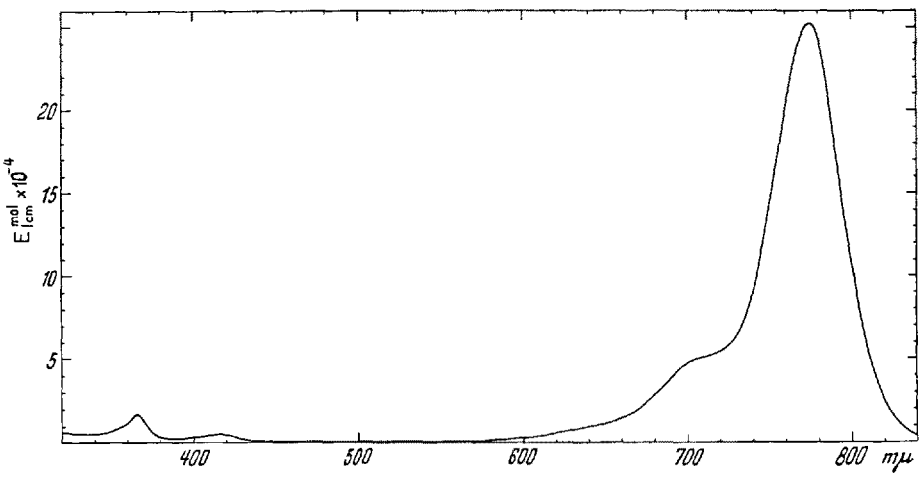
Werte etwas kleiner sind als die theoretisch gewonnenen Beträge. Dieser Befund dürfte... zum Teil damit im Zusammenhang stehen, dass nicht alle Polymethinketten der einzelnen Farbstoffmolekeln in Trans-Konstellation angeordnet sind, dass vielmehr ein Gemisch verschiedener cis-trans-isomerer Verbindungen vorliegt, ähnlich wie dies nach ZECHMEISTER u.a. im Falle der Polyene anzunehmen ist.»

meter between eluates of the respective pigment zones. Thus, in numerous experiments, the same spectral curve was obtained from any form (Fig.)[1].

In accordance with KUHN's concept, we believe that solutions of at least the member studied in the cyanine dye series contain a stereoisomeric equilibrium mixture, in which, evidently, not all possible cis-trans forms are present in equal quantities but a few configurations



Molecular extinction curve of the $n = 3$ representative of the symmetrical cyanine dye series (in ethylene chloride).

Considering the theoretical importance of the point made by KUHN, we wish to report briefly on some unpublished experiments concerning stereoisomerism of the $n = 3$ member of the above series.

First, according to FISHER and HAMER[1], the quaternary iodide was prepared which did not reveal more than a single zone on the chromatographic column. The iodide was converted into the corresponding chloride simply by a short heating of its ethylene dichloride solution. This solution was purified by chromatographing on calcium carbonate and the upper main zone was cut out. Upon elution with a mixture of ethylene chloride and methanol, the latter was eliminated by washing with concentrated saline solution. The ethylene chloride was then evaporated *in vacuo*, the cyanine chloride taken up in some methylene chloride and crystallized by the addition of hexane (rectangular prisms).

When a fresh ethylene chloride solution of these crystals was adsorbed on calcium carbonate and developed with ethylene chloride containing 10 per cent acetone, three sharply differentiated dark blue zones appeared although no marked interzones were observed. The uppermost zone, very probably containing the all-trans form, showed the darkest shade. When any of the three zones was cut out, eluted as described above, transferred into ethylene chloride and rechromatographed, exactly identical chromatograms, composed of the three zones obtained. The colorimetrically measured ratio of the three pigment forms was practically constant, viz. 82:13:5 (from top to bottom).

The spontaneous interconversion of the stereoisomers at room temperature, during elution and transfer operations, took place at such high rates that refluxing, exposure to sunshine, etc., did not essentially alter the ratio mentioned. Neither was it possible, for the same reason, to differentiate in the BECKMAN spectrophoto-

predominate as had been observed earlier in the field of diphenylpolyenes and carotenoids[2].

L. ZECHMEISTER and J. H. PINCKARD

California Institute of Technology, Gates and Crellin Laboratories of Chemistry, Pasadena, California, No. 1744, October 15, 1952.

Zusammenfassung

Chromatographische Beobachtungen stützen die auf spektraltheoretische Berechnungen basierte Annahme von H. KUHN, dass in gewissen Zyaninfarbstofflösungen ein Gemisch von cis-trans-isomeren Formen vorliegt.

