The crystal structure of insulin

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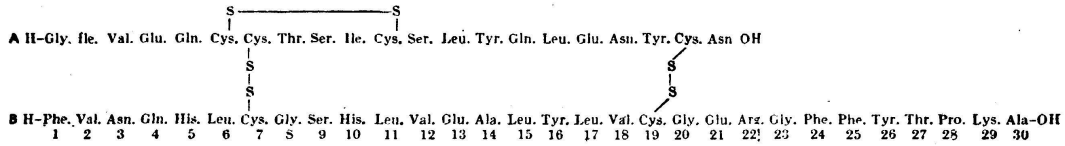
The hormone, insulin, has been found to crystallise in a number of different polymorphic modifications depending on pH, or the presence of added salts or other molecules. The crystals we can now describe in some detail are rhombohedral and were obtained in the first crystallisation of insulin by J.J. ABEL in 1925 [1]. For the next few years the crystals proved a little erratic in appearance until D.A. SCOTT [2] showed that they ordinarily contained zinc and were formed in the presence of zinc or certain other similar divalent metals. It was a sample of Boots' insulin, crystallised by SCOTT's method and given to me by Sir ROBERT ROBINSON in 1935, that led to my own interest in the insulin crystal structure.

The first X-ray photographs of insulin showed that the unit cell in the crystal was a flat rhombohedron of edge, when dry, of 47.6 Å, containing a molecular weight of protein of about 36000, sensibly the same as the weight of the insulin molecule found in solution by SVEDBERG in the ultracentrifuge [3]. As the years wore on, it became clear that this mass was composite. The crystallographic evidence first divided it, at least, by three, and then detailed work on the chemical structure by SANGER and his colleagues proved that it must be divided by six. The formula (I) [4] was



verified by synthesis but with some difficulty. The first synthetic preparation showed only small biological activity; it seemed clear that there were problems involved in obtaining the molecule in the correct three-dimensional arrangement. This is not surprising in view of the intricate organisation of the peptide chains in insulin now found in the crystal structure.

The detailed X-ray analysis of insulin was taken up a little slowly as SANGER's chemical structure determination was reaching conclusion. Two observations made at this stage proved helpful. First, an examination of the distribution of the intensities showed that the arrangement of insulin molecules in the unit cell had approximately the symmetry 32 [5], with limited two-fold axes perpendicular to the three-fold axis, placed as shown in figure 1. Secondly, SCHLICHTKRULL, through very careful studies of the

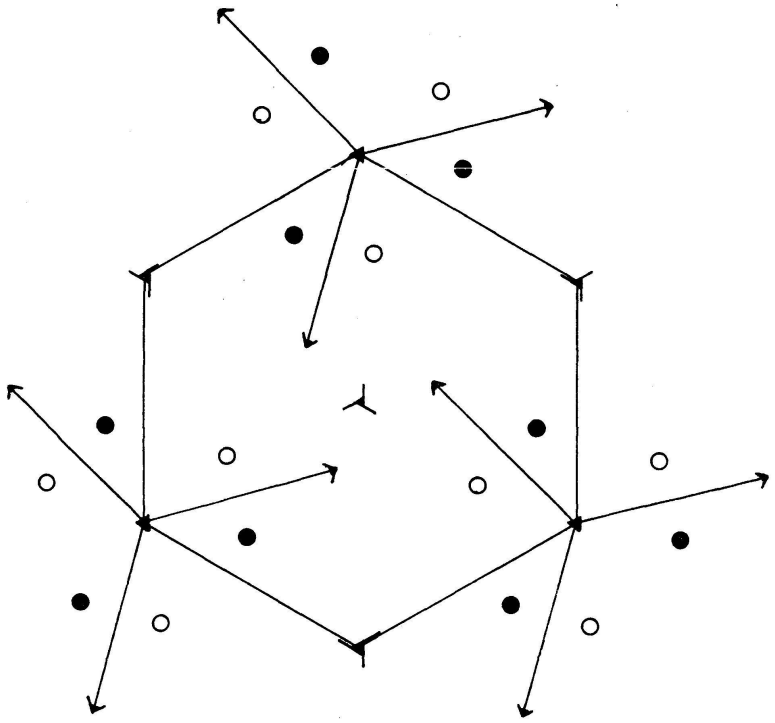


Figure 1
The symmetry elements of 2-zinc insulin crystals

crystallisation of insulin, designed to provide preparations for clinical use, found that there were two zinc insulin modifications; the one normally obtained contained two zinc ions per six molecules while the other, prepared in higher sodium chloride concentrations, contained four zinc ions per six molecules [6]. The crystal symmetry required the two zinc ions to be placed along the three-fold axis, sensibly related to one another by the local two-fold axes.

