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X-Ray Analysis and the Structure of Insulin

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In a symposium on endocrinology it seems proper to begin by looking at insulin as it exists in the β granules of the pancreas. There have been a number of observations in the past made with the light microscope of single crystals, probably of insulin, visible in the granules, particularly in the pancreas of the dog. With the electron microscope it is possible to see dense, apparently crystalline, aggregates in the β granules of many animals and within these, compact particles, regularly arranged. A very good example, photographed by Greider, Howell and Lacy (1969), shows roughly spherical particles in lines about 50 Å apart in a β granule in rat pancreas. It may well be that the actual formation of crystals is a consequence of partial drying of the granules on isolation or preparation for electron microscopy; the individual particles of

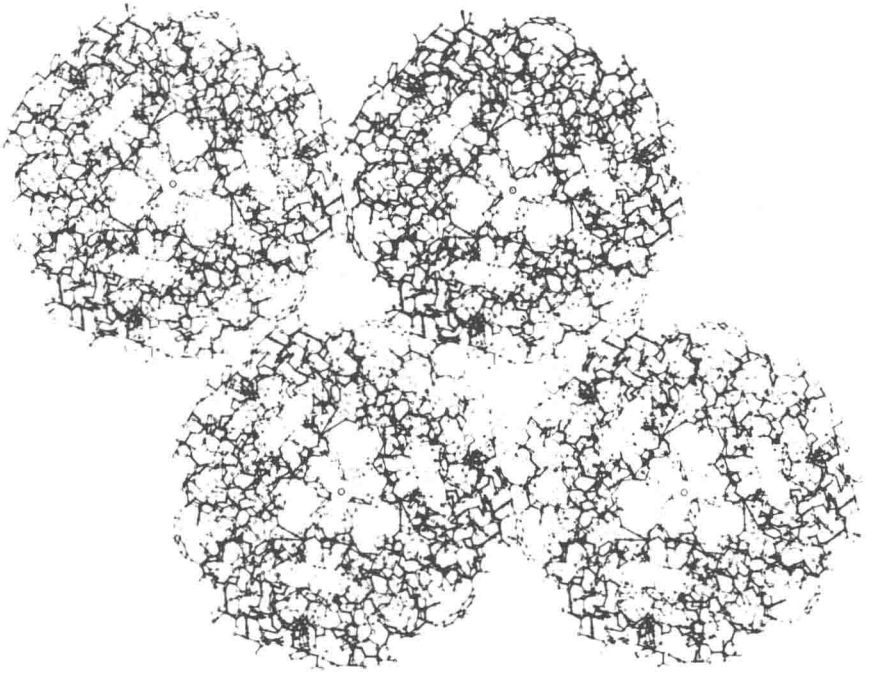


Fig. 1. Projection of the atomic positions found in the crystal structure of rhombohedral insulin crystals along the three fold axis. The atoms (small circles) are grouped in four hexamer units at relative z heights, z, $z + 1/3$, $z + 2/3$.

insulin that appear would however be almost certainly normally present in the living tissue.

The size of a single particle visible within the β granules is very similar to that of the hexamer of insulin molecules, 48 Å across, found in rhombohedral pig insulin crystals by X-ray analysis. Hexamers are shown in Fig. 1 in projection along the three fold axis of the rhombohedral crystals; the view is not unlike that of the rat islet 'crystal' though this may well have a different structure in detail from pig insulin. The appearance of hexamers both in the crystals and in the islets would be expected from experiments on insulin in solutions which show that, in the presence of zinc, six insulin molecules aggregate around two zinc ions; in most creatures, except perhaps the guinea pig and coypu, zinc is present in the β granules.