[1] N. I. FISHER and F. M. HAMER, J. chem. Soc. (London) *1933*, 189.

[1] For a curve of the iodide in methanol, taken between wave lengths, 540–820 mμ cf. L. G. S. BROOKER, R. H. SPRAGUE, C. P. SMYTH, and G. L. LEWIS, J. Amer. Chem. Soc. *62*, 1116 (1940); cf. also A. L. SKLAR, J. chem. Phys. *10*, 521 (1942).

[2] L. ZECHMEISTER, Chem. Reviews *34*, 267 (1944).

Phospholipid-Cholesterol Complex in the Structure of Myelin

When the principal long spacings in the X-ray diffraction pattern of fresh normal nerve are compared with those of specimens which have been modified either by previous drying and re-wetting with RINGER solution[1], or by freezing and thawing[2], or by treatment with lipid solvents[2], it is seen that the modifications of structure apparently lead to increases in diffraction spacings. Similarly, when "residual complex" spacings of normal dried

[1] J. ELKES and J. B. FINEAN, Discussions of The Faraday Society, No. 6 (Lipoproteins), p. 134 (1949).

[2] J. ELKES and J. B. FINEAN, Exp. Cell Res. (in press).

nerve are compared with those of nerve which have been modified before drying, it is again found that the modified specimens show the higher diffraction spacings.

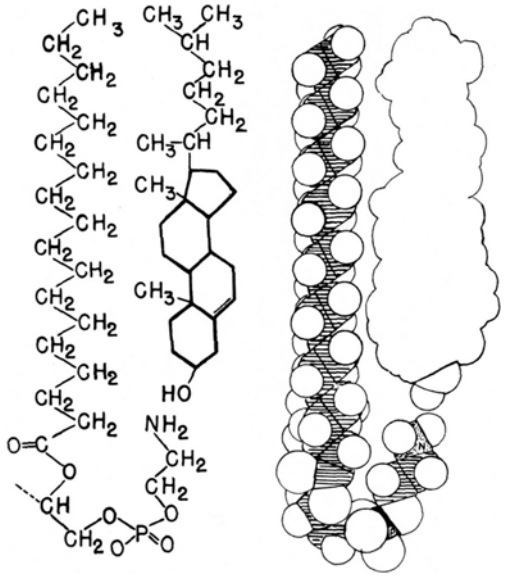


Fig. 1.

A detailed study of these diffraction patterns indicates that the most probable structure for myelin is one consisting of alternate layers of lipid and a non-lipid material which may be protein. The fundamental repeating unit of the structure probably contains two bimolecular leaflets of lipid which must be very similar in constitution but differ in geometrical arrangement, and two layers of protein, the whole system building up to a spacing of about 171 Å. In the lipid layer, the longest lipid molecules appear to be tilted or curled in some way so that they do not contribute their fully extended length to the thickness of the leaflet. The curled form would appear to be more in keeping with the radial orientation data derived from polarised light[1] and X-ray diffraction studies[2], and some way of stabilizing these long molecules individually in a curled form would seem to be necessary. A consideration of the molecular structures of individual lipid components of myelin indicates the possibility of a stable complex formation between cholesterol molecules and the longer phospholipid or cerebroside molecules. Cholesterol is present in myelin in approximately equimolecular proportions with phospholipid, and furthermore it is found only as free cholesterol, a fact which suggests that the –OH group may be important in binding the molecule into the myelin structure. A possible arrangement for the complex is illustrated in Figure 1. Here, each long-chain lipid molecule is stabilized individually by a cholesterol molecule. The hydrocarbon part of the cholesterol lies alongside the long chain of the phospholipid or cerebroside, and adheres by VAN DER WAAL'S attractive forces. The polar end group of the long chain lipid curls around so as to come into close association with the –OH group of the cholesterol. In the case of a phospholipid molecule such as phosphatidyl ethanolamine (used in the illustration) or phosphatidyl serine, which has a terminal amino group, there is the possibility of hydrogen bond formation, but in the case of other molecules simply a general ionic interaction is postulated. Such a complex, in which the longest and shortest lipid molecules in myelin are bound together to form a single stable unit, is suggested as a possible component of myelin. The highest bimolecular leaflet spacings obtained in the fractionation of nerve lipids are of the order of 65 Å. If a lipid giving such a spacing is associated with cholesterol (bimolecular leaflet about 35 Å) in the manner indicated, then the layer thickness would be effectively reduced to about 50 Å. The X-ray diffraction data from modified nerve structures indicates that the protein layers may be about 30 Å thick. If the protein layers of fresh myelin have a thickness of this order, and the lipid layers are about 50 Å, then the water layers may contribute as little as 10 Å to the repeating unit of 170 Å. Such a structural unit for myelin, incorporating the cholesterol-phospholipid (or cerebroside) complex and these proposed layer thicknesses, is illustrated in Figure 2. The necessary difference in geometrical arrangement between the two lipid layers shown here is such that one is a mirror image of the other about the plane of the protein layer. This would appear to be one of the simplest ways of representing the difference between the lipid layers, but it would place certain limitations on the free rotation of the lipid molecules and complexes about their long axes.

