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Review Article

Crystallography. Part I

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"The impossible we do at once—miracles take a little longer." (1)

INTRODUCTION

WILLIAM H. WERKMEISTER, a philosopher, once compared the advances of the sciences with those of the humanities (2). He showed that advances in travel could be illustrated by plotting velocity of travel by man or machine with date of discovery. The resulting plot was roughly exponential. The advances in the humanities with time, he suggested, would represent a zig-zag line which does not show the long range upswing. Since the discovery of crystallography, advances with time would fit the exponential-type curve. With little or no imagination, one concludes that X-ray crystallographers in the near future will be performing routine studies in structural analysis without the use of chemical properties. With increasing effort they will help to predict or substantiate hypotheses in reaction mechanisms. They have already helped to solve the riddle of life; scientists recognize the worth of the crystallographer when discussing RNA and DNA. X-ray crystallographers, through their reports, will allow scientists to utilize imaginations with increasing tempo. It is little wonder then that Grenville-Wells reminded the readers of the optimistic advertisement quoted above (1).

Cannon recorded a discussion with Gamgee, a British biological chemist who visited Cannon's laboratories at Harvard University at the turn of the century (3). After Cannon apologized for the physical facilities, Gamgee responded, "I have never noticed that the nature of the cage determined the singing of the bird." Today, the pharmaceutical crystallographer has been termed a "rare bird" (4), but this bird is the first to admit that the cage is important. The refinement of methods and X-ray equipment and the development of computors have contributed to a most rapid advance.

Crystallography was first developed as a branch of mineralogy. It was concerned with the study of three-dimensional bodies and the laws which governed their growth, shape, and geometric character. Crystallography soon reached such proportions that it became a separate science. To review crystallography in total would require volumes. Faced with this dilemma, the reviewer took the easy way out by limiting the pages of words to some aspects of X-ray and optical crystallography. To review just two of the total number of different phases of crystallography and application to pharmaceutical research does not allow one to conclude that there are but two important phases—it just provides an opportunity for other experts to write review articles.

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Editor's note: Other important aspects of crystallography, including optical crystallography and crystalline properties, will be discussed by Dr. Biles in Part II of this review which will appear in the July issue of THIS JOURNAL.

NOMENCLATURE

An ideal crystal is a regular polyhedral solid bounded by plane faces which represents an extended array of atoms arranged in a definite order in all directions. This body contains a unit cell or unit of structure, repetition of which, in three dimensions, produces the crystal. Each unit cell is the same size for the specific crystal and contains the same number of particles similarly arranged. Even though size and shape of such a crystal may vary, the angles between the faces will remain constant. At times many of the crystal faces may not be evident, but there still remains an orderly internal arrangement of the atoms. A measurement of the interfacial angles reveals a number of faces of the same type. Faces of the same type are referred to as a form. In some instances, one form is all that is needed to define the three-dimensional solid. In other instances and at times demanded, two or more forms are present. The crystals of a given substance may vary in size, relative development of given faces, and the number and kind of faces or forms present. Crystals showing such variations have different habits. Thus, some crystals are described as needles, others are tabular, equant, columnar, or lamellar. The habit acquired depends upon the solvent used, the temperature, concentration, impurities, and the rate of precipitation.

The crystal faces are identified using Miller indexes. These indexes consist of a series of whole numbers which have been derived from the parameters by their inversion and, if necessary, the subsequent clearing of fractions. The numbers are so given so that the three numbers refer to the *a*, *b*, and *c* crystallographic axes, respectively. The possible faces from various planes of molecules or atoms, as identified by the Miller indexes may be illustrated by either two- or three-dimensional figures. Imagine looking down the *c* axis of a crystal and at the *a-b* plane of the crystal. Some different crystal faces which parallel and do not intersect the *c* axis are identified by Miller indexes, as shown in Fig. 1.

Crystal faces intersect at definite angles to each other and the faces of the crystal parallel the internal planes of the crystal. Thus, one may compare Fig. 1 with the observation of the density of trees in the various rows of trees in a fruit orchard. The next time you are driving down the highway and pass a fruit orchard, try to visualize the different planes and guess the Miller indexes for each of the rows. Perhaps, though, it would be simpler to consider the

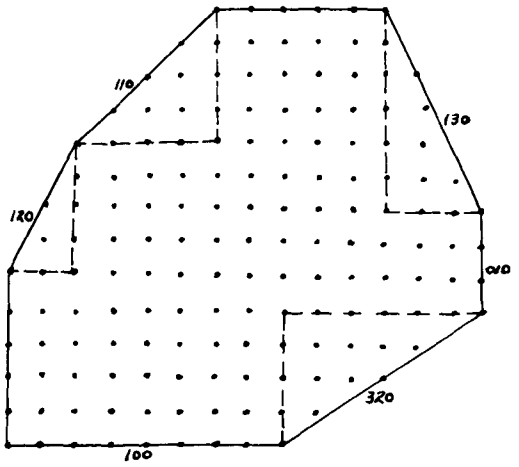


Fig. 1—A cross section of a crystal perpendicular to the *c* axis.

