

DOROTHY CROWFOOT HODGKIN

## The X-ray analysis of complicated molecules

*Nobel Lecture, December 11, 1964*

I first met the subject of X-ray diffraction of crystals in the pages of the book W. H. Bragg wrote for school children in 1925, 'Concerning the Nature of Things'. In this he wrote: "Broadly speaking, the discovery of X-rays has increased the keenness of our vision over ten thousand times and we can now 'see' the individual atoms and molecules." I also first learnt at the same time about biochemistry which provided me with the molecules it seemed most desirable to 'see'. At Oxford, seriously studying chemistry, with Robinson and Hinshelwood among my professors, I became captivated by the edifices chemists had raised through experiment and imagination-but still I had a lurking question. Would it not be better if one could really 'see' whether molecules as complicated as the sterols, or strychnine were just as experiment suggested? The process of 'seeing' with X-rays was clearly more difficult to apply to such systems than my early reading of Bragg had suggested; it was with some hesitation that I began my first piece of research work with H. M. Powell on thallium dialkyl halides, substances remote from, yet curiously connected with, my later subjects for research.

A series of lucky accidents (a chance meeting in a train between an old friend of mine, Dr. A. F. Joseph and Professor Lowry was one) took me to Cambridge to work with J.D.Bernal in 1932. There our scientific world ceased to know any boundaries. In a sub-department of Mineralogy, changed during my stay into one of Physics, we explored the crystallography of a wide variety of natural products, the structure of liquids and particularly water, Rochelle salt, isomorphous replacement and phase determination, metal crystals and pepsin crystals, and speculated about muscular contraction. Our closest friends were biologists and biochemists. I left Cambridge with great reluctance to try to settle down academically and to try to solve at least one or two of the many problems we had raised.

I do not need here to give a detailed account of the theoretical background of structure analysis by the X-ray diffraction of crystals since this was done long ago by W.L.Bragg<sup>1</sup> and again two years ago, very beautifully, by Perutz and Kendrew<sup>2</sup>. The experimental data we have to employ are the

X-ray diffraction spectra from the crystal to be studied, usually recorded photographically and their intensities estimated by eye. These spectra correspond with a series of harmonic terms which can be recombined to give us a representation of the X-ray scattering material in the crystal, the electron density. The calculation involves the summation of a Fourier series in which the terms have the amplitudes and phases of the observed spectra; both depend on the positions of the atoms in the crystal, but only the amplitudes are easily measurable. As Perutz and Kendrew explained, the introduction of additional heavy atoms into a crystal under investigation at sites which can be found, may make it possible to calculate phase angles directly from the observed amplitudes of the spectra given by the isomorphous crystals. One is then in the position that, from a sufficient number of measurements, one can calculate directly the electron density and see the whole structure spread out before one's eyes. However, the feat involved in the calculations described two years ago was prodigious - tens of thousands of reflections for five or six crystals were measured to provide the electron-density distribution in myoglobin and haemoglobin. More often, and with most crystals, the conditions for direct electron-density calculation are not initially met and one's progress towards the final answer is stepwise; if some of the atoms can be placed, particularly the heavier atoms in the crystal, calculations, necessarily imperfect, of the electron density can be started from which new regions in the crystal may be identified; the calculation is then repeated until the whole atomic distribution is clear. At the outset of my research career, two essential tools became available, the Patterson synthesis and Beevers and Lipson strips. Patterson showed that a first Fourier synthesis calculated directly from the raw data, without phase information, represented the inter-atomic vector distribution in the crystal structure<sup>3</sup>. This was capable, in simple structures, of showing the whole atomic arrangement and, in more complicated ones, at least of indicating the positions of heavy atoms. Beevers and Lipson strips<sup>4</sup> provided the means for a poor crystallographer to start calculating - each strip represents the wave-like distribution corresponding with a single term. I still have the letter Beevers and Lipson wrote offering me a box for £ 5 - I bought it.

Our early attempts at structure analysis now seem to be very primitive. The crystal structures of cholesteryl chloride and bromide proved not sufficiently isomorphous to solve by direct-phase determination. We moved over to cholesteryl iodide, where the heavier atom was both easier to place in the crystal from the Patterson synthesis (Fig. 1) and contributed more to the scattering<sup>5</sup>. Harry Carlisle showed it was possible to place the atoms in three

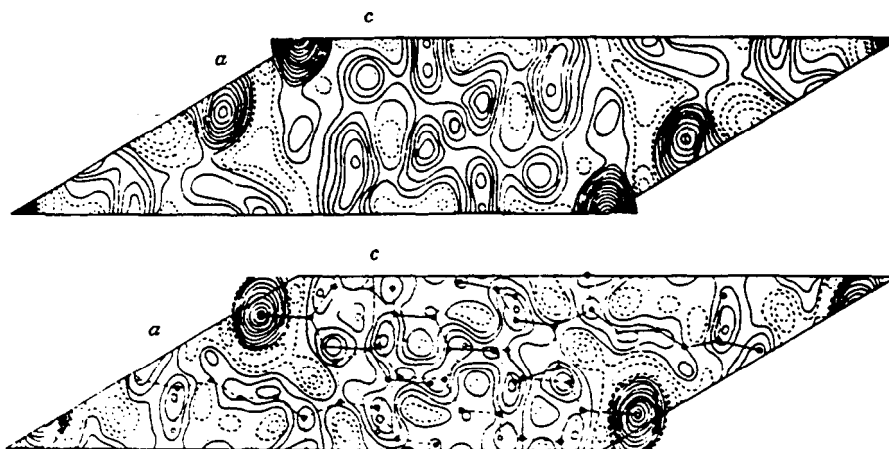


Fig. 1. The initial stages of the X-ray analysis of cholesteryl iodide. Above Patterson projection along *b*. The heavy peaks indicate I - I vectors. Below, electron-density projection calculated with terms given the phase angles of the iodine contributions. The outlines of two sterol molecules related by a two-fold screw axis are drawn in.

dimensions by calculating sections and lines in the three-dimensional electron-density distribution with phases derived at first from the iodine contributions alone; it took him months to make calculations on Beevers-Lipson strips which now would take fewer hours. The atomic arrangement found completely confirmed the sterol formula as revised by Rosenheim and King and Wieland and Dane, following Bernal's first X-ray measurements<sup>6</sup>. We sought for a compound of more unknown structure.

