

teria, have constantly shown minute Gram negative cocco-bacilliform bodies. They are commonly extracellular; occurring singly, in pairs, or in loosely formed aggregates, but may also be found intracellularly within both phagocytic and epithelial cells. They vary somewhat in size, with an estimated range of 0.1 to 0.5 μ , many of them approaching the limit of visibility with the ordinary microscope. Early in the disease the bodies are generally numerous, but in some cases their detection requires a prolonged search. The same bodies are also present in films made from the nasal passages of birds injected with unfiltered exudate. Their detection is often more difficult in this case, due to the presence of numerous bacteria.

All attempts to cultivate these bodies, either aerobically or anaerobically, in artificial media have met with failure. In several instances, however, there has been isolated a minute Gram-bacillus which tends to form compact clumps in fluid cultures. This organism, which is not pathogenic, bears a superficial morphological resemblance to the cocco-bacilliform bodies, and like them is capable of passing through certain Berkefeld V candles. Whether or not it is in any way related to these bodies can not be stated at present.

The injection of exudate from birds infected with the coryza of rapid onset into birds which have recovered from the coryza produced by injection of *Hemophilus gallinarum* is often followed by a coryza of slow onset which may be reproduced in series. *Hemophilus gallinarum* is not present in the exudate in these cases. Stained films, however, show cocco-bacilliform bodies indistinguishable from those which characterize the more naturally produced disease. These findings suggest that the coryza of rapid onset and long duration is in reality a mixed infection in which both *Hemophilus gallinarum* and the present virus, of undetermined nature, are operating. Such a relationship would offer an adequate explanation for the previously noted discrepancies in the coryzas produced by exudate and culture, respectively.

Gibbs² has recently stated that the causative agent of a fowl coryza with which *Hemophilus gallinarum* was not associated was capable of passing through certain celloidin membranes and estimated that the diameter of the agent was between 80–120 m μ . Filterability is not, however, a trustworthy characteristic for the classification of an infective agent. The present bodies may also be considered as filterable. The smaller forms certainly do not exceed 100 m μ in diameter. They are morphologically similar to the bodies which characterize fowl pox, vaccinia and psittacosis, but also resemble the Rickettsiae of typhus and allied diseases. The present knowledge

of the coryza bodies is, however, too meager to warrant a statement as to their nature. It is realized that the implied relationship of them to the etiology of coryza rests on purely circumstantial evidence and that a final appraisal must await their recovery or cultivation in a pure state.

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THE ROLE OF LIPOIDS IN THE X-RAY DIFFRACTION PATTERNS OF NERVE

In order to obtain greater detail with respect to the 40–45 Å equatorial spacings previously reported for medullated nerve,¹ photographs were made with very small pinholes (0.2 mm), with well-centered small beads and with distances of 10–20 cm from specimen to plate (copper K α radiation). These photographs revealed large well-oriented spacings previously unsuspected for animal tissues. Because of the light which this work throws on the interpretation of nerve diffraction patterns and because of the biological significance of the lipoids, to which these large spacings apparently are due, we wish to describe briefly these results.

The rôles of the myelin and the axis cylinder in the diffraction patterns of nerve were investigated by studying photographs obtained from the lipid and protein constituents of nerve separately. When rubbed up with water the solids obtained by evaporation of a benzene-alcohol extract of dried cow spinal cord gave patterns similar to those of fresh medullated nerve as exemplified by corpus callosum or motor roots. The dried lipid patterns are similar to those of dried medullated nerve, with the exception of the equatorial points at 11.5 Å observed in the latter. This spacing was duplicated by fibers spun from nucleoprotein extracts of cow spinal cord and lobster claw nerves and presumably corresponds to the side-chain distance of these protein macromolecules.²

The lipid constituents of the myelin sheath of fresh medullated nerve appear to be organized in a manner such as to form a single type of fluid crystal which gives a spacing in its long direction of approximately 171 Å. This is strongly indicated by the fact that the spacings observed are 85.5, 56.8, 42.7 and 34.2 Å with the even orders strongest and the odd orders weakest; each of the rings has strong equatorial accentuation. Upon drying nerve yields principally

¹ F. O. Schmitt, G. L. Clark and J. N. Mrgudich, *SCIENCE*, 80: 567, 1934.