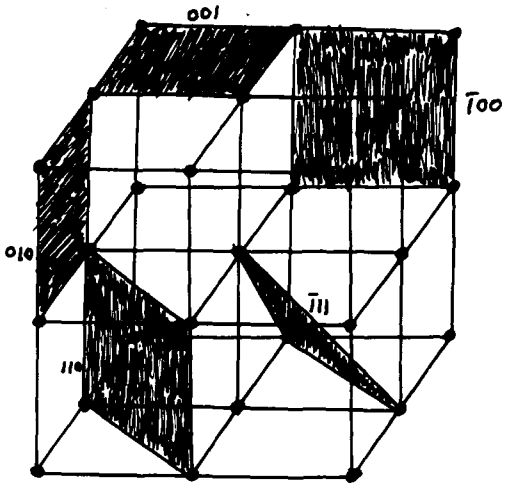


Fig. 2—Some possible crystal faces of the sodium chloride crystal.

structure of the sodium chloride crystal in which one sodium ion is surrounded by six chloride ions, and each chloride ion surrounded by six sodium ions. The possible faces could be identified by the three-dimensional diagram illustrated in Fig. 2.

Examining Fig. 2, it is observed that there is a perfectly regular arrangement of the cations and anions. The three-dimensional network of sodium ions represents the space lattice of the sodium ions in the crystal of sodium chloride. Similarly, there is a space lattice of chloride ions. The space lattice of sodium chloride represents the interpenetration of the sodium and chloride lattices. Each ion in the lattice has exactly the same environment as any other point representing the same ion. The space lattice of the crystal is built up of a three-dimensional basic pattern

which is called the unit cell. The external appearance of the crystal is determined by the shape and dimensions of the unit cell.

By the application of geometry, it has been shown that only 14 different kinds of simple space lattices are possible. That is, there are only 14 ways in which similar points can be arranged in a regular three-dimensional order. By taking combinations of the various lattices that are possible for each crystallographic system, there have been built up 230 different space groups. The space-group symbols and the procedure for deducing the space groups are described in crystallography textbooks (5). For purposes of review, most of the readers recall that crystals vary in their angular relationships and symmetry. On this basis, a crystal is classified into one of six crystal systems. Each system is defined in terms of the crystallographic axes, which are imaginary lines used to describe the position of the plane faces in space. The systems, depending on the symmetry, are subdivided into 32 classes.

X-RAY CRYSTALLOGRAPHY

The increasing importance of this science can be felt by reading the excellent reviews. One could read the fascinating reports of molecules in crystals (6), the motion of the atoms in crystals (6, 33), crystallographic studies of compounds of biological interest (34, 35), the application of crystallography to chemistry (36), the use of X-ray crystallography in the pharmaceutical industry (37, 38), and British achievements in X-ray crystallography (39). Should the scientist become a convert to this science, in a few sentences Lonsdale could tell him the required background (40). If a few hundred pages are necessary to convert the scientist, then out of the many excellent textbooks written, the books of Bunn (41) and Robertson (42) would prove interesting material.

The Unit Cell

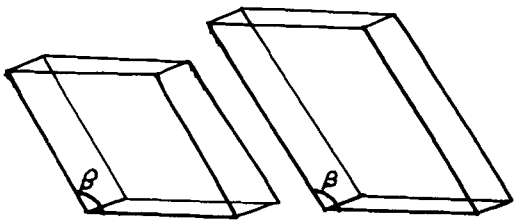
The arrangement of the molecules in the space lattice and the relationship of the molecules to the crystal shape was predicted before the use of X-ray procedures. Hodgkin (6) and Robertson (7) have called attention to the observations of Hooke in 1664. Hooke noted the crystal shape of alum. He considered that the shape resulted from "three or four several positions or postures of globular particles . . . by these kinds of texture or position of globular bodies, may you find out all the variety of regular shapes into which the smooth surfaces of Alum are form'd. . . nor does it hold in superficies but in solidity also, for it's obvious that a fourth Globule laid upon the third in this texture, composes a regular Tetrahedron, which is a very usual Figure of the Crystals of Alum."

By X-ray analysis, it has been determined that each unit cell contains four aluminum ions, four potassium ions, 48 water molecules, and eight sulfate ions. Hodgkin commented that, remarkably enough, the relative positions of the positive ions in the crystal conform with Hooke's suggestions but, in total, the structure of alum is considerably more complicated than Hooke's drawings propose (6).

One of the first insights into the unit cell structure was presented by W. H. Bragg when he measured the unit cells of naphthalene and anthracene (8). The measurements were also reported by Robertson in 1933 (9, 10). The cell dimensions for the two compounds are shown in Table I and Fig. 3.

