disturbing effect on human metabolism. Nevertheless, choline plays an important role both as a structural component of tissues and in biological methylation reactions. Dietary deficiency of it leads to gross pathology in several species of animals.

Chemistry—Choline is (β-hydroxyethyl)trimethylammonium hydroxide. Since it is completely dissociated, it is comparable to alkali hydroxides as a base. Consequently, it does not exist as a base at body pH but rather as a salt; the anion is that present in its immediate biological environment. The β-(hydroxyethyl)trimethylammonium cation is the biologically important moiety. The cation is incorporated into phospholipids, such as lecithin and sphingomyelin, and acetylcholine, a substance released at cholinergic nerve junctions during transmission of nerve impulses. Acid hydrolysis of phospholipids yields the free

choline salt, which is very soluble in water and to a lesser extent in ethanol. Assay for choline is accomplished with a microbiological method using a mutant strain of *Neurospora*.



Choline

Metabolic Functions, Dietary Requirement, and Food Sources—Besides its vital function as a precursor of acetylcholine, which is important in the sequence of nerve-muscle stimulations, choline is an important contributor of methyl groups needed for the *in vivo* synthesis of metabolites and perhaps some hormones. The biogenesis of choline appears to be universal in nature and is the result of the three-step transfer of methyl groups to an acceptor, which may be either free aminoethanol or phosphatidyl aminoethanol. Such transfers require methionine as a methyl donor (actually, *S*-adenosylmethionine). Choline is indirectly a source of methyl groups; it is first oxidized to betaine, which then may transfer a methyl group to homocysteine to form methionine. By thus regenerating methionine lost in transmethylation reactions, exogenous choline can spare the amino acid for use in protein synthesis. Methionine is an essential amino acid.

Choline has the property of preventing the deposition of excess fat or of causing the removal of excess fat from the liver of experimental animals fed high-fat diets and, because of this, it is often classified as a *lipotropic agent*. The lipotropic action probably relates to the incorporation of choline into phosphatidylcholine (lecithin), which, in turn, is incorporated into phospholipids and lipoproteins. The lipotropic action is independent of the function of choline as a reservoir of methyl groups.

There is presumptive evidence from nutritional and metabolic studies and teleological considerations that choline is important, if not essential, for the infant. It is appropriate to ensure, therefore, that choline is present in infant formulas at least to the level found in human milk. This is about 90 mg/L. Most infant formulas contain about 1 1/2 times this amount. It is equally appropriate to include choline in chemically defined diets to be used as the sole source of nutrients for critically ill patients.

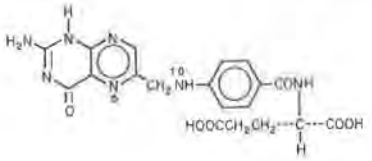
An average mixed diet consumed by man in the US has been estimated to contain 500 to 900 mg of choline a day, an amount known to be adequate when compared with animal requirements. Foods that supply large amounts of choline are liver, kidney, brain, muscle meats, fish, nuts, beans, peas, and eggs. Moderate amounts exist in cereals, milk, and a number of vegetables.

FOLIC ACID (FOLACIN)

The vitamin derives its name from the Latin word *folium*, leaf. It was first isolated from spinach leaves where it is now known to occur in relatively minute amounts compared with other food sources. Several apparently unrelated factors had been isolated in various laboratories before realization that they had in common the same parent compound, pteroyl-L-glutamic acid: Factor U (a chick growth factor), vitamin M (a factor for monkeys), vitamin B_c (a chick antianemia factor), liver and yeast *L casei* factors (bacterial growth factors), and others. In 1972 the International Union of Nutritional Sciences Committee on Nomenclature decided that the term folacin should be used as the generic descriptor for folic acid pteroylmono-L-glutamic acid. However, the USP continues to call pteroylglutamic acid by the descriptor folic acid, and medical and biochemical practice usually does the same.

Chemistry and Assay—Pteroylglutamic acid crystallizes from cold water, in which it is only slightly soluble, as yellow spear-shaped platelets. It is readily destroyed by boiling in acid solution, and its solutions will deteriorate in sunlight. It is insoluble in alcohol or the usual organic solvents but readily dissolves in dilute solutions of alkali hydroxides and carbonates. The characteristic UV absorption spectrum of pteroylglutamic acid in dilute NaOH is used to aid in identification and measurement of the compound.

A series of compounds with several molecules of glutamic acid attached to the first glutamic acid radical in peptide linkage have been synthesized. Compounds with one, two, three, and seven glutamic acid groups have been isolated. The latter three are known as conjugates. Some animals and man can utilize them as a source of pteroylglutamic acid, presumably because appropriate digestive enzymes can hydrolyze them. Microorganisms can use them to only a variable and limited extent unless they are first hydrolyzed to the free form with liver, kidney, or pancreatic enzymes, called conjugases.



The functional form of this vitamin group is basically the 5,6,7,8-tetrahydrofolic acid in which a formyl group (-CHO), when present, is attached at either or both the N^5 or N^{10} positions. The hydrogenated N^5-formyl compound, formerly called *folinic acid*, or leucovorin, is available, as is the monosodium salt of folic acid, as a discrete pharmaceutical preparation. It properly is termed 5-formyltetrahydrofolic acid. These compounds similarly serve as standards during assay of the vitamin. A USP Reference Standard Folic Acid is available. Separately, the three moieties that make up the folic acid molecule (pteroic acid, *p*-aminobenzoic acid, and glutamic acid) have no vitamin activity.

The quantitative assay of folacin in natural products is mainly by biological or microbiological methods. In the chick assay, the birds are placed on a folic acid–free diet until they became anemic, after which folic acid supplements and the test material are administered. The degree of recovery is related to the quantity of reference folic acid fed. The two organisms most used in the microbiological method are *Lactobacillus casei* and *Streptococcus faecalis*. The method is based on the fact that pteroylglutamic acid is a required growth factor for each; however, the assay is complicated when biological material is analyzed, because naturally occurring folic acid derivatives do not all have the same biological activity for the two organisms.

Folic acid can be determined by either of two physicochemical methods, provided the compound is present in relatively pure form. One method is the spectrophotometric measurement of the extinction maxima of the UV absorption curve; the other is the spectrometric measurement after oxidative fission of folic acid to 4-aminobenzoylglutamic acid followed by diazotization and coupling to give an azo dye. Folic acid also can be determined with high-pressure liquid chromatography.

Metabolic Functions—Folic acid is one of the important hematopoietic agents necessary for proper regeneration of the blood-forming elements and their functioning. Although the mechanism whereby folic acid performs this vital role is not understood, much is known about the involvement of folic acid as a coenzyme in intermediary metabolic reactions in which one-carbon units are transferred. These reactions are important in interconversions of various amino acids and in purine and pyrimidine synthesis. This role is in contrast to that of choline in furnishing and transferring so-called labile methyl groups in transmethylation reactions. The biosynthesis of purines and pyrimidines is linked ultimately with that of nucleotides and ribo- and deoxyribonucleic acids, functional elements of all cells.

The concept of antivitamins or vitamin antagonists is exemplified in a particular aspect of folic acid metabolism. By virtue of its structural similarity, sulfanilamide competes with *p*-aminobenzoic acid in the biological synthesis of folic acid. The organism is thus deprived of needed folic acid. Sulfonamides act, therefore, as growth inhibitors of certain pathogenic organisms, a competitive antagonism that is responsible for the antibacterial action of sulfa drugs. Since mammals use preformed folic acid, sulfonamides do not disrupt the host metabolism.

Numerous analogs of pteroylglutamic acid have been prepared that exhibit potent antifolic acid activity. Several compounds, notably aminopterin (4-aminopteroylglutamic acid) and methotrexate (4-amino-N^{10}-methylpteroylglutamic acid), compete with folic acid in nucleic acid synthesis and have been used in the treatment of various cancers, psoriasis, and certain immune disorders. The antimicrobial drugs trimethoprim and pyrimethamine are also antifolate drugs.

Dietary Requirement and Food Sources—Folic acid deficiency results in megaloblastic anemia, glossitis, diarrhea, and weight loss. A deficiency is best diagnosed by the demonstration of low levels of the vitamin in serum or blood by microbiological assay or by the hematological response to a physiological dose of folic acid, 50 to 200 μg intramuscularly a day for 10 days. The condition of megaloblastic anemia arising as a result of dietary deficiency of folacin occurs most frequently after the age of 65, in persons suffering from malabsorption syndromes, in women during the last trimester of pregnancy, and in infants receiving unfortified proprietary formulas or goat's milk. In the treatment of megaloblastic or macrocytic anemia, folic acid should be administered as the sole therapeusis only when the possibility of pernicious anemia and other primary diseases of the small bowel has been excluded absolutely, a restriction necessitated by the vitamin's ability to mask other diagnostic signs of these conditions.

In recent years folic acid has been linked as a possible agent in lowering the risk of rare but serious defects in fetal development of the

brain and spinal cord, including spina bifida and anencephaly. These conditions generally are referred to as neural tube defects (NTDs). In some interventional and observational studies in which women of childbearing age were given folic acid supplements, lower levels of NTDs were observed than with placebo controls. It should be noted that these studies were accomplished in areas where the pretreatment rates of NTDs were near or above 2 per 1000 live births, and supplemental levels of folic acid were between 0.4 and 4 mg/day. Also, data obtained for populations in which folic acid intakes were exceedingly low showed no relationship with the rates of NTDs, and therefore, the condition does not appear to be caused by classic folic acid deficiency. Furthermore, research with animals has not shown any increase in NTDs with folic acid–deficient diets.

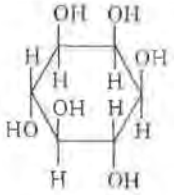
No mechanism for the observed relationship of folic acid consumption and NTD rates in humans has been proposed. The US Public Health Service has recommended that all women who are capable of becoming pregnant should consume 0.4 mg of folic acid per day throughout their childbearing years for the purpose of reducing their risk of an NTD pregnancy.

This recommendation was made after the 1989 revision of the RDAs was released by the Food and Nutrition Board of the NAS. The 1989 RDAs are 0.2, 0.18, and 0.4 mg for men, women, and pregnant women, respectively.

A balanced American diet for adults contains approximately 0.2 to 0.6 mg of total folic acid activity, and the intestinal microflora also provide some absorbable amounts of the vitamin. The best food sources of folic acid are liver, kidney, dry beans, asparagus, mushrooms, broccoli, and collards. Other good sources include spinach, peanuts, lima beans, cabbage, sweet corn, chard, turnip greens, lettuce, milk, and whole wheat products.

INOSITOL

Inositol is hexahydroxycyclohexane (1,2,3,4,5,6-cyclohexanhexol; *i*-inositol; *myo*-inositol; *meso*-inositol). Actually, there are nine stereoisomeric cyclohexanols, all of which now are referred to commonly as inositols. Several occur in nature; the isomer described above is by far the most prevalent and is the only one that is biologically active.



Inositol

Inositol occurs normally in nearly all plant and animal cells, either free or combined, suggesting that it is an essential cell constituent. In animal tissues it occurs as a constituent of phospholipids. In plants it usually is found as *phytic acid*, the hexaphosphate ester of inositol. There has as yet been no demonstration of need for inositol in human nutrition. In fact, large amounts of phytic acid in the diet interfere with the absorption of minerals, especially calcium, zinc, and iron.

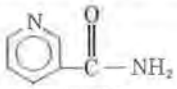
Although inositol possesses weak lipotropic activity, it is not as effective as methionine or choline. There is no valid therapeutic use of the compound. It may, however, be important to ensure its presence, at levels customarily found in human milk, in foods that are fed to infants and critically ill patients as the sole item of the diet. Inositol is measured by a microbiological assay.

NIACIN (NICOTINIC ACID AND NICOTINAMIDE)

Nicotinic acid (niacin) and nicotinamide (niacinamide) have identical properties as vitamins. Both compounds had been known for approximately 20 years before their biological significance was realized. In 1867 nicotinic acid was synthesized by the oxidation of nicotine with nitric acid. But it was not until 1937 that it was isolated from biological sources and found to be effective in the cure of black tongue in dogs and, later, pellagra in humans. The vitamin has none of the pharmacological properties of nicotine, however. In the 1940s the term *niacin* was adopted as a synonym for food labeling purposes to avoid association with the nicotine of tobacco. The term *niacin* is used generically to include both nicotinic acid and nicotinamide.

Chemistry and Assay—Nicotinic acid is pyridine-3-carboxylic acid. The structures of nicotinic acid and nicotinamide are shown below.

Nicotinic Acid



Nicotinamide

Niacin, the most stable of the vitamins, is not destroyed by heating in acid or alkaline solution. It withstands mild oxidation and retains its biological activity during the processing of food and the preparation and storage of pharmaceuticals. It is readily soluble in water or alcohol but insoluble in ether or chloroform. Niacinamide, on the other hand, may be extracted from water solution with ether. The amide is hydrolyzed readily to the free acid by heating in acid or alkaline solution.

The usual commercial synthesis of nicotinic acid used in foods and drugs is by the oxidation of quinoline with potassium permanganate or manganese dioxide, and monodecarboxylation of the purified quinolinic acid with controlled heating. Nicotinamide usually is prepared by esterifying nicotinic acid with methanol followed by ammonolysis.

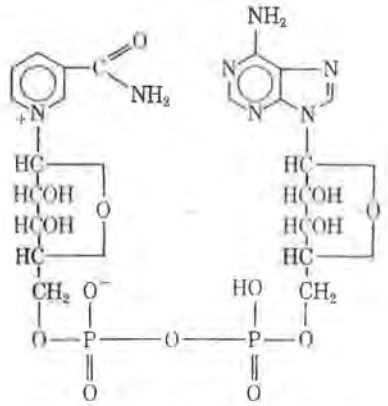
The activity of both forms of the vitamin is expressed in milligrams of the chemically pure substance. Because they have identical biological activity and their molecular weights are nearly identical, they are equivalent on a weight basis. Reference Standard Niacin and also Niacinamide Reference Standard are available from the USP.

Niacin may be determined in food, drugs, and biological materials by microbiological assay or by chemical methods. No animal biological method exists. The chemical determination involves reaction of the pyridine ring with cyanogen bromide and coupling of the fission product with an aromatic amine. The yellow polymethine dye that is formed is measured in a spectrometer at 436 nm. In natural products niacin occurs mainly in combined form as a coenzyme and must be liberated by acid hydrolysis before assay.

The microbiological assays employ *Lactobacillus arabinosus* as the test organism. A quantitative discrimination between nicotinic acid and nicotinamide in a sample is possible by assaying with both this organism, which uses both forms, and *Leuconostoc mesenteroides*, which can use only nicotinic acid.

Metabolic Functions—In the body niacin is converted to niacinamide, which is an essential constituent of coenzymes I and II that occur in a wide variety of enzyme systems involved in the anaerobic oxidation of carbohydrates. The coenzyme serves as a hydrogen acceptor in the oxidation of the substrate. These enzymes are present in all living cells and take part in many reactions of biological oxidation.

Nicotinamide adenine dinucleotide (NAD) is the inner salt of the 5′-ester of 3-carbamoyl-1-D-ribofuranosylpyridinium hydroxide with adenosine 5′-pyrophosphate and has the structure shown below. Nicotinamide adenine dinucleotide phosphate (NADP) differs only in that the adenosine moiety is esterified at its 2′-position with phosphoric acid.



NAD

These coenzymes are synthesized in the body and take part in the metabolism of all living cells. Since they are of such widespread and vital importance, it is not difficult to see why serious disturbance of metabolic processes occurs when the supply of niacin to the cell is interrupted.

The observations of numerous nutritionists that the daily requirement for niacin is influenced by the amount and kind of dietary protein led to the discovery that the amino acid tryptophan functions as a potential precursor of niacin. The efficiency of the conversion indicates that 60 mg of dietary tryptophan is equivalent to 1 mg of niacin. This relationship has given rise to the use of the term *niacin equivalent*, which is defined for the purpose of estimating the adequacy of diets in this vitamin as 1 mg of niacin or 60 mg of dietary tryptophan.

Niacin is absorbed readily from the intestinal tract, and large doses may be given orally or parenterally, with equal effect. Niacin, as nicotinic acid, is prescribed widely by physicians in gram amounts for the purpose of lowering blood cholesterol levels. The mechanism for this action is not fully understood; however, the effect is known to occur as

a result of decreased cholesterol synthesis in the liver. Only the nicotinic acid form of the vitamin provides the effects. The use of such high doses of nicotinic acid can have serious side effects, including impairment of liver function. Nicotinic acid at these levels should be used only in conjunction with appropriate monitoring of normal liver function.

The principal excretory product of niacin in the urine is *N*-methylnicotinamide, a fluorescent compound formed in the liver. On a normal diet approximately one-fourth of the niacinamide ingested is excreted as *N*-methylnicotinamide. With increased levels of niacin intake the percentage of ingested niacin excreted as the fluorescent substance is decreased.

Dietary Requirement and Food Sources—Pellagra, which means rough skin, is the primary deficiency disease due to lack of sufficient niacin in the diet, and it appears only after months of dietary deprivation. The condition involves the GI tract, the skin, and the nervous system. Loss of weight, anorexia, weakness, insomnia, headache, and diarrhea are common and appear without obvious cause. Other early symptoms may include abdominal pain, nervousness, and mental confusion.

