acid[3] in distilled water was dropped into the noses of 114 mice, three times a day for three successive days, and 0.05 cc of 1:250 dilution of virus-infected mouse brain, or at least 1,000 minimal lethal doses, on the fourth day. Only six of the mice succumbed to experimental encephalomyelitis; whereas of 153 untreated, control animals that received the same virus at the same time, 142 died. The results are more remarkable when considered in contrast with the general ineffectiveness, in 76 mice, of other substances substituted for tannic acid. They include normal saline solution, normal and antivirus rabbit serum, merthiolate (1:5000), hexylresorcinol (in S. T. 37, 1:3), ephedrine (1:100), and formalin (1:50). The formalin was administered to induce especially increased nasal secretion; it is noteworthy that the excessive mucoid discharge so produced did not hinder local infection with the virus. On the contrary, the tannic acid solution caused no grossly visible damage to the nasal mucosa.[4]

The chemical effects of the acid on tissue, as they are now known, are dehydration, precipitation of the soluble proteins of superficial cells and secretions and combination of all proteins with it to form a material which, among other properties, shows a greater degree of resistance to the destructive action of ordinary bacteria and enzymes.[4, 5, 6, 7] Histological studies of nasal membranes of tannin-treated mice reveal either no effect or slight shrinkage of the lining cells, accompanied by a deposition of more or less uniform precipitated material covering the ciliary surfaces. The open nasal spaces contain a slightly increased cellular exudate. By following the method of Clark,[8] who reported the transit, within 3 hours, of Prussian blue particles from the nasal cavity to the brain of the rabbit, by way of the olfactory nerve, we found that in normal mice there occurred within this time a generalized dispersion of the dye throughout the epithelium and invasion of the nerve by finer blue granules. In tannin-treated animals, however, the dye was less evident in the epithelial layers and not visible in the olfactory rootlets and nerve.

The preventive action of tannic acid is exerted locally: Tannin-treated mice are as susceptible to lethal infection after cerebral inoculation of virus as are normal animals. Another point showing that a barrier, as it were, may be set up against the infectious agent at a portal of its entry is that virus, if dropped into the nose first and tannin later, even in so short a time as 15 minutes, loses none of its activity.

It appears, besides, that a sufficient amount of tannic acid is necessary to insure its action in warding off virus infection. The method as described above was shown to yield protection in 94.7 per cent. of the treated mice, but when 0.25 instead of 0.5 or 1 per cent. of the chemical was employed, resistance was induced in 75 per cent. of the animals. Again, when the acid was administered in the usual dose of 0.5 or 1 per cent. but instilled on one or two, instead of three successive days, 39 and 62 per cent., respectively, of 23 mice in each instance, were found to resist the virus given intranasally on the day following the last instillation of the chemical.

The duration of protection afforded by tannin is, however, transient; the nasal passages approach their normal condition of sensitiveness to virus infection on the eleventh day after the beginning of the tannic acid instillations. Thus, when virus was dropped into the nose of mice on the fourth day, 94.7 per cent. of the animals were protected; on the fifth, 87.5; sixth, 83; seventh, 58.8; eighth, 30 per cent. and on the ninth and tenth days, only an occasional mouse was found to be refractory to infection. Thereafter all tannin-treated mice were normally reactive to the virus. Nevertheless, experimental results indicate that the treatment can be repeated in the same animal so as to bring about a similar temporary virus-resistant state.

To conclude, it has been found possible to induce a transient resistance in mice to intranasal infection with two strains of equine encephalomyelitis virus by prior applications of tannic acid to the nasal mucosa.

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[3] An unknown quantity is lost at each instillation through insufficient sniffing by the mouse, for the method consists in depositing the fluid employed drop by drop at the nasal orifices by means of a 27 gauge needle. The animal then inhales the material, requiring 2 minutes for the procedure. By this method injury to the nasal mucosa is avoided. However, for reasons as yet unknown, the same technique, when applied to guinea pigs, has thus far failed to protect these animals against intranasal infection with the same virus.

[4] For the innocuousness of tannic acid, especially in the amounts given, see W. A. Bastedo, "Materia Medica," etc., W. B. Saunders Co., Philadelphia, 1933, 3rd edit.

[5] H. P. Kruyt, "Colloids," John Wiley and Sons, New York, 1930.

[6] H. Gnamm, "Die Gerbstoffe und Gerbmittel," Wiss. Verlags., M.B.H., Stuttgart, 1933.