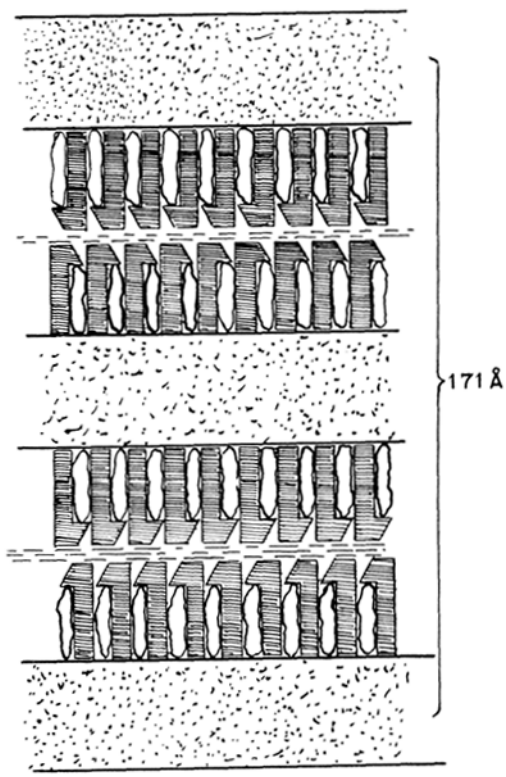


Fig. 2.

A more detailed discussion of this structure and the extent to which it helps to explain X-ray diffraction data on the modification of myelin will be published soon.

[1] W. J. Z. SCHMIDT, Zellf. mik. Anat. *23*, 657 (1936).

[2] J. ELKES and J. B. FINEAN, Discussions of The Faraday Society No. 6 (Lipoproteins), p. 134 (1949). – F. O. SCHMITT, R. S. BEAR, and K. J. PALMER, J. Cell Comp. Physiol. *17*, 353 (1941). – G. BOEHM, Koll. Z. *62*, 22 (1933).

I am grateful to Professor A. C. FRAZER and Professor J. ELKES for their continued interest in this work and to the Medical Research Council for financial assistance.

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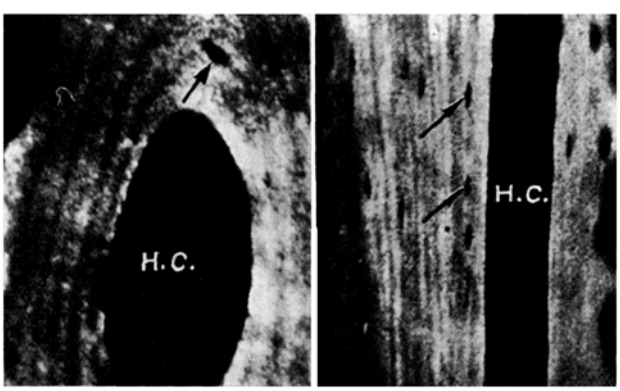
Department of Pharmacology, The Medical School, Birmingham 15, September 12, 1952.

Zusammenfassung

Auf Grund von Untersuchungen mit Röntgen-Kleinwinkelstreuung an myelinhaltigen Nervenfasern wird ein erweitertes und verfeinertes Molekül-Modell beschrieben.

Lamellar Structure of Osteons Demonstrated by Microradiography

Microradiography with soft X-rays has been used to demonstrate the content of mineral salts in the different structures of bone[1]. It has been possible to prove that the osteons have a varying content of mineral salts and that within an osteon the mineralization has its highest value close to the HAVERSIAN canal and diminishes towards the periphery. In general the young osteons formed in the resorption cavities have a much lower content of mineral salts than the old osteons (fragments of old bone). There is a continuous increase of the amount of mineral salts in the osteon with its increasing age[2].



In a series of investigations dealing with electron microscopy of bone[3] the lamellae of the osteons have been demonstrated at high magnification. Based on electron microscopic investigations of replicas of bone tissue the conclusion was drawn that the osteons are built up of alternating "fibrillar" and "cemented" lamellae[4].