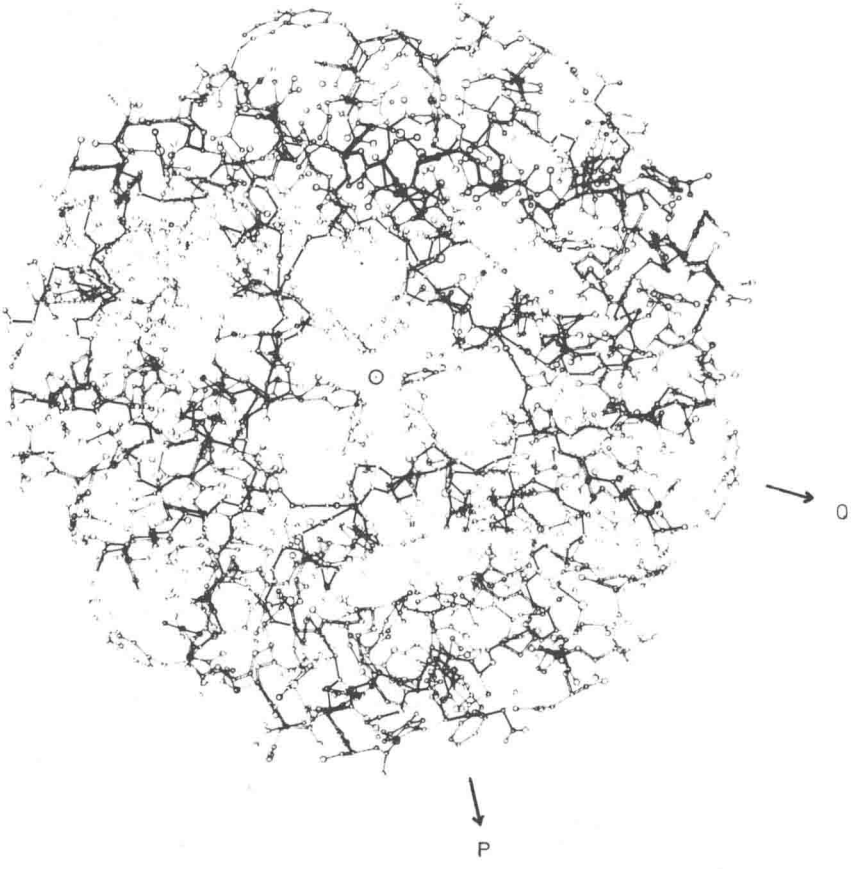


Fig. 2. Projection along the three fold axis of the atomic positions in a single insulin hexamer. the atoms zinc (along the three fold axis), sulphur, oxygen, nitrogen and carbon are shown by circles in decreasing order of size and are joined by lines representing chemical bonds. Hydrogen atoms are omitted.

Fig. 2 shows a single hexamer of insulin, again observed in projection along the three fold axis of a rhombohedral insulin crystal. All of the atoms, except hydrogen atoms, are shown by circles varying in size with atomic number in the order zinc, sulphur, oxygen, nitrogen and carbon. Their positions are not at all precisely defined. The experimental evidence on which they are based – our next order of observation on insulin – is an electron density map derived by calculation from the intensities of spectra obtained by diffraction of X rays passing through the insulin crystals (Adams *et al.*, 1969, Blundell *et al.*, 1971). The spectra extend to spacings of 2·8 Å and the electron density map calculated accordingly provides a very blurred representation of the atomic positions. Its character can be demonstrated by plotting to scale contours of equal calculated electron density on sheets of perspex and stacking the sheets together to cover the unit volume in the insulin crystal. Groups of atoms, such as benzene rings, appear as single peaks, while peptide chains are represented by strands with higher density in the neighbourhood of, for example, carbonyl groups. A few overlapping sections of the electron density map are shown in Fig. 3 while Fig. 4 indicates how a part of the chemical structure of insulin is fitted within the electron density outlines in a single section of the map.