We were encouraged to try our operations on penicillin by Chain and Abraham before ever the antibiotic itself was crystallised; I grew crystals for X-ray analysis from 3 mg of the sodium salt flown over during the war from the Squibb Research Institute to Sir Henry Dale; the crystals were grown under the watchful eyes of Kathleen Lonsdale, who brought them to me from London. Later, we also grew crystals of potassium and rubidium benzylpenicillin, hoping again for an isomorphous series. But first the sodium salt was not isomorphous with the other two, then the potassium and rubidium ions were in such positions in the structure that they did not contribute to many of the reflections. The solution followed from a comparison of very imperfect maps calculated for the two series. But the methods by which these maps were obtained were more a consequence of the ingenuity of my collaborators, Charles Bunn and Barbara Low, combined with our low computing

power, than general processes for structure solving today<sup>7</sup>. This little structure would now be handled quite differently, by heavy-atom methods, using one crystalline form alone. For example, by biosynthetic methods it is easy to introduce a heavy atom such as bromine into the molecule; the heavy atom can be placed unambiguously in three dimensions by the calculated Patterson distribution; the remaining atomic positions appear with no difficulty at all in the following three-dimensional electron-density distribution and, on refinement, the atoms appear beautifully clearly. The example shown in Fig. 2 is actually bromophenoxymethylpenicillin<sup>8</sup>, prepared for a study of the differences between benzylpenicillin and the acid-stable penicillins by Dr. Margreiter. But with molecules of this sort it is really not necessary to introduce an

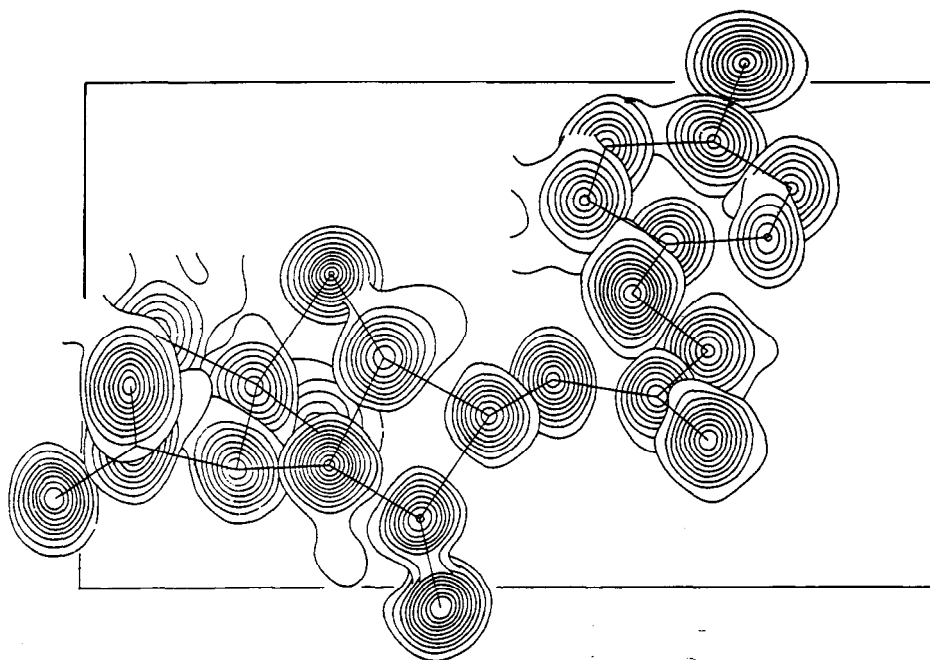


Fig. 2. *p*-Bromophenoxymethylpenicillin. Electron density near the atomic centres shown projected on the *c* plane. The contours are drawn at intervals of  $1 e/A^3$  (K.J. Watson).

extra heavy atom at all. The sulphur atom present is itself relatively heavy enough to operate for structure finding purposes in sodium benzylpenicillin only a little less effectively than bromine. As Maslen and Abrahamsson showed, in relation to penicillin V itself<sup>9</sup> and cephalosporin C (ref. 10), with

only a little more trouble one can place the sulphur atom unambiguously in the crystal structure and use the vector distribution relative to this to find the remaining atoms. Fig. 3 shows the electron density in the crystal of cephalosporin Cc, a very interesting antibiotic prepared by Abraham and Newton. Here it is easy to see the chemical structure, a four-membered  $\beta$ -lactam ring attached to a six-membered sulphur-containing ring-derived most probably like the corresponding penicillin, from  $\delta$  - ( $\alpha$ -aminoadipoyl)-csteinyll valine but in an oxidised form. The natural antibiotic, cephalosporin C, loses on hydrolysis an acetyl group to give the more stable Cc molecule with the carboxyl group combined in a five-membered lactone ring. It crystallises with one molecule of acetic acid of crystallisation, fitting in between the long chains in the crystal".

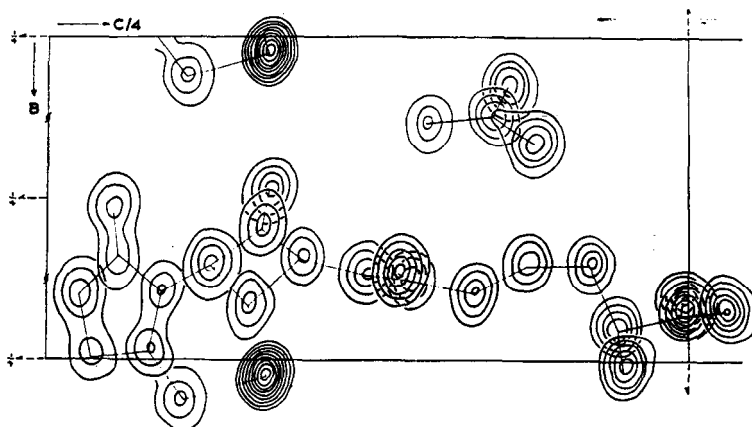


Fig.3. Cephalosporin Cc. Electron density near the atomic centres shown projected along *a*. Contours at intervals of  $2 e/A^3$  (R.Diamond).

The X-ray scattering effect of sulphur is roughly one sixth of that of the rest of the molecule of cephalosporin C, approximately the same as that of cobalt in relation to vitamin B<sub>12</sub>, cyanocobalamin. At the time that Dr. Lester Smith brought us his first red crystals of this, the antipemicious anaemia factor, shortly after its first isolation by Dr.Folkers and his colleagues, we knew nothing at all about the molecule. Two X-ray photographs, taken overnight, showed that it had a molecular weight of the order of 1500. It is of such complexity that even its analytical formula follows best from its X-ray analysis. Formally, the process of structure determination followed the course

outlined earlier - 3D Patterson, 3D Fourier, atom sorting in rounds of calculation - the outline hardly gives an accurate impression of the stages of confused half knowledge through which we passed. Again an important part in the analysis was played by our having a view of the corrin nucleus surrounding the cobalt atom in quite different crystals, the cyanocobalamin crystals and also some, or perhaps I should say one, crystal of a hexacarboxylic acid derived by degradation from them by Cannon, Johnson and Todd<sup>12</sup>. And we were greatly helped by friends with computers; on a particularly happy day Kenneth Trueblood, on a casual summer visit to Oxford, walked into the laboratory and offered to carry out any additional calculations we needed on a fast computer in California, free and for nothing and with beautiful accuracy. Extracts from the calculations he carried out still provide some of the best examples of the processes I have been describing, of the gradual appearance of precise peaks marking atomic positions through stages in the electron-density calculations<sup>13</sup>. Examples are given in Fig. 4.