² The protein extraction and fiber spinning was performed as described by F. O. Schmitt and R. S. Bear, *Proc. Soc. Exper. Biol. and Med.*, 32: 943, 1935.

² C. S. Gibbs, *SCIENCE*, 81: 345, 1935.

a strong equatorial point at 71 Å and a ring with equatorial accentuation at 43 Å; a faint equatorial point occurs also at approximately 140 Å. Apparently drying causes a separation of lipid components, one remaining oriented while another becomes somewhat disoriented. Another indication of the tendency of drying to separate the lipid components of the myelin sheath is observed in the meridionally sickled 4.7 Å ring of medullated nerve. Upon drying the nerve this ring becomes resolved into a number of rings, of which the most prominent are those at 4.2, 4.7, 5.2 and 5.9 Å. These spacings are found also in dried lipoids extracted from cow spinal cord. The 4.2 and 4.7 Å rings are typical of dried lecithin, those at 5.2 and 5.9 Å of cholesterol. Moreover, such a separation of mixed lipid crystals upon drying has been shown to occur in artificial mixtures of lipoids. The patterns obtained from such mixtures show considerable variability, depending upon the degree of hydration and the relative proportions of the individual constituents.

Although it was previously demonstrated³ that the

15.5 Å equatorial sickles of fresh nerve are not due to connective tissue, as claimed by Boehm,⁴ the present work shows that this spacing can be duplicated by wet nerve lipoids and is, therefore, interpreted best as a higher order reflection from the extremely well-oriented lipoids in the myelin sheath. This interpretation is strengthened by the fact that with drying this spacing fades out both in medullated nerve and in nerve lipoids.

The well-known tendency of lipoids to combine tenaciously with proteins,⁵ their frequent association in living cells, and the wealth of diffraction spacings given by lipoids, as illustrated above, all indicate the necessity of distinguishing carefully between the lipoids and proteins in diffraction pictures of biological materials.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

METHOD FOR STUDY OF CRYSTALS FOUND IN AMOEBA BY MEANS OF X-RAYS

THE following method seems to present a very interesting way for finding out the nature of the crystals which are present in some of the vacuoles in Amoeba.

The crystals were prepared for the x-ray picture by putting a few Amoebae which had been carefully washed in several changes of redistilled water on a thin collodion membrane which was stretched over a small round hole which had been drilled in one end of an ordinary glass slide. After the amoeba had settled to the bottom, the excess water was drained off with filter paper, and the amoebae fixed with absolute alcohol. Another thin layer of collodion was added to cover these.

The x-rays were directed on one of these amoebae

and then enlarged by means of a pinhole camera. The important features of the photograph obtained are presented in the diagram. On the photograph three or four faint Laue spots are present and the three most distinct ones are represented in the diagram by the spots A, B and C. These seem to indicate that there are one or more definite crystals present which can be investigated further by this method, but are insufficient for identification purposes. The faint outer circle surrounding the central part is probably due to the presence of the collodion membranes used to support the crystals. These investigations are now being continued and preparations of millions of crystals used instead of a few crystals. In this way it is hoped that we will get a series of diffraction spots and thus enable us to identify the crystals if they are like any that are already known.

The author is greatly indebted to Dr. Maurice Huggins for supervising the taking of the x-ray pictures and explaining the results obtained.

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VARIABLE TENSION CLAMPS FOR PHYSIOLOGY EXPERIMENTS

THREE marking pens record nicely on the smoked paper, but the fourth drags. The fourth is fixed and

⁴ G. Boehm, *Koll. Zeitschr.*, 62: 22, 1933.

⁵ See, for example, S. P. L. Sørensen, *Koll. Zeitschr.*, 53: 102, 170, 360, 1930.

³ SCIENCE, 80: 567, 1934.

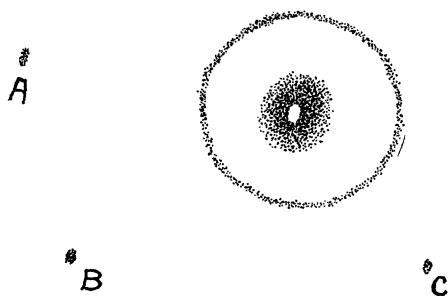


FIG. 1.