TABLE I.—UNIT CELL DIMENSIONS OF SOME AROMATIC HYDROCARBONS

	a, Å.	*b*, Å.	*c*, Å.	*β*
Naphthalene	8.34	6.05	8.69	122°49′
Anthracene	8.58	6.02	11.18	125°
Diphenyl	8.22	5.69	9.5	94.8°
p-Diphenylbenzene	8.08	5.60	13.59	91.9°
p-Diphenyl-diphenyl	8.05	5.55	17.81	95.8°



(A) (B)

Fig. 3—The unit cell dimensions for naphthalene (A) and anthracene (B).

It should be noted that two sides of the unit cells in Fig. 3 are almost the same distance. Also the angle beta (obtuse angle) for both cells are very close. The two cells differ with respect to the *c* axis, which is 2.49 Å. longer in anthracene than in naphthalene. It may be recalled that the diameter of the benzene ring is almost this distance (8).

The unit cell dimensions have also been determined for diphenyl (11), *p*-diphenylbenzene (12), and *p*-diphenyldiphenyl (13). The unit cell dimensions for these compounds are also listed in Table I. Again two sides of the cells are very similar (also similar to those of naphthalene and

anthracene); the angle beta for the three compounds are similar, but the difference lies along *c*. The bond distance between the benzene rings was found to be 1.48 Å. Adding the 1.48 Å. to the 2.49 Å., the sum is found to be approximately the same value as the differences between the values along *c*.

The unit cell dimensions of long chain organic compounds have been the subject of discussion for approximately 30 years. Francis, Collins, and Piper (14) determined the unit cell dimensions for a number of saturated fatty acids, alcohols, alkyl iodides, and ethyl esters, as well as some dicarboxylic acids. A definite correlation was obtained when the molecular weight of the compounds of each of the homologous series was plotted against the melting point. A straight line relationship was also obtained when the crystal spacings were plotted against the carbon content. Francis, *et al.*, reported that when the fatty acids containing the even numbered carbon atoms were crystallized from glacial acetic acid, C-type crystals were obtained. The specific spacings were noted. When the same fatty acids were crystallized from either benzene or acetone, different spacings were noted. These were referred to as B crystals. When the fatty acids containing odd numbered carbon atoms were crystallized from glacial acetic acid, acetone, or benzene, only spacings corresponding to B crystals were observed. Abrahamsson and von Sydow (15) reported the variation of unit cell dimensions of long chain fatty acids of form C. For the acids with 12, 14, 18, 22, and 26 carbon atoms, it was found that all dimensions in the monoclinic cell were found to be dependent on the number of carbon atoms in the chain. The *a* and *b* axes and angle beta decreased asymptotically with increasing chain length. The *c* axis and long spacings, *d* (001), obeyed linear laws.

Warren and Matthews (16) noted the straight line relationship of *d* spacings and molecular weight for alcohol derivatives of the xanthates. They used the strong inner line as the *d* distance of choice. They also investigated the unit cell dimensions of the anilides of acids from formic acid through stearic acid (17). The patterns of the even members of series C_8 to C_{18} show a marked similarity, suggesting a uniformity of crystal structure which may be described as isostructural. Their results were in agreement with Slagle and Ott (18). Matthews, Warren, and Michell continued this line of investigation (19). When the first-order reflections of the silver salts of fatty acids were plotted against the number of carbon atoms in the fatty acid, a straight line was obtained. The equation was determined to be

$$X = 9.85 + 2.44\,(y - 2) \qquad \text{(Eq. 1)}$$

The value of 2.44 represented the increment of the —CH_2 group, and the value of 9.85 represented the combined value for the —CH_3 and —COOAg group. Similarly, in a study of the amides of the fatty acids, the straight line relationship of the first-order reflection was given by

$$X = 6.25 + 1.85\,(y - 2) \qquad \text{(Eq. 2)}$$

The value of 1.85 for the amides represented the increment for the —CH_2 group.

Merritt and co-workers identified alkyl halides as the alkyl 6-nitrobenzothiazolyl-2-sulfides and sulfones (20). Their differentiation of the halides was made by observation of the three strongest reflection lines. Wurz and Sharpless studied the amides of saturated aliphatic acids (21). A straight line relationship was correlated for the odd and even series. The line for the even series had a greater *y* intercept than that for the odd series. Garska, *et al.*, recently reported that when the fatty alcohol could not be identified with gas chromatography or mass spectrometry, the alcohol benzoate could be prepared and X-ray analysis performed (22). They too reported an apparent correlation existing between length of the carbon chain attached to the 3,5-dinitrobenzoate radical and the size of the largest *d* spacing in the diffraction pattern of the ester.