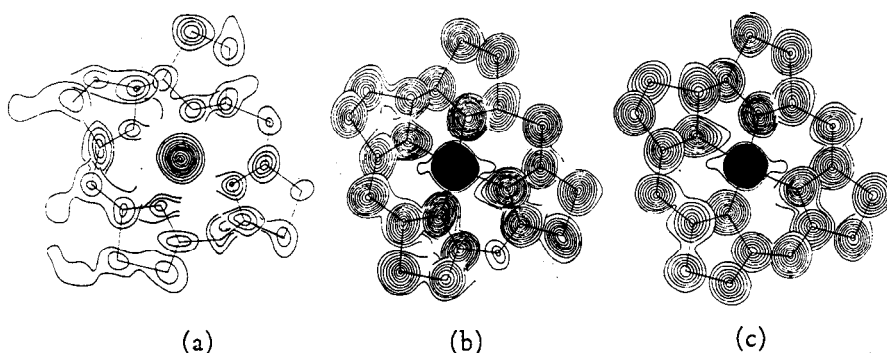


Fig.4. Electron-density peaks over the corrin nucleus in the hexacarboxylic acid at different calculation stages. Terms phased on contributions calculated for (a) cobalt, (b) with the nucleus atoms less C-10, (c) cobalt with all the nucleus atoms. Contours at  $1 e/\text{\AA}^3$ , except over Co.

Today our best evidence for the structure of the nucleus in  $B_{12}$  comes from the X-ray analysis of cobyrinic acid, Factor V Ia, orange-red crystals isolated in very small quantities from sewage sludge by Bernhauer, Wagner and Wahl (ref. 14). The X-ray analysis was achieved by what still seems to me a remarkable operation. The crystals are monoclinic,  $P2_1$ , with two molecules in the unit cell, and X-ray photographs, taken of them with copper  $K\alpha$  -radiation

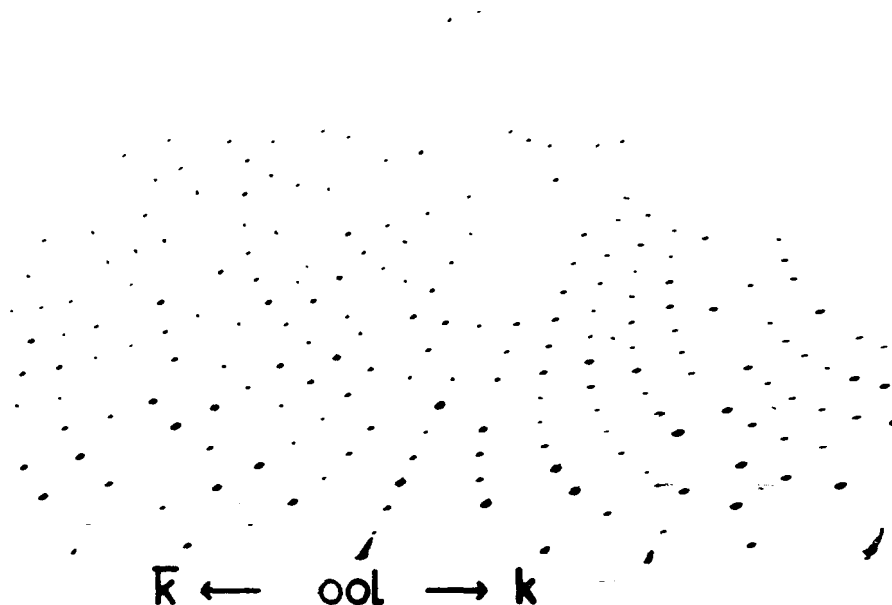


Fig.5.  $okl$  reflections from Factor V Ia. Note dissymmetry across line marked  $\longleftrightarrow$ .

show very markedly the effects of anomalous dispersion - compare Fig. 5,  $F_{hkl} \neq \overline{F_{hkl}}$ . The effects are due to a small phase change introduced by the scattering at the cobalt atom which has an absorption edge near the wave length of copper  $K\alpha$ -radiation. They make it possible to use yet another method of phase angle determination first suggested by Bijvoet, Ramachandran and others<sup>15,16</sup>, and illustrated in Fig. 6. By measuring the intensities of both  $F_{hkl}$  and  $\overline{F_{hkl}}$  reflections, Dale and Venkatesan were able to assign rather accurate phase angles to 1994 reflections - about half the total observed. The calculation requires a knowledge of the cobalt atom position, easily found from a Patterson synthesis. The first three dimensional electron density map calculated with just these 1994 terms showed the whole molecule and crystal structure clearly defined; the chemical formula of cobyric acid (I) could have been written with very little hesitation from this map alone although, strictly, it shows only part of each atom (Fig. 7a). With further rounds of calculation the full electron density is introduced; even many of the hydrogen atom positions then appear as individual peaks as in Fig. 7b. It is clear that the molecule is present in the form of an aquo- or hydroxy-cyanide, where the cyanide group

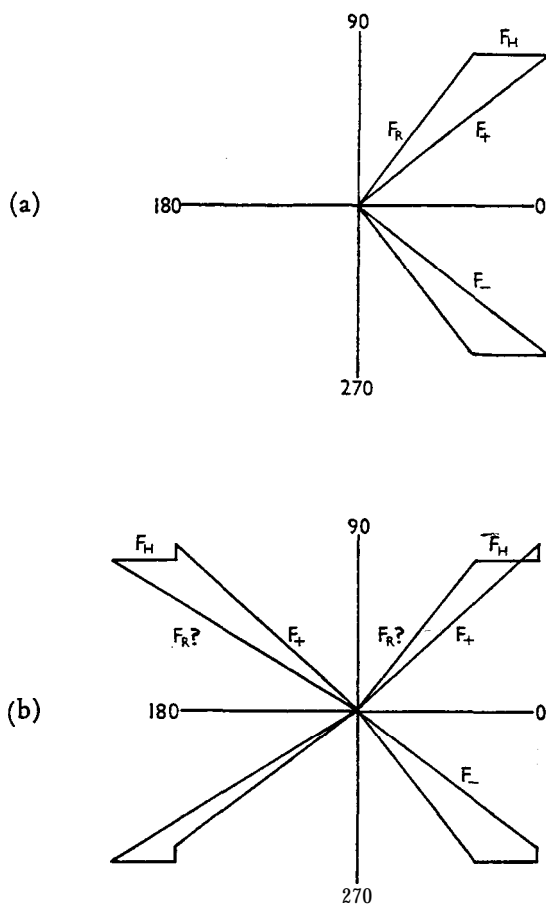
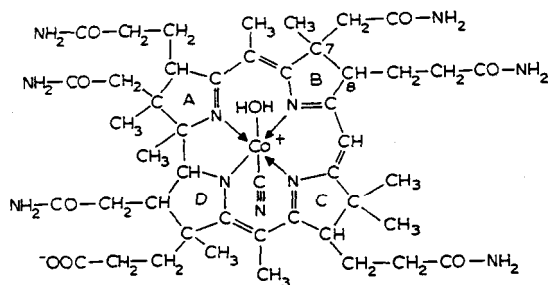


