X-Ray Single Crystal Photographs of Insulin

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SINCE insulin was first prepared crystalline[1] in 1926, several efforts have been made to obtain X-ray photographs of the crystals. The first attempts of W. H. George[2] by the powder method failed to show any pattern indicative of a crystal structure, and though later long spacings were reported by G. L. Clark and K. E. Korrigan[3], it was impossible to base any unambiguous interpretation on their results. The fact that pepsin could be made to give a single crystal X-ray diffraction pattern[4] suggested that the problem of insulin, which is in many respects a more stable crystalline species, could be attacked in the same way if large enough crystals could be grown. This was made possible by D. A. Scott's study of the crystallisation of insulin in the presence of salts of zinc and of other metals[5].

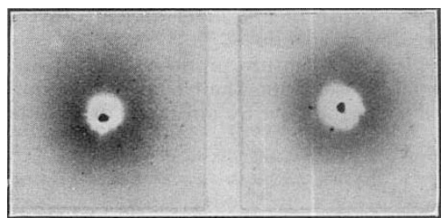
The crystallisation was therefore carried out by a modification of Scott's method from a phosphate buffer solution containing a little acetone and some zinc chloride at a *p*H of 6·2–6·5. The solution was cooled very slowly from 50° to room temperature over a period of three days, at the end of which time sufficiently large crystals had grown.

The crystals have the form of very flat rhombohedra which often grow in pairs united at the ends of their trigonal axes. The larger ones present the appearance of six lobed stars and are as much as 0·2 mm. across and 0·05 mm. thick. These show a positive uniaxial figure when viewed along the trigonal axis. The crystals prove to be perfectly stable in air (unlike pepsin) with unchanged birefringence and reflecting power, and it was accordingly possible to examine them dry by X-ray methods.

Three series of X-ray photographs have been taken on three separate crystals, one rotating about the trigonal axis and the others about the normals to $(10\bar{1}0)$ and $(11\bar{2}0)$. Examples are shown in Fig. 1. Copper $K\alpha$-radiation was used and exposure times of about 15 hours for a single 5° oscillation photograph with a plate distance of 6 cm. The crystals have so far proved unaltered by exposure for more than 100 hours to X-radiation. The photographs taken indicate a simple rhombohedral cell of a 44·3 A. and $\alpha = 115°$ correct to about 2 per cent. This referred to hexagonal axes corresponds to a cell three times as large with $a = 74·7$ A., $c = 30·6$ A., which shows no halvings but those required by the rhombohedral lattice. The structure may also be described in terms of a pseudocubic body-centred cell twice the size of the primitive cell with $a = 47·7$ A., $\alpha = 103°\ 6'$. No planes of symmetry are present and the space group is therefore $R3$. The cell molecular weight calculated for the primitive rhombohedral cell and the density 1·315 measured by Dr. Eyer[6] is 39,300 ± 800. (Density measurements on the actual crystals used gave 1·306 ± 0·003.) As Abel has measured the water lost by heating the crystals at 104° in a vacuum at 5·35 per cent of the air-dried weight[7], the weight of insulin in the cell (cell molecular weight—water of crystallisation) may be deduced as 37,200, which is very close to the weight of one molecule of insulin reported by The Svedberg[8].

It therefore appears that the crystal unit cell contains only one molecule of insulin, although a cell containing $3n$ sub-molecules is not excluded by the X-ray data. The laws of crystal symmetry rigorously applied would require this molecule to have trigonal symmetry, but it is possible also that the crystal attains apparent trigonal symmetry by a statistical regularity of arrangement of molecules about the lattice points, or that the X-ray effects so far observed are first approximation mass effects to which further work may add a fine structure, due to the arrangement of the atoms within the molecules, which our methods are as yet insufficiently delicate to detect. The measurements obtained do, however, fix quite definitely the arrangement of the molecules with respect to one another and their approximate size and shape, since this follows directly from the crystal lattice, while the variation of the intensities of the spectra strongly suggests that the arrangement of atoms within the molecules is also of a perfectly definite kind.

The crystal structure of insulin is of an eight co-ordination type, as the possible reference to the pseudo-cubic body-centred cell of twice the size most clearly indicates. Each insulin molecule is



(*a*) (*b*)

FIG. 1. X-ray photographs of crystalline insulin. (*a*) Rotation axis normal to $(11\bar{2}0)$, beam direction 0°–5° from ‖ $(10\bar{1}0)$. (*b*) Rotation axis [0001], beam direction 0°–5° from ‖ $(10\bar{1}0)$.

surrounded by eight others, two at the short distance of 30 A. above and below along the trigonal axis, and six at the longer distance of 44 A. along the edges of the primitive rhombohedron. The shape of the molecule therefore appears approximately as an oblate spheroid of diameters 44 A. and 30 A. D. A. Scott gives the atomic percentage of zinc in insulin crystals as 0·00795, or 3 atoms of zinc per molecule of insulin[9]. It therefore seems reasonable to suggest that these atoms are required to bind the molecules into nets parallel to the c plane, one between each pair of insulin molecules along the six points of contact, the closer linkage along the c axes being due to other causes. This would provide some explanation of the rôle played by zinc and other bivalent metals in promoting the crystallisation of insulin.

