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The Absorption Spectrum of Medullated and of Non-medullated Nerves.*

BETTY R. MONAGHAN AND FRANCIS O. SCHMITT.

From the Department of Zoology, Washington University.

That the conduction of the nerve impulse depends in part at least upon processes of oxidation and reduction is becoming constantly more apparent. It has already been demonstrated that somewhere in the cycle of reactions which produce the nerve impulse, oxygen activated by Warburg's respiratory enzyme is required (Schmitt[1]). The present investigation was undertaken with the purpose of determining the possible rôle of the intracellular hemin compounds (Keilin[2] and Warburg[3]) in nerve function.

The freshly dissected nerves were placed on a slide or in a vessel especially constructed for the purpose and examined with a Zeiss spectromicroscope. The nerves (usually 4 to 8 for each examination) were piled in a heap in order to provide sufficient thickness of material. The claw nerves of the Homarus and of Limulus served as types of non-medullated nerves while green frog sciatics were used as examples of medullated nerves.

* The experiments upon non-medullated nerves were performed for the greater part at the Marine Biological Laboratory, Woods Hole, Mass. The expense was defrayed in part by a grant to Washington University by the Rockefeller Foundation.

[1] Schmitt, *Am. J. Physiol.*, 1930, **95**, 650.

[2] Keilin, *Proc. Roy. Soc. Ser. B.*, 1930, **106**, 418.

[3] Warburg, *Biochem. Z.*, 1930, **227**, 245.

It was found that the absorption spectrum of the nerves of Homarus and of Limulus resembled each other closely, each presenting 2 distinct bands. The spectrum consisted of one fairly dark band extending from 552 to 562$\mu\mu$ with its center approximately at 557$\mu\mu$, and a second band which, when observable, was located at 525$\mu\mu$. For the same thickness of material the bands were much more intense in Limulus nerves than in Homarus nerves.

Such a two-banded spectrum is characteristic of hemochromogen compounds and from the position of the bands it might be supposed that they represent one component of cytochrome. This seems unlikely, however, since the bands may be observed in fresh nerves in sea water immediately after removal from the animal without the addition of a reducer. Unfortunately, in order to see the bands at all it is necessary to pile the nerves and it is entirely possible this renders them somewhat anaerobic, in which case one would expect the bands of reduced cytochrome to appear. On the other hand, placing the nerves in pure oxygen or treating them with 5% ethyl urethane, which should poison the reducing mechanism, had no noticeable effect on the spectrum. Nor could any consistent change be observed when the nerves were placed in pure nitrogen or when treated with strong reducing agents. While it cannot definitely be stated the absorption spectrum of non-medullated nerves is not that of cytochrome, it is clear that the hemochromogen present in these nerves does not react to reagents as does cytochrome. In contradistinction to nerve, however, muscles of Limulus, Homarus, or frog clearly show four-banded spectra typical of cytochrome, although the action of reagents is not in all cases typical of cytochrome as described by Keilin.

When freshly dissected sciatic nerves of green frogs are examined in the manner described, 2 faint bands make their appearance, which, upon the addition of strong alkali and a reducer are transformed into the more intense bands (at 561 and 525$\mu\mu$) of globin hemochromogen. It can be demonstrated that these bands are due to hemoglobin in the capillaries supplying the nerve fibers rather than to any compound in the fibers themselves. If for a short time preliminary to dissecting the nerves the frogs are perfused with Ringer solution no bands appear even in the presence of alkali and reducer.

Neither cytochrome nor any hemochromogen having a similar spectrum is present in frog nerve in detectable concentrations. The spectrum differs from that of non-medullated nerves also in that there is very marked general absorption in the blue approximately

to 522$\mu\mu$. Treating these nerves with pure nitrogen, with a reducer, or with sodium cyanide in concentrations from N/1000 to N/1 has no effect on the spectrum. Ethyl urethane likewise has no effect.