Sharma and Biswas reported the X-ray diffraction study of *n*-alkyl malonic acids (23). The melting points of the acids and the characteristic long spacings were recorded. Their work is quite similar to those reported by Francis, *et al.* (14). The saw tooth arrangement of the melting points and long spacings for the odd and even membered series was reported. The spacings in the alkyl fatty acids corresponding to the length of the molecules suggest that the molecules are interlinked as dimers. Sharma and Biswas reported that such spacings in the alkyl malonic acids were approximately equal to the calculated extended length of a single molecule. They suggested that the alkyl malonic acids existed as a single unit, presumably due to internal saturation through intramolecular hydrogen bonding. Additional reports on relationships between the unit cell dimensions and chain length include those of Brock and Hannum (24), Lutz and co-workers (25), Guertin, *et al.* (26), and W. S. Singleton (27).

Pharmaceutical scientists could very well apply the knowledge obtained from unit cell dimensions. Such data can be used for purposes of

identification. The crystallographer may compare his diffraction pattern with the values in the A.S.T.M. file (28). In one of the laboratories it was reported that one of the liquid multivitamin preparations produced had a wine base as a major constituent. Upon changing the source of supply for the wine, a precipitate began to appear in the finished product. X-ray patterns were taken of the precipitate and checked against an A.S.T.M. file. The precipitate was identified as calcium tartrate tetrahydrate (29). Purity determinations can be determined. Concentration and stability has been suggested for suspensions (30). Shell states, since the crystalline material has a definite size unit cell, that this fact can be used as a general quantitative method of analysis which offers a combination of specificity and precision. The completeness or progress of a chemical reaction could be followed by X-ray diffraction methods since the unit cell dimensions of the product would, in all probability, be different from those of the reactants (31).

Mott has discussed the role of vacant lattice sites in crystalline solids. He believes that the vacant lattice site allows the diffusion in solids, and particularly when two metals are welded together. During the welding, a region intermediate between the two metals is formed which is occupied by an alloy of the two. Diffusion has taken place, which means that two adjacent atoms in a crystalline solid are able to change places if the temperature is high enough. This exchange can come about only with the help of a vacant lattice site. It was also expressed that vacancies play a role in metallic fatigue (32).

X-Ray Diffraction

In 1949, Kaufman and Fankuchen, reviewing the reports on X-ray diffraction, stated that in a matter of 10 to 15 years diffraction had been established as a most useful tool for analytical purposes. The tool was not self-sufficient; it was incapable by itself of replacing chemical analysis; but it had proved useful for identification of crystalline substances (43). Later reviews were written by Fankuchen, Kaufman, and Post (44–47). Post and Fankuchen also reported on low temperature X-ray crystallography (48). In 1936 there was concern about the filing of diffraction data. Hanawalt and Rinn suggested an effective use of a large library of standard X-ray diffraction patterns for the identification of unknowns (49). In 1938 Hanawalt, *et al.*, published information concerning classification and use of diffraction patterns. They included data on 1000 compounds (50). Frevel, Rinn, and Anderson then tabulated the diffraction data for 705 cubic substances (51), 447 tetragonal substances (52), and 1287 hexagonal substances (53). Matthews (54) recommended the use of a punched card system, and Ashley and Newton (55) suggested the use of a Kardex system. The American Society of Testing Materials (A.S.T.M.) card index file of X-ray diffraction data is widely used (56). Recently, Beukelman discussed the efficient use of an I.B.M. file of A.S.T.M. powder X-ray diffraction data (57). The literature sources for the X-ray diffraction patterns for different series of compounds of pharmaceutical interest are listed in Table II.

TABLE II.—REFERENCES TO X-RAY DIFFRACTION PATTERNS OF COMPOUNDS OF POSSIBLE PHARMACEUTICAL INTEREST

Compound Type	Compound Type
Aromatic hydrocarbons (58)	Azoic coupling components (77)
Aromatic hydrocarbons-trinitrofluorenone (59)	Cobalt ammine azides (78)
Alkyl halides (20)	Guanidine derivatives (79)
Aldehydes and ketones (60)	Explosives (80)
Surface-active agents (61)	Penicillins (81)
Fatty acids (27, 62)	Dyestuffs (82)
Fatty acid silver salts (26)	Barbiturates (83–85)
Aliphatic acid amines (21)	Sedatives and anticonvulsants (86)
Alkyl dithiol esters of sebacic acid (25)	Boron compounds (87)
Alcohol benzoate esters (22)	Phosphorous nitrogen compounds (88)
Substituted benzoic acids (63)	Sodium phosphates (89)
Aliphatic and aromatic amines (64)	Calcium phosphates (90)
Sterols and their digitonides (65, 66)	Strontium phosphates (91)
Steroids (67–70)	Crystalline ferrocenes (92)
Fructose dinitrophenylhydrazone (71, 72)	Copper chelates (93)
Phenols (73, 74)	Porphyrins (94)
Tetrazole derivatives (75)	Alcohols (95)
Oxadiazoles (76)	Bacterial viruses (117)

