possesses four triangular faces and four hexagonal faces. This polyhedral configuration is in agreement with Svedberg's deduction that insulin is a "globular"

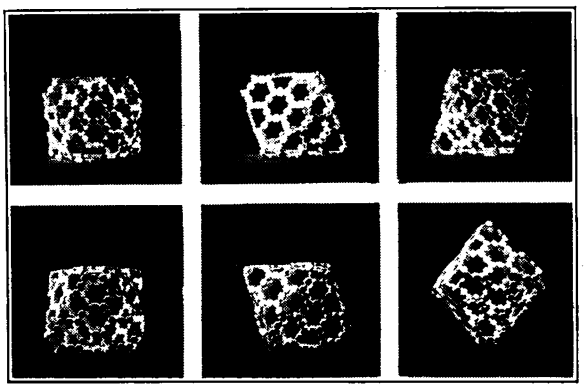

FIG. 2

molecule with low asymmetry number[6] and in fact offers an interpretation of the nature of this "globularity," which may be useful in the future in a quantitative interpretation of the asymmetry numbers of megamolecules in general. Further, since the C_2 structure (like all C_n structures) is a condensation of amino (or imino) acid molecules, no prosthetic group is required, in accordance with the chemical evidence.

The most stringent test of any proposed structure is afforded by the x-ray findings.[5] In the first place the insulin lattice has space group R3, and the unit cell is rhombohedral and contains one molecule only. Strictly interpreted, this means that the molecule itself has trigonal symmetry. Now for all the space-enclosing cyclols, the median network (which is to be regarded as a shorthand notation for the molecular structure, from which its essential features can be deduced) has four trigonal axes, if the distribution of different amino acids be left out of account. This symmetry requirement can then be met by any C_n and at the same time interpreted to mean that in the insulin molecule one hexagonal and one triangular face have the various residues trigonally arranged, while the three other hexagonal faces and also the three other triangular faces have identical arrangements of residues.

Next, the unit rhombohedral cell has $a = 44.3$A and $\alpha = 115°$. On working out the detailed geography of the structure proposed, it is found that the cyclol molecule C_2 (whose median network is shown in Fig. 2) fits easily and elegantly into this cell; furthermore, its structure suggests actual mechanisms of coordination in this megamolecular lattice. Thus the coordination between a molecule and its neighbors above and below at a distance 30.2A along the trigonal axis appears to be due to the simultaneous linking of a number of peptide hydroxyls. On the other hand each molecule appears to be linked severally to its six neighbors at distances 44.3A along the edges of the primitive rhombohedron by means of groups belonging to side chains, probably by the phenolic groups of tyrosine residues, which are held together by zinc (or other) cations. This mechanism accounts for the data of Scott which establish the fact that in insulin crystals there is a stoichiometric relation between the insulin content and the content of zinc, of cadmium or of cobalt.[7] The proportion is three cations to one molecule of insulin, which is in accordance with the mechanism of coordination suggested above, assuming that each insulin molecule has a half share of the six cations, located on the rhombohedral edges. The present suggestions thus fit in with and explain the view that crystalline insulin contains the metals as chemically combined constituents and not as mere impurities, and throws light also upon the fact[7] that the best acidity for the crystallization of insulin in the presence of certain metals is pH 6.0 to 6.2 on the alkaline side of the isoelectric point[8] pH 5.0 – 5.5.

Full details of the work will appear shortly.

D. M. WRINCH

MATHEMATICAL INSTITUTE
OXFORD

THE DIFFUSION COEFFICIENT AND MOLECULAR SIZE OF VISUAL PURPLE

IN order to secure some notion of the molecular dimensions of visual purple, we have determined its diffusion coefficient by the method of Northrop and Anson.[1] This involves measuring the rate with which a dissolved substance passes from an enclosed solution into an outer solvent through a porous glass or alundum disk calibrated with substances of known diffusion coefficient.

The two basic properties of visual purple are its color and its light sensitivity. We have therefore relied on these criteria for measuring its diffusion. Using three different glass disks and four preparations of visual purple, we have obtained apparent diffusion coefficients of 0.0153, 0.0125, 0.0161 and 0.0152, with an average of 0.0148 sq. cm. per day at 6° C.

These values are probably not the real diffusion coefficients, because the glass disks become clogged during the manipulations. The clogging happens rapidly and then stops; after diffusion equilibrium with visual purple has become established over night the rate usually remains constant for as long as we have measured it, in one case for a week.

To estimate this clogging factor we calibrated the disks as usual with 2 M NaCl before the visual purple

[6] T. Svedberg and I.-B.- Eriksson-Quensel, *Tabulae Biologicae Periodicae*, 5: 351, 1935–36.

[7] D. A. Scott and A. M. Fisher, *Biochem. Jour.*, 29: 1048, 1935.

[8] F. O. Howitt and E. B. R. Prideaux, *Proc. Roy. Soc. Lond. B.*, 112: 13, 1932.