From the above it is evident that it would be of great interest if microradiographs could be registered with such high resolving power that the mineral content of the lamellae could be demonstrated by X-ray absorption.

Cross and longitudinal sections 5 to 10 μ in thickness were prepared from human femur (compacta). The sections were ground on glass plates under absolute ethanol[1]. These sections were placed in close contact with a fine grained LIPPMAN photographic emulsion and exposed with 4 kV X-rays in vacuo. The X-rays were filtered in 9 μ of aluminium. Thus an 1 : 1 scale image of bone section was produced by the X-rays on the LIPPMAN emulsion. This image was enlarged by photomicrography. In the figure there are shown two such enlarged microradiagrams registered from cross and longitudinal sections. The whiter a structure is in the figure the higher its content of mineral salts. The arrows point to osteocytes. The osteocytes are not mineralized. H.C. indicates the HAVERSIAN canal. *From the pictures it is evident that the X-ray absorption varies in the different lamellae.* In agreement with earlier investigations[2] we again find a higher mineralization in the part of the osteon that is closest to the vascular channel, and that the young osteons are less mineralized than the old parts of the bone. At the wavelengths used, the major X-ray absorption is caused by both the organic and inorganic components of the bone tissue. The greatest part of the radiation generated at 4 kV and filtered in 9 μ Al lies at wavelengths between 8 and 12 Å with a small portion with wavelengths about 3 Å[3]. The former wavelength range lies beyond the K-absorption edges of calcium and phosphorus, which somewhat reduces the inorganic contributions to the absorption. For X-rays of 8·32 Å wavelength the mass absorption coefficients for protein and tricalciumphosphate are 1000 and 1400 (to the nearest hundred). A 5 μ thick section of protein transmits about 60% of 8·32 Å X-rays while a 5 μ thick layer of tricalciumphosphate transmits about 12%. Some complementary experiments were performed with 3 Å X-rays and the lamellar structure could be seen also with this radiation although not so clear as with the 8–12 Å X-rays. At 3 Å the mass absorption coefficient for calcium is about 1000 but for protein only about 50. In this wavelength (3 Å), therefore, practically all absorption is caused by the mineral salts.

From the experiments and consideration presented above it is clear that the X-ray absorption varies in different lamellae. *Thus there are lamellae with a high content of substances (organic + inorganic material) alternating with those having less substance.* The ratio inorganic to organic material is probably higher in the lamellae with a high X-ray absorption than in those with less absorption. The latter conclusion is in agreement with electron microscopic observations[4].

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Zusammenfassung

Die Lamellen des Haverschen Systems wurden durch Röntgenmikroradiographie demonstriert. Lamellen mit hoher Röntgenabsorption wechseln mit solchen niedriger Absorption ab.

[1] R. AMPRINO and A. ENGSTRÖM, Acta Anat. *15*, 1 (1952). – A. ENGSTRÖM and R. AMPRINO, Exper. *6*, 267 (1950). – R. AMPRINO, Z. Zellforsch. *37*, 144 (1952).

[2] R. AMPRINO and A. ENGSTRÖM, Acta Anat. *15*, 1 (1952).

[3] CH. ROUILLER, L. HUBER, ED. KELLENBERGER, and E. RUTISHAUSER, Acta Anat. *14*, 9 (1952). – L. HUBER and CH. ROUILLER, Exper. *7*, 338 (1951). – E. RUTISHAUSER, L. HUBER, E. KELLENBERGER, G. MAJNO, and CH. ROUILLER, Arch. Sci. (Soc. Phys. Hist. nat. Genève) *3*, 175 (1950).

[4] CH. ROUILLER, L. HUBER, ED. KELLENBERGER, and E. RUTISHAUSER, Acta Anat. *14*, 9 (1952).

[1] R. AMPRINO, Z. Zellforsch. *37*, 144 (1952).

[2] R. AMPRINO and A. ENGSTRÖM, Acta Anat. *15*, 1 (1952). – A. ENGSTRÖM and R. AMPRINO, Exper. *6*, 267 (1950). – R. AMPRINO, Z. Zellforsch. *37*, 144 (1952).

[3] A. ENGSTRÖM and B. LINDSTRÖM, Biochim. biophys. Acta *4*, 351 (1950).

[4] CH. ROUILLER, L. HUBER, ED. KELLENBERGER, and E. RUTISHAUSER, Acta Anat. *14*, 9 (1952).