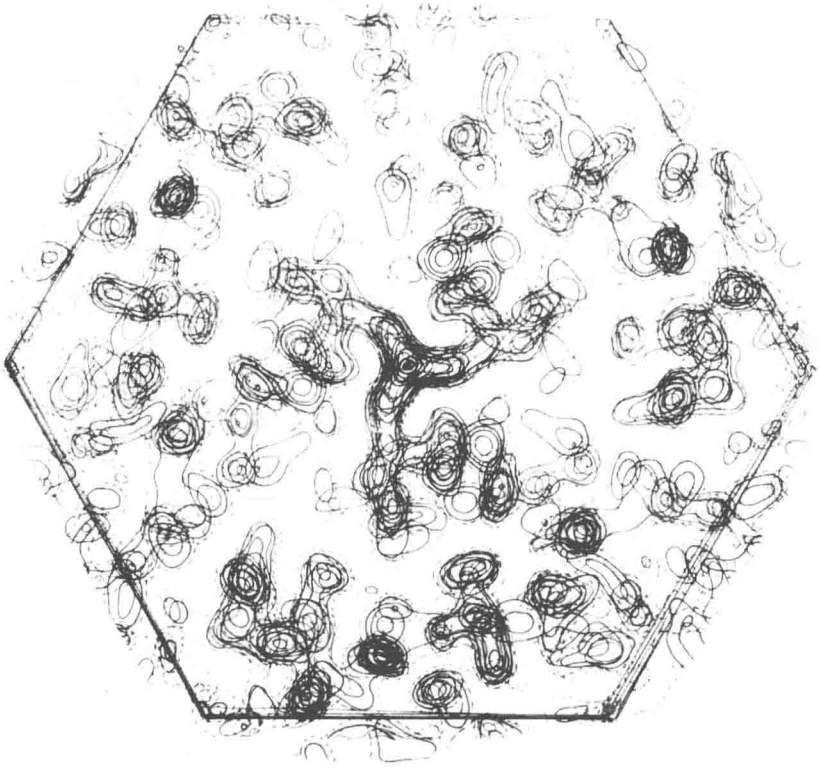


Fig. 3. Photograph of part of the electron density model showing sections between 10/48 and 5/48 in z. The contours are drawn on each sheet at intervals of 0·1 e/A^3.

It will be clear from the character of the electron density map that while our knowledge of the atomic positions is, in detail, very imprecise, certain features of the arrangement of the peptide chains within the insulin molecules and of the six insulin molecules within the hexamer are very clear. Our confidence that many of the details of atomic positions now deduced are also reasonably correct depends on the close correlation of the electron density map with the chemical structure of pig insulin derived by Ryle, Sanger, Smith and Kitai (1955), shown in Table 1. It is also helped by other evidence, particularly on the titration of insulin in the presence and absence of zinc, which made it very probable that one of the histidine residues was attached to the zinc ions.

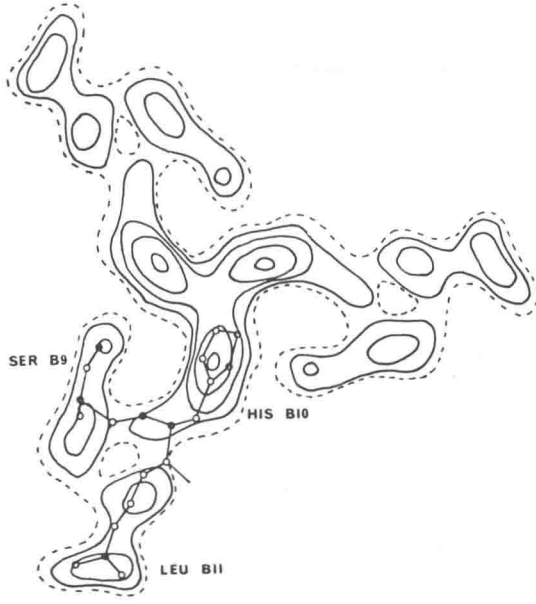


Fig. 4. Electron density contours in the section z = 9/48 with superimposed the positions of the histidine, leucine and serine residues. Filled circles represent atoms close to section, open circles atoms within 1 Å of section.

The electron density map shows that the two zinc ions are arranged along the three fold axis of the crystal and are about 17 Å apart. The insulin molecules are linked in threes around them through the B 10 histidine residues. Though the linking of the histidine residues to the zinc ions is similar, it does not appear quite identical in the two triplets in the projected view along the three fold axis. The molecules in one triplet are arranged relative to those in the second triplet nearly but again not quite exactly as required by two fold symmetry axes along the lines OP and OQ of Fig. 2 normal to the three fold axis. In certain regions the packing together of the six molecules in the hexamer is as close as is the packing of the amino acid residues and peptide chains within a single molecule. As a whole, accordingly the hexamer presents a compact spheroidal appearance to the external world, 48 Å in diameter, 35 Å in height. Viewed down the three-fold axis its circumference appears smoothly circular but the upper and lower