Fig. 6. Phase determination in a monoclinic crystal such as cobyric acid. (a) In a pair of isomorphous crystals. Here where  $F$  values for the substituted and unsubstituted crystals and for the heavy atoms ( $F$ ,  $F_R$  and  $F_H$  respectively) can all be found there is an ambiguity in the phase-angle determination about the line 0-180 in the Argand diagram. (b) In a single crystal containing an anomalous scatterer,  $F_{hkl}$  and  $\overline{F_{hkl}}$ ,  $F_+$  and  $F_-$  can be distinguished, but in general  $F_R$  is unknown, and the phase angle is ambiguous about the line 90-270°. In the study of cobyric acid, the phase angle nearer  $F_H$  was chosen.

replaces the nucleotide in cyanocobalamin and the water molecule the cyanide group<sup>17</sup>.

Many details of the crystal structure of factor V Ia are interesting in relation to its chemistry (Fig. 8). Thus theamide groups on the periphery of the molecule are all hydrogen-bonded to those of neighbouring molecules either



directly or through water molecules, except one, that on ring B. This is turned inwards to the hydrogen bond with the water molecule on the cobalt atom; the forces involved are sufficient to distort the position of the  $\beta$ -carbon atom, C-7, to which it is attached. At the same time, the amide nitrogen atom is brought close to C-8 with which it could readily react to form the lactam

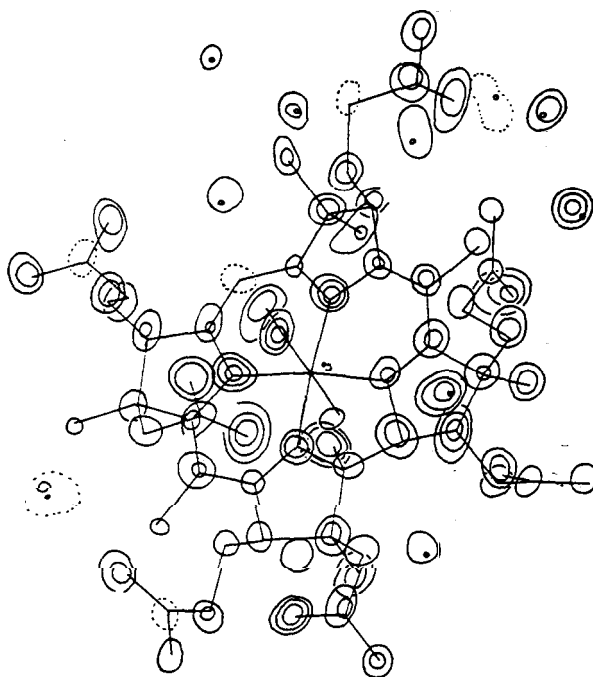


Fig. 7a. Electron density peaks from  $\rho_1$  calculated for cobyrinic acid, factor V 1a. The contours are at intervals of  $1 \text{ e}/\text{A}^3$ , starting with the 2 electron contour. The 1-electron contour is dotted where necessary to define an atomic position.

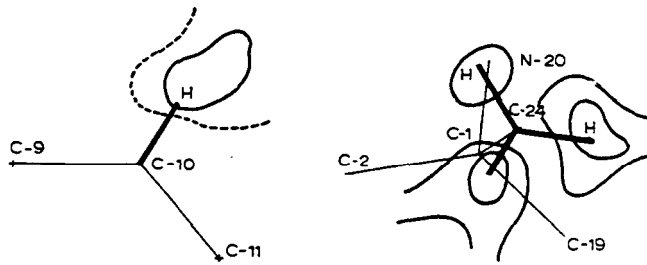


Fig. 7b. Part of difference map  $\Delta\rho_6$ , calculated for cobyric acid showing density due to hydrogen atoms attached to C-24 and C-10.

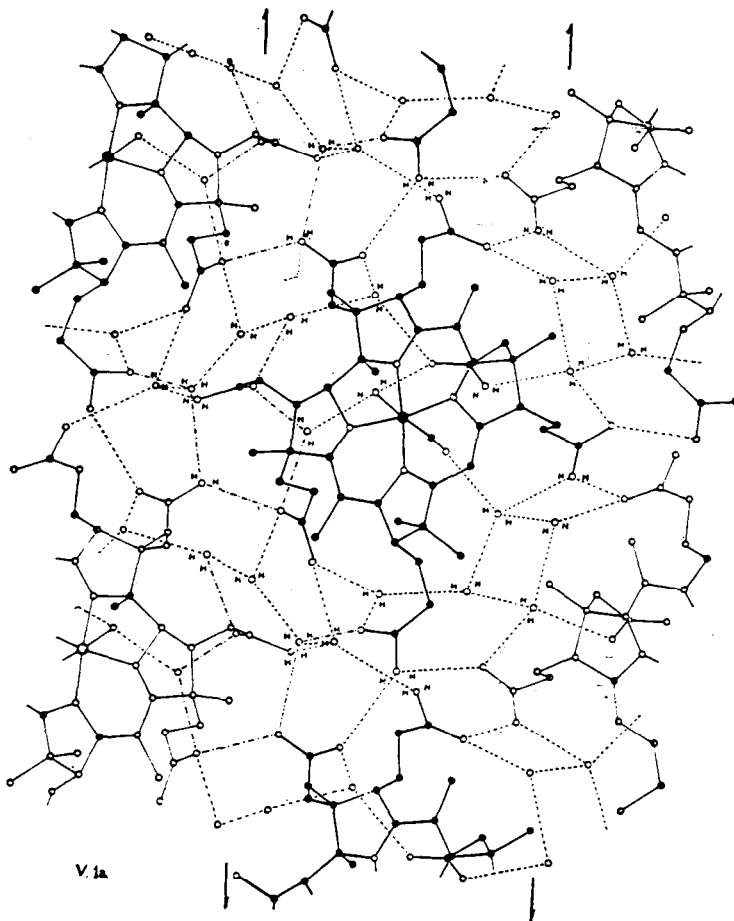
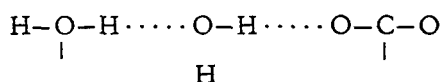
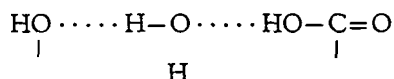


Fig. 8. The crystal structure of cobyric acid projected along  $a$ . Molecules centred at  $x$  are shown in strong lines, those at  $\bar{x}$  in thin lines. Hydrogen bonds are dotted.

ring observed in the hexa acid, mentioned earlier. That the oxygen atom attached to cobalt is part of a water molecule, not a hydroxyl group, is suggested by the fact that these orange-red crystals separate at acid pH (*cf.* the haemoglobins and myoglobin). In our crystal this oxygen atom makes a second contact through a water molecule with the carboxyl group attached to a neighbouring molecule. It would, in fact, be very easy to change the system



to



by a movement of two hydrogen atoms within the crystal; it would be interesting to see if this movement occurs, as in Rochelle salt, under the influence of an electric field.