That the crystal structure of insulin might be solved by isomorphous replacement of the zinc by other ions was suggested very early in the 1930's by J. D. BERNAL [7] and by J. M. ROBERTSON [8]. Our first attempts were designed to replace the zinc in the crystallising liquid by mercury, thallium or lead. An initial apparent success with lead could not be repeated. Only much later, at the suggestion of Dr. STRANDBERG and Dr. TILÅNDER, we found a method of making lead insulins. Well formed crystals of 2-zinc insulin were left over night in dilute 0.1% EDTA acetate buffer. Under these conditions the zinc is removed from the crystals which appear a little cloudy and crazed but still give good diffraction effects, showing intensity differences from 2-zinc insulin. Over a second night, the zinc free crystals, left in lead acetate solutions, take up lead. The lead ions occupy the original two zinc sites and also additional sites, both between the zinc ions and on the outskirts of the molecule. Their distribution is illustrated in figure 2 which shows, in addition, the positions of other heavy atoms which may be introduced into the crystal by soaking ex-

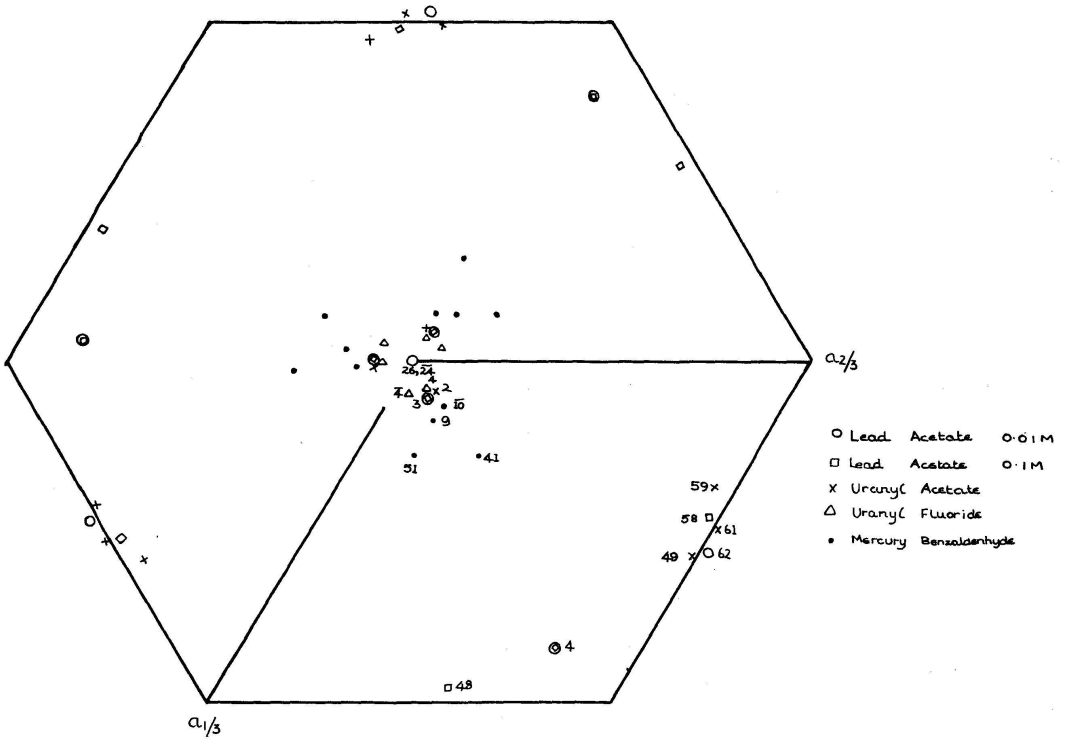


Figure 2
Heavy atom positions found in different insulin derivatives projected onto the c plane