Typical manifestations of pellagra in a well-advanced stage are diarrhea, dermatitis, and dementia. GI difficulties vary in severity, and absence of gastric secretion is a common finding. In the more advanced state, diarrhea is severe. Dermatitis has a characteristic appearance and occurs at those sites subject to exposure or irritation. The skin lesions are usually bilaterally symmetrical and appear first as erythematous patches, changing to brown pigmented areas, followed by desquamation and thickening. Glossitis is common; it is characterized by swelling and redness at the margins and tip of the tongue. Because of inflammation and superficial desquamation, the tongue, gums, and lips appear scarlet and smooth. Mental symptoms vary in occurrence and intensity; they include irritability, mental depression, and emotional instability. A confused mental state with hallucinations, mania, and delirium is seen in advanced stages of the disease. Pellagra is a complex deficiency, and symptoms of riboflavin, thiamine, and folacin deficiency frequently complicate the clinical picture.

Treatment of the disease requires immediate change to a nutritionally adequate diet and the administration of niacin or niacinamide. When neurological symptoms are present, use of thiamine and riboflavin may be necessary as well. Recovery from the acute condition is dramatic in most instances and occurs within 24 to 48 hr. Small doses given frequently during the day have been found to be more effective than a single large daily dose. Niacinamide is preferable to niacin because it does not produce vasodilation in the skin with sensations of itching, burning, or tingling. With severe nausea and diarrhea, intravenous injection of niacinamide is of additional advantage.

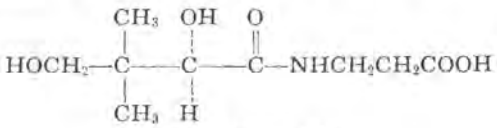
In considering dietary requirement and the foods that contribute to it, one must consider the content of preformed niacin and the niacin available by conversion from tryptophan, an essential amino acid present in all good-quality proteins. The minimum requirement to prevent pellagra is the equivalent of about 4.4 mg of niacin/1000 kcal/day. The recommended dietary allowance of the Food and Nutrition Board is 6.6 mg per 1000 kcal and not less than 13 mg at caloric intakes below 2000 kcal. Most diets consumed in the US supply from 500 to 1000 mg or more of tryptophan a day and 8 to 17 mg of preformed niacin, equivalent to 16 to 33 mg of niacin.

Poultry, meats, and fish constitute the most important single food group source of niacin. Organ meats are somewhat superior to muscle tissue. Potatoes, legumes, and some green leafy vegetables contain moderate amounts of preformed niacin, as do whole grains. An important public-health nutrition practice, begun in the 1940s, is the nutrient enrichment of cereal products: wheat flour, farina, corn products, rice, macaroni and noodle products, and bread. Niacin, thiamine, riboflavin, and iron are mandatory ingredients in products that are labeled *enriched*. The level of enrichment for niacin is such that a significant proportion of the daily requirement is obtainable from a generous serving of these foods.

PANTOTHENIC ACID

Knowledge of the identity and importance of pantothenic acid grew principally from experimental studies on microorganisms and chicks. Because of its wide distribution in nature it was named *pantothenic* (Greek, *pantothen*, from all sides). The terms vitamin B_3 and chick antidermatitis factor once were applied to variously purified concentrates of the factor, but they are now obsolete. No known therapeutic value exists for pantothenic acid, except perhaps in the treatment of frank or suspected cases of combined nutritional deficiencies.

Chemistry and Assay—Pantothenic acid is optically active (chiral). Maximum vitamin activity resides only in the D-form, and it is readily available as either the sodium or calcium salt, which are crystalline substances. Another commercially available form used in liquid preparations is D-pantothenyl alcohol (panthenol). Chemically, pantothenic acid is a composite structure of β-alanine and 2,4-dihydroxy-3,3-dimethylbutyric acid γ-lactone, connected in peptide linkage.



D-Pantothenic Acid

The free acid is fairly stable in neutral solution but sensitive to acids, bases, and heat. The salts are somewhat more stable, but even these are destroyed by autoclaving.

Pantothenic acid, its salts and alcohol, can be assayed by both chemical and microbiological methods. A chick growth method has been used, but it is time-consuming and has been replaced since suitable methods are available for releasing the bound vitamin (a protein enzyme) from its firm combination in plant and animal tissue. The first step in chemical assay is acid or alkaline hydrolysis. This cleaves the molecule at the peptide linkage into an alanine part and a pantoic acid part. These fission products then can be determined photometrically by suitable color reactions. In addition both gas-liquid chromatography and high-pressure liquid chromatographic methods now exist. *Saccharomyces carlsbergensis* and *Lactobacillus plantarum* are used for the microbiological assay of pantothenic acid and its salts. There is available a USP Reference Standard Calcium Pantothenate.

Metabolic Functions, Dietary Requirement, and Food Sources—Pantothenic acid is of the highest biological importance because of its incorporation into coenzyme A (CoA), which is involved in many vital enzymatic reactions transferring a two-carbon compound (the acetyl group) in intermediary metabolism. It is involved in the release of energy from carbohydrate, in the degradation and metabolism of fatty acids, and in the synthesis of such compounds as sterols and steroid hormones, porphyrins, and acetylcholine. CoA is composed of one mole each of adenine, ribose, and β-mercaptoethylamine and three moles of phosphate for each mole of pantothenate.

Many microorganisms depend on the same metabolic pathways for their growth and reproduction as do animal species and humans and thus also require pantothenic acid. Some have the ability to synthesize pantothenic acid at a life-sustaining rate from proper precursors. Synthesis by the bacterial flora of the intestine in humans appears to be an important source of the vitamin and is the probable explanation, in part, of why pantothenic acid deficiency in humans is seldom encountered. A deficiency syndrome has been experimentally induced in human volunteers by the oral administration of a pantothenic acid antagonist, ω-methylpantothenic acid, imposed on a pantothenic acid–deficient diet. It has been impossible so far to induce an isolated deficiency of the vitamin in less than at least 9 months on anything resembling a natural diet alone, because of the occurrence of significant amounts of pantothenic acid in such a wide variety of foods.

The symptoms that appear to be specific for a lack of available pantothenic acid from the studies using the antivitamin are neuromuscular disorders (paresthesias of the hands and feet and cramping of the legs and impairment of motor coordination), loss of normal eosinopenic response to adrenal corticotrophic hormone (ACTH), heightened sensitivity to a test dose of insulin, and, in concert with pyridoxine, a loss of antibody production. Fatigue, malaise, headache, sleep disturbances, nausea, abdominal cramps, epigastric distress, occasional vomiting, and an increase in flatus were subjective observations of the pantothenic acid–deficient human volunteers.

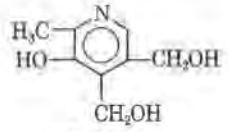
Usual diets of adult Americans furnish about 10 to 15 mg of pantothenic acid a day, with a probable range of 6 to 20 mg. A daily intake of 5 to 10 mg is probably adequate for children and adults, and there is no evidence for or against a greater requirement during pregnancy or lactation. Human milk contains about 2 mg/L; cow's milk, about 3.5 mg/L. Liver and other organ meats and eggs are particularly good sources. Broccoli, cauliflower, white and sweet potatoes, tomatoes, and molasses are quite high in pantothenic acid. Muscle tissue of beef, pork, lamb, and chicken also is a good source.

PYRIDOXINE (VITAMIN B_6)

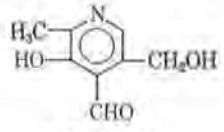
Vitamin B_6 does not denote a single substance but is rather a collective term for a group of naturally occurring pyridines that are metabolically and functionally interrelated; namely, pyridoxine, pyridoxal, and pyridoxamine. They are interconvertible *in vivo* in their phosphorylated form. There is no information on the relative biological activity of the three compounds in humans, and since pyridoxine is the most stable, it probably contributes the most vitamin activity to the diet.

Chemistry and Assay—Pyridoxine as the free base has a bitter taste and is readily soluble in water, alcohol, or acetone. It crystallizes as the hydrochloride and is prepared in this form for commercial use. Pyridoxine is one of the more stable vitamins and in the alcohol form withstands heating in acid or alkaline solution. Pyridoxal and pyridoxamine are less stable, however, and are known to undergo destruction in the more severe heat treatments sometimes used in food processing. Under most conditions of processing and storage of foods and pharmaceutical preparations, the vitamin is retained well.

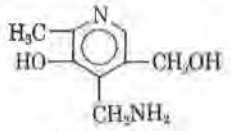
The structures of the three active forms of the vitamin and the phosphorylated form of one of them, pyridoxal phosphate, are shown below.



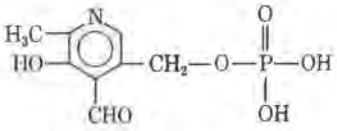
Pyridoxine



Pyridoxal



Pyridoxamine



Pyridoxal Phosphate

The biological activity of the vitamin is expressed in milligrams of the chemically pure substance, usually pyridoxine hydrochloride, for which a USP Reference Standard is available. Chicks and rats have been used for the biological assay of vitamin B_6 by placing the animals on a deficient basal diet that, when supplemented with known amounts of the test vitamin, supports a degree of growth related to the amount present. It is necessary to measure the three forms of vitamin B_6 to determine accurately the total biological activity. This can be accomplished with a high-pressure liquid chromatographic method. Microbiological assays also can discriminate between the individual vitamin B_6 components. A very useful technique employed in this type of assay is the preliminary separation of the different vitamin forms by a column chromatographic procedure using an ion exchanger. The column eluates then are analyzed by procedures suited to the vitamin form present in the eluates. The organisms most commonly used are *Saccharomyces carlsbergensis, Lactobacillus casei,* and *Streptococcus faecalis.*

Metabolic Functions, Dietary Requirement, and Food Source—Vitamin B_6 in the form of pyridoxal phosphate or pyridoxamine phosphate functions in carbohydrate, fat, and protein metabolism; its major functions are most closely related to protein and amino acid metabolism. The vitamin is a part of the molecular configuration of many enzymes (a coenzyme), notably glycogen phosphorylase, various transaminases, decarboxylases, and deaminases. The latter three are essential for the anabolism and catabolism of proteins.

The biological activity of vitamin B_6 seems to be a function of the molecule as a whole, since small changes in structure render it inactive. Deoxypyridoxine, a derivative of the vitamin in which one of the methanol groups is reduced to a methyl group, has potent antivitamin activity, but it is of limited experimental use in man because of its toxicity. The antivitamin isonicotinic acid hydrazide (isoniazid) has been used widely in the treatment of tuberculosis. It is chemically related to pyridoxine and acts also as an antagonist, thus requiring physicians to be alert to the pyridoxine nutriture of patients so treated. A similar antagonism is possible during treatment of hypertension with the drug hydralazine.

No classic syndrome of pyridoxine deficiency exists, probably because it is distributed widely in nature and unique or unusual dietary habits have not so far produced an uncomplicated deficiency. That it is essential for the growth of animals and human infants is well-established. Other manifestations of deficiency in humans are probably an acrodynia-like syndrome characterized by edema and loss of hair, nerve degeneration resulting in behavioral changes, and, in infants, convulsive seizures. The latter symptom was shown to result when infants were fed a proprietary milk-based formula, unsupplemented with pyridoxine, in which the natural vitamin content was destroyed inadvertently during sterilization. In this instance, marked changes in electroencephalogram patterns of the infants were produced, and they returned to normal minutes after pyridoxine administration.

In infants, although daily requirements of the vitamin are met by consumption of adequate quantities of normal breast milk, the protein–vitamin B_6 relationship is critical. General experience with proprietary formulas suggests that metabolic requirements are satisfied if the vitamin is present in amounts of 0.015 mg/g of protein, or 0.04 mg/100 kcal. The recommended dietary allowances of the Food and Nutrition Board for adolescents and adults, including conditions of pregnancy and lactation, range from 2.3 to 2.6 mg a day.

The best food sources of vitamin B_6 are muscle meats, liver, green vegetables, and whole-grain cereals. The bran from the cereal grains has especially large amounts. Nuts, corn, eggs, and milk are also good sources.

If large doses of vitamin B_6 are ingested for long periods of time, peripheral neuropathies develop. In most observations these involve levels in excess of 500 mg a day; however, one case with levels as low as 250 mg a day was reported.

RIBOFLAVIN

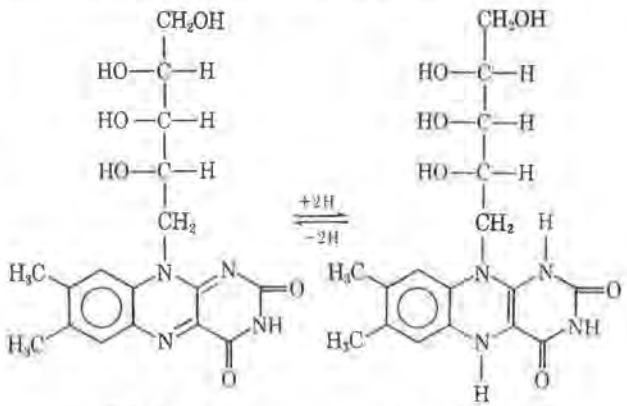
Riboflavin was formerly known as vitamin B_2 or G and lactoflavin. It owes its discovery as one of the components of the B-vitamin group to its characteristic fluorescence and pigmenting quality in such common foods as milk and egg yolk. Isolation and characterization of the yellow protein enzyme originally from yeast led to studies on the essential nature of the flavin pigment part of the enzyme in human metabolism, growth, and health.

Chemistry and Assay—Riboflavin is a yellow to orange-yellow, crystalline powder with a slight odor. When dry, it is not appreciably affected by diffused light.

In alkaline solution it is readily soluble but quite unstable to heat and to light, forming lumiflavin, a fluorescent degradation product that is without biological activity. Riboflavin is more stable to heat in acid solution, particularly from pH 1 to 6.5, but upon irradiation forms lumichrome, also biologically inactive. Photodegradation occurs in the skin, and infants with kernicterus who are treated with UV light may become riboflavin-deficient. Riboflavin is adsorbed readily from acid or neutral solution on such agents as frankonite, fuller's earth, and certain zeolites and eluted with acetone or pyridine solutions. Adsorbates have been used in pharmaceutical preparations, but from some of these the vitamin has been found to be unavailable to the human because of difficulty of elution in the intestinal tract.

Solutions of riboflavin have a characteristic yellow-green fluorescence that has a maximum absorption at 565 nm in the acid pH range. This property is made use of in the chemical determination of riboflavin. It is reduced rapidly by hydrosulfite, or by hydrogen in the presence of zinc in acid solution, to the leuco form, which is colorless and nonfluorescent. The leucoriboflavin is reoxidized easily by shaking in air. This oxidation-reduction property (see below) is the probable basis for the biological importance of riboflavin in the respiratory enzyme systems.

One gram dissolves in 3000 to about 20,000 mL of water, the variations in the solubility being due to differences in the internal crystalline structure of the riboflavin; it is more soluble in isotonic sodium chloride or alkaline solution than in water and less soluble in alcohol. It is insoluble in most lipid solvents. Derivatives such as the phosphate or acetate have been prepared for use in pharmaceutical preparations when higher concentrations are desired.



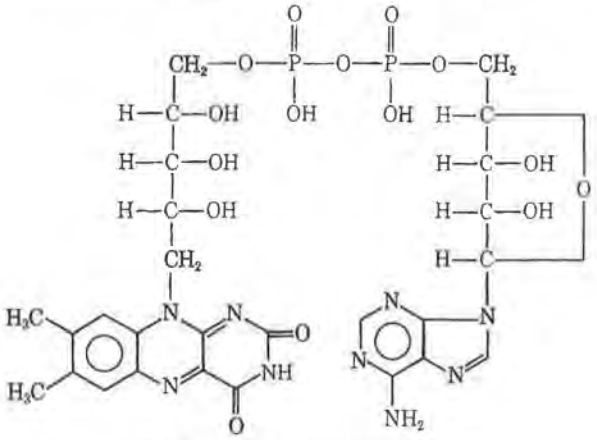
Riboflavin **Leucoriboflavin**

The activity of riboflavin is expressed in milligrams of the chemically pure substance, and a USP Reference Standard Riboflavin is available for assay purposes. In early work, the riboflavin content of substances was measured by a rat growth bioassay method, but this has been replaced by both physicochemical and microbiological methods.

Chemical determinations are based on colorimetric and fluorometric procedures. Straightforward measurement of the intrinsic yellow color of riboflavin is often sufficient for assaying pharmaceutical preparations. The fluorometric method is more sensitive and free of interferences and is therefore more suited to the assay of the vitamin in foods. It depends upon the extraction of the vitamin with dilute acid, filtration, treatment of the filtrate with permanganate and hydrogen peroxide to destroy interfering pigments, and measurement of the fluorescence. Assays also can be accomplished using high-pressure liquid chromatography and a fluorometric detector.

Lactobacillus casei is used as the test organism for microbiological assay of riboflavin. It is determined by measurement of the growth stimulation of the organism or by alkaline titration of the acid produced during incubation.