The interatomic distances in the inner ring of factor V Ia, which has been called the corrin ring, conform very closely with the distances proposed<sup>12</sup> for a structure containing six resonating double bonds, so closely as to leave almost no doubt of the correct formulation of its chemical structure (*cf.* Fig. 9). It was all the same, quite a moment in my life when J. D. Dunitz showed last summer at the Royal Society very closely similar figures derived by the X-ray analysis of the nickel corrin derivative synthesized early this year by Eschenmoser and his coworkers<sup>18</sup>. The molecule as a whole is much smaller (Fig. 10) but the identity of the nucleus with that in cobyric acid is certain.

Very recently, Dunitz and Meyer have refined the nickel corrin structure through several more stages; their latest interatomic distances shown in Fig. 11 are now so close to the earlier proposed theoretical figures that one begins to feel that the small remaining deviations are likely to be real, e.g. in C-N 9-21 and 11-22. There is a tendency in the natural series also for these bonds to be longer than the distances given by the simple-theory at first proposed.

One of the features of the corrin nucleus in the natural compounds is that even the inner ring is not quite planar. The same is true of the synthetic nucleus. In nickel corrin, the distortion is small but very regular and tetrahedral in character in relation to the nickel atom; alternate nitrogen atoms, bonded to it, lie above and below the least squares plane passing through the nickel and four nitrogen atoms. The non-planarity of the system as a whole no doubt derives from the stereochemistry of the five-membered ring C-1, C-19,

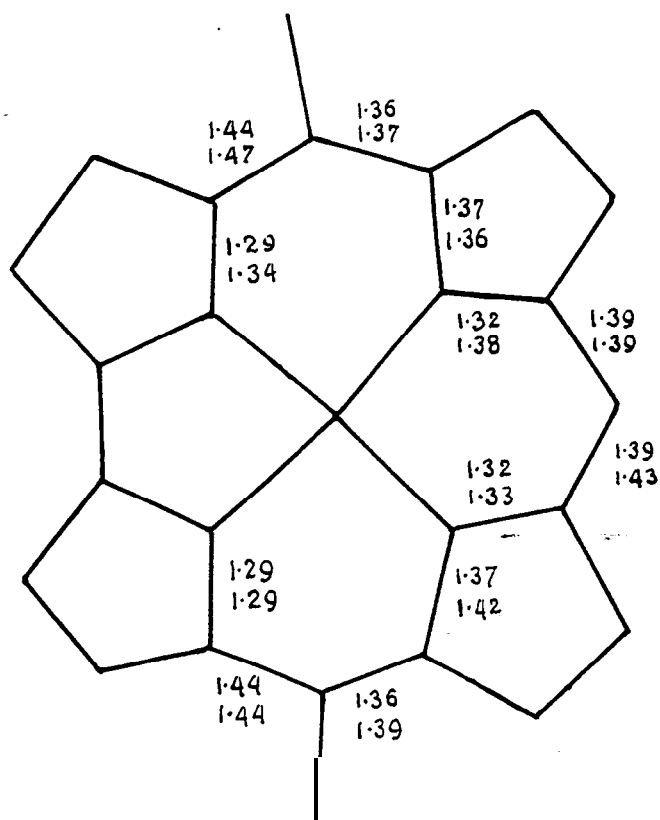


Fig. 9. Interatomic distances (below) found from  $\rho$  for cobyric acid compared with those suggested for a system containing six resonating double bonds.

N-23, Ni, N-20. In the natural corrins, the deviations are rather different in compounds with or without the nucleotide. In the unsubstituted compounds, and particularly in cobyric acid, the deviations are in the same sense as in the nickel corrin derivative and as in this molecule, C-5 and C-15, which carry C-35 and C-53 respectively, are on opposite sides of the plane containing the cobalt and four inner nitrogen atoms. In the nucleotide-containing compounds these two atoms are on the same side of the inner plane. In both series, the most marked deviations occur in the region of C-35 which is in a very overcrowded situation.

Cobyric acid is the natural precursor of the most remarkable molecule of our series, Co-5'-deoxyadenosyl-cobalamin, the coenzyme discovered by Barker, which ought, most properly to be called vitamin B<sub>12</sub>. Crystals of

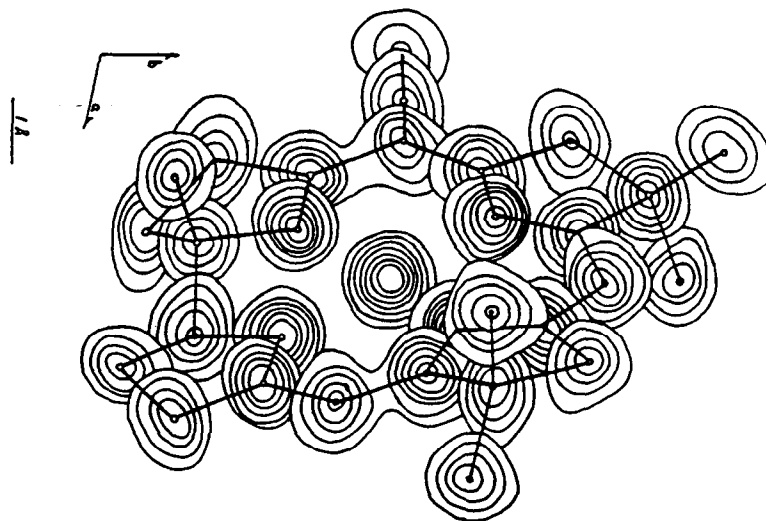


Fig. 10. Electron-density peaks over the atoms in the nickel corrin derivative (Dunitz and Meyer).

this compound were grown from water in capillary tubes by Dr. Galen Lenhert in 1960 from material supplied by Dr. Barker, and X-ray photographs were taken of them *in situ*, in their mother liquor. Again the intensities of the X-ray diffraction spectra were measured, Patterson and electron-density distributions were calculated, atoms belonging to the best known parts of the cobalamin molecule being placed first in the calculations. At this point, I should pause to say that a great advantage of X-ray analysis as a method of chemical structure analysis is its power to show some totally unexpected and surprising structure with, at the same time, complete certainty. Fig. 12 illustrates the structure we found - first, the electron-density map calculated over the region of uncertain structure, with the known part of the molecule placed - then the whole atomic arrangement that is derivable from the completed map. Clearly, in this molecule, cobalt was shown to be attached direct to the 5'-carbon atom of the adenosyl residue as in formula II<sup>9</sup>. There followed directly an explanation of the observed great instability of the molecule to light and cyanide ions - instability which had led to the failure of earlier investigations to recognise its existence and to isolate, in its place, cyanocobalamin.