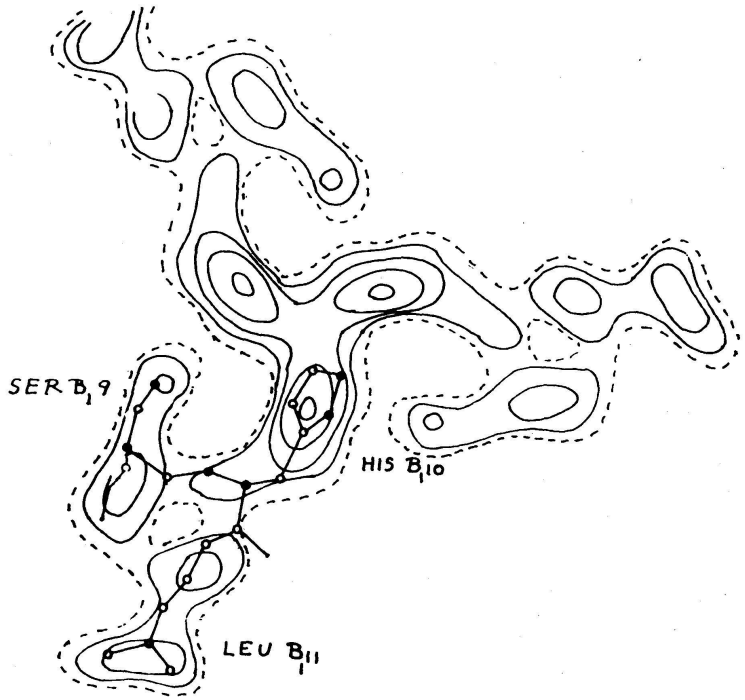


Figure 3
Electron density contours in section of three-dimensional distribution at 14/48 c showing fitting of residues to the density. Full circles show atoms nearest the section

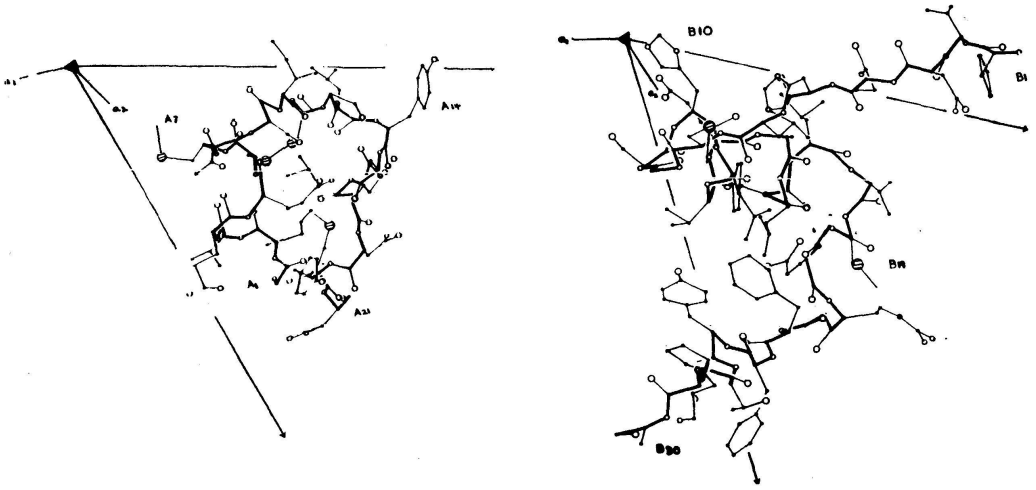


Figure 4
Projections along the c axis of the atomic positions derived for atoms in a) the A chain b) the B chain of one insulin molecule

periments. The structure analysis depended on measuring diffraction effects from all the different crystals shown in figure 2, placing the heavy atoms initially by difference Patterson calculations, followed by difference Fouriers and least squares calculations. Always the substitution pattern and its unravelling proved complicated, but the set of phase angles evaluated nevertheless produced an electron density distribution which, at 2.8 Å resolution, provided an interpretable map of the atomic positions in insulin.