Metabolic Functions—Riboflavin plays its physiological role as the prosthetic group of a number of enzyme systems that are involved in the oxidation of carbohydrates and amino acids. It functions in combination with a specific protein, either as a mononucleotide containing phosphoric acid (FMN) or as a dinucleotide combined through phosphoric acid with adenine (FAD).



Flavin-adenine dinucleotide (FAD)

The specificity of each of the enzymes is determined by the protein in the complex. By a process of oxidation-reduction, riboflavin in the system either gains or loses hydrogen. The substrate, either carbohydrate or amino acid, may be oxidized by a removal of hydrogen. The first hydrogen acceptor in the chain of events is NAD or NADP, the di- or trinucleotide containing nicotinic acid and adenine. The oxidized riboflavin system then serves as hydrogen acceptor for the coenzyme system and in turn is oxidized by the cytochrome system. The hydrogen finally is passed on to the oxygen to complete the oxidative cycle. A number of flavoprotein enzymes have been identified, each of which is specific for a given substrate.

There is evidence now that some of the flavin enzymes contain metallic constituents. These metalloflavoproteins may contain iron, copper, or molybdenum. Succinic dehydrogenase, for example, contains iron, and xanthine oxidase contains molybdenum as well as iron.

After phosphorylation, riboflavin is absorbed from the intestinal tract and excreted in the urine. A human adult on an ordinary diet excretes from 0.5 to 1.5 mg in 24 hr, depending on the content of the diet. Of a 10-mg dose taken by mouth, 50 to 70% is excreted within 24 hr. In riboflavin deficiency there is little or none found in the urine. Measure of excretion has been used as a diagnostic sign of deficiency. Riboflavin, like thiamine, is stored to a limited extent, and constant dietary supply is needed to maintain normal body levels. Liver, kidney, and heart tissues contain relatively large amounts of riboflavin because of their high enzyme content.

Dietary Requirement and Food Sources—Symptoms of human ariboflavinosis include cheilosis (reddening of the lips and the appearance of fissures at the corners of the mouth), characteristic changes in color of the mucous membranes, inflammation of the tongue, and denuding of the lips. Lesions of a seborrheic nature also have been observed as a result of riboflavin deficiency. Ocular manifestations that appear in man and animals are characterized chiefly by corneal vascularization, in which the cornea is extensively invaded by small capillaries. This usually is accompanied by sensations of itching, burning, and roughness of the eyelid and lacrimation, photophobia, and visual fatigue. Some of these conditions may, of course, arise from other causes and do not necessarily indicate riboflavin deficiency.

Riboflavin deficiency in humans has not been found to be widespread in any part of the world, but is undoubtedly a complicating factor in other deficiency diseases such as pellagra. For therapeutic purposes, doses of 1 to 10 mg a day have been given. Rapid disappearance of symptoms of ariboflavinosis occurs with 10-mg doses, and some question the need for administering amounts larger than this.

Studies dealing with the quantitative riboflavin requirement of the human indicate that it is related to body size, metabolic rate, and rate of growth. The parameter used to express these most closely is metabolic body size, represented as kilograms of body weight taken to the ¾ power. The recommended daily dietary allowance of the Food and Nutrition Board for riboflavin is 0.4 to 0.6 mg for infants, 0.8 to 1.2 mg for children up to 10 years, 1.0 to 1.7 mg for adolescents and adults, and slightly higher for women during pregnancy and lactation. In general, the minimum requirement for riboflavin is about 0.3 mg for adults and 0.8 mg for infants on a 1000-kcal-intake basis. From a physiological point of view, an intake of more than 0.5 to 0.6 mg/1000 kcal may be of little extra value in normal adult persons.

Riboflavin is widely distributed in nature, in both plants and animals, as an essential constituent of all living cells, and therefore is found widely distributed in small amounts in foods. It is quite stable during the processing of food, except when there is excessive exposure to light. Because of its water solubility, there is moderate loss of riboflavin in cooking when the cooking water is discarded. This loss, however, is generally smaller than that of thiamine, niacin, or ascorbic acid.

Foods that make important contributions of riboflavin to the diet are liver and other organ tissues, milk, and eggs. Vegetables and fruits furnish a small but constant supply.

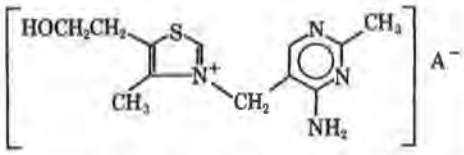
Many species of microorganisms are capable of synthesizing riboflavin, and because of the extensive bacterial growth in the human intestinal tract, this may form an important and constant source of supply of riboflavin and may account for the limited occurrence of deficiency in humans.

When it was recognized that cereal products would be a good vehicle to use to improve the content of riboflavin in many diets, its mandatory addition as an enriching ingredient was adopted. In concert with thiamine, niacin, and iron, riboflavin is present in nutritionally significant amounts in enriched wheat flour, farina, corn products, bread, macaroni, and noodle products. Because of certain cooking habits and the apparent unacceptability of the unnatural yellow color, the enrichment of rice with riboflavin has been resisted.

THIAMINE

Concentrates of thiamine, often termed vitamin B_1, were given the latter name by early workers in this country who recognized that at least two accessory dietary factors were needed for normal growth of laboratory rats, one in butter fat and the other in *milk sugar*. The names they suggested for these factors were fat-soluble vitamin A and water-soluble vitamin B. It was shown subsequently by a number of investigators that the latter consisted of a group of substances rather than a single compound, but vitamin B_1 was finally the first pure compound of the group to be laboriously isolated from rice polishings. In the pioneer studies on this substance it was found that a thiamine concentrate prevented polyneuritis in chickens, which later was found to be caused by the absence of thiamine in their diet. Deriving from this observation, an early name for the factor is aneurin (from antineuritic), which has persisted in some countries.

Chemistry and Assay—Thiamine is a generic term applied to all substances possessing vitamin B_1 activity, regardless of the anion attached to the molecule. The cationic portion of the molecule, which is the part that may properly be called *thiamine*, is made up of a substituted pyrimidine ring connected by a methylene bridge to the nitrogen of a substituted thiazole ring. In the general structural formula, *A* is any appropriate anion but usually chloride (see structure below). In addition, ammonium salts may be formed with the amine substituent on the pyrimidine ring. The common nomenclature is confusing, but in general, the term mono, as in thiamine mononitrate or thiamine monophosphate, designates the thiazolium type salt. Thiamine chloride hydrochloride is the ammonium salt formed by reacting thiamine chloride with hydrochloric acid (see page 1816).



Thiamine compounds are usually readily soluble in water or in alcohol but insoluble in fat solvents. They are stable in acid solution and may be heated without decomposition but are unstable in neutral or alkaline solution. At neutral or alkaline pH, splitting occurs at the methylene bridge upon heating in the presence of moisture. Splitting of the molecule takes place quantitatively in the presence of bisulfite ions, a reaction that is made use of in preparing dietary constituents free of thiamine for bioassay purposes.

Thiamine is oxidized in alkaline solution to thiochrome, a biologically inactive, highly fluorescent substance. This reaction is the basis for the chemical method of estimating thiamine. The pure vitamin is not readily oxidized in air.

An alternate commercial form of vitamin B_1, widely used because of its greater stability than the hydrochloride, is the mononitrate.

The activity of the vitamin is expressed in milligrams of the chemically pure substance, and a USP Reference Standard Thiamine Hydrochloride is available.

The determination of thiamine in food, biological materials, and pharmaceutical products is done almost exclusively by the thiochrome fluorometric method. On oxidation with ferricyanide in alkaline solution, thiamine is transformed into thiochrome, which has a strong blue fluorescence. It is a very sensitive method and correlates well with bioassay results. The sequence in the determination involves extraction of the vitamin, enzyme hydrolysis, adsorption, elution, and oxidation to thiochrome, which is extracted with isobutanol and determined fluorometrically.

Before the development of suitable physicochemical methods, thiamine was determined in a typical rat-growth assay that is based on the growth response of young thiamine-depleted rats to supplemental doses of a reference standard and to the test material either fed in or separate from the diet or injected parenterally.

Metabolic Functions—In a phosphorylated form, thiamine (thiamine pyrophosphate; cocarboxylase) serves as the prosthetic group of enzyme systems that are concerned with the decarboxylation of α-ketoacids. For example, pyruvic acid is decarboxylated to form a two-carbon residue. This process of decarboxylation is catalyzed by the pyruvic acid decarboxylase enzyme system, which consists of a specific protein, manganese ions, and diphosphothiamine. An α-hydroxyethyl group (the *acetaldehyde* residue of the decarboxylated pyruvic acid) attaches to the 2-carbon of the thiazole ring. The hydroxyethyl group (active *acetate*, active *acetaldehyde*, or two-carbon fragment) attaches to one of the sulfur atoms of lipoamide, from which it is removed by coenzyme A. Pyrophosphorylated thiamine is effective in the decarboxylation of other α-ketoacids as well. Some decarboxylation processes are reversible, so synthesis (condensation) may be achieved; thus, thiamine also is important to the biosynthesis of keto-acids. It is involved in transketolase reactions.

Thiamine is absorbed readily in aqueous solution from both the small and large intestine and then is carried to the liver by the portal circulation. In the liver, as well as in all living cells, it normally combines with phosphate to form cocarboxylase. It may be stored in the liver in this form or it may combine further with manganese and specific proteins to become active enzymes known as carboxylases.

Thiamine is excreted in the urine in amounts that reflect the amount taken in and the amounts stored in the tissues. Measurement of the urinary excretion of thiamine after giving a small dose of thiamine is useful in determining whether body stores are adequate or deficient.

Dietary Requirement and Food Sources—Polyneuritis (dysfunctioning of the nervous system) or beriberi is the frank disease associated with thiamine deficiency in humans. Peripheral neuritis is a pathological condition of the nerves of the extremities; usually both legs are affected and sometimes the arms as well. The symptoms include loss of sensation, muscle weakness, and paralysis. In beriberi this condition also is associated with edema and abnormal electrocardiogram patterns.

Severe cases of beriberi are commonly found in the Orient among people whose diets consist principally of milled or polished rice, from which the vitamin, contained in the bran and germ of the cereal, is largely removed during the milling process. American dietaries generally furnish sufficient thiamine to meet requirements, and with the use of a varied diet, including whole-grain cereals or enriched bread or flour, the adequacy of thiamine in most instances is beyond question. Symptoms of thiamine deficiency have been observed among chronic alcoholics, who use alcohol in place of food as a source of energy. Deficiency also occurs in cases of chronic diarrhea, in which absorption is interfered with over a period of time, and during pregnancy complicated with anorexia and nausea.

In the diagnosis of thiamine deficiency, symptoms to be noted in particular are anorexia, fatigue, loss of weight, sensation of burning in the soles of the feet, tenderness in calf muscles, muscle cramps, and general muscular weakness. Such signs are not in themselves specific, however, without supplementary laboratory findings that indicate a reduced thiamine content in blood and urine.

For treatment of beriberi or thiamine deficiency in humans, the first requisite is a nutritionally complete, well-balanced diet. Good diet is essential, because beriberi in most instances results from a complex or multiple deficiency, and administration of thiamine alone may precipitate a condition resulting from a lack of other water-soluble factors. Doses of 10 to 100 mg of thiamine have been used in severe cases to bring about a cure, but evidence of superiority of the larger doses is lacking. As size of the dose is increased, the proportion of thiamine retained rapidly decreases, the excess being excreted rapidly in the urine. Frequent small doses are to be preferred to a single large daily dose. Only in the most severe cases or in patients with impaired intestinal absorption does parenteral administration appear advantageous. Pharmaceutical preparations of many types and potencies are available commercially.

It is generally assumed that thiamine need is related to caloric need, particularly calories derived from carbohydrate. The Food and Nutrition Board considers that 0.5 mg/1000 kcal will maintain satisfactory thiamine nutriture under normal conditions in the US. As the caloric allowance varies with age, so does the recommended daily dietary allowance for thiamine; for infants, 0.3 to 0.5 mg; for children up to 12 years, 0.7 to 1.4 mg; for adolescents and adults, 1.0 to 1.5 mg, the highest allowance being for boys and men 15 to 22 years. The literature on thiamine needs in maternal and child nutrition suggests an increased need for thiamine during pregnancy, and an additional 0.3 mg a day is recommended in accordance with the increased caloric recommendation.

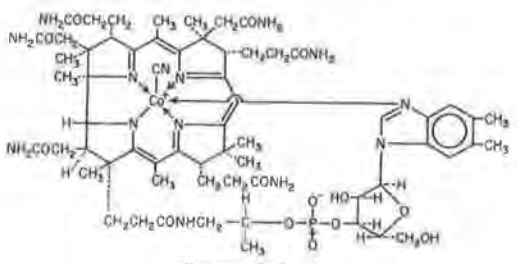
Thiamine is found widely distributed in foods. Thiamine is found in all plants and is synthesized by some microorganisms, particularly yeasts. No one food can be considered of particular importance above all others, although the cereal grains, milk, legumes, nuts, eggs, and pork probably furnish the larger proportion of thiamine in diets used in this country. Sophistication and processing of foods generally tend to reduce the thiamine supply. For example, in the preparation of wheat flour, separation of the bran coat and germ removes 3/4 or more of the thiamine present in the whole wheat. This is true for other cereal grains as well. Much of the white flour, corn grits, and rice used in this country is enriched to approximate the whole-grain level. Because of the lability of thiamine to heat, cooking and baking processes reduce the raw food content of the vitamin.

The loss of thiamine in home cooking is not considered excessive, except with foods cooked in large amounts of water that then is discarded. Because of its solubility, the thiamine content of the cooking water is always appreciable.

VITAMIN B_{12}

Vitamin B_{12}, the most recently discovered of the B group, was isolated from liver fractions in crystalline form in 1948 and was soon after shown to be specific for the treatment of Addisonian pernicious anemia. It was established that vitamin B_{12} is the active principle in extracts of liver, employed for more than 30 years in the control of pernicious anemia. Liver continues to be an important dietary source of the vitamin, but liver injection is no longer used in the treatment of pernicious anemia, because of the ready availability of crystalline forms of the vitamin.

Chemistry and Assay—Vitamin B_{12} is a complex water-soluble compound that crystallizes as small red needles that have a specific rotation in dilute aqueous solution of −59°. Characteristic absorption maxima occur at 278, 361, and 550 nm. The crystalline substance blackens without melting at 300°. The compound is a cobalt coordination complex, in which the cobalt is trivalent and has a coordination number of six. The complex is neutral. Vitamin B_{12} is composed of two heterocyclic systems, a benzimidazole and a modified porphyrin nucleus, with the following structure:



Cyanocobalamin

Actually, the cyanide group coordinated to the cobalt is not a part of the true vitamin but rather is an artifact caused by isolation of the vitamin on charcoal; in the liver the ligand is 5'-deoxyadenosyl anion. Nevertheless, by strict organic chemical definition, by virtue of the fact that the cyanide was the first form of the vitamin to be isolated, cyanocobalamin *is* vitamin B_{12}. When the ligand is hydroxide instead of cyanide, the compound is *vitamin* B_{12a} (hydroxocobalamin); when it is water, the substance is *vitamin* B_{12b}; (aquocobalamin); when it is nitro, the compound is *vitamin* B_{12c}; *the 5'-deoxyadenosyl form is coenzyme* B_{12}; if the ligand is methyl, the compound is *methyl* B_{12}. Sulfito- and thiocyanatocobalamins also are known. In practice, all of these compounds are vitamin B_{12}. A similar situation obtains with respect to the name *cobalamin*, which strictly is synonymous with cyanocobalamin but in loose practice applies to any active compound containing the α-(5,6-dimethylbenzimidazoyl) corrin nucleus. *Cobamides* is a generic term that has been used for these compounds.

Vitamin B_{12} (cyanocobalamin) in an atmosphere of hydrogen with a platinum catalyst is reduced to a red crystalline compound with slightly changed UV-absorption maxima, and a reduced stability to heat. Vitamin B_{12a} results from such reduction. Vitamin B_{12b}, another reduced form, occurs in natural sources.

Commercially, vitamin B_{12} is obtained from fermentation by *Streptomyces griseus*. The vitamin is precipitated from aqueous solutions saturated with ammonium sulfate by 1-butanol. Purification is achieved by chromatography, using bentonite or aluminum silicate as the adsorbent. Sharply defined red bands are formed during the development of the chromatograms, indicating the location of the vitamin. The red band is separated mechanically and eluted with water. The concentrated water solution on addition of acetone gives the crystalline vitamin, which can be purified further by recrystallization from aqueous acetone.

The USP provides a Reference Standard Cyanocobalamin for use in assay of the vitamin. A physicochemical method for determining vitamin B_{12} involves measurement of light absorbance at certain specific wavelengths characteristic for cyanocobalamin. This method is only applicable to relatively concentrated solutions of the compound, such as pharmaceutical preparations. Vitamin B_{12} also can be determined with high-performance liquid chromatography.

Vitamin B_{12} is one of the most active biological factors known; its activity for bacteria is measured in terms of millimicrograms. Because of this sensitivity of some bacteria to such low levels of the vitamin and the fact that foods contain exceptionally low concentrations of the vitamin, microbiological methods are widely used. The following three organisms, which require vitamin B_{12} for growth, are used: *Lactobacillus leichmannii*, *Ochramonas malhamensis*, and *Euglenia gracilis*.