Some comments concerning the reports listed in Table II are warranted. Hofer and Peebles (58) reported that impurities lowering the melting point of the hydrocarbons as much as 15° did not modify the diffraction patterns appreciably. Rose and Van Camp (63) showed that benzoic acid derivatives having the same empirical formulas and same melting points could be differentiated with X-ray diffraction. Gaebler, Parsons, and Beher (65) detected differences in the X-ray diffraction analysis of digitonin polymorphs, whereas the infrared spectra of the samples used were virtually identical below 9.2 and above 9.7 μ. In the second study, Beher and co-workers reported that X-ray diffraction definitely established sterol digitonides as chemical com-

pounds, whereas infrared analysis did not distinguish between a simple mixture and a corresponding digitonide. Baker, Beher, and Parsons (67–70) reported the diffraction patterns for 138 steroids. Moore and Burkardt (75) stated that failure of X-ray patterns of the same compound to correlate may arise from polymorphism or preferential orientation of sample particles. In the reporting of the *d* distances for the various barbiturates, Williams (83) criticized the values reported by Penprase and Biles (84). Williams was justified in so doing if the *d* distances are required for pure solids, or for inclusion in the A.S.T.M. file. However, the report of Penprase dealt with a rapid identification of barbiturates using specific methods. With such methods polymorphism is evident and is, in fact, an aid for rapid identification. North and Rich (117) examined the T2 virus which has a very substantial tail structure and the T7 virus which has a small tail. Experiments suggested that some of the DNA inside the bacteriophage T2 virus head is oriented parallel to the tail. Furthermore, evidence suggested that the DNA may be organized into crystalline domains about 140 Å. across in T2 and 120 Å. in T7. The reports of the diffraction patterns of the various phosphates are indicative of the complexity of phosphate chemistry (89–91). The diffraction patterns of the calcium phosphates and strontium phosphates can undoubtedly be utilized in the study of strontium deposition in the bone. The review of the X-ray work of the fatty acids by O'Connor should be considered a masterpiece considering the complexity of the subject (62).

Quantitative Diffraction Method

Space will not permit the discussion of the use of X-ray diffraction analysis in a number of different analytical techniques. These techniques include emission spectroscopy (96–102), absorption (103, 104, 108), analysis combining absorption and diffraction (105), photoelectron spectra for surface analysis (106), low angle scattering analysis of particle size (107), and scattering methods (109,110). The selected references listed above are offered as a substitute for a discussion.

Single Crystal Analysis

Kaufman and Fankuchen (43) stated that single crystal diagrams may give more information than powder diagrams. In addition to a set of *d* spacings, one can obtain the unit cell size rather simply and, from a consideration of the systematic extinction, determine the space group. Bunn (41) has stated that the unit cell dimensions can be determined from powder diffraction data for the crystals of high symmetry. However, it is quite difficult to determine the unit cells from powder data for crystals of low symmetry. Nevertheless, Azaroff and Buerger have made available directions for the determination of unit cell dimensions from powder diagrams (111). This text is written in a manner which is easily understood. There are limitations in determining the unit cell dimensions. These limitations depend on the amount of data collected, particularly for a series of compounds. Examination of Table I will indicate the limitations and the value obtained from the unit cell determinations. Structural analysis is both much more complex and much more revealing.

Single crystal analysis is of great value in the determination of molecular weight. Douglass and Cook (112) reported that there was a question whether the lycoctonine monohydrate was represented by the empirical formula of $C_{25}H_{40}NO_7 \cdot H_2O$ or twice this value. The molecular weight determination by single crystal analysis was used to clarify the mystery. Straumanis (113) reported that the molecular weight determination by X-ray analysis may surpass the accuracy values obtained by chemical methods. The molecular weight of beta-lactoglobulin was determined by X-ray (114). Crystallographers in the pharmaceutical industry have been called upon to determine molecular weights by X-ray single crystal analysis (115, 116). Shell has described a method which requires a relatively small investment of time, yet with errors usually no greater than one hydrogen atom (116).

Structural Analysis

With continued refinement of methods used in the study of structural analysis, compounds have been restudied. It has become common in the past few years to calculate the electron density in crystals in three dimensions. With this study, the positions and modes of vibration of the atoms in the crystal may be studied (6). Cox, *et al.*, in a series of papers, studied the crystal structure of benzene (118–120). The positions of the hydrogens were determined and it was reported that the benzene molecule, as a whole, was oscillating with a root mean square amplitude of approximately 7.9° about the sixfold axis. Such a movement is allowed by the nondirected character of the intermolecular forces and the cog-wheel packing of the molecules in the crystal (6). The crystal structures of 3,4-benzpyrene (121) and 9,10-dimethyl-1,2-benzanthracene (122) were studied. Both molecules were found not to be planar. It was found that two ab-

normally short bonds and one very long bond were present in 3,4-benzpyrene. In 9,10-dimethyl-1,2-benzanthracene, ring D was found to be bent down out of the general plane by about 20° and atom C_{15} up by about 12°. On the other hand, in a study of the crystal structure of 1,2-benzanthracene, the molecule was found to be planar within experimental error (123). Perhaps a relationship can be established from X-ray analysis concerning carcinogenic activity of these type compounds.