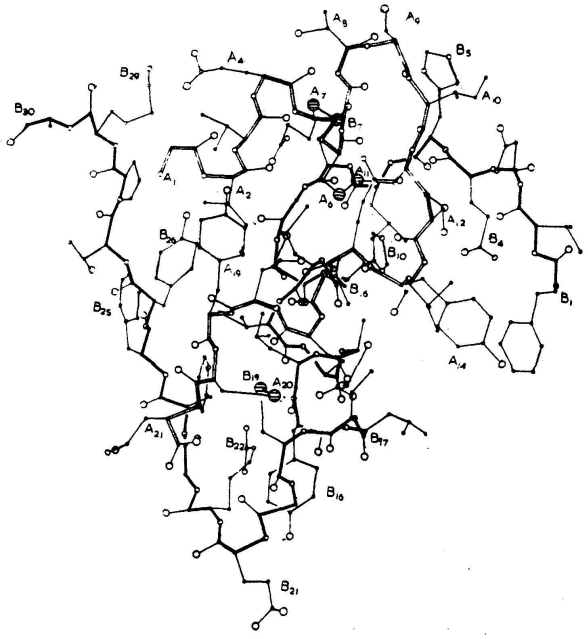


Figure 5
Projection of the atomic positions in one insulin molecule perpendicular to the c axis

The recognition of the arrangement of the atoms began from the zinc ions outwards and is illustrated by figure 3. It depends essentially on the projection of scale models against scaled drawings of the electron density, at first at 1 cm = 1 Å, later at 2 cm = 1 Å. The zinc ions were surrounded by three elongated peaks which could be identified as B 10 histidine, following the correlation of the neighboring peaks with shapes expected for B 9 serine and B 11 leucine. Much of the residue identification was very straightforward. Only in one region, between A 12 and A 18 has the first solution reached been substantially modified by later, more precise, model building. The placing of the individual atoms is necessarily still imprecise and may be improved as more data extending the limit of resolution are introduced.

Figures 4, 5, and 6 illustrate the arrangement of the atoms in detail in the insulin crystal structure. Within each molecule the A chain has a very

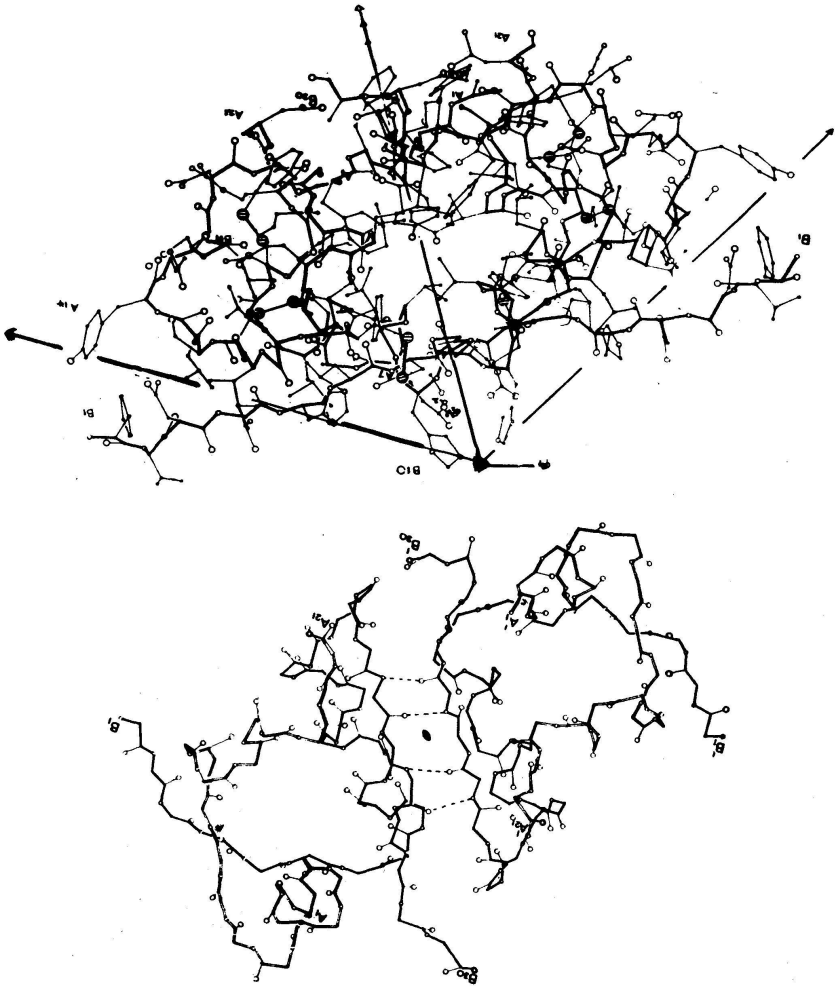


Figure 6

a) The positions of the atoms in the insulin dimer projected along the c axis
b) The peptide chains in the dimer projected along the two-fold axis. Hydrogen bonds dotted