The detailed geometry of the coenzyme molecule as a whole is fascinating in its complexity. The peculiar form of the corrin ring with the direct link

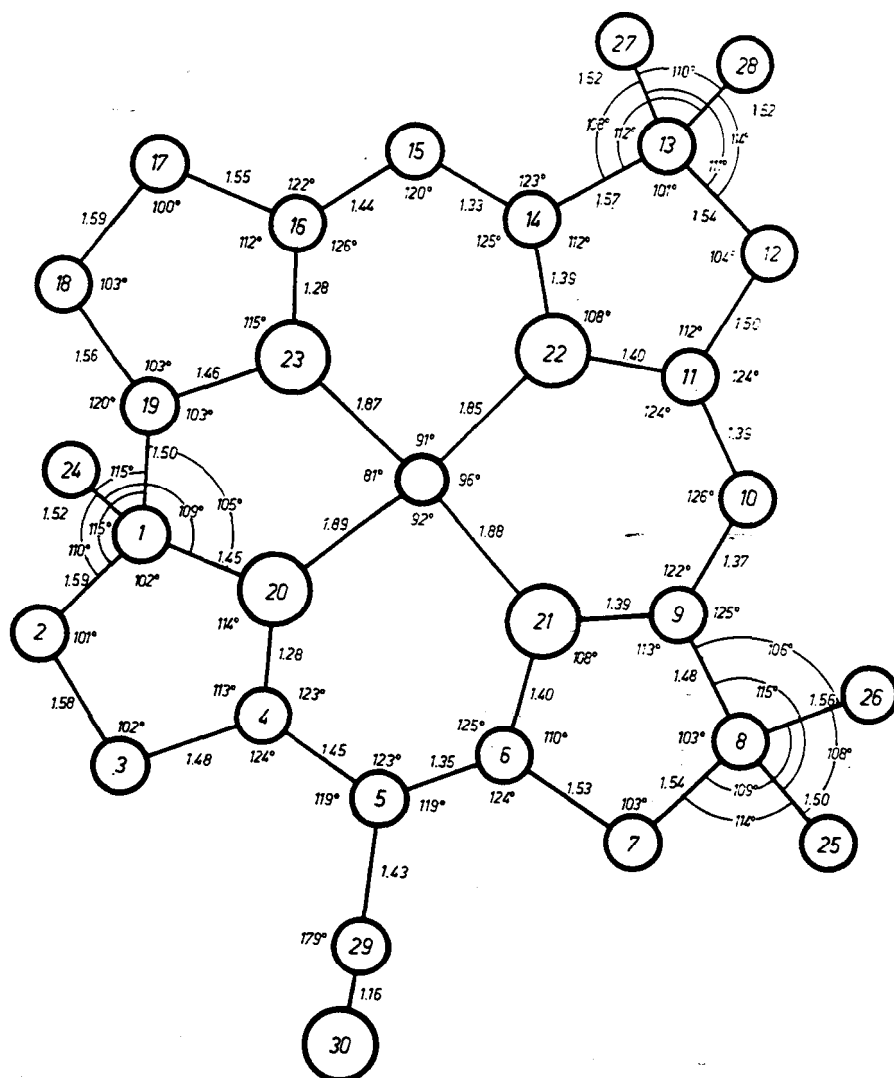


Fig.11. Interatomic distances measured in the nickel corrin derivative (Dunitz and Meyer).

between rings A and D and the position of the methyl group at C-24 make the two sides of the corrin ring system very different stereochemically and the differences are reinforced by the positions of the methyl group and acetamide and propionamide residues attached at the carbon atoms (*cf.* Fig. 13). Approach to the cobalt atom from the lower side of the molecule is hindered by

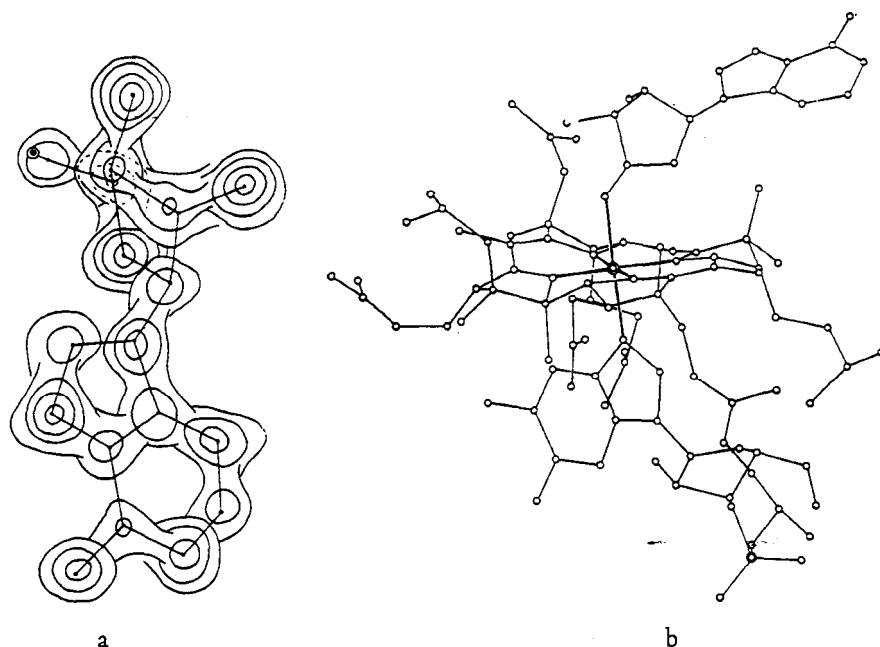


Fig. 12. (a) Electron-density peaks over the adenosyl residue calculated from X-ray data on dimethylbenzimidazole cobamide coenzyme. The calculation illustrated was one in which phase angles were computed from the positions of all the atoms in the molecule except those shown. Although only part of the electron density appears, the relative weights of the atoms are in agreement with their chemical structure. (b) The atomic positions found for the coenzyme molecule projected along the crystallographic *b* axis.

the methyl group C-24 and all groups attached here are rather loosely bound and easily displaced. At the upper site, on the other hand, attachment of a wide variety of ligands is possible; once in position they are enclosed by non-polar groups, the methyl and methylene groups projecting normal to the plane of the ring. Here they may be positively protected from immediate reaction with the surrounding solvent, for use when required in different biochemical transformations. In terms of this structure, one can begin to understand some of the uses to which the cobalamin nucleus is put in nature, for example, its part in the transfer of methyl groups. In the laboratory, methylcobalamin can be made by a series of reactions involving the reduction of aquocobalamin to a compound, probably cobalamin hydride, which easily exchanges with methyl compounds such as diazomethane or methyl sulphate; in nature, reduction also seems necessary for methyl transfer; the experiments of D. D.