[7] M. Bergmann, personal communication.

[8] W. E. Le G. Clark, "Reports on Public Health and Medical Subjects," Ministry of Health, London, 1929, pp. 1–27.

X-RAY DIFFRACTION STUDIES ON NERVE

ANALYSIS of x-ray diffraction photographs of nerve made by us during the past few months indicates what appears to be a fundamental similarity between the fine structure of the axis cylinder of nerve and that of other animal fibers, such as hair, tendon and

muscle. In this preliminary note we wish to describe briefly the most prominent features of these photographs and their probable interpretation in terms of nerve structure. A detailed report will appear in another place.

For this work the Kα radiation of copper was used, the average exposure time being ten minutes. While the patterns are not as sharp as those from more perfectly oriented structures, such as hair and tendon, the spacings are clear enough to permit fairly satisfactory measurement. In fresh sciatics the following spacings are distinguishable: an equatorial point at 40–45 Å and another at 14–17 Å, a ring at 4.6–4.8 Å, with clearly defined meridianal sickles, and an outer ill-defined ring at 2.8–3.1 Å. The inner equatorial point very close to the central spot can be observed only by careful centering of the primary beam and by using very small pinholes and beads. This pattern seems to be typical for fresh medullated nerve, since it has been obtained from the sciatic, motor and sensory roots and spinal cord of the frog and cat. The smaller equatorial spacing decreases with drying to 11–12 Å. Tension tends to sharpen the picture somewhat, but, as in the case of muscle, produces no new pattern.

Because of its similarity to the diagram of α-keratin it seems likely that the molecular configuration producing the diagram in nerve corresponds to a system of oriented protein primary valence chains lying parallel to the fiber axis. The equatorial spacing of 17 Å corresponds to the direction of the side chain rungs (0, 0, 1 spacing of Astbury and Street[1]) and the meridianal spacing probably corresponds to the reflection from double amino-acids residues along the fiber axis. A large equatorial spacing has been observed in keratin diagrams, but its significance is uncertain.[1] We have entertained the view that this spacing in nerve and perhaps also in other animal fibers may correspond to the lateral distance between micelles. Since it is almost completely absent in pictures from nerves subjected to long soaking in alcohol, it is conceivable that this lateral distance is maintained by lipid or other fatty molecules acting as lateral spacers and oriented perpendicularly to the long axis of the micelles. Since the 4.6 Å spacing shows relatively imperfect fibering and the side chain spacing tends to appear as elongated points there must be considerable random orientation of primary valence chains. Intermicellar protein chains are also evidenced by the diffuse ring at 3.1 Å.

Boehm,[2] who also observed the 17 Å equatorial and the 4.8 Å meridianal spacings, attributed the former to connective tissue micelles and the latter to radially oriented fluid crystals of the myelin sheath. Axis cylinder, according to him, produces no pattern. However, we find no correlation between the presence of connective tissue and the 17 Å spacing; this spacing has been observed not only in sciatic nerve but also in corpus callosum, spinal cord, motor and sensory roots, and also in lobster and crab nerves. We have, moreover, observed a spacing of 4.8–5.0 Å in lobster nerve, and in a few instances, meridianal sickles at 2.5 Å, which are presumably second order reflections. There can be no doubt that the primary valence chains in lobster and crab claw nerves are very highly solvated and exist for the greater part, in the fresh tissues, as unoriented chains. The fresh nerve usually shows only one or two very diffuse rings. But by careful drying under tension, or better by very slow dehydration with increasing concentrations of alcohol up to absolute, a very clear equatorial spacing appears at 11–14 Å, and second order meridianal sickles are often visible at 2.4–2.5 A. From these considerations it seems more reasonable to believe that the pattern observed with nerve is essentially that of a single system of partially oriented primary valence chains probably admixed with unoriented intermicellar protein chains. Histological evidence seems fairly conclusive that the site of this fibrous structure is the axis cylinder. This explanation fits well with data of thermal shortening and on solvation and desolvation of nerve.[3] Since the radiation required to produce these patterns has no appreciable effect on the irritability of the nerves and if further analysis of the pictures confirms the view given above this constitutes the first evidence for the existence of a typically fibrous condition in the axis cylinder of a "normal" nerve. Moreover, a means is now available for a direct attack upon the problem of the rôle of the axis cylinder proteins in nerve phenomena.

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