compact form: only in a small initial region does the chain follow an α helical form. It rests in a hollow enclosed on three sides by the B chain. The latter begins with an extended region for the first ten residues, passes into a rigid α helix, which provides a backbone to the molecule between B 10 and B 20 and then ends with a further long extended region. The two molecules in the asymmetric unit are similar but not exactly identical in atomic distribution. Part of the differences may arise through their interaction to form a close dimer around the local two-fold axis. The terminal B chains are here in contact and interact to form a hydrogen bonded pleated sheet between the peptide chains belonging to residuse 24, 25, and 26. This kind of structure has two-fold symmetry, but the symmetry is not maintained by the actual residue arrangement; in particular, one of the phenyl groups, B 25, turns across the "two-fold" axis to pack closely with the second B 25 phenyl group, and other similar deviations can be observed in the contacts made by the two valine residues at B 12

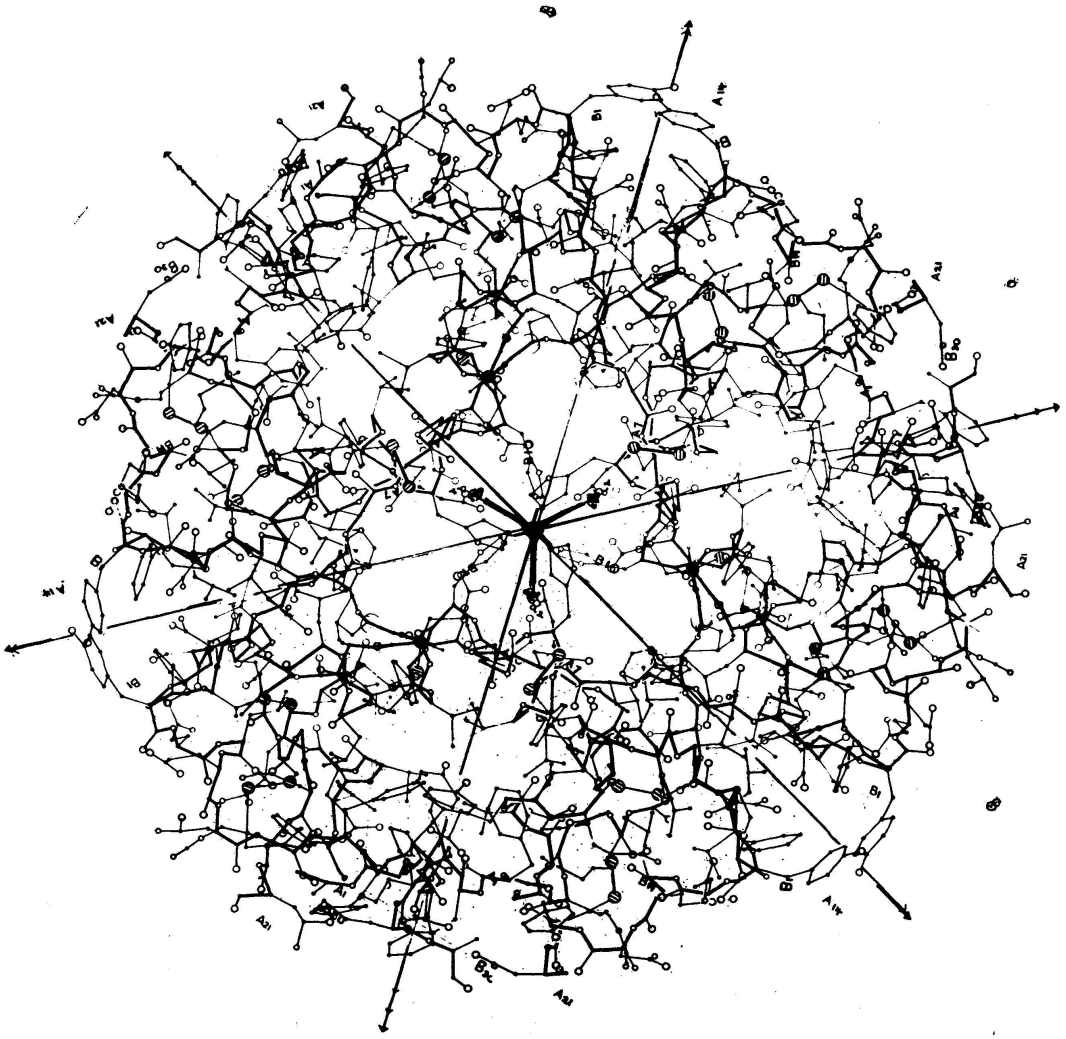


Figure 7
Atomic positions found in the insulin hexamer projected along the c axis