Aromatic hydrocarbon complexes have been studied (59, 124–127). Aromatic nitro compounds have been found to complex with hydrocarbons. The stability of the complex has been ascribed to "polarization bonding" (125) between components. The complexation of 1,3,7,9-tetramethyl uric acid, T.M.U.A., and pyrene has been studied by De Santis and co-workers. The T.M.U.A. solubilizes polycyclic aromatic hydrocarbons in water to form 1:1 and 1:2 crystalline complexes from organic solvents. It was found that the T.M.U.A. molecules in pure form in the crystal state stack up with alternate molecules similarly arranged. Neighboring molecules are not mirrored but rotated 180° with respect to each other. When a 1:1 complex of T.M.U.A. and pyrene crystallized out, the molecules were found to be stacked in alternating fashion. Every other molecule in a stack was a T.M.U.A. molecule. The orientation of these molecules was similar. A 1:2 complex has been isolated. The author would anticipate that the arrangement in the crystal will be a 1:2:1:2 arrangement. Of course it would be interesting to see if a 1:3 complex and a 2:3 complex could be isolated.

De Santis (127), *et al.*, suggested, that in the T.M.U.A. crystal the second molecule paired was rotated from the other molecule by 180° since the $\overset{+}{N}—\overset{-}{O}$ bonds (dipoles) are roughly perpendicular to the long axis of the molecules and that interaction between dipoles is the main factor responsible for pairing. Thus dipole-dipole interaction may predominate over the hydrogen bonds. When a methyl or a keto group is attached to the nitrogen, the dipole bond is weakened. The report of Sutor (128, 129) may be related to this discussion. It was found by Sutor that caffeine and theophylline molecules pair up in the same plane. The paired molecules are illustrated in Fig. 4. The arrows identify the dipoles.

Since the methyl group on the nitrogen reduces the strength of the dipole, and since the unit cell of caffeine is slightly greater in volume than that of theophylline, the greater solubility in water

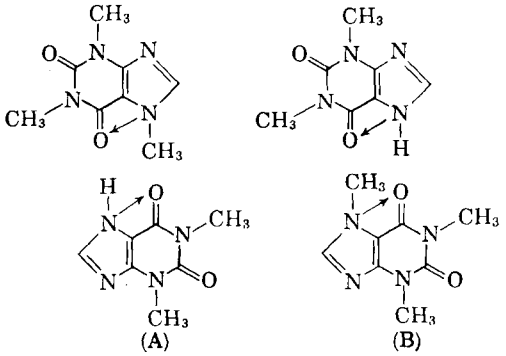


Fig. 4—The paired molecules of theophylline (A) and caffeine (B).

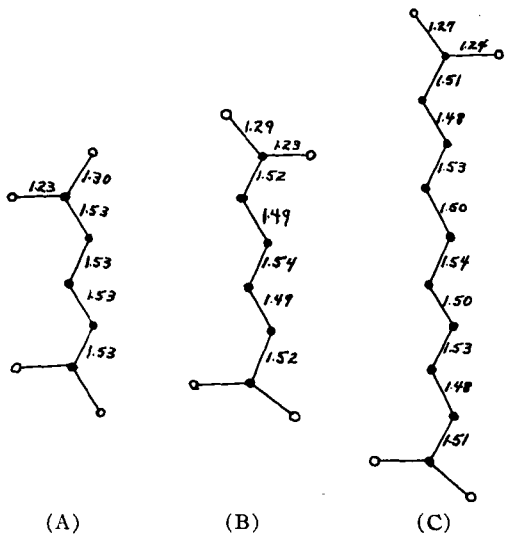
and lower melting point of caffeine, in comparison to theophylline, is better appreciated. Sutor points out that the disordering of the molecules along the *c* (of the unit cell) is the difference in the strength in which water is held. Theophylline does not effloresce even though there are chains of water molecules running through the structure. If the water molecules are disordered in their arrangement along *c*, only a fraction of them would be hydrogen bonded to the caffeine molecule. Thus, water is more firmly linked to a theophylline molecule. Gerdil and Marsh suggested an alternate proposal for the placement of water in the caffeine structure (130). With the information proposed concerning the relative positions of the molecules of caffeine and theophylline and the effect of the dipoles, the reader might want to make a comparison with the study of caffeine complexation by the Higuchi group (131–135).