Metabolic Functions, Dietary Requirement, and Food Sources—The vitamin is essential for the normal functioning of all cells, but particularly for cells of the bone marrow, the nervous system, and the GI tract. It appears to facilitate reduction reactions and participate in the transfer of methyl groups. Evidence exists that vitamin B_{12} is involved in protein, carbohydrate, and fat metabolism, but its chief importance in mammalian tissues seems to be, together with folic acid, in the anabolism of deoxyribonucleic acid in all cells. Coenzyme forms of vitamin B_{12}, in which the vitamin is linked to adenine and a sugar, which catalyze specific reactions in intermediary metabolism, have been isolated from bacterial cultures and probably have similar vitamin roles in mammalian cells.

The biochemical fault in pernicious anemia, a condition caused by a prolonged deficiency of vitamin B_{12}, is a failure of elaboration of the intrinsic factor, normally secreted by the parietal cells of the stomach mucosa. This intrinsic factor, which is essential for the absorption of the vitamin through the intestinal wall, forms a complex with vitamin B_{12}. Intrinsic factor is a glycoprotein of 45,000 daltons.

Vitamin B_{12} is a requisite for normal blood formation, and certain macrocytic anemias respond to its administration. In pernicious anemia, unless accompanied by intrinsic factor, the vitamin is not absorbed orally in effective amounts and must be administered parenterally in microgram quantities. Preparations containing vitamin B_{12} and intrinsic factor concentrate are now available for oral use and have been shown for short-term use at least to be equivalent in value to the injections. Clinical studies indicate that if milligram amounts of the vitamin are administered orally in the absence of intrinsic factor, enough of the vitamin passes through the intestinal wall to be effective in maintaining the pernicious anemia patient. However, the injectable form of vitamin B_{12} continues to be the drug of choice because of the desirability of regular attention of a physician to the condition of the patient.

The evidence indicating that vitamin B_{12} is the antipernicious anemia factor is complete. In treating pernicious anemia, vitamin B_{12} administered intramuscularly produces a maximal reticulocyte response in 4 to 9 days and a restoration of red- and white-cell counts in 4 to 6 weeks. The change in bone marrow from a megaloblastic to a normoblastic state is dramatic and occurs within a few hours after the injection of as little as 1 μg of the vitamin. Vitamin B_{12} is considered to be the extrinsic factor of Castle, the absorption of which from the intestinal tract is facilitated by the intrinsic factor present in normal gastric juice. The biochemical defect in pernicious anemia, then, is a failure of elaboration of the intrinsic factor. Because of this relationship, vitamin B_{12} given orally is much less effective in the pernicious anemia patient and entirely ineffective if there is complete absence of intrinsic factor.

The vitamin is effective in preventing the occurrence of neurological changes common to pernicious anemia. These symptoms are observed more frequently among the elderly because absorption of vitamin B_{12} has been shown to decrease among this population. However, it is not uncommon to identify women with neurological changes caused by vitamin B_{12} deficiency in their mid-thirties to late thirties. Acute symptoms of combined-system disease have been found to disappear rather promptly after B_{12} administration, but recovery appears to depend more on the chronicity of the disease than on the extent of neurological involvement, and conditions of long standing are less apt to show recovery.

Osteoblast activity probably also depends upon vitamin B_{12}.

A simple nutritional concept of pernicious anemia that seems valid is that of essentially an uncomplicated deficiency of vitamin B_{12} conditioned by the lack of intrinsic factor and, hence, the inability to absorb the vitamin from ingested food. This validation rests on several types of evidence; particularly convincing is the comparison of the clinical development of vitamin B_{12} deficiency in vegans, in patients who had total gastrectomy (resulting in removal of intrinsic factor and interference with absorption of the vitamin), and the relapse following withholding of therapy from previously adequately treated patients with pernicious anemia. Simple experimental dietary deficiency of vitamin B_{12} has not yet been produced in the adult human under conditions of careful continuous observation. It seems probable that the requirements for parenterally administered (or absorbed) vitamin B_{12} by the patient with pernicious anemia or gastrectomy are similar to the requirements of the normal subject.

The recommended daily dietary allowance of the Food and Nutrition Board for vitamin B_{12} ranges from 0.5 to 3 μg; the lower value is for infants, and the higher value is for women during pregnancy.

Vitamin B_{12} occurs in meat and dairy products but is not present to any measurable extent in plants or cereal grains. It is probable that indigenous bacteria in plant foods synthesize sufficient vitamin B_{12} to meet the requirement of those individuals whose dietary habits preclude the use of animal food sources.

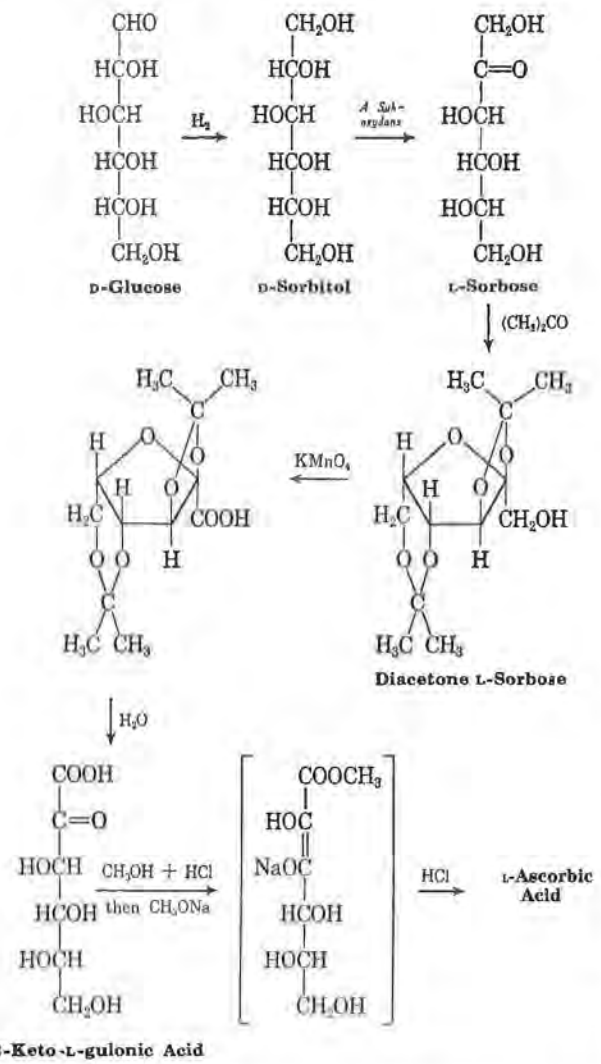
WATER-SOLUBLE VITAMIN PREPARATIONS

AMINOBENZOIC ACID—page 1214.

ASCORBIC ACID

L-Ascorbic acid [50-81-7] $C_6H_8O_6$ (176.13). See page 1805.

Preparation—The article in commerce is produced exclusively by synthesis. Sorbitol, a hexose sugar, occurring in several fruits but commercially obtained by hydrogenating dextrose in the presence of a Cu-Cr catalyst, is the raw material for the production of ascorbic acid. The D-sorbitol in aqueous solution is converted by the action of the organism *Acetobacter suboxydans* to L-sorbose, which is a ketose. The L-sorbose then is condensed with acetone by means of sulfuric acid to form diacetone sorbose. The object of the acetonation is to protect the hydroxyl group from oxidation in the subsequent steps. The diacetone sorbose, after suitable purification, is oxidized by potassium permanganate and then hydrolyzed, forming 2 keto-L-gulonic acid. This acid is esterified with methanol, and an intermediate sodio compound is formed with sodium methoxide. Hydrolysis with aqueous HCl removes the methyl group and sodium and lactonizes it to form ascorbic acid. The process is illustrated as follows.



Description—White or slightly yellow crystals or powder; odorless and on exposure to light gradually darkens; in the dry state, reasonably stable in air, but in solution rapidly deteriorates in the presence of air; melts at about 190°; specific rotation (1 in 10 aqueous solution) between +20.5 and +21.5°; aqueous solution has the acidic properties of a monobasic acid, and it forms salts with metallic ions. pK_a 4.2 and 11.6.

Solubility—1 g in about 3 mL water or 40 mL alcohol; insoluble in chloroform, ether, or benzene.

Incompatibilities—Stable in the dry state but in solution oxidizes rapidly in the presence of air. The reaction is accelerated by *alkalies and certain metals,* especially *copper;* it is retarded by acids. Aqueous solutions are strongly acidic, with a pH of 2 to 3.

Comments—In addition to the uses described on page 1806, it is sometimes given with iron salts in the treatment of iron-deficiency anemia; it functions to keep the iron in the ferrous state and hence to improve absorption. Apart from coadministration of vitamin C and iron preparations, a few cases of hypochromic anemia improve upon increasing the intake of vitamin. For additional information, see the general statement on *Ascorbic Acid.*

It also is used as a urinary-acidifier to enhance the effectiveness of methenamide by lowering the pH of the urine and thus aiding in the formation of formaldehyde.

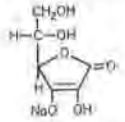
The effect of megadoses (10 to 15 times the RDA) has not been proved, and large overdoses should be discouraged.

Numerous, unapproved uses for ascorbic acid have been claimed, such as in the prevention and treatment of cancer, for infections of the gingiva, hemorrhagic states, mental depression, dental caries, acne, collagen disorders, ulcers of the skin, hay fever, and the common cold.

No more than the RDA should be given to the pregnant woman; the metabolism of the fetus adapts to high levels of the vitamin, and scurvy may develop after birth when the intake drops to normal levels.

SODIUM ASCORBATE

L-Ascorbic acid, monosodium salt; Cevalin



Monosodium L-ascorbate [134-03-2] $C_6H_7NaO_6$ (198.11).

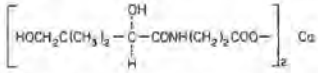
Description—White or very faintly yellow crystals, or crystalline powder; odorless or practically odorless; relatively stable in air; on exposure to light it gradually darkens; pH (1 in 10 solution) between 7.5 and 8.

Solubility—1 g in 1.3 mL of water; very slightly soluble in alcohol; insoluble in chloroform or ether.

Comments—A pharmaceutical necessity for *Decavitamin Capsules* and *Decavitamin Tablets.* It also is used as an antioxidant in fruit and vegetable canning and in the processing of meat.

CALCIUM PANTOTHENATE

β-Alanine, *(R)-N*-(2,4-dihydroxy-3,3-dimethyl-1-oxobutyl)-, calcium salt (2:1); Dextro Calcium Pantothenate



Calcium D-pantothenate (1:2) [137-08-6] $C_{18}H_{32}CaN_2O_{10}$ (476.54); the calcium salt of the dextrorotatory isomer of pantothenic acid.

Preparation—Several syntheses are available. In one, isobutyraldehyde is converted to the lactone of 2,4-dihydroxy-3,3-dimethylbutyric acid, the D-enantiomer of which, obtained by resolution, is combined with β-alanine to form D-pantothenic acid and then converted to the calcium salt.

Description—Slightly hygroscopic, white powder; odorless, has a bitter taste and is stable in air; unstable to heat both in the dry state and in acid or alkaline solution; most stable at pH 5.5 to 6.5, and its solutions may be autoclaved at this pH for a short time without appreciable loss; solutions are neutral or slightly alkaline to litmus, with a pH of 7 to 9; specific rotation (calculated on the dried basis and in a 5% solution) +25 to +27.5°.

Solubility—1 g in about 3 mL water; soluble in glycerin; practically insoluble in alcohol, chloroform, or ether.

Comments—See the general statement on *Pantothenic Acid.* Since a deficiency of pantothenic acid alone is virtually unknown, the primary indication for use is a general nutritional deficiency. Clinical cases have been too few to supply creditable data on dosage; consequently, the dose that follows is more customary than meaningful.

CYANOCOBALAMIN

α-5,6-Dimethylbenzimidazolylcobamide cyanide; Vitamin B_{12}

Vitamin B_{12} [68-19-9] $C_{63}H_{88}CoN_{14}O_{14}P$ (1355.38).

Preparation—Vitamin B_{12} can be isolated from aqueous liver extracts and from *Streptomyces griseus* fermentation. Commercially, it is obtained from the latter source (see page 1813).

Description—Dark red, hygroscopic crystals or amorphous or crystalline powder; when the anhydrous compound is exposed to air it may absorb about 12% water.

Solubility—1 g in 80 mL water; soluble in alcohol; insoluble in acetone, chloroform, or ether.

Comments—This and other forms of vitamin B_{12} are used to treat various megaloblastic anemias, especially *pernicious anemia* and other anemias in which the secretion of the intrinsic factor is impaired, as in *gastric cancer, gastric atrophy, total* or even *subtotal gastrectomy.* It also may be used to treat the megaloblastic anemias of *tropical sprue, idiopathic steatorrhea, gluten-induced enteropathy, regional ileitis, ileal resection, malignancies, granulomas, strictures or other structural disorders of the ileum* in which vitamin B_{12} absorption is impaired; in most of these folic acid deficiency is even more severe, and combined therapy is indicated. Its deficiencies untreated for periods of more than 3 months may result in permanent degenerative spinal cord lesions. The megaloblastic anemia associated with *fish tapeworm infestation* also responds to the vitamin. The megaloblastic anemias of pregnancy, infancy, alcoholism, and poverty usually are due to folic acid deficiency and only infrequently respond to it. The vitamin is *not useful* in the treatment of infectious hepatitis, multiple sclerosis, trigeminal neuralgia, anorexia, miscellaneous neuropathies, thyrotoxicosis, retarded growth, aging, and various psychiatric disorders, and claims to the contrary and promotion therefore represent an abuse. It should not be administered intravenously and is contraindicated in patients who are

sensitive to it or cobalt. Patients with Leber's disease have been found to suffer severe and rapid opticatrophy when treated with it. Either cyanocobalamin or hydroxocobalamin may be used for a loading dose in the Schilling test for malabsorption of the vitamin in diseases that affect the lower bowel, such as *sprue*.

A nasal spray has been developed that is said to provide significant absorption in the nasal mucosa and may supplant the parenteral dosage forms.

In addition to intrinsic factor, GI absorption requires an alkaline pH. In the presence of pancreatic disease it may be necessary to administer the oral vitamin with bicarbonate or give the vitamin parenterally.

For additional information about cyanocobalamin see the general statement on *Vitamin B_{12}*.

HYDROXOCOBALAMIN

Cobinamide, dihydroxide, dihydrogen phosphate (ester), mono(inner salt), 3′-ester with 5,6-dimethyl-1-α-D-ribofuranosyl-1*H*-benzimidazole; Vitamin B_{12a}

Cobinamide dihydroxide dihydrogen phosphate (ester), mono(inner salt), 3′-ester with 5,6-dimethyl-1-α-D-ribofuranosylbenzimidazole [13422-51-0] $C_{62}H_{89}CoN_{13}O_{15}P$ (1346.37); an analog of *Cyanocobalamin* in which a hydroxyl radical has replaced the cyano radical.

Preparation—Cyanocobalamin in solution is hydrogenated at room temperature with the aid of Raney nickel. The solution then is exposed to air and diluted with acetone. Oxidation takes place, and upon standing, the hydroxocobalamin crystallizes.

Description—Dark red crystals or red crystalline powder; odorless or has no more than a slight acetone odor; anhydrous form is very hygroscopic; pH (2 in 100 solution) between 8 and 10.

Solubility—1 g in 50 mL water, 100 mL alcohol, 10,000 mL chloroform, or 10,000 mL ether. It is preferable to make aqueous solutions in acetate buffer at a pH between 3.5 and 4.5 in which 1 g dissolves in about 100 mL water.

Comments—See *Cyanocobalamin*.

FOLIC ACID

L-Glutamic acid, *N*-[4-[[(2-amino-1,4-dihydro-4-oxo-6-pteridinyl)-methyl]amino]benzoyl]-, PGA; Folacin; Pteroylglutamic Acid; Folvite

N-[*p*-[[(2-Amino-4-hydroxy-6-pteridinyl)methyl]amino]benzoyl]-L-glutamic acid [59-30-3] $C_{19}H_{19}N_7O_6$ (441.40). See page 1807.

Preparation—Commercial syntheses use different processes. In one of these, 2,3-dibromopropionaldehyde, dissolved in a water-miscible organic solvent (alcohol, dioxane), is added to a solution of equal molecular quantities of 2,4,5-triamino-6-hydroxypyrimidine and *p*-aminobenzoylglutamic acid, maintaining a pH of about 4 by the controlled action of alkali as the reaction progresses. The scheme of the reaction is analogous to that described for *Methotrexate* (1498), the only difference being the starting pyrimidine compound.

Description—Yellow or yellowish orange, odorless, crystalline powder.

Solubility—Very slightly soluble in water; insoluble in alcohol, chloroform, or ether; readily dissolves in dilute solutions of alkali hydroxides or carbonates and is soluble in hot diluted hydrochloric or sulfuric acid, forming very pale yellow solutions.