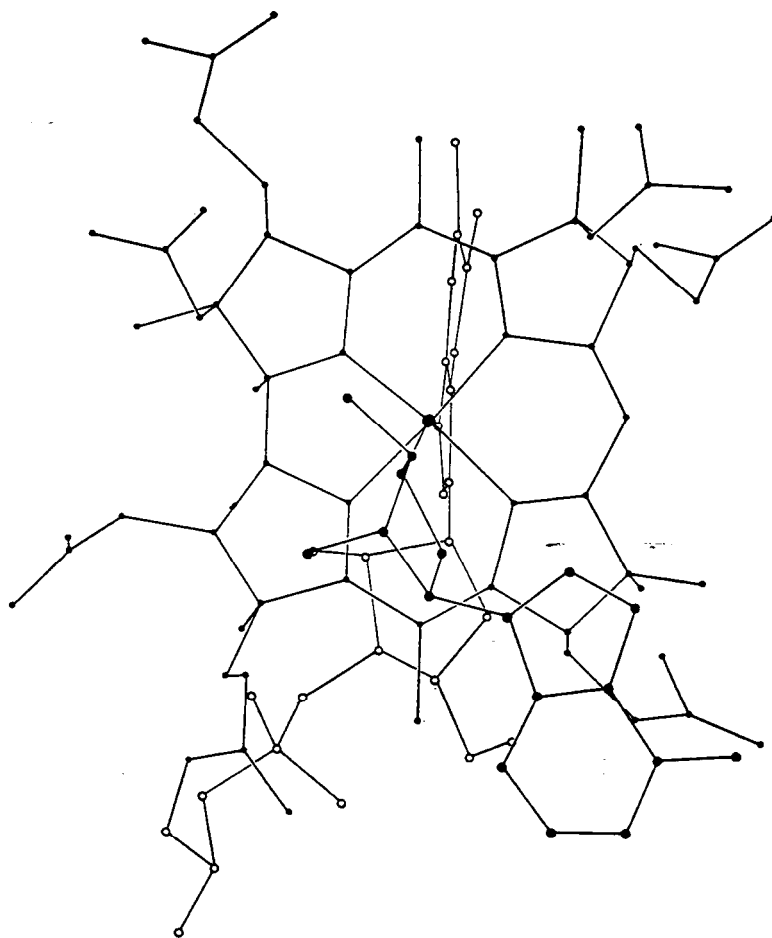


Fig. 13a. The positions found for the atoms in the coenzyme molecule projected (a) onto the least square squares plane passing through cobalt and the four inner nitrogen atoms, (b) and (c) across this plane.

Woods and his colleagues strongly suggest that methylcobalamin is the actual intermediate in one of the pathways by which methyl groups are transferred from methyltetrahydrofolate to homocysteine to form methionine<sup>20</sup>. other effects of the B<sub>12</sub> coenzymes are still not easy to explain in detail, particularly the part they play in the isomerisation reactions which led to their discovery. How so complex a system can effect the simple and fundamental migration process that changes methylmalonate to succinate, for example, is still a problem. One can see features of the system that might be important, the variable

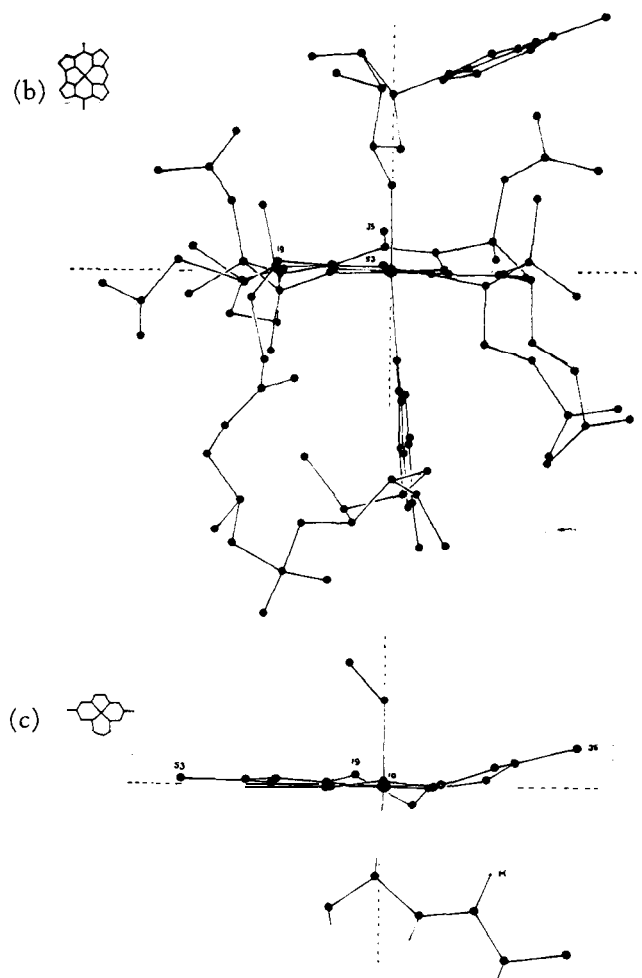


Fig. 13b and c.

valency of cobalt and the possibility of changes within the corrin nucleus, the fact that the cobalt to carbon bond readily breaks to give rise to free radicals<sup>21</sup>. But one would like to be able to observe the molecule in action, held in the meshes of the necessary enzyme, and this may be both possible and necessary for the detection of unstable intermediates in the reaction path.

There have been some practical results following from our knowledge of the structure of the molecules so far described. Penicillin V has been synthesised by Sheehan and Henery-Logan<sup>22</sup>. The coenzyme, a few years ago obtainable with difficulty in milligram quantities, can now be prepared very easily from

cyanocobalamin - Dr. Lester Smith has made crystals 0.5 cm across. Soon, and no doubt very beautifully, cyanocobalamin itself will be synthesised by R.B. Woodward. But microorganisms are even more efficient than chemists at synthesis of molecules of this magnitude and will most likely continue to provide the main supplies of these compounds to be used in medicine. What we most hope to gain from knowledge of the structure and synthesis of these molecules is a complete understanding of their biogenesis and the part they play in metabolism. This should enable natural processes to be controlled when they go astray.

I should not like to leave an impression that all structural problems can be settled by X-ray analysis or that all crystal structures are easy to solve. I seem to have spent much more of my life not solving structures than solving them. I will illustrate some of the difficulties to be overcome by considering our efforts to achieve the X-ray analysis of insulin.