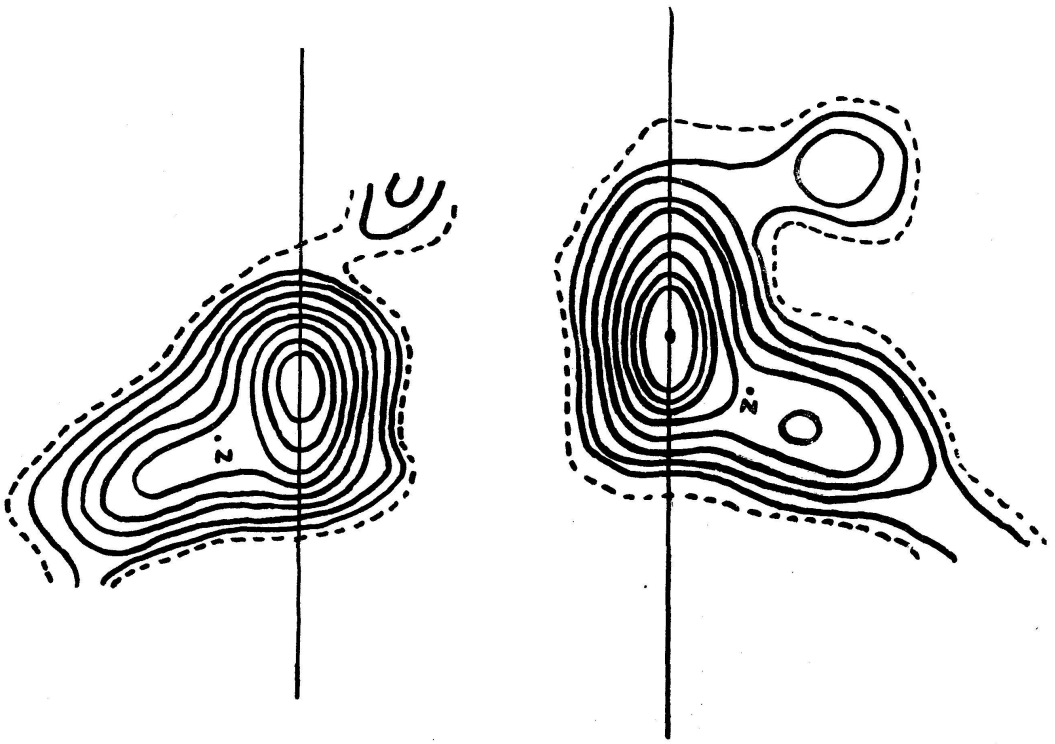


Figure 8
Electron density contours over the zinc ions and attached atoms, drawn on sections passing through the three-fold axis and the proposed histidine nitrogen positions

and glutamic acid residues at B 13. The latter residues surround a central pool in the crystal structure into which pass many of the heavy ions introduced in soaking experiments.

Around the three-fold axis of the crystals three insulin dimers are held together by the two zinc ions to form a very compact spheroidal hexamer illustrated in projection in figure 7. It seemed interesting to consider the coordination of the atoms around these zinc ions compared with that found in the basic zinc chlorides studied by Professor NOWACKI and others. In $Zn_5(OH)_8Cl_2 \cdot 1\ H_2O$, for example, there are two types of zinc ion, I, octahedrally surrounded by oxygen atoms, II, tetrahedrally surrounded by three oxygen atoms and one chlorine atom [10]. At first we thought it most probable that the zinc in the insulin crystals was surrounded by six atoms—three histidine nitrogen atoms and three water oxygen atoms—represented by small elongated peaks in the electron density map. Detailed drawings given in figure 9 of the peaks defining the zinc ions show however that both are elongated in the direction of the c axis and could conceivably conceal in their outlines tetrahedrally attached water molecules, hydrogen bonded to three surrounding water molecules. Alternatively the lengthening of the zinc peaks might be due to uncertainty in their parameters in the c direction. We can only hope that further refinement and extended data will help to resolve the problems of both zinc ion coordination spheres.