Comments—The only valid therapeutic use is in the treatment of a deficiency of the vitamin or prophylactically in instances in which the folacin requirement is increased, as in the third trimester of pregnancy. *Megaloblastic anemias* in which folic acid deficiency occurs may result from malabsorption syndromes, such as *sprue, idiopathic steatorrhea, celiac disease, intestinal reticulosis, regional jejunitis, jejunal diverticulosis, blind loop syndrome, and gastroenterostomy* and from antacid use in the elderly. Megaloblastic anemia of infancy is generally the result of generalized malnutrition, as is nutritional megaloblastic anemia. In all of the above-named megaloblastic anemias vitamin B_{12} deficiency often coexists, and folic acid, alone, may be inadequate. Pernicious anemia should be ruled out, lest the vitamin mask the disease (see below). In the megaloblastic anemias of deficiency, a low serum folic acid level will obtain. However, in megaloblastic anemias consequent to treatment with pyrimethamine, phenytoin and related substances, or methotrexate, the serum folic acid levels may be normal; the signs of deficiency result from the antimetabolite effects of the drugs, and they may be overcome competitively by increasing its intake. It is not effective in the treatment of aplastic anemia, leukemia, anemias of infection and nephritis, and general reduction in bone marrow activity of unknown origin.

The vitamin usually is absorbed readily from the GI tract and from parenteral sites of administration. The portion of administered folic acid that is excreted in the urine varies directly with the dose; only a small fraction appears in the urine following the oral ingestion of 0.1 mg, but up to 90% may be excreted by the kidney when a single dose of 15 mg is ingested. The fate of the unrecovered vitamin is unknown. The indications for parenteral use are rare. A solution in water for injection, prepared with the aid of sodium hydroxide or sodium carbonate, is the preferred form for injection.

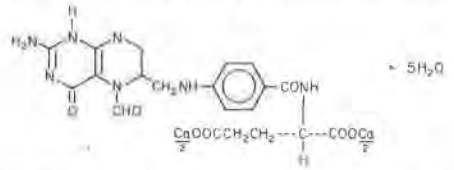
It is capable of bringing about an incomplete and temporary hematopoietic response in pernicious anemia, which may cause the physician to overlook the basic disorder. But it does not affect the progressive neurological lesions of the disease, which may appear explosively and in an irreversible stage. Doses that will correct a deficiency but do not generally cause a remission in pernicious anemia are on the order of 0.1 to 0.4 mg.

Infants fed on a goat milk formula should have a 50 μg a day supplement of folic acid.

For additional information concerning folic acid see the general statement on *Folic Acid*.

LEUCOVORIN CALCIUM

L-Glutamic acid, *N*-[[(2-amino-5-formyl-1,4,5,6,7,8-hexahydro-4-oxo-6-pteridinyl)methyl]amino]benzoyl]-, calcium salt (1:1), pentahydrate; Folmic Acid; Citrovorum Factor



Calcium *N*-[*p*-[[(2-amino-5-formyl-5,6,7,8-tetrahydro-4-hydroxy-6-pteridinyl)methyl]amino]benzoyl]-L-glutamate (1:1) pentahydrate [6035-45-6] $C_{20}H_{21}CaN_7O_7 \cdot 5H_2O$ (601.58); *anhydrous* [1492-18-8] (511.51).

Preparation—Folic acid simultaneously is hydrogenated and formylated in 90 to 100% formic acid under the influence of platinum oxide catalyst at low temperature and atmospheric pressure to yield leucovorin. Conversion to the calcium salt may be accomplished by dissolving the leucovorin in NaOH solution, treating with $CaCl_2$, and precipitating with ethanol.

Description—Yellowish white or yellow, odorless powder; pK_a 3.8, 4.8, and 10.4.

Solubility—Very soluble in water; practically insoluble in alcohol.

Comments—Leucovorin is folinic acid (see *Folic Acid*, page 1807). The calcium salt is a convenient pharmaceutical form that is preferred for intramuscular injection. Consequently, its uses and limitations in the *treatment of the megaloblastic anemias* are the same as those for folic acid. However, it is superior to folic acid in *counteracting the excessive effects of the folic acid antagonists (methotrexate*, see page 1498), since the antagonists competitively antagonize the conversion of folic acid to leucovorin and not the leucovorin itself and also since leucovorin is an excellent competitor for the inward transport system.

NIACIN

3-Pyridinecarboxylic acid; Nicotinic Acid

Nicotinic acid [59-67-6] $C_6H_5NO_2$ (123.11). See page 1808.

Preparation—Niacin may be variously prepared, as by oxidation of nicotine with nitric acid or potassium permanganate, by oxidation of quinoline, or synthesis from pyridine.

Description—White crystals or crystalline powder; odorless or with a slight odor; melts at about 235°; pK_a 4.85.

Solubility—1 g in about 60 mL water; freely soluble in boiling water, boiling alcohol, or also solutions of alkali hydroxides or carbonates; practically insoluble in ether.

Comments—Chiefly in the treatment of pellagra, a disease common among the poor in subtropical countries because of diet deficiency. It also has been found useful in conjunction with vitamin B_1 and riboflavin in the treatment of nutritional deficiency in chronic alcoholism.

In doses of 20 mg or more in humans, niacin elicits a vasodilator effect that occurs a few minutes after oral ingestion or immediately after intravenous injection and lasts for a few minutes to an hour. Symptoms of flushing, itching, burning, or tingling occur, along with an increased skin temperature and increased motility and gastric secretion. Nicotinyl alcohol also shares this vasodilator property, and at one time both nicotinic acid and the alcohol popularly were used in the treatment of peripheral vascular disease and senility (as a cerebral vasodilator). These uses are obsolete and now are but an annoying side effect of large doses. The vasodilator effect of the oral drug is lessened if it is given with a meal.

Larger doses lower blood cholesterol, phospholipids, triglycerides, and free fatty acids, and the drug is used in the treatment of hypercholesterolemia, mostly in combination with cholestyramine, colestipol, or clofibrate. Nicotinamide does not possess the hypolipemic or the vasodilator property.

Large doses, especially those over 3 g a day, cause abnormalities in liver function, including jaundice.

Niacin is absorbed well orally, and the oral and parenteral doses are the same. With large doses, a considerable amount is excreted into the urine, so it is advisable to give several small doses during the day rather than one large one.

For additional information see the general statement on *Niacin*.

NIACINAMIDE

3-Pyridinecarboxamide; Nicotinamide; Nicotinic Acid Amide

Nicotinamide [98-92-0] $C_6H_6N_2O$ (122.13). See page 1808.

Preparation—From niacin by various methods, as by reaction with thionyl chloride followed by treatment with ammonia, or by interaction of ammonia gas with molten niacin.

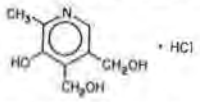
Description—White, crystalline powder; odorless or nearly so, and with a bitter taste; solutions are neutral to litmus paper; melts between 128 and 131°.

Solubility—1 g in 1.5 mL water, 5.5 mL alcohol, or 10 mL glycerin.

Comments—See page 1808 and *Niacin*. This drug lacks the vasodilator, GI, hepatic, and hypolipemic actions of niacin. Consequently, it is preferred to niacin in the treatment of deficiency.

PYRIDOXINE HYDROCHLORIDE

3,4-Pyridinedimethanol, 5-hydroxy-6-methyl-, hydrochloride; Vitamin B_6 Hydrochloride



Pyridoxol hydrochloride [58-56-0] $C_8H_{11}NO_3 \cdot HCl$ (205.64).

Preparation—Several processes are available. One may be viewed as a cyclizing dehydration of ethyl glycinate (I), ethyl pyruvate (II), and 1,4-diethoxy-2-butanone (III) followed by saponification and decarboxylation at position 2 and cleavage of the three ethoxy groups with HI or another suitable reagent. Reaction of the base with HCl yields the hydrochloride. US Pats 2,904,551, 3,024,244, and 3,024,245.

Description—Colorless or white crystals or a white, crystalline powder; stable in air and slowly affected by sunlight; solutions are acid to litmus, with a pH of about 3; melting range 202 to 206°, with some decomposition.

Solubility—1 g in 5 mL water or 115 mL alcohol; insoluble in chloroform or ether.

Comments—Deficiency in adults is extremely difficult to induce, and the therapeutic need for this vitamin, alone, in the adult is of rare occurrence. However, it is justified to give it along with other B vitamins when there is evidence of a *multiple B-vitamin deficiency*. It may be used prophylactically to prevent, or to treat, peripheral neuritis in *patients treated with isoniazid*. It has been claimed that the vitamin controls the *nausea and vomiting of pregnancy* or of *radiation sickness*, but unequivocal proof has never been presented. In infants with *convulsive seizures due to pyridoxine dependency*, administration of the vitamin promptly corrects the condition (see the general statement on *Pyridoxine*). It has been claimed to be medically effective in treating the carpal-tunnel syndrome; however, more data are required to substantiate this claim. Extremely high doses (600 to 3000 mg per day) have been administered to schizophrenics, autistic children, and children exhibiting hyperkinesis. However, clear evidence of benefit has not been established. Caution needs to be exercised with these levels of administration because of reports of severe sensory-nervous-system dysfunction after daily consumption of 2 to 5 g. It may be effective in correcting hypochromic or megaloblastic anemia in patients with adequate levels of iron who have not responded to other hematopoietic agents. Since it antagonizes levodopa, patients with Parkinson's disease treated with the latter drug should not take multivitamin supplements containing pyridoxine (see *Levodopa*, page 1341).

RIBOFLAVIN

Lactoflavin; Vitamin B_2

Riboflavine [83-88-5] $C_{17}H_{20}N_4O_6$ (376.37). See page 1810.

Preparation—Mostly by synthesis. In one method, 1-(6-amino-3,4-xylidino)-1-deoxy-D-ribitol (I) is condensed with alloxan (II) in acetic acid with boric acid as a catalyst. Among other ways, I may be prepared by condensing D-ribitol with 4,5-dimethylphenylenediamine. US Pat 2,807,611.

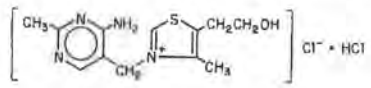
Description—Yellow to orange-yellow, crystalline powder with a slight odor; melts at about 280°; saturated solution is neutral to litmus; when dry not appreciably affected by diffused light, but when in solution, light induces quite rapid deterioration, especially in the presence of alkalies.

Solubility—Very slightly soluble in water, alcohol, or isotonic sodium chloride solution; very soluble in dilute solutions of alkalies; insoluble in ether or chloroform.

Comments—To treat ariboflavinosis (riboflavin deficiency) and also to supplement other B vitamins in the treatment of pellagra and beriberi (see the general statement on *Riboflavin*).

THIAMINE HYDROCHLORIDE

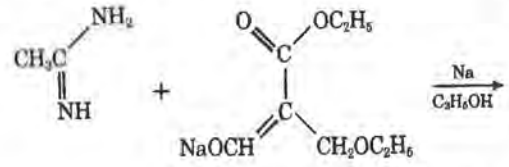
Thiazolium, 3-(4-amino-2-methyl-5-pyrimidinyl)methyl-5-(2-hydroxyethyl)-4-methyl-, chloride, monohydrochloride; Vitamin B_1 Hydrochloride; Aneurine Hydrochloride



[67-03-8] $C_{12}H_{17}ClN_4OS \cdot HCl$ (337.27).

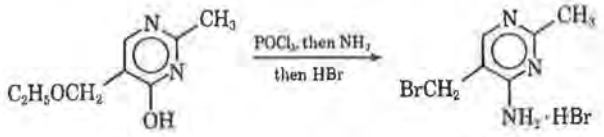
Preparation—This vitamin consists of two ring systems, a pyrimidine portion and a thiazole portion joined by a methylene bridge. The *pyrimidine* may be prepared by several processes, one of which is as follows: Ethyl acrylate [CH_2=$CHCOOC_2H_5$] is heated with ethyl alcohol, forming β-ethoxypropionic ester [$C_2H_5OCH_2CH_2COOC_2H_5$], which is condensed in the presence of sodium metal with formic acid to form ethyl sodioformyl-β-ethoxypropionate I. This then is condensed with acetamidine, yielding 2-methyl-5-ethoxymethyl-5-hydroxypyrimidine, II. This compound is treated with phosphorus oxychloride, thereby replacing the OH on carbon 6 with Cl, and by reacting the resulting chloro derivative with ammonia, the Cl is replaced by NH_2. Finally, on treating the latter product with HBr, 2-methyl-5-bromomethyl-6-aminopyrimidine hydrobromide, III, is produced.

The *thiazole* portion of the thiamine molecule may be built up in the following matter: Ethyl acetoacetate IV is treated with ethylene oxide [C_2H_4O] and the resulting acetyl-butyryl lactone, when reacted with sulfuryl chloride, yields chloroacetyl butyrolactone. This compound is decarboxylated when heated with HCl, splitting off CO_2 and forming 3-chloro-5-hydroxy-2-pentanone, V. The latter, when condensed with thioformamide yields the thiazole, 4-methyl-5-hydroxyethylthiazole, VI.

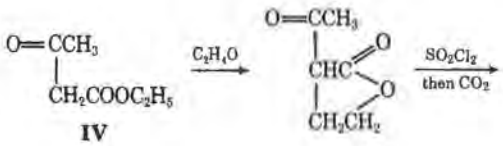


Acetamidine — Enol form of Ethyl Sodioformyl-β-ethoxypropionate

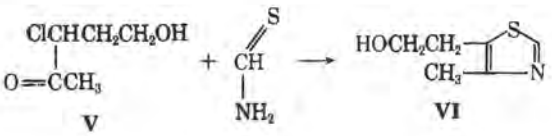
I



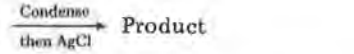
II — III



IV



V — VI



The final step in this process is the combination of the pyrimidine and the thiazole to form a thiazolium halide. Since this is a simple addition of an alkyl halide, the (bromomethyl) pyrimidine, to a tertiary amine, the thiazole, it is readily effected by bringing the two compo-

nents together in a suitable solvent. The vitamin-bromohydrobromide so obtained is transformed into the corresponding chlorine compound, thiamine, with freshly precipitated silver chloride. The silver combines with the bromine to form the less soluble silver bromide, and the chloride from the silver chloride replaces the bromine.

Description—Small white crystals or a crystalline powder usually with a slight, characteristic odor; when exposed to air, the anhydrous product rapidly absorbs about 4% water; solutions are acid to litmus paper; pH (1 in 100 solution) between 2.7 and 3.4; melts, with some decomposition, at about 248°.

Solubility—1 g in about 1 mL water or about 170 mL alcohol; soluble in glycerin; insoluble in ether or benzene.

Incompatibilities—In the dry state, it is stable. Acidic solutions having a pH below 5.5, preferably from 5 to 3.5, are also relatively stable. *Alkalies* destroy it. It is precipitated from solution by several of the *alkaloidal reagents* such as *mercuric chloride, iodine, picric acid, tannin,* and *Mayer's reagent.* It is sensitive to both *oxidizing* and *reducing agents.*

Elixirs of thiamine hydrochloride are necessarily acid in reaction and are, therefore, incompatible with any acid-neutralizing substance. *Phenobarbital sodium* has been an occasional offender in this respect, the result frequently being such as to cause precipitation of the phenobarbital as well as a partial lowering of the acidity of the mixture, with consequent deterioration of the vitamin. Phenobarbital, not the sodium derivative, may be dispensed in such an instance, provided that sufficient alcohol is present to keep it in solution. If a part of the elixir is replaced with alcohol for this purpose, an amount of thiamine hydrochloride equivalent to that contained in the volume so replaced must be added to the product.

Comments—To treat *beriberi* and also *general B-vitamin deficiency.* The fact that it cures the neuropathologies of beriberi has given rise to a widespread use of the vitamin in nearly any type of neuropathology. Although such indiscriminate use can do no organic harm to the patient, it constitutes an unnecessary expense; the promotion of the vitamin for such promiscuous use constitutes an abuse. For additional information see the general statement on *Thiamine.*

OTHER WATER-SOLUBLE VITAMIN PREPARATIONS

Carnitine [L-(3-Carboxy-2-hydroxypropyl)trimethylammonium hydroxide inner salt; [461-06-3] Vitamin B_T; $C_7H_{15}NO_3$ (161.20); Carnitor]—*Preparation:* See Wolf G, ed. *Monograph: Recent Research on Carnitine,* Cambridge MA: MIT Press, 1965. It may be isolated from meat extracts or prepared synthetically. *Description and Solubility:* White, very hygroscopic solid melting at about 197°. Readily soluble in water or hot alcohol; practically insoluble in most organic solvents. *Comments:* Required in mammalian energy metabolism and has been shown to facilitate long-chain fatty acid entry into cellular mitochondria, therefore providing the substrate for β-oxidation and subsequent production of energy. It is synthesized in the liver from lysine. Deficiency may occur from impaired hepatic synthesis or transport from liver to muscle. Carnitine deficiency may lead to elevated triglyceride and free fatty acid concentrations, diminished ketogenesis, and lipid infiltration of muscle and liver.

Choline Bitartrate [(2-Hydroxyethyl)trimethylammonium Bitartrate; $C_9H_{19}NO_7$ (253.25)]—*Preparation:* See *Choline Chloride,* below. *Description and Solubility:* A white, hygroscopic, crystalline powder with an acidic taste; odorless or may have a faint trimethylamine-like odor. Freely soluble in water, slightly soluble in alcohol, and insoluble in benzene, chloroform, or ether. *Comments:* As a nutrient or dietary supplement.