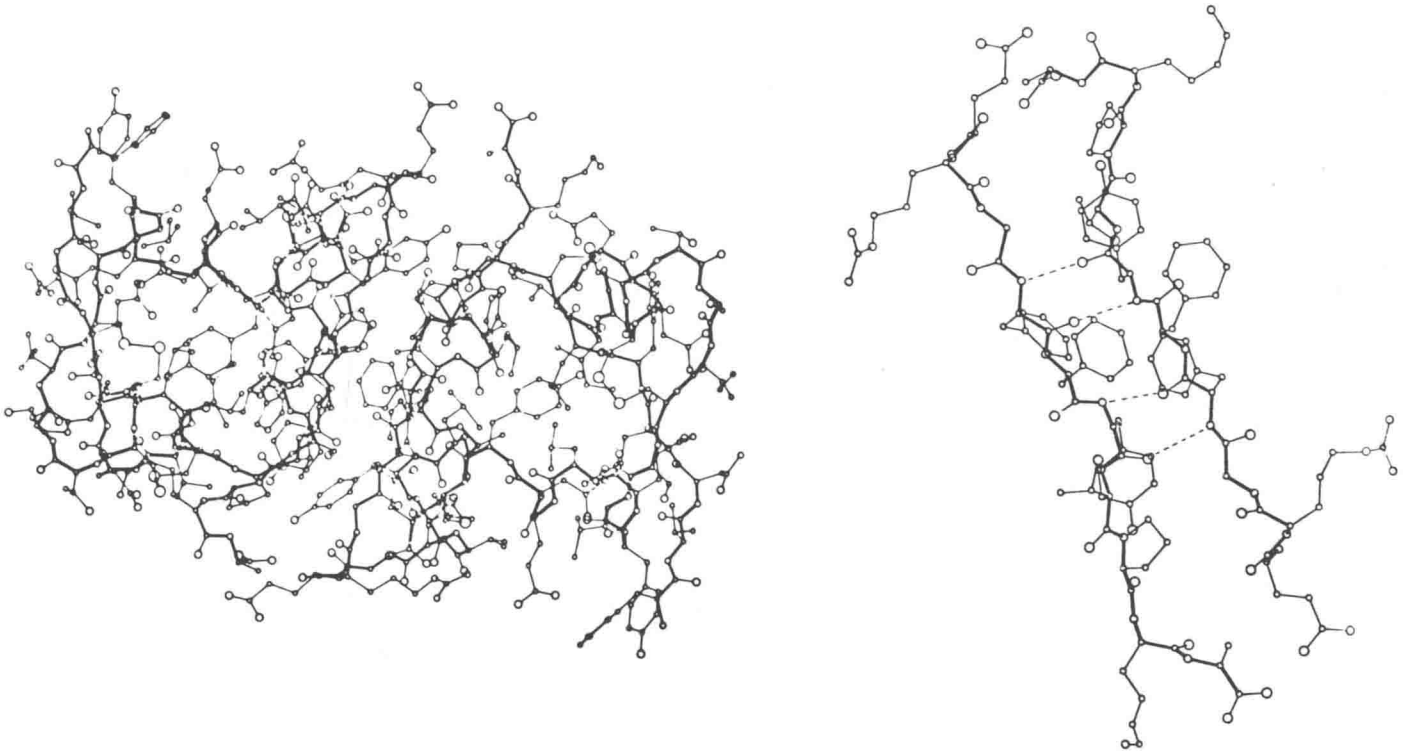


Fig. 5. (a) The atomic positions in the insulin dimer viewed along the 'two' fold axis. (b) The last eight residues of each molecule within the dimer in antiparalleled arrangement; hydrogen bonds dotted.

surfaces of the spheroid are in fact pitted by deep grooves between projecting residues of the A chain loops.

Around one of the two fold axis, 0P of Fig. 2, the contacts between the insulin molecules appear to be very close; it seems almost certain that these are the contacts responsible for the dimeric character frequently observed in molecular weight measurements of insulin in solutions in the absence of zinc. They are illustrated in Fig. 5 which shows a view of the atomic positions in the dimer along the 'two' fold axis. They include both non polar van der Waal's interactions between, for example, the valine B 12 and phenyl alanine B 24 groups of the two molecules, and also hydrogen bonded contacts between the peptide carbonyl and NH groups, B 24 and B 26. The latter appear as part of a β-pleated sheet type of structure, formed by the antiparallel arrangement of the terminal residues of the B chain in the two molecules. In Fig. 5b, where this system is isolated, one can see that one reason for geometrical differences