[1] J. H. Northrop and M. L. Anson, *Jour. Gen. Physiol.*, 12: 543, 1929.

was introduced into the diffusion cell; then the calibration was repeated after the establishment of a steady state of diffusion, with the visual purple still in the cell. With two preparations of visual purple the first calibration gives an average diffusion coefficient of 0.0156, while the second calibration makes it 0.0190 sq. cm. per day. The latter is probably more nearly correct, because the second calibration records the actual state of the disk during the diffusion of the visual purple.

Efforts to eliminate the clogging factor by using alundum disks were not satisfactory because they trapped air easily, and the individual determinations varied widely (from 0.0108 to 0.0177 sq. cm./day) even in a single run. However, the values are of the same order of magnitude as with the glass disks.

To derive the molecular size of visual purple from diffusion data involves the application of Einstein's[2] equation which relates the diffusion coefficient to the radius of the molecule by way of the coefficient of friction and Stokes's law. Such an application is quite reasonable,[3] provided one is interested only in an order of magnitude. Taking the diffusion constant as 0.0148 sq. cm./day, the equations yield 8.04×10^{-7} cm. as the radius of the molecule of visual purple; using the more probable value of 0.0190 sq. cm./day, the radius becomes 6.26×10^{-7} cm. Either of these values shows visual purple to be a large molecule of the size heretofore found only for proteins. Its molecular volume computed from the smaller and more probable radius is 623,000; and if its specific gravity is 1.3 like that of most proteins, its molecular weight is 810,000.

Molecular weights computed from diffusion coefficients are frequently higher, often by a factor of 2 or even 3, than those computed from osmotic pressure or sedimentation data. The real molecular weight of visual purple may therefore be half or even a third of the value here given. For our purposes, this is of minor importance because even so it would still be a very large molecule of the kind found only among the proteins.[4]

This is powerful evidence that visual purple is really a protein, and agrees with Kühne's[5] inability to separate visual purple from the neurokeratin of the rods, and with his demonstration of the high temperature coefficient of its thermal destruction. Adding Wald's contribution[6] that visual purple liberates a carotenoid when it is bleached by light or acted upon by chloroform, it definitely places visual purple among the conjugated proteins—the carotenoid proteins.

[2] A. Einstein, *Z. Elektrochem.*, 14: 235, 1908.
[3] M. L. Anson and J. H. Northrop, *Jour. Gen. Physiol.*, 20: 575, 1937.
[4] T. Svedberg, *Trans. Faraday Soc.*, 26: 740, 1930.
[5] W. Kühne, in *Hermann's Handbuch d. Physiol.*, 3 (1): 235, 1879.
[6] G. Wald, *Jour. Gen. Physiol.*, 19: 351, 1935.

The visual purple solutions were prepared as previously described,[7] and were buffered with borate-KCl to a pH of 9. The glass disks of porosity 4G had a diameter of 30 mm and were about 0.5 mm thick. The inner diffusion cell contained 10 cc of visual purple solution, and the outer cell contained 7 cc of solvent—either digitonin or bile salts solution—also buffered to pH 9. When the disks were to be calibrated after the diffusion had reached equilibrium, NaCl was added at the beginning to both the visual purple and the outside solutions to make a concentration of 2 M; then when the diffusion rate had become constant, we determined the passage of the NaCl into an outer digitonin solution without NaCl. The entire apparatus was immersed in a water bath at 6° C. in a dark cold-room kept at the same temperature. The water bath was carefully mounted on rubber and then on a heavy concrete block to avoid undue vibration. The concentration of visual purple was determined with a photoelectric spectrophotometer, using as a measure the difference in photometric density at 500 mμ between the unbleached and subsequently bleached solution. The pH of 9 is to avoid any regeneration after bleaching. The experiments were made during the past year, and were aided by a grant from the Rockefeller Foundation.

SELIG HECHT
AURIN M. CHASE
SIMON SHLAER

LABORATORY OF BIOPHYSICS
COLUMBIA UNIVERSITY

RATE OF MATURATION OF YOUNG RED CELLS IN CANARIES

THE erythrocytes that are present in the peripheral blood of birds may be separated into three types, all of which are nucleated.

(1) Nucleated basophilic erythrocytes are spheroidal in shape, free from hemoglobin and stain only with basic dyes. The nucleus is large and spheroidal, and the chromatin is coarsely reticulated. Only a fraction of one per cent. of these occur in the peripheral blood of canaries.

(2) Nucleated amphophilic erythrocytes are spheroidal or elongate in shape, contain a small amount of hemoglobin and stain with either acid or basic dyes. The nucleus is large and oval in shape and the chromatin is present in the form of irregularly shaped clumps of various sizes; such nuclei are called polychromaphilic. Usually from one to 6 per cent. of these occur in the peripheral blood of canaries.

(3) Nucleated oxyphilic erythrocytes are spheroidal or elongate in shape, and contain hemoglobiniferous or oxyphilic cytoplasm. The nucleus is small, being

[7] S. Hecht, A. M. Chase, S. Shlaer and C. Haig, SCIENCE, 84: 331, 1936.