Choline Chloride [(2-Hydroxyethyl)trimethylammonium chloride; [67-48-1 $C_5H_{14}ClNO$ (139.62)]—*Preparation:* For the preparation of choline, see *Choline Dihydrogen Citrate. Description and Solubility:* White, deliquescent crystals; a 10% aqueous solution has a pH of about 4.7. Very soluble in water or alcohol. *Comments:* For the metabolic effects of *Choline,* see page 1806. The salt is used to reduce fatty infiltration of the liver and thus supposedly to prevent degeneration and cirrhosis. Such infiltration may occur after exposure to certain chemical intoxicants, such as carbon tetrachloride, chloroform, and various other halogenated hydrocarbons (including several general anesthetics), divinyl ether, etc. Moderate-to-severe ethanol intoxication and habitual ingestion of ethanol also predispose to fatty infiltration of the liver. Patients who are acutely ill and cannot eat or persons on a high-fat diet frequently develop fatty livers, for which this vitamin may be given. In none of these conditions has there been clearly demonstrable efficacy. Furthermore, a high-protein diet, especially one that includes eggs, meat, liver and milk, not only provides some of this vitamin but also methionine, which promotes the endogenous synthesis of *Choline* (see page 1806). Once cirrhosis occurs, it is probably too late for any possible benefits. There is no evidence that it is helpful in infectious hepatitis. For the above reasons, there is no longer any official preparation of it. Since the anion is irrelevant to the metabolic effects, the chloride is neither superior nor inferior to other salts.

Choline Dihydrogen Citrate [(2-Hydroxyethyl)trimethylammonium Dihydrogen Citrate; $C_{11}H_{21}NO_8$ (295.29)]—*Preparation:* By treating aqueous trimethylamine with ethylene oxide. Conversion to the dihydrogen citrate is conveniently effected by dissolving the base in a suitable solvent such as ethanol and treating with an equimolar portion of citric acid. *Description and Solubility:* Colorless, translucent crystals, or a white, granular to fine, crystalline powder; odorless or may have a faint trimethylamine odor and has an acidic taste; hygroscopic when exposed to air; melts between 103 and 107.5°; 1 g dissolves in 1 mL water or 42 mL alcohol; very slightly soluble in ether, chloroform, or benzene. *Comments:* See *Choline Chloride,* above.

Sodium Folate [Monosodium Folate [6484-89-5] $C_{19}H_{18}N_7NaO_6$ (463.38); Folvite Sodium]—For the structure of the acid, see page 1807. *Preparation:* Folic Acid is reacted with $NaHCO_3$. *Description and Solubility:* Clear, mobile liquid with a yellow or orange-yellow color; pH between 8.5 and 11. *Comments:* Has the actions of *Folic Acid* (page 1807); however, the salt is preferred for parenteral use.

Thiamine Mononitrate [Thiazolium, 3-[(4-amino-2-methyl-5-pyrimidinyl)methyl]-5-(2-hydroxyethyl)-4-methyl-, nitrate (salt); thiamine Nitrate; Vitamin B_1 Mononitrate; thiamine nitrate [532-43-4] $C_{12}H_{17}N_5O_4S$ (327.36)]—*Preparation:* In one method thiamine hydrochloride is reacted with sufficient NaOH to remove the HCl and replace the chloride ion by OH, and the resulting thiamine hydroxide is neutralized with nitric acid. *Description:* White crystals or crystalline powder, usually with a slight, characteristic odor; pH (1 in 50 solution) 6 to 7.5. *Solubility:* 1 g in about 44 mL water; slightly soluble in alcohol or chloroform. *Comments:* More stable than the hydrochloride; solutions of the nitrate are practically neutral, while those of the hydrochloride are acid. Its vitaminergic actions and uses are identical to those of the hydrochloride. See *Thiamine Hydrochloride.*

MULTIVITAMIN PREPARATIONS

In the preceding text and in various monographs, attention was called in several instances to the fact that it is desirable at times to administer more than one vitamin for what appear to be the symptoms of a single deficiency. The quotation "In the shadow of pellagra walks beriberi" has considerable substance in fact. Diets deficient in niacin are frequently also deficient in thiamine and certain other B vitamins of similar dietary source. The same relationship holds frequently for folacin and vitamin B_{12}. Malabsorption syndromes affect the assimilation of several vitamins. Furthermore, the repair of a deficiency of one vitamin may increase the requirement for another; for example, repletion of thiamine increases the need for riboflavin. Diseases in which there is increased metabolism, such as thyrotoxicosis, increase the need for more of the vitamins, as do periods of hard physical work, stress, pregnancy, and lactation. Therefore, multivitamin therapy is often rational. Multivitamin therapy also is recommended for individuals, who on restricted diets for weight control or lacks vitality, those who are debilitated, and those working in hazardous environments. Use of multivitamin supplements for infants and preschool children should be done on the advice of a pediatrician.

OTHER NUTRIENTS

AMINO ACIDS AND PROTEINS

NUTRITIONAL ROLE—Protein hydrolysates, in which proteins have been reduced to short-chain peptides and amino acids, long have been used orally or in relatively dilute solutions intravenously as supplementary nutrients for patients unable to metabolize intact protein adequately. For patients in whom oral or tube feeding is contraindicated or inadequate, good nutrition may be achieved and maintained, for several

months if necessary, by the procedure of intravenous feeding known as *total parenteral nutrition (TPN),* sometimes called *intravenous* or *parenteral hyperalimentation.* Such feeding provides essential nutrients in a sufficiently concentrated form that does not exceed normal daily fluid requirements; this necessitates formulation of markedly hypertonic solutions (2000 mOsm/L and higher). Such solutions must be infused at a constant rate throughout the entire day into a large-diameter *central* vein where rapid dilution by high blood-flow minimizes vascular damage and the risk of phlebitis or thrombosis that is likely to occur on injection into a peripheral vein. The infusion route is generally through a surgically placed subclavian catheter into the superior vena cava, but in infants and small children it may be through a catheter in the jugular vein.

The most critical component in TPN is a nitrogen source available for repletion and/or maintenance of lean body mass and proteins essential for wound healing, tissue repair, and growth. Protein hydrolysate injections, sometimes supplemented with amino acids, are used as nitrogen sources, but in most hospitals solutions of mixed crystalline L-amino acids have replaced the former. Crystalline L-amino acids appear to be more efficiently metabolized and better tolerated in the body than are the peptides of protein hydrolysates. Also, individual acids may be readily and reproducibly formulated to meet specific requirements of patients, such as those with renal failure and infants who are premature.

So that amino acids may be used for protein synthesis and to achieve positive nitrogen balance and weight gain in debilitated patients it is necessary to provide the equivalent of at least 150 nonprotein calories per gram of nitrogen administered. When relying on TPN it is preferable to use intravenous fat emulsion to provide energy needs (see *Fats and Oils,* below).

A 10% soybean oil emulsion (Intralipid), developed and used in Europe since 1961, has an osmolarity of 280 mOsm/L (essentially isotonic with blood) and can be administered through peripheral veins. The fat particles of this egg-yolk phospholipid emulsion are less than 0.5 μm in diameter, similar in size to naturally occurring chylomicrons. The emulsion is a useful source of calories and also will prevent and correct essential fatty acid deficiencies that may develop during long-term parenteral nutrition using nonlipid calorie sources.

If an intravenous emulsion is not used, large amounts of dextrose are required to achieve caloric balance and to avoid the fluid overload that would result from use of weaker solutions; markedly hypertonic concentrations of dextrose (25%—five times the isotonic concentration—or higher) must be supplied. As solutions so concentrated are prone to produce thrombosis when injected into a peripheral vein, they must be infused into a central vein, as described above.

In addition to dextrose and amino acids, TPN solutions may contain vitamins and electrolytes (often added to meet individual patient requirements). Various solutions for TPN use are commercially available, as are kits that include, for example, a 1-L bottle containing 500 mL of 50% dextrose solution under vacuum, a 500-mL bottle of 8.5% solution of a crystalline amino acid mixture composed of 8 essential and 7 nonessential amino acids in biologically usable proportion (FreAmine III), and a transfer set and additive cap for aseptic preparation of the final solution.

The composition of FreAmine III, in g/100 mL is *Essential Amino Acids:* L-isoleucine 0.59; L-leucine 0.77; L-lysine acetate 0.87; L-methionine 0.45; L-phenylalanine 0.48; L-threonine 0.34; L-tryptophan 0.13; L-valine 0.56. *Nonessential Amino Acids:* L-alanine 0.60; L-arginine 0.81; L-histidine 0.24; L-proline 0.95; L-serine 0.50; aminoacetic acid 1.19; L-cysteine HCl <0.02. The calculated osmolarity of the solution is approximately 850 mOsm/L. Aminosyn (*Abbott*), a preparation of crystalline amino acids containing a somewhat different proportion of the same essential acids and, with the exception of L-tyrosine replacing L-cysteine, the same nonessential amino acids, is supplied in concentrations of 5%, 7%, and 10% of the total acids, with calculated osmolarities of approximately 500, 700, and 1000 mOsm/L, respectively.

TPN solutions, which often require extemporaneous addition of compatible vitamins and/or electrolytes to solutions such as described above, should be prepared by a pharmacist experienced in parenterals production, using aseptic techniques performed under a laminar-flow, filtered-air hood (see Chapter 42).

In recent years certain free amino acids have been prescribed for a variety of medical conditions for which neither drug nor food approval have been obtained. Regulations on the food-additive use are limited to providing protein requirements. Therefore, these uses of single amino acids are without approved status. Consumption of high levels of single amino acids has been associated with severe metabolic and medical consequences.

CHEMISTRY—The USP has provided monographs of standards and tests for each of the crystalline amino acids used in amino acid dosage forms. For comparative purposes the formulas and chemical names of the L-amino acids are given in Chapter 26 and other chemical data are provided in Table 106-4.

Each of the amino acids is synthesized readily, by a variety of methods, but always as a DL-mixture. While resolution to obtain the L-form can in some cases be conveniently accomplished, often it is easier and more economical to isolate individual acids from the mixed amino acids obtained by hydrolysis of selected proteins. Chromatographic fractionation of amino acids in such hydrolysates has generally replaced the tedious fractional precipitation and derivative distillation methods formerly employed.

The articles that follow describe certain amino acids that are used for certain nonnutritional purposes as well as components of nutritional formulations; also included are brief articles on *Protein Hydrolysate Injection* and *Oral Protein Hydrolysates.*

ARGININE HYDROCHLORIDE

R-Gene 10

L-Arginine monohydrochloride [1119-34-2] $C_6H_{14}N_4O_2 \cdot HCl$ (210.66). For the structural formula of arginine, see Chapter 26.

Preparation—Arginine is present in the hydrolysis products of many proteins; for a method of separating it from gelatin hydrolysate see *J Biol Chem* 1940; 132: 325. It is converted to the hydrochloride by reaction with HCl.

Description—White crystals or crystalline powder; practically odorless.

Solubility—Soluble in water; slightly soluble in hot alcohol.

Comments—Arginine has been variously used in clinical practice. Intravenous administration in the symptomatic management of severe encephalopathies associated with ammoniacal azotemia, on the theory that arginine combines with ammonia to form asparagine, has not been of value in significantly reducing blood ammonia levels or in improving the clinical status of patients, and use of the amino acid for this purpose is no longer approved by the FDA. Oral administration to patients with cystic fibrosis to correct malabsorption and steatorrhea and by inhalation as a mucolytic have not been effective. It is used as a nutritional supplement in conditions in which its dibasic amino character or possible blood ammonia–reducing power is useful.

It stimulates pituitary release of growth hormone and prolactin and pancreatic release of glucagon and insulin, and arginine hydrochloride is used diagnostically to evaluate pituitary growth hormone reserve and detect deficiency of the hormone in various conditions. It is administered by intravenous infusion, and blood samples are taken at 30-min intervals after beginning infusion, for 2.5 hr; the plasma growth hormone levels in these samples and in others taken 30 min before and at the start of infusion are determined and diagnostically evaluated.

GLYCINE

Aminoacetic Acid; Glycocoll

NH_2CH_2COOH [56-40-6] $C_2H_5NO_2$ (75.07).

Preparation—Aminoacetic acid is a constituent of many proteins. It may be synthesized by many processes; industrially it is prepared by interaction of ammonia with chloroacetic acid.

Description—White, odorless, crystalline powder, with a sweetish taste; solution is acid to litmus; pK_a 9.78.

Solubility—1 g in 4 mL water or 1254 mL alcohol; very slightly soluble in ether.

Table 106-4. L-Amino Acids

AMINO ACID[a]	MOLECULAR FORMULA	MOLECULAR WEIGHT	SOLUBILITY IN WATER	pK VALUES
L-Alanine 56-41-7	$C_3H_7NO_2$	89.09	1 g in 6 mL	pK_1 3.34 pK_2 8.17
L-Arginine 74-79-3	$C_6H_{14}N_4O_2$	174.20	1 g in 5 mL	pK_1 2.18 pK_2 9.09 pK_3 13.2
L-Aspartic acid 56-84-8	$C_4H_7NO_4$	133.10	1 g in 200 mL	pK_1 1.88 pK_2 3.65 pK_3 9.60
L-Cysteine 52-90-4	$C_3H_7NO_2S$	121.16	Freely soluble	pK_1 1.71 pK_2 8.33 pK_3 10.78
L-Cystine 56-89-3	$C_6H_{12}N_2O_4S_2$	240.30	1 g in 9000 mL	pK_1 1 pK_2 2.1 pK_3 8.02 pK_4 8.71
L-Glutamic acid 56-86-0	$C_5H_9NO_4$	147.13	1 g in 115 mL	pK_1 2.19 pK_2 4.25 pK_3 9.67
L-Histidine 71-00-1	$C_6H_9N_3O_2$	155.16	1 g in 24 mL	pK_1 1.78 pK_2 5.97 pK_3 8.97
L-Hydroxyproline	$C_5H_9NO_3$	131.13	1 g in 3 mL (α-form)	pK_1 1.82 pK_2 9.65
L-Isoleucine[b] 73-32-5	$C_6H_{13}NO_2$	131.17	1 g in 25 mL	pK_1 2.36 pK_2 9.68
L-Leucine[b] 61-90-5	$C_6H_{13}NO_2$	131.17	1 g in 42 mL	K_a 2.5×10^{-10} K_b 2.3×10^{-2}
L-Lysine[b] 56-87-1	$C_6H_{14}N_2O_2$	146.19	Freely soluble	pK_1 2.20 pK_2 8.90 pK_3 10.28
L-Methionine[b] 63-68-3	$C_5H_{11}NO_2S$	149.21	Soluble	pK_1 2.12 pK_2 9.28
L-Phenylalanine[b] 63-91-2	$C_9H_{11}NO_2$	165.19	1 g in 34 mL	pK_1 2.16 pK_2 9.18
L-Proline 147-85-3	$C_5H_9NO_2$	115.13	1 g in 0.7 mL	pK_1 1.99 pK_2 10.60
L-Serine 56-45-1	$C_3H_7NO_3$	105.09	1 g in 20 mL	pK_1 2.19 pK_2 9.21
L-Taurine 107-35-7	$C_2H_7NO_3S$	125.14	1 g in 16 mL	pK_1 1.50 pK_2 8.74
L-Threonine[b] 72-19-5	$C_4H_9NO_3$	119.12	Freely soluble	pK_1 2.15 pK_2 9.12
L-Tryptophan[b] 73-22-3	$C_{11}H_{12}N_2O_2$	204.22	1 g in 88 mL	pK_1 2.38 pK_2 9.39
L-Tyrosine 60-18-4	$C_9H_{11}NO_3$	181.19	1 g in 2200 mL	pK_1 2.20 pK_1 9.11 pK_2 10.07
L-Valine[b] 72-18-4	$C_5H_{11}NO_2$	117.15	1 g in 12 mL	pK_1 2.32 pK_2 9.62

[a] The number below the name of each amino acid is its *Chemical Abstracts Service* (CAS) Registry Number. For structures and nomenclature see Chapter 26.
[b] Essential amino acids.

Comments—As an irrigating fluid in transurethral resection of the prostate. The acid also is used in an antacid preparation, as a complex salt. However, its limited buffering capacity does not warrant the expense of such a preparation. It is used primarily in admixture with other amino acids in TPN formulations.

SUGARS

Sugars are carbohydrates that are sweet to the taste and highly soluble in water. They may be either monosaccharides or disaccharides. The chemistry of the sugars is discussed in Chapter 26. In the section below are listed only those sugars that are used in medicine as aliments. Some of the sugars also have important uses as pharmaceutical necessities, in parenteral fluids, as diuretics, as osmotic *stuffing* for injection of other drugs, etc; consequently, the monographs of certain nutrient sugars may be found elsewhere in this volume.

DEXTROSE—page 1043.

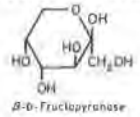
DEXTROSE INJECTION—page 1248.

DEXTROSE AND SODIUM CHLORIDE INJECTION—page 1249.