The crystal structures of uracil (136), adenine hydrochloride (137), guanine hydrochloride (139), and thymine monohydrate (138) have been reported. Parry draws attention to the close similarity between the dimensions of the pyrimidine ring in adenine hydrochloride and in uracil. Broomhead reported that the cell dimensions of guanine hydrochloride and adenine hydrochloride are different, but the structures are strikingly similar. The structures of various nucleotides have been reported (140–142). Bryan and Tomita (143) emphasized the value of the study of purines and pyrimidines for use in the formulation of structures for nucleic acids. For this formulation the identification of sites of protonation of the bases at acid pH, knowledge of the molecular geometry of the various bases, and a possible model for the geometry of interbase hydrogen bonding in polynucleotides would be most valuable. The reading of Spencer's report on the stereochemistry of DNA should be a must for

those interested in cell development (144). The Crick and Watson model has suggested a fascinating mechanism for the process of heredity (39). The structure analysis of proteins has been reviewed by Kendrew and Perutz (35). A most recent review on proteins by Kendrew was published (145).

The difference in solubility in water and melting points for the aliphatic dibasic acids has been the subject of reports by X-ray crystallographers (146–150). Morrison and Robertson (150) stated that several factors could account for the saw tooth type arrangement. The carboxyl groups in beta-glutaric acid deviate further from the plane of the carbon atoms than in the even numbered series studied. The end-to-end hydrogen bridge connections between the carboxyl groups are 2.69 Å., which is almost the same as in the even structures, but the lateral connection is 3.6 Å., which is much weaker than in the even series. In addition, the bond lengths are more closely normal in beta-glutaric acid than those found in the even series. The bond distances of some of the dibasic acids studied (147–150) are shown in Fig. 5.

Bragg, Perutz, *et al.* (151–153), examined the structure of hemoglobin. They reported that the pleochroism of sheep hemoglobin varied considerably. On this basis they suggested that structural differences are evident between fetal and adult sheep hemoglobin (152). In a later paper they reported that reduced, oxy, carboxy, or methemoglobin existed in three polymorphic forms, whereas the hemoglobin of normal adult man crystallizes in six different forms. Patterson projections of horse and human hemoglobin possess similarities which imply a close relationship between the internal architecture of these two proteins (153). Partial X-ray crystallographic studies have been reported for the sterols (154) and three trimethylsteroids (155). The structural analysis has not been performed for these complex molecules. The unit cell dimensions have been published. This is also true of prednisolone acetate (155). Gafner, *et al.*, reported that the trimethyl steroids had many similarities. He cautioned, however, that the intensities of the diffraction patterns could be misleading since powder samples were not free from preferred orientations (155).



(A) (B) (C)

Fig. 5—The bond distances for glutaric acid (A), adipic acid (B), and sebacic acid (C).

A major accomplishment in X-ray structural analysis was performed by the Hodgkin group in England with the help of White, Prosen, and Trueblood in the United States (157–160). They determined the structure of vitamin B_{12} without the use of chemical analysis. The various techniques used in determining the structure would prove interesting reading.

X-ray structural analysis has been used to aid in the determination of molecule stability and in explaining organic reactions. Three examples will be discussed (161–166). The structure of benzene and *p*-chlorobenzene iododichloride has been studied (161, 162). Archer and van Schalkwyk reported that the ICl_2 group in benzene iododichloride was linear and symmetrical. The direction of this group is approximately at right angles to the *ac* plane. Bekoe and Hulme (162) stated that the electrophilic group in the *para* position gave rise to a dipole moment directed away from the iodine atom. It is possible that a tendency of electrons to drift away from the iodine atom partially increased the positive charge on the iodine and so makes a greater resonance stabilization of the molecule. In the linear ICl_2 group the third valence is at right angles to the halogen group. Presumably dsp^3 or d^3sp bonds are formed, but not fully used. The main cohesive forces in the crystal are between the iododichloride groups. Watson, in studying the crystal stuctures of 1-chloro-2,4-dinitrobenzene and 1-bromo-2,4-dinitrobenzene stated that the relative positions of the nitro group should be studied since this group affected the rate of reaction for nucleophilic aromatic substitution (163). A maximum activation through electron transfer is attained if the nitro group is planar with the benzene ring. It was reported that the nitro group in the 1-chloro derivative was rotated 39° and the nitro group was rotated 40° for the 1-bromo derivative.

Thus, the activating effect of the nitro group is greatly diminished by this steric effect and may result in a net deactivation. Donohue and co-workers have examined the polymorphs of sulfur (164–166). Donohue (164) suggested that the geometrical considerations of some of the allotropic forms of sulfur should be included in discussions concerning reactions of compounds containing the S—S bond, especially with regard to transition states or activated complexes, even though the precise energetics are not known. Thus the structural data of sulfur will undoubtedly be of increasing importance.