It is of particular interest to compare this crystal structure of insulin with that which may be deduced for pepsin from the X-ray measurements previously reported. It has been found that the true cell of pepsin has a c dimension three times as long as that at first suggested, namely, 461 A., and that the structure should probably be referred to rhombohedral axes[10] $a = 162$ A., $\alpha = 23°\ 50'$. The first order of the reflections from the c plane to occur is, however, the 45th, while the strongest order is the 48th, which indicates that the most marked periodicity along c is one of only 9·6–10·2 A., very much the

same as the distance—10 A.—between layers of atoms along the c axis of insulin. It seems significant, further, that the length of a in pepsin—67 A.—referred to the original hexagonal axes, is so similar to that of insulin—74·7 A.—when given hexagonal axes. These two dimensions define a crumpled layer structure in which the molecules are arranged in networks of six-sided rings of the non-planar cyclohexane type which occurs, for example, in diamond and wurtzite. From the side of such a ring projected on to (0001), $a/\sqrt{3}$, or 38·7 A. in pepsin, and the thickness of the order of 10 A., a length for one radius of the pepsin molecule may be calculated = 20 A. In insulin, the layers are so arranged that atoms in one fall as nearly as possible into the spaces of the one below, which makes a very compact structure. In pepsin we may imagine that the layers of rings are slid relatively to one another, to bring atoms of the lower ring directly beneath those of the upper ring in such a way that each is approximately tetrahedrally co-ordinated. The effective depth of a single layer is then equal to the thickness of the ring system plus the diameter of a single molecule, and may be calculated to be 51·2 A., with a spheroidal pepsin molecule of diameter 41·2 A. in this direction. A combination of the crystallographically possible ways of sliding the ring systems is able to give the required length of c, nine times that of the depth of one layer and fifteen times the c dimension of insulin.

This kind of structure proposed for pepsin is of a very much looser type than that of insulin. Each molecule is surrounded by only four others and there are large channels through which free movement of water and dissolved substances may occur within the crystal structure. On drying, such a structure would collapse, in agreement with the fact that, in contradistinction to insulin, the crystals of pepsin lose their birefringence on exposure to air and only show crystalline X-ray diffraction effects when immersed in the mother liquor. Various observations[11] suggest that loose 4 co-ordination structure of this kind may be general among certain classes of protein crystals which belong either to a hexagonal type with an axial ratio about 2·3 similar to that of pepsin, or to a cubic type which shows diamond cleavages. Wherever the attraction between the adjacent protein molecules is of the same order of magnitude as that between the protein molecules and the medium, a low co-ordination structure type may be expected. In insulin, on the other hand, where the molecules can be strongly attracted together with the assistance of metal atoms, the structure is very much more condensed and shows a high co-ordination number.

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History of the Menthols

AT a joint meeting of the Chemical Society, and the Glasgow Sections of the Society of Chemical Industry and the Institute of Chemistry, held in the Royal Technical College, Glasgow, on March 15, Prof. John Read, of the University of St. Andrews, gave a lecture entitled "From Governor Phillip to *d*-neoisoMenthol: the Story of a Research, 1788–1934".

Prof. Read said that it was his intention to select a research paper and show what a rich background it possessed when given its proper setting in the world of things, men and affairs. The paper in question closed a chapter, or perhaps more correctly a book, in the history of the important chemical family of menthols. The usual source of ordinary menthol is the essential oil of the peppermint plant, *Mentha piperita*, which has been cultivated in Japan for more than two thousand years. The first mention of crystalline menthol was made in 1771 by Gambius, a Dutch botanist. It is now known that this so-called 'mint camphor' is a member of the first of four series of menthols. Prof. Read and Dr. Grubb completed the tale of these four series in the University Chemical Laboratories at St. Andrews on Christmas Day, 1933.

In tracing the trend of events which led up to this chemical climax, Prof. Read reminded his audience that Capt. Cook landed in eastern Australia, hitherto unknown, on April 29, 1770. In his "Journal" he wrote of the landing-place: "The great quantity of New Plants, etc., Mr. Banks and Dr. Solander collected occasioned my giving it the name of Botany Bay". From the earliest days, indeed, the unique vegetation of this isolated land attracted the interested attention of visitors and settlers. Two-thirds of the native Australian flora belongs to the family Myrtaceæ, which is represented in Europe by a single species. *Eucalyptus*, the outstanding Australian genus of this family, is a specialised form adapted to the barren and extra-tropical Australian areas; it developed after the separation of Australia from the tropical lands. Typically Australian, it is virile, aggressive, and an excellent colonist, with all the characteristics of youth.

Some graphic extracts from Dr. John White's "Journal of a Voyage to New South Wales" followed. Dr. White was surgeon-general to the First Settlement, under Governor Phillip, who reached Botany Bay with his fleet of marines, officials and convicts on January 20, 1788, after a voyage lasting eight months. The "Journal" shows that the voyage had its romantic aspects as well as its hardships and notes of grimness: "May 28. Departed this life, Ismael Coleman, a convict, who, worn out by lowness of spirits and debility, brought on by long and close confinement, resigned his breath without a pang. August 31. James Baker, a private marine, received 200 lashes for endeavouring to get passed on shore by means of one of the seamen, a spurious dollar, knowing it to be so. . . . Many of these young ladies [in a convent in Rio de Janiero] were very agreeable both in person and disposition; and by frequently conversing with them at the grate, we formed as tender an intercourse as the bolts and bars between us would permit of."

The fleet lingered for a month at Rio, before weighing for the Cape of Good Hope. It is said, although Dr. White does not endorse the statement,