FRUCTOSE

D(−)-Fructose; Levulose

D(−)-Fructose; Levulose



β-D-Fructopyranose

D-Fructose [57-48-7] $C_6H_{12}O_6$ (180.16); a sugar usually obtained by the inversion of aqueous solutions of sucrose and subsequent separation of fructose from glucose.

Preparation—Sucrose is inverted by treatment with dilute acid at moderate temperature, and the fructose is separated by precipitation of

the lime-fructose complex. Fructose is released from the complex with carbon dioxide, which precipitates the calcium as carbonate. After filtering, the fructose solution is purified with activated carbon and ion-exchange resins and evaporated to dryness.

Description—Colorless crystals or a white, crystalline or granular powder, which is odorless and has a sweet taste; specific rotation, −89 to −91°.

Solubility—1 g in about 15 mL alcohol or about 14 mL methanol; freely soluble in water.

Comments—A ketohexose used parenterally as a carbohydrate nutrient. It is converted to liver glycogen and metabolized more rapidly than dextrose, without requiring insulin, and thus may be used in diabetic patients. It is indicated in patients requiring fluid replacement and caloric feeding but contraindicated in hypoglycemia, for which dextrose should be used. It also is contraindicated in patients with hereditary fructose intolerance.

LACTOSE—page 1044.

LIQUID GLUCOSE—page 1044.

SUCROSE—page 1025.

SYRUP—page 1027.

OTHER SUGARS

Invert Sugar

[8013-17-0]—An equimolar mixture of glucose and fructose, produced by hydrolysis of sucrose. Forms clear, colorless solutions with a pH of 3.5 to 6. *Comments:* Instead of dextrose, for parenteral administration of carbohydrate. While it has the same caloric value as dextrose (4 kcal/g), invert sugar is utilized more rapidly and may be administered intravenously twice as fast as dextrose.

FATS AND OILS

The role of fat in the nutritional physiology of humans is both complex and contradictory. The unique and essential part it plays in metabolic processes and in the palatability of food points out its importance. Stored fat (adipose tissue) as well as dietary fat are concentrated sources of energy that the body can use efficiently for physical activity and in times of physical stress. Fat when oxidized to carbon dioxide and water yields 9 kcal/g, whereas protein and carbohydrates both yield approximately 4 kcal/g. Energy from food, consumed in excess of metabolic needs is stored in the body as fat and represents the major body reserve of energy during periods of low caloric intake. Certain components of fat, called polyunsaturated fatty acids, are essential dietary components for tissue biosynthesis of prostaglandins, which perform vital hormone-like activities in the transmission of genetic information in all cells. Food fats are carriers, to varying degrees, of fat-soluble vitamins (A, D, E, and K). Also, a diet too restricted in fats lacks flavor and satiety value.

That fats also are involved or indicated in such significant pathologies as obesity and atherosclerosis or the syndrome called coronary heart disease (CHD) is well known. Epidemiological, experimental, and clinical investigations have identified a number of *risk factors* associated with susceptibility to CHD that may be controlled. These include an elevation in plasma lipids, especially plasma cholesterol, high blood pressure (hypertension), heavy cigarette smoking, obesity, and physical inactivity. Persons falling into *risk categories* on the basis of their plasma lipid levels can be made aware of this during a physician's examination and appropriate professional dietary advice then can be followed. For such persons it is important, in addition to maintaining a desirable body weight, to decrease substantially the intake of total fat and saturated fat and to lower cholesterol consumption. Recent studies not yet conclusive indicate that so-called ω-3-fatty acids contained in oils obtained from fish harvested in cold-water regions and also found in lesser amounts in soybeans and rapeseed oils may have beneficial effects in lowering plasma low-density lipoprotein (and cholesterol) triglycerides and lowering the tendency for platelet aggregation.

There are many abnormal conditions in which faulty digestion and absorption of fat occur and excessive amounts of fat are present in the feces. When these conditions exist, there is fecal fat loss, poor absorption of other nutrients, and diarrhea. As a result, there may be substantial weight loss and general malnutrition.

In recent years, it has been shown that the digestion and absorption of short- and medium-chain triglycerides (MCTs) are different from those of the long-chain triglycerides that are characteristic of most food fat. The hydrolysis and absorption of MCTs are faster than those of long-chain triglycerides, and it is possible for MCTs to be absorbed directly into the intestinal mucosa without first being hydrolyzed, making it possible to absorb MCTs in the absence of pancreatic juice and bile. Coconut oil contains more medium-chain fatty acids than other fats and oils and is used as a source for fractionation and preparation of MCTs. MCTs are commercially available as relatively pure 8-carbon or 10-carbon triglycerides and as a 4:1 mixture.

MCTs have been found to be useful in conjunction with the usual therapy in the treatment of such diseases as pancreatic insufficiency, cancer of the pancreas, cystic fibrosis of the pancreas, obstruction of the bile duct, certain abnormalities in the lymphatic system, regional enteritis, and postoperative cases involving the removal of much of the stomach or small intestine. The most consistent beneficial effects reported from the use of MCTs are a decrease in the fecal loss of fat and less diarrhea. In recent years fat emulsions have gained wide use in providing the energy needs of critically ill patients, particularly those with severe burns or those who must rely on TPN for long periods of time. These intravenous fat emulsions were developed in the early 1960s, in Europe, and typically contain soybean oil, egg yolk phospholipids, glycerin, and water for injection. The fat particles are less than 0.5 μm in diameter, similar in size to naturally occurring chylomicrons. These emulsions are available in 10 and 20% suspensions and provide the essential fatty acids. The levels of use have been shown to be safe up to 35 to 40% of calorific needs.

OTHER FATS

Intravenous Fat Emulsion [Liposyn; Intralipid]—*Description:* Water emulsions of 10 and 20%; osmolarity, approximately 300 to 350 mOsm/kg of water, 260 to 268 mOsm/kg emulsion; particle size less than 0.5 μm in diameter. *Comments:* As source of calories and essential fatty acids, usually for patients requiring parenteral nutrition for more than 5 days.

CORN OIL—page 1029.

OLIVE OIL—See RPS-18, page 1309.

PEANUT OIL—page 1029.

TRACE ELEMENTS

The trace elements are those inorganic nutrients that are required in small or *trace* amounts, a few micrograms to a few milligrams per day for humans or per kilogram of diet for an experimental animal. The essentiality of several trace elements was established for animals and humans during the 1930s. A resurgence of interest in this area has occurred because of technological advancements in analytical methodology and development of highly purified diets and *clean* environments for experimental animals.

Fourteen elements now are thought to be essential; however, evidence to support required functions in animals and humans is still incomplete for nickel, silicon, tin, and vanadium. It is expected that all 14 of these, and possibly others, will be shown to be required by human beings. There also is evidence that boron may be essential. Some pertinent chemical and biological information on these elements is shown in Table 106-5. Some elements, notably manganese and chromium, can exist in several oxidation states; however, only one or two are compatible with a biological environment and function.

The amount of each element in a normal 70-kg adult man may vary considerably, depending on requirement and whether or not the element can be stored in certain tissues. Daily requirements *(allowances)* have been established for a few of the trace elements (Table 106-2). Ranges of typical daily intakes of the other elements by healthy individuals provide a very rough guide to maximal needs. These values are based on limited data.

Information on trace-element distribution in foods is presented in Table 106-6. This is an attempt to indicate important sources of the elements or the level, particularly if low, in important foods. This table is of rather limited usefulness because it is based on so little information. At present, too little is known about the effect of agricultural practices and manufacturing processes on trace-element content.

Our understanding of trace-element function in humans is less complete than that of vitamins. Study of a deficiency syndrome in animals often precedes recognition of deficiency or metabolic problems in humans, particularly as related to a disease. For this reason, deficiency syndromes in animals are described for each element known to be essential.

Similarly, our knowledge of trace-element toxicity in humans is limited, and we must rely on animal data. Two problems must be considered. One is the effect of long-term supplementation with a *moderate* excess above requirement. For children and adults the FDA regulations on dietary supplements for each of four trace elements permit an excess of 50% above the US RDA (see Table 106-2). It is important to consider not only the amount of a single trace element, but also the balance among all required elements. This area requires periodic review as knowledge increases. The other toxicity problem relates to short-term intake of multiple recommended doses, either accidentally or purposefully. This must be regarded as undesirable, depending on the excess intake level. It is well known to be very serious in the case of infants swallowing capsules containing ferrous sulfate.

Inorganic elements are very different from the various organic nutrients in that they cannot be destroyed or converted into another substance by the metabolic processes in the animal. In most cases the trace elements are bound to an organic ligand. This is the means for effecting elemental transport and function and minimizing toxicity. The binding may be very loose or very firm. Many of the elements are part of metalloenzymes. Nucleic acids also bind metal ions in a consistent pattern; however, the significance of this is not established. Other mechanisms of function are described for individual elements below.

Many pairs or larger groups of essential elements may have chemical properties that are closely similar. This can result in competition for binding sites that may alter transport, storage, excretion, and function.

There are many elements in biological systems that have no known essential function but that have some chemical properties similar to those of required elements. These elements can become a health threat when they are present in sufficient quantity to replace a required element or to bind excessively to some organic ligand and cause a physiological aberration. Modern industrial technology has effected translocation of large quantities of many minerals from their native stores in the ground to the air, the water, and ultimately the food supply. Three elements that have caused concern and some isolated severe problems for humans are mercury, cadmium, and lead. The nutritional status of an exposed person can modify the severity of adverse response to a toxic level of an element. A deficiency of certain nutrients can result in a more severe adverse effect, while a moderate excess of other nutrients can afford some protection. The possibility must be kept in mind that elements now regarded only as toxic may have an essential function at a very low level of intake.

Analysis of trace elements can be accomplished by both chemical and physical techniques. Modern advances such as

Table 106-5. Biological Data for the Essential Trace Elements

ELEMENT	AMOUNT IN 70-kg HUMAN (mg)	DAILY HUMAN INTAKE RANGE[a] (mg)
Chromium	6.6	0.06–0.36
Cobalt	1.1	0.015–0.160
Copper	75–150	0.75–1.2
Fluorine	2600	0.5–1.7[b]
Iodine	10–20	0.3–0.7
Iron	4000–5000	10–17
Manganese	12–20	1.5–3.0
Molybdenum	9.3	0.1–0.2
Nickel	10	0.10–0.15
Selenium	—	0.6–1.0
Silicon	18,000	—
Tin	17	1.5–3.5
Vanadium	10–25	0.01–0.02
Zinc	1400–2300	8–16

[a] Values from FDA total diet study.
[b] Excludes high-fluoride areas.

Table 106-6. Distribution of Essential Trace Elements in Foods[a]

ELEMENT	FOOD SOURCE CONTENT: AVERAGE TO HIGH	FOOD SOURCE CONTENT: LOW
Chromium	Dried brewers' yeast, bran and germ of cereal grains, molasses, liver	Refined cereals, refined sugar
Cobalt	Leafy vegetables	Milk, refined cereals
Copper	Liver, kidney, shellfish, nuts, dry legumes, whole-grain cereals	Milk, muscle meat, eggs, fruit, vegetables
Fluorine[b]	Seafish, red meat, eggs, tea	Milk
Iodine[b]	Seafish, shellfish, iodized salt, milk	
Iron	Liver, kidney, shellfish, muscle meats, poultry, heart, egg yolk, dried legumes, cane molasses, nuts	Milk, refined sugar
Manganese	Whole-grain cereals, dried legumes, tubers, fruits, nonleafy vegetables	Milk, poultry, fish
Molybdenum	Liver, kidney, dried legumes, whole-grain cereals, leafy vegetables	Fruits, root and stem vegetables, muscle meats, milk
Nickel	Whole-grain cereals, vegetables	Muscle meats, fats, eggs, milk
Selenium	Liver, kidney	
Silicon	Whole-grain cereals, chicken skin, beer	Animal foods
Tin[d]	Cereals, muscle meats	Milk
Vanadium[b]	Liver, muscle meats, fish, bread, some cereal grains, nuts, a few root vegetables, oils from corn and soybeans	Milk, most vegetables
Zinc	Meat, egg yolk, whole-grain cereals, oysters, fowl, milk	Fruits, fish, vegetables

[a] Bioavailability is not taken into consideration; see text for individual elements.
[b] Most foods are highly variable.
[c] Selenium content is markedly affected by available selenium during growth of the plant or animal food. Cooking losses can occur.
[d] The tin content is markedly increased by exposure to tin-plated containers.

induction coupled plasma, atomic absorption spectrometry, and neutron activation analysis provide rapid, accurate, and low-cost measurements.

CHROMIUM

Deficiency Syndrome and Function—The principal defect in chromium deficiency is an impairment of glucose utilization; however, disturbances in protein and lipid metabolism also have been observed. In the young animal, growth rate may be reduced. Corneal lesions have been observed in rats deficient in both chromium and protein; no lesions have been seen with either single deficiency.

Impaired glucose utilization occurs in many middle-aged and elderly human beings. In experimental studies, significant numbers of such persons have shown improvement in their glucose utilization after treatment with chromium. There also have been improvements in diabetic children and infants with kwashiorkor.

For biological activity, chromium must be trivalent. The most active form of chromium is that which is incorporated into a low-molecular-weight organic molecule that occurs in many foods. Its structure is not known yet. This compound has been designated GTF (glucose tolerance factor). From a variety of biochemical studies, it appears that the presence of insulin is required for all functions of chromium. GTF is the only one of many compounds tested that passed through the rat placenta into the fetus.

Metabolism and Bioavailability—Chromium is transported by transferrin in the plasma and competes with iron for binding sites. The main excretory route is through the urine; however, some chromium is excreted in the bile and by the small intestine. The newborn animal has large stores of chromium that decline with age.

Toxicity—In animals, a wide margin of safety separates toxicity from the nutritional requirement of chromium (III).

COBALT

Deficiency Syndrome, Function, and Metabolism—The only known essential function of cobalt is as a component of vitamin B_{12} (see page 1812).

Cobalt salts are absorbed poorly. Excretion is via the bile and through the intestinal wall. Cobalt is widely distributed in the body, with the highest concentrations in the liver, kidney, and bone.

Toxicity—High levels of cobalt can produce a polycythemia in many species, an effect that is unrelated to vitamin B_{12}. Cobalt usually is considered relatively nontoxic; however, severe cardiac failure and some deaths in humans have resulted from consumption of large amounts of beer containing 1.2 to 1.5 ppm of cobalt. The element was added to the beer to promote optimal foam stabilization.

COPPER

Deficiency Syndrome and Function—The most common defect observed in copper-deficient animals is anemia. Other abnormalities include growth depression, skeletal defects, demyelination and degeneration of the nervous system, ataxia, defects in pigmentation and structure of hair or wool, reproductive failure, and cardiovascular lesions, including dissecting aneurysms. Copper deficiency occurs very infrequently in human beings. A deficiency has been observed in some South American infants and a few in the United States receiving an artificial formula diet deficient in copper.

Several copper-containing metalloproteins have been isolated from animal tissues, including tyrosinase, ascorbic acid oxidase, laccase, cytochrome oxidase, uricase, monoamine oxidase, δ-aminolevulinic acid dehydrase, and dopamine-β-hydroxylase. Copper functions in the absorption and utilization of iron, electron transport, connective tissue metabolism, phospholipid formation, purine metabolism, and development of the nervous system. Ferroxidase I (ceruloplasmin), a copper-containing enzyme, effects the oxidation of Fe (II) to Fe (III), a required step for mobilization of stored iron. There is evidence that a copper-containing enzyme is responsible for the oxidative deamination of the epsilon amino group of lysine to produce desmosine and isodesmosine, the cross-links of elastin. In copper-deficient animals the arterial elastin is weaker, and dissecting aneurysms may occur.

Metabolism and Bioavailability—Copper is absorbed from the small intestine. Most of the copper in the plasma is in ceruloplasmin; however, significant amounts are loosely bound to albumin, the fraction is important in transport. The plasma copper level increases in acute infections, in pregnancy, and in women taking birth-control pills. Small amounts of copper are excreted in the urine, but the major excretory pathway is via bile and feces.

Copper is present in high concentrations in the brain, liver, heart, and kidney, with the highest levels occurring at birth. It is important that pregnant women receive adequate copper during pregnancy, so that the infant will have adequate stores of copper at birth.

A variety of salts of copper have been found to be available to experimental and domestic animals. These include the sulfate, nitrate, chloride, carbonate, oxide, hydroxide, iodide, glutamate, glycerophosphate, aspartate, citrate, nucleinate, and pyrophosphate. Elemental copper and copper sulfide are utilized poorly. The chemical form of copper in food is largely unknown. The absorption of copper can be decreased by large amounts of phytic acid, ascorbic acid, calcium, and zinc.

Toxicity—Wilson's disease, a genetic disease in humans, leads to excess copper accumulation in the brain, liver, and kidney, which results in mental and neurological abnormalities. The disease is treated by administration of a chelating agent, penicillamine (β,β-dimethylcysteine), which removes excess copper from the tissues and results in its excretion.

FLUORINE

Deficiency Syndrome and Function—The most important relationship of fluoride to health is that of preventing dental caries. Fluoride has been shown to enter the hydroxyapatite of teeth to form a more perfect crystal that resists acid attack more effectively. (See *Sodium Fluoride*, page 1217.) In areas where the fluoride content of the drinking water is unusually high, osteoporosis and calcification of the aorta of elderly persons are less than in control population groups not receiving high fluoride. In these areas the effective fluoride concentration is high enough to cause mottling of the tooth enamel in young children.