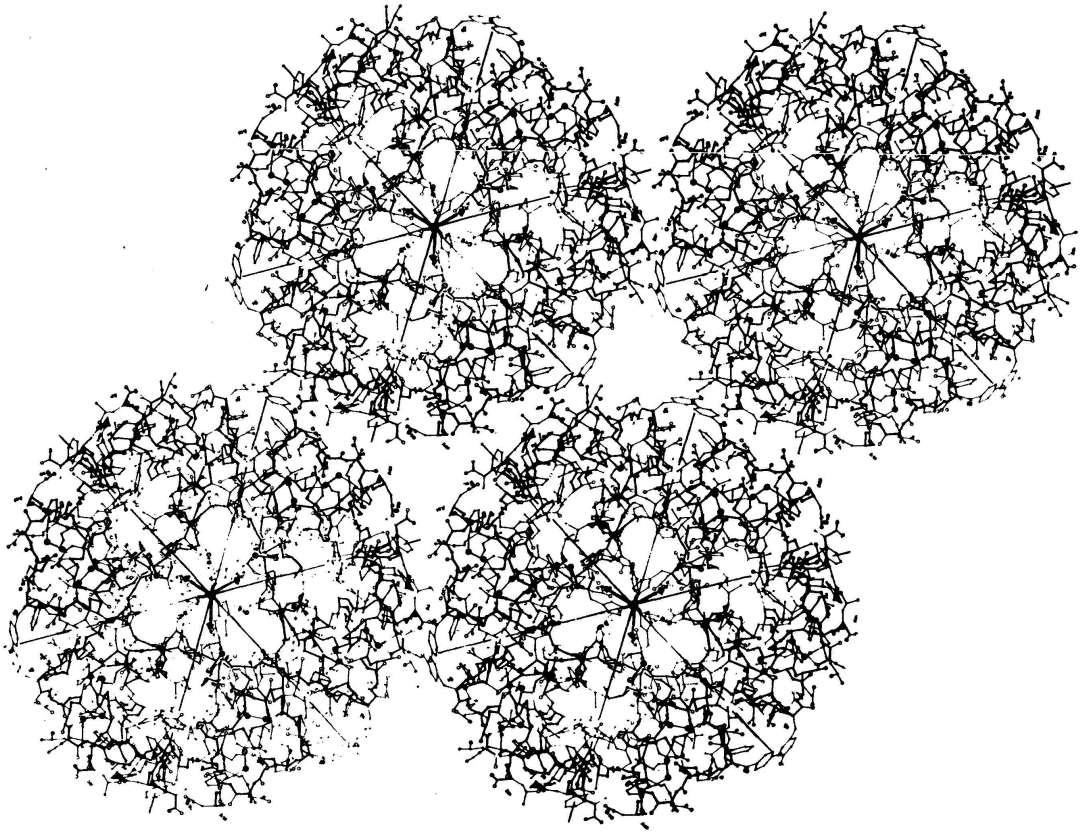


Figure 9
Four insulin hexamers seen projected along the c axis as in an insulin crystal

The detailed arrangement of residues over the surface of the insulin molecules ought to be suggestive in relation to the biological activity of insulin, the way in which the hormone affects the metabolism of glucose. Both polar and non polar residues are present on its surface; polar residues include some, such as asparagine A 21, the removal of which seems to destroy or lower the biological activity of insulin. The non polar residues are largely buried in the insulin hexamer but might be released to interact, for example, with membranes, if, as seems likely, the insulin molecule or dimer is released in the bloodstream. Since we still do not know exactly what molecules interact with insulin, we cannot carry our speculations very far. To our present view the molecule could act either as an enzyme or as a carrier. But at least it is interesting that almost certainly the zinc insulin crystal structure provides the method of storing the active hormone adopted in the pancreas. Electron microscope pictures of insulin in the storage granules in a number of different creatures show small bodies which look like crystals; in the rat, particularly [11], faint lines can be traced across the granules 50 Å apart, sensibly the same distance as the diameter of the hexamers in the crystal structure of zinc insulin.

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