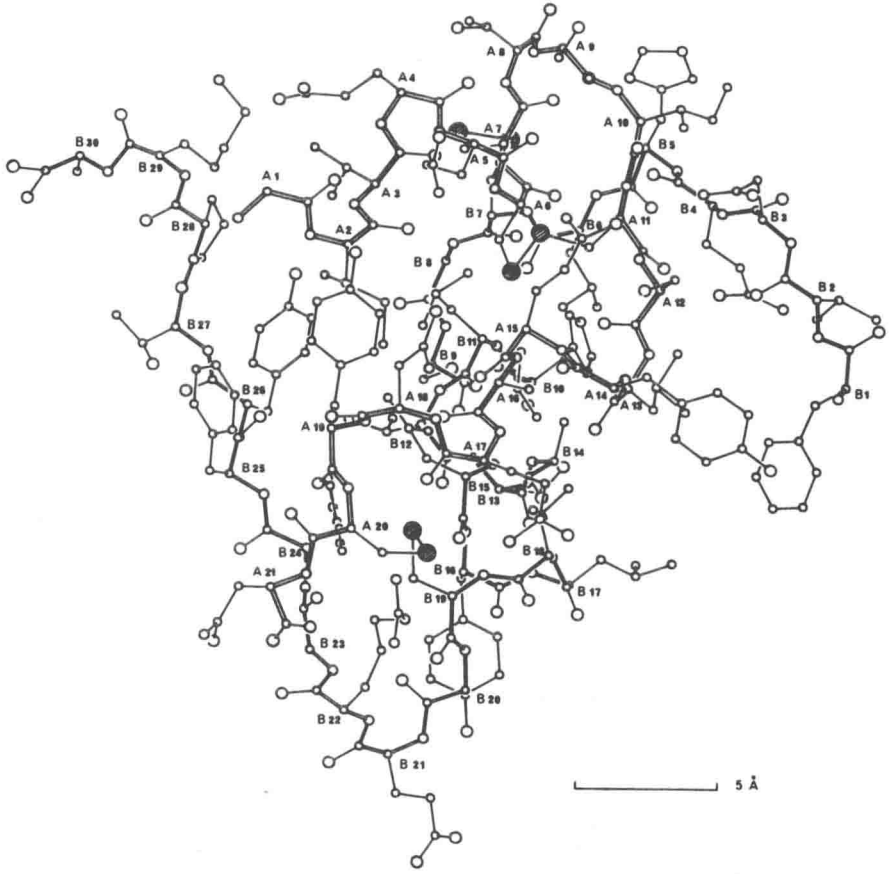
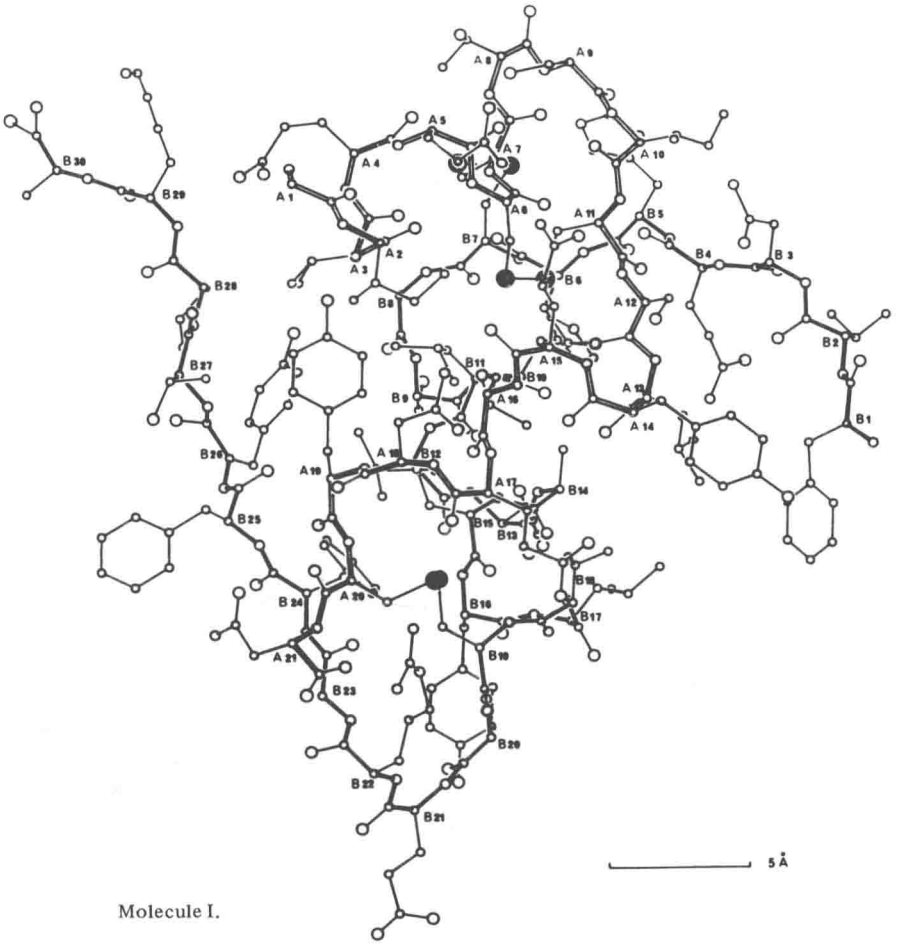