In an effort to determine the nature of the substances responsible for the general absorption in frog nerve, an ether extract of the dried nerves was made. This extract exhibited the same spectrum as was observed in the intact nerves. An excess of acetone was then added to the ether extract and the solution filtered. The precipitate so obtained, consisting chiefly of the nerve phosphatids, was examined and found to give a spectrum very similar to that of the original extract, though much fainter. The filtrate was evaporated to dryness and examined spectroscopically. This fraction exhibited 2 distinct bands located in the region of 500 and 465$\mu\mu$, respectively. The substance responsible for these bands is doubtless a carotinoid pigment, possibly carotin or xanthopyll. The total absorption extending to about 520$\mu\mu$ observed in the intact nerves is due, therefore, to the 2 wide bands of the carotinoid pigments superimposed upon the general absorption of the phosphatids and allied substances.

Using the method devised by Connor[4] for the quantitative determination of the carotin content of tissues it was estimated that nerve contains approximately 0.5 mg. of carotin per gram dry nerve substance. If a solution of this pigment is allowed to stand for any length of time it loses its yellow color and the characteristic absorption bands. This bleaching is due to the spontaneous absorption of oxygen by the carotin (Willstätter and Escher[5]).

Arnaud[6] suggested that carotin may function in plants in a manner similar to that in which hemoglobin functions in blood. The possibility that it may act as an oxygen transferring substance is strengthened by the fact that, despite its great affinity for oxygen, it remains at a relatively constant level of reduction in the living cell. Willstätter and Mieg,[7] as a result of their work on the oxygen capacity of carotin, arrived at conclusions similar to that of Arnaud. The evidence, however, that carotin may be reversibly oxidized and reduced in cells is as yet very meager. If such evidence can be obtained, the presence of these pigments may be of considerable importance in the oxidative processes in medullated nerves.

[4] Connor, *J. Biol. Chem.*, 1928, **77**, 619.

[5] Willstätter and Escher, *Z. Physiol. Chem.*, 1910, **64**, 47.

[6] Arnaud, *Compt. Rend. Soc. Biol.*, 1889, **109**, 911.

[7] Willstätter and Mieg, *Ann.*, 1907, **355**, 1.

That portion of the total absorption in the blue end of the spectrum of frog nerves which is not due to carotinoid pigments is probably to be attributed to the partial oxidation of the unsaturated groups in the phosphatids and related substances of the myelin sheath. Commercial (partially oxidized) lecithin, for example, shows a very similar spectrum. *Pure* linoleic acid shows little absorption in this region. If allowed to stand in air, however, general absorption up to about 520$\mu\mu$ soon appears, resulting from the oxidation, and perhaps polymerization of the unsaturated acid.

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Changes in Morphology of Giardia Canis as Affected by Diet.

H. TSUCHIYA. (Introduced by J. Bronfenbrenner.)

From the Department of Bacteriology and Public Health, Washington University School of Medicine.

The study on the *Giardia canis* (Hegner, 1922) of dog revealed the fact that there were at least 2 diverse strains, as has been found with *Giardia* in man.[1] These strains differed one from another with respect to the dimensions and shapes of their cysts and trophozoites. Differences were also noted in the ease of obtaining trophozoites and of producing excystation *in vitro*. Differences in size and shape of cysts remained constant as long as the diets were the same.

When infected animals (young puppies) were kept on a diet rich in carbohydrates, the mean dimensions of cysts were 11.47$\mu \pm .03\mu$ in one and 10.37$\mu \pm .03\mu$ in the other. However, when a carbohydrate diet was replaced by a high protein diet, the respective mean dimensions were found to be 12.05$\mu \pm .03\mu$ and 11.38$\mu \pm .03\mu$. These changes were transitory in nature and were easily reversed by corresponding changes in the diet. In view of the fact that diets affected at the same time change in the bacterial flora from an aciduric to a putrefactive type and vice versa, the question arises as to whether the variations in *Giardia* were due to the changes in diet directly or indirectly as result of changes in the bacterial flora.

With respect to the power to invade the tissues, the strains exhibited no differences—in no instance were there any trophozoites present within the tissues.

Incidentally, in the materials examined during the course of the

[1] Tsuchiya, H., *Am. J. Hyg.*, 1930, **12**, 467.