Metabolism and Bioavailability—The absorption of fluoride from the GI tract is rapid and complete. Even the water-insoluble forms are absorbed fairly well. Fluoride can cross membranes easily, and it passes readily from the plasma into the tissues; however, the mammary gland and the placenta offer some resistance to transport. Excess fluoride is excreted in the urine.

Bones typically have high concentrations of fluoride, which gradually increase throughout life to about age 55 years. Fluoride supplementation increases bone density but is reported to increase brittleness. Of the soft tissues, the kidney is highest in fluoride. Calcium and aluminum can decrease the absorption of fluoride, and sodium chloride can depress the skeletal uptake of fluoride.

Toxicity—Toxic doses of fluoride cause loss of appetite and body weight, muscular weakness, clonic convulsions, pulmonary congestion, and respiratory and cardiac failure.

Chronic exposure to fluoride most often comes through consumption of drinking water, usually from deep wells drilled through or near fluoride-containing rocks. Levels of fluoride around 2 ppm or higher produce a permanent brownish mottling of tooth enamel when the exposure is during the time of tooth formation.

IODINE

Deficiency Syndrome, Function, and Metabolism—The iodine-deficiency disease is goiter (see *The Thyroid Hormones*, page 1378). In iodine-deficient young, growth is depressed and sexual development is delayed, the skin and hair are typically rough, and the hair becomes thin. Cretinism, feeble-mindedness and deaf-mutism occur in a severe deficiency. There is reproductive failure in the female and decreased fertility in the male.

Goiter has been observed in human beings in many areas of the world, with incidence in women and children usually higher than in the adult male. As a public-health measure, use of iodized salt has markedly reduced the incidence of goiter. Goitrogens also can cause goiter (see *Antithyroid Compounds*, page 1380).

The only known function of iodine is for the production of the thyroid hormones, which regulate cellular oxidation.

The absorption of iodide can occur at all levels of the GI tract. Iodinated amino acids can be absorbed as such but less efficiently than iodide. Excretion of iodine is primarily via the urine, and the amount is a reasonably good indicator of thyroid status. Iodine in saliva is reabsorbed.

IRON

Deficiency Syndrome and Function—Hypochromic microcytic anemia is the characteristic result of iron deficiency. Depending on the severity, the anemia is accompanied by listlessness and tiredness, palpitation on exertion, sore tongue, angular stomatitis, dysphagia, and koilonychia.

Iron is an essential component of several important metalloproteins. These include hemoglobin, myoglobin and many oxidation-reduction enzymes. In iron deficiency, there may be reduced concentrations of some of the iron-containing enzymes, such as cytochrome c in liver, kidney, and skeletal muscle and succinic dehydrogenase in the kidney and heart.

Metabolism—Iron is absorbed from the small intestine; however, the exact mechanism regulating the amount absorbed is still a matter

of controversy. The proportion of dietary iron absorbed is greater in iron-deficient anemic individuals. Iron is transported via the blood, in which it is bound to transferrin, a β_1-globulin.

The iron from deteriorated red blood cells is reused. Under normal circumstances, the loss of iron from the body is very small, about 1 mg a day for men and an additional average daily loss of 0.5 mg a day by menstruating women. Iron is stored in the bone marrow, intestinal wall, liver, and spleen, with the latter organs containing the largest amounts.

Bioavailability—The recognition of anemia as a major public-health problem for menstruating women and young children throughout the world has focused on the need for more-extensive and better fortification of foods. This has stimulated a great deal of research on the availability of iron from foods and inorganic sources. Iron compounds that are utilized readily by experimental animals and humans are ferric ammonium citrate, ferrous sulfate, ferrous gluconate, ferrous fumarate, and ferrous ammonium sulfate. Average to poor sources of iron are reduced iron, ferric chloride, and ferric pyrophosphate. Very poor sources are ferric oxide, ferrous carbonate, sodium iron pyrophosphate, and ferric orthophosphate. The availability of iron from foods can vary also.

Several dietary components can affect the availability of iron from many sources. Phytic acid and antacids can decrease iron absorption. The availability of iron is increased by a variety of reducing compounds such as ascorbic acid and molecules with sulfhydryl groups, as well as histidine and lysine. The smaller the particle size of elemental iron, the greater is the intestinal absorption and use. Heme iron is absorbed as such. Very high intakes of zinc, copper, manganese, and cadmium can decrease the absorption of iron. Many additional studies are needed to evaluate adequately the availability of iron as influenced by composition of the diet and method of food preparation.

Toxicity—Because iron absorption is regulated by the body, moderate excess above the RDA was considered harmless. Recent epidemiological data suggest that continued high intake of iron may raise the risk for chronic disease occurrence, particularly those that are increased with free radical formations, such as cancer. Deaths have occurred, however, in children who swallowed capsules or tablets containing a readily available source of iron, such as ferrous sulfate. Acute effects include vomiting, hematemesis, hepatic damage, tachycardia, and peripheral vascular collapse.

Some individuals have a metabolic defect such that their iron absorption is not carefully controlled, and even a normal iron intake can lead to excess tissue accumulation. A disease known as hemochromatosis results. It usually can be controlled by phlebotomy at periodic intervals; however death can result if the disease is not treated.

MANGANESE

Deficiency Syndrome and Function—Manganese deficiency has been produced experimentally in many animals. Characteristics of the deficiency include growth depression of the young animal, skeletal abnormalities (ranging from mild rarefaction to crippling deformities), mortality of the young, perosis (slipping of the Achilles tendon and accompanying joint deformity) in birds, depressed reproduction of both males and females, nutritional chondrodystrophy of the chick embryo, and ataxia in newborn mammals, with head retraction, tremor, abnormal otoliths, and semicircular canals in the ears. Newborn manganese-deficient guinea pigs have aplasia or marked hypoplasia of the pancreas. Manganese deficiency never has been recognized in humans.

Manganese is required for the synthesis of mucopolysaccharides of cartilage and for the conversion of mevalonic acid to squalene. Glucose utilization is impaired in manganese deficiency. Pyruvate carboxylase is a manganese metalloenzyme.

Metabolism and Bioavailability—The homeostatic mechanism for regulating the concentration of manganese in the body is very precise. Manganese is absorbed from the small intestine and then is transported via the blood in the trivalent form bound to a β_1-globulin, transmanganin. Manganese is excreted in the bile and through the intestinal wall. The latter constitutes the principal mechanism for regulating the amounts of manganese in the tissues. With a high manganese intake, the element also is excreted in the pancreatic juice. The amount excreted in the urine is very small.

High levels of manganese occur in bone, liver, kidney, pancreas, and the pituitary, whereas the concentration in the skeletal muscle is very low. The manganese in bone cannot be mobilized to meet a need. The stores of manganese, in the order of their importance, are found in the liver, skin, and skeletal muscle. There is not a special store in the newborn.

In chick studies it was found that manganese was equally available from the oxide, carbonate, sulfate, and chloride. High dietary intakes of calcium and phosphorus can decrease manganese absorption.

Toxicity—Miners exposed to manganese oxide dust for long periods of time develop psychiatric abnormalities that resemble schizophrenia. This is followed by crippling neurological disorders similar to those found in Parkinson's disease. Most young animals are unaffected by 1000 ppm of manganese in the diet.

MOLYBDENUM

Deficiency Syndrome, Function, and Metabolism—Adverse effects due to simple deficiency of molybdenum in humans and in experimental animals have never been observed. Xanthine oxidase is an important molybdenum-containing enzyme. Due to a variety of indirect evidence and the importance of xanthine oxidase, molybdenum is considered to be an essential trace mineral for humans, probably required in very small amounts. No RDA has been established.

Molybdenum supplied by water-soluble salts is absorbed readily. The element crosses the mammary gland easily. Excretion is into both urine and feces. The liver and kidney have the highest soft-tissue concentrations of molybdenum. Changes in level of dietary intake can be reflected in the concentrations in liver, kidney, skin, bones, and hair. The newborn does not have special stores of the element. Sulfate can affect the absorption, tissue distribution, and excretion of molybdenum. The content of molybdenum in erythrocytes decreases in many types of anemia.

Toxicity—The tolerance of animals to high intakes of molybdenum varies with species, age, and the level of numerous other dietary components. The toxicity is decreased by copper, inorganic sulfate, and the sulfur amino acids.

NICKEL

Evidence that nickel is an essential element is based on abnormalities produced in chicks and rats fed diets containing 3 to 4 ppb of nickel. Lipid metabolism was affected. Rats maintained through successive generations on the nickel-deficient diet had increased fetal mortality.

Absorption of nickel is small from ordinary diets. Excretion is primarily through the feces; however, significant amounts can be lost in sweat. Phytate can form a very stable complex with nickel, so it is possible that phytate may decrease absorption of nickel. Further studies are required to establish clearly the essentiality of nickel and its significance to human health.

A low level of toxicity has been established for nickel in rats, mice, monkeys, and chicks.

SELENIUM

Deficiency Syndrome and Function—Depending on species, age, and specific diet composition, a deficiency of selenium can lead to one or more of the following abnormalities: growth depression, muscular dystrophy, degeneration of the myocardium, neurological lesions, liver necrosis, pancreatic fibrosis, exudative diathesis, ceroid-pigment deposition in adipose tissue, and death. Deficiency occurs in domestic animals with intakes below 0.02 to 0.05 ppm. Deficiency in humans has only been demonstrated in China, where extremely low intake causes a cardiomyopathy in children (Keshan disease). The NAS safe and adequate daily dietary intakes of selenium are 10 to 80 μg for children and 50 to 200 μg for adults.

Most deficiency syndromes responsive to selenium also respond favorably to vitamin E. An exception is pancreatic fibrosis, which occurs only in selenium deficiency. Selenium is an essential component of the enzyme glutathione peroxidase. This provides a link between the antioxidant properties of vitamin E and the biological function of selenium in preventing most of the same selenium-deficiency problems. Animal studies have indicated that selenium may be useful as a chemoprevention agent, but studies in humans have not been accomplished. Experimentally, selenium has been shown to provide protection to pulmonary oxygen toxicity similar to that observed for vitamin E.

Metabolism—Selenium is absorbed from the duodenum. It can be metabolized to a variety of compounds and lost from the body via the bile, pancreatic and intestinal secretions, and ultimately through the feces, urine, and expired air. Selenium can replace sulfur in the normal sulfur amino acids, and selenite also can bind to sulfur amino acids. It also is incorporated into selenonucleosides and may be involved in genetic translation. The highest tissue concentrations of selenium occur in the kidney, pancreas, pituitary, and liver.

Toxicity—Acute selenium toxicity is characterized by abdominal pain, excess salivation, grating of the teeth, paralysis, and blindness. Eventually, disturbed respiration leads to death.

Selenium is one of the most toxic of the essential nutrients, and the quantitative separation of required and chronic toxic levels is not very large. The source of selenium has a significant impact on the level that will cause toxicity to develop. Organic compounds containing selenium enhance absorption and, therefore, are toxic at lower levels. For domestic animals, the requirement is about 0.1 to 0.2 ppm, and 3 to 4 ppm in

the diet are beginning levels for chronic toxicity. Intakes above 500 μg for long periods of time are considered to present a risk of toxicity in man. A reported carcinogenicity for selenium is an elusive association that has not been clarified finally.

SILICON

With highly purified diets it has been possible to produce a deficiency of silicon in chicks and rats. The deficiency affected growth rate, bones, and integumental tissues. The primary biochemical lesion in the deficient animals was an effect on the cartilage matrix.

Silicon (as silicates) is absorbed easily from the intestinal tract and excreted readily in the urine, in part as SiO_2. Silicon is distributed widely in soil, plants, and animal tissues. It is relatively nontoxic; however, siliceous kidney stones have been reported in persons who live in regions with water high in silicate concentration or who chronically ingest magnesium trisilicate antacids.

TIN

Through rigid exclusion of environmental and dietary tin, it has been possible to produce growth retardation responsive to this element in rats. A maximal growth effect was obtained with 1 ppm of tin in the diet, a level similar to that found in many foods.

Tin is absorbed poorly and most of that in the diet is excreted in the feces. Tin has a low order of toxicity.

VANADIUM

Chicks and rats fed a diet containing less than 10 ppb of vanadium had slow growth, defective bones, and altered lipid metabolism. Vanadium is a rather toxic element. The addition of 25 to 50 ppm of vanadium to the diet of rats causes diarrhea and mortality.

ZINC

Deficiency Syndrome and Function—Zinc is required for growth of every animal species studied; therefore, growth depression of young animals is invariably observed if the zinc deprivation is severe enough. Other characteristics of deficiency include skin lesions, alopecia, abnormal feathering in birds, deformed and poorly mineralized bones, hyperkeratinization of the esophagus, reduced numbers of circulating lymphocytes, impaired reproduction in males and females, fetal abnormalities, and decreased learning ability. Persons with impaired taste acuity and discrimination and delayed healing of wounds and burns have responded favorably to therapeutic doses of zinc in some cases.

Nutritional dwarfism has been studied extensively in the Middle East. The syndrome includes delayed sexual development, reduced height and weight, hepatosplenomegaly, spoon nails, and usually anemia. Although the subjects were deficient to some degree in several nutrients, zinc was required to correct the hypogonadism and growth depression. The syndrome occurs in both males and females. Indolent ulcers and delayed wound healing in patients with low plasma zinc levels have been reported, and both systemic and topical administration of zinc compounds are followed by accelerated healing. There is limited evidence that some young children and elderly persons in the United States do not receive adequate zinc.

Zinc is known to occur in many important metalloenzymes. These include carbonic anhydrase, carboxypeptidases A and B, alcohol dehydrogenase, glutamic dehydrogenase, D-glyceraldehyde-3-phosphate dehydrogenase, lactic dehydrogenase, malic dehydrogenase, alkaline phosphatase, aldolase, and others. Impaired synthesis of nucleic acids and proteins has been observed in zinc deficiency. There is some evidence that zinc may be involved in the secretion of insulin and in the function of the hormone. It appears to be a modulator of neurohumoral transmission.

Metabolism and Bioavailability—Zinc can bind readily to sulfhydryl groups, amino groups, and imidazole groups of proteins, amino acids, and other organic molecules.

Zinc is absorbed primarily from the duodenum. It binds to all proteins of the plasma; however, it is bound most loosely to albumin, and this may be important for transport to and from tissues. The concentration of zinc in plasma decreases rapidly when a low-zinc diet is fed, and it is reduced in pregnancy and in women taking birth control pills. The principal route of excretion is via the feces. Small amounts of zinc are excreted daily in the urine; these increase when there is tissue catabolism such as occurs in burns and in fasting. Significant losses of zinc also can occur in the sweat.

Zinc is present in all tissues, with very high concentrations in the prostate and choroid of the eye. Generally, tissue concentrations are not affected greatly by zinc deficiency. The stores of zinc in the body are thought to be small.

Zinc is equally available to normal animals from a wide variety of inorganic salts as well as metallic zinc. Phytic acid can markedly decrease absorption of zinc, particularly in the presence of large amounts of calcium. Consumption of whole-wheat bread, which contains phytic acid, has been shown to be primarily responsible for the zinc-deficiency dwarfism observed in the Middle East. The toxic effects of cadmium are probably partially related to interference with the normal physiological pathways and functions of zinc.

Toxicity—The taste threshold for a soluble salt of zinc in water is 15 ppm of zinc, whereas 40 ppm have a very definite taste. A dose of 225 to 450 mg of zinc has an emetic effect in an adult man. Acute toxicity of zinc is characterized by dehydration, electrolytic imbalance, stomach pain, lethargy, dizziness, muscular incoordination, and renal failure. High zinc intakes are known to lower copper absorption; therefore zinc supplements should be taken only with adequate intakes of copper. Zinc has been used successfully to treat Wilson's disease.

ZINC SULFATE—See RPS-19, page 1271.

BIBLIOGRAPHY

Berdanier CD, White TKA. *CRC Desk Reference for Nutrition* (CRC Desk Ref Series). Boca Raton, FL: CRC Press, 1998.

Composition of Foods Raw–Processed–Prepared. USDA Handbook 8. Washington, DC: USGPO, 1976–1998.

Diet and Health:Implications for Reducing Chronic Disease Risk. Washington, DC: NAS-NRC National Academy Press, 1989.

Escott-Stump S. *Nutrition and Diagnosis-Related Care.* Philadelphia: Lippincott, 1997.

Nollet NML. *Handbook of Food Analysis.* New York: Dekker, 1996.

Novara T, ed. *Encyclopedia of Vitamins, Minerals, and Supplements.* Facts on File, 1996.

Official Methods of Analysis, ed 16. Washington, DC: Assoc Off Anal Chem , 1990.

Pennington JAT. *Bowes' & Church's Food Values of Portions Commonly Used,* ed 17. Philadelphia: Lippincott-Raven, 1997.

Stubbs ME, Olson JA, Stuke M. *Modern Nutrition in Health and Disease.* Baltimore: Williams & Wilkins, 1998.

The US Guide to Vitamins and Minerals. Bethesda, MD: USPC, 1996.