Recently, developments in techniques have allowed a reinvestigation of bond distances. Refinement of the structure of anthraquinone was reported by Murty (167). Refinement was done utilizing two-dimensional synthesis, three-dimensional synthesis, and three-dimensional differential synthesis. The hydrogen atoms were detected and their positions verified. Thus the hydrogen carbon bond distance was measured and compared to that obtained by other methods. The structure of chlortetracycline hydrochloride was also analyzed by X-ray procedures (168). From the analysis of this antibiotic Hirokawa, *et al.*, proposed that (*a*) the fourth ring takes a partial quinone structure, (*b*) C_{11} and O_{11} are single bonded, (*c*) C_{10a}—C_{12} is conjugated and no double bond is localized on the C_{11a}—C_{12} bond, (*d*) carbon sequence C_1—C_3 is also conjugated, (*e*) carbon sequence C_4—C_6 is completely saturated, (*f*) the dimethylamino group takes the polar configuration with respect to the first ring. Point (*f*) represents an exception to the structure found on chemical grounds. Thus the X-ray structure corresponds to the conformation assigned on chemical grounds to epi-chlortetracycline. The organic portions of the molecules exhibit only van der Waals contacts except for the hydrogen bonds around the anion. Only intramolecular hydrogen bonds are found. The chloride ion, which lies near the apex of the pyramid made by the dimethylamine group and C_4 is surrounded by six atoms which form a distorted octahedron. Those six atoms are N_4, O_6, $CH_{3(6)}$, O_{12}, O_{12a}, and N_{amide}.

Crystallographic studies of the biuret reactions have been made by Freeman and co-workers (169). They report that the metal-peptid complexes appear to be involved *inter alia* in the transition states of metal-activated proteolytic enzyme reactions. The metal contributes to the color formation. In some studies for metal binding the peptid nitrogen atoms were the donor atoms while in other studies the donor atoms were the peptid oxygens. When copper II was chelated and involved the nitrogen atom, the complex was anionic divalent; when chelation occurred with the oxygen, the complex was cationic divalent. The crystal structure of isosteric compounds was studied. Giacomello and co-workers determined the orientations and stucture of 2,4-dihydroxybenzoic acid and 2-hydroxy-4-aminobenzoic acid. It was found that even though the compounds were isosteric, crystallization occurred and different lattices were formed. The relationship of the isosteres in the crystalline state was illustrated. The reader should refer to the original reference for full appreciation (170, 171). X-ray studies of additional compounds of pharmaceutical interest are listed in Table III.

TABLE III.—SOME COMPOUNDS OF PHARMACEUTICAL INTEREST ANALYZED BY X-RAY

Acridine polymorphs (172–174)	Chelates (179)	Penicillin (185,186)
dl-Alphaprodine HCl (175)	Colchicine (180)	Strychnine (187,188)
Antihistamines (176)	Cycloseri ne(181)	Liquid water (189)
Biotin (177)	Fatty acids (62, 191)	Ice (190)
Cellobiose (178)	Formic acid (182)	
	Glycine polymorphs (183, 184)	

The X-ray analysis of colchicine (180) was helpful in the determination of the structure of the molecule. von Sydow (191) illustrates the various forms of the fatty acids referred to as A, A′, B, B′, C. Malenkow proposed that in liquid water the importance of the dodecahedron formation from water molecules is evident.

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Research Articles

Influence of the Absorption Rate of Tolbutamide on the Rate of Decline of Blood Sugar Levels in Normal Humans

By EINO NELSON, E. L. KNOECHEL, W. E. HAMLIN, and J. G. WAGNER

The absorption of tolbutamide and several of its salts was studied by means of carboxytolbutamide excretion rate measurements following the oral ingestion of the drugs in nearly constant surface dosage forms by normal adult humans. Lowering of blood sugar levels produced in normal adult humans after the oral ingestion of the same preparations was also studied. Initial rate of decline of blood sugar level could be correlated with either the absorption rate of tolbutamide or the amount of tolbutamide in the body 1 hour after drug ingestion; increasing with increasing absorption rate or increase in amount of tolbutamide in the body. In most instances, increases in *in vitro* dissolution rate were reflected in increases in rate of decline of blood sugar level, absorption rates, or amounts of tolbutamide in the body at 1 hour.

THE RELATIONSHIP between the rate of absorption of the antidiabetic drug tolbutamide[1] and resulting depression in blood sugar level has not beeen previously studied. The availability of several salts of tolbutamide with different dissolution rates and, hence, different absorption rates, made possible the present study which was concerned with the nature of this relationship.

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Tolbutamide is 1-butyl-3-*p*-tolylsulfonylurea.

EXPERIMENTAL

Materials.—Pharmaceutical grade tolbutamide and its sodium salt were used.

The 2-amino-2-methyl-1-propanol salt of tolbuta-