Fig. 6. Molecule II. The two molecules of insulin found in the crystal viewed in equivalent directions perpendicular to the three fold axis. Molecule I is seen in the orientation corresponding with Fig. 5; molecule II has been rotated around the two fold axis by 180°.

between the two molecules in the dimer may be the necessity for close packing of residues; thus the two phenyl alanine B 25 groups pack together, destroying the exactly symmetrical relation between them which might have appeared from the chain arrangement.

The two insulin molecules in the dimer are therefore very similar but not geometrically identical in every detail. They are illustrated in Fig. 6 in which they have been set side by side, for comparison. In each, the B chain starts out in an extended conformation from B 1 – B 8, turns sharply into an α helix from B 9 – B 20, and then through a U turn involving residues 21 – 23, ends in a further long extended region from 24 – 30. Within the rigid framework provided by these structures, the A chain forms a small compact unit. The exact conformation in certain regions has proved difficult to define; it follows a more involved course after an initial α helical turn, to make the loop bridged by an internal disulphide bond. From A 13 – A 19 it takes a loosely helical course,



Molecule I.

placing the long hydrophilic side chains on the outside of the molecule. It is held in position within the B chain cavity by the disulphide bridges to either end of the B chain α helix; there are strategic internal contacts, both polar and non polar, between the chains.

If we knew the receptor system with which insulin interacts, it might now be possible to try experiments within the crystal structure to locate the precise region or regions in the molecule that are most closely involved in the biological activity. In our present state of ignorance about the nature of the reactions concerned, we have to follow, by more devious routes, whatever clues are available. These include various experiments on the inactivation of insulin under different circumstances and the variation of the residues in the insulin molecule with species.

So far sequences have been determined for some twenty different insulins (Smith, L.F., 1966) – not a great number, really, in view of the complexity of nature. They are summarized in Table 1. The residues that have up to now been found to be invariant are circled; one might suppose that most, if not all, of these are important to our understanding of the biological activity of the molecule; it is interesting to see how they are arranged geometrically in relation to the observed structures of insulin, as monomer, dimer and hexamer. From Fig. 7 one can see straight away that a large number, all the cystine residues, glycine B 8 and B 23, leucine B 6, B 11, B 15 and A 16, valine B 18 and isoleucine A 2, are concerned with composing the relative arrangement of the two insulin chains in space; both by chemically defining their junction, enabling particular geometrical turns to be made, and encouraging defined internal residue relations. Certain other groups, valine B 12, tyrosine B 16 and phenyl alanine B 24, play a similar role in the surface within the dimer, encouraging particular contacts between molecules. A few 'invariant' residues only, A 1 glycine, A 5 glutamine, A 19 tyrosine, and A 21 asparagine are close together in the same region of the surface in all states of aggregation of the molecule. Among the different reactions which inactivate insulin it is perhaps significant that removal of either glycine A 1 or asparagine A 21 leads to almost total inactivation (Carpenter, 1966).

So we have a possible picture emerging of particular residues held on a surface in an arrangement which is essential to activity but there are additional complexities in the situation which make us hesitate to say this is all that is required. The characteristics of the insulin dimer seem to be important in relation to the distribution of invariant residues. This might be either because the dimer presents an essential extended active surface or because the residues in the monomer monomer interface are themselves involved in interactions and are at some stage released. At the concentrations at which insulin circulates in the blood it is probably present as a mixture of monomers and dimers; with traces of zinc around even hexamers may occur, so that any of the three might well be the active species; it would certainly be interesting, as suggested on another occasion, to test the activity of dimers internally cross linked to inhibit dissociation to the monomer.

At present it seems most likely that the insulin hexamer provides a useful but not essential method of storing insulin in the β granules – not essential because the B 10 histidine, necessary for its construction, is absent in the guinea pig and