Insulin is a molecule of weight about 6000, larger than any so far described, though small if considered as a protein and compared with myoglobin and haemoglobin. Although its complete chemical structure is now known from Sanger's researches<sup>23</sup>, it is quite unclear what exactly the molecule does that makes it so necessary to life. Our hope, following the kind of reasoning outlined above, is that a complete knowledge of the molecular geometry, how the peptide chains fit together within the molecule and the molecules within crystals, may make it possible for us to understand and control its behaviour. It crystallises in a number of different modifications and their very different degrees of complexity seem significant. In acid insulin salts the molecule appears to be dimeric, the two insulin molecules in one dimer being related to one another by two-fold axes of symmetry<sup>24</sup>. Crystallographic two-fold axes also relate insulin molecules in a cubic metal-free form of insulin first observed by Abel<sup>25</sup> in 1927. These crystals have so far only been obtained as extremely small rhombic dodecahedra; their habit and symmetry suggest that here six insulin dimers may be grouped in a larger aggregate, with symmetry 23, reminiscent of structures proposed for the smaller viruses<sup>26</sup>. But it is difficult to get sufficient X-ray diffraction effects from these crystals to check the hypothesis. In all insulin crystals and in solutions which contain adequate zinc, or some other similar bivalent metal ions, a definite aggregate of six insulin molecules appears; having, indeed, the molecular weight 36000 first recorded by Svedberg<sup>27</sup> in 1935. This insulin hexamer corresponds with the unit cell in the rhombohedral crystals first investigated and is the asymmetric unit in the monoclinic form which appears in the presence of phenol. The proportion of

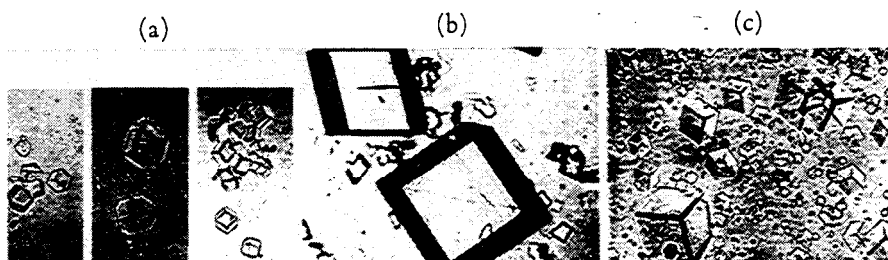
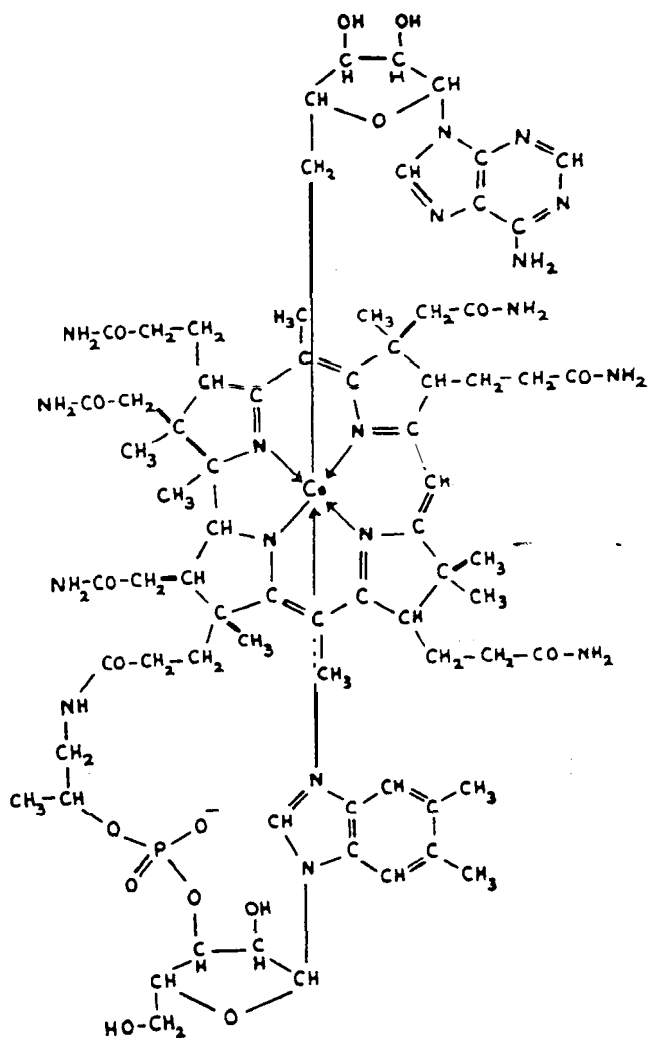


Fig. 14. Crystals of insulin (a) cubic; (b) monoclinic and (c) rhombohedral (taken from Schlichtkrull, *Insulin Crystals*, 1958).

zinc to insulin molecules is 2:6 and the symmetry relations strongly suggest that the zinc is situated on the three-fold axes around which are arranged insulin molecules related to one another both by two- and three-fold axes. The symmetry relations have been explored by the use of the functions described by Rossman and Blow<sup>28</sup>. In the presence of halide, a slightly different packing is adopted with 4 Zn:6 insulin molecules. All these crystals give beautiful X-ray diffraction effects from which it ought to be possible to solve the structure to atomic resolution. Here the zinc present is much too light, about 0.01 in scattering effect, to be used for phase determination as we used cobalt in B<sub>12</sub>. We need to introduce additional heavy atoms into the crystal structure. This is not difficult to do in quantity. But it seems very difficult to limit the crystal uptake to the one per insulin molecule which should be easy to place by X-ray methods, either by chemical reaction or by cocrystallisation, the methods adopted by Kendrew and Perutz. We are driven either to try to solve an initially complex problem, concerned with the heavy atom distribution in the crystals, or to do more chemistry in the hope of binding one heavy atom alone to each insulin molecule. In practice, I suppose, we shall attempt both. There are encouraging features of our present experiments that lure us on - some of our heavy atom containing crystals show very marked anomalous absorption effects, from which some initial phasing evidence can be obtained. But the electron-density maps we have so far calculated are far too imperfect and difficult to interpret for me to present them today\*.

It will be clear from all that I have said so far that my research owes a debt I cannot adequately pay to the work of others, my colleagues who have provided many of the ideas I have used and many interesting examples of similar analyses, my collaborators, without whose brains and hands and eyes very little would have been done. I should also like to remember here today many whose friendship and encouragement I have greatly enjoyed. I will name three particularly, W. T. Astbury, I. Fankuchen and K. Linderström-Lang, because they would themselves so much have enjoyed this occasion.

\* Subsequent examination suggests these maps are not so imperfect as I supposed and are interpretable.

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