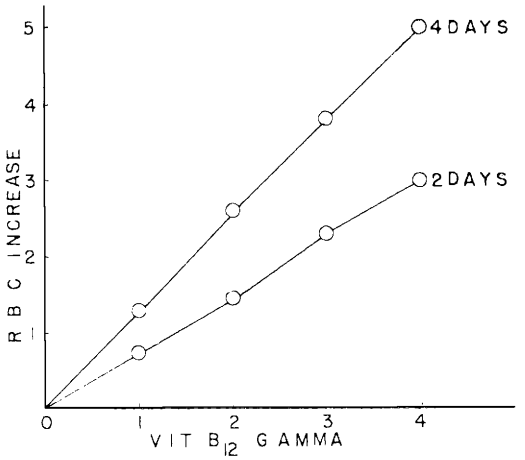

FIG. 2.

Increase in R.B.C. (red blood cells) over control at different levels of vit. B_{12}. R.B.C. count expressed as millions per cu. mm. Each value represents the average from a group of 6 animals.

the anemia. It is of interest that on the sixth day the red blood cell count of the test animals increased to values higher than normal. As indicated the concentration of hemoglobin was not influenced by the administration of vit. B_{12}. Presumably the size of the erythrocytes newly formed under the influence of vitamin B_{12}, the hemoglobin content of the cells, the total blood volume and possibly other factors account for these findings which are in striking contrast to the effects of vit. B_{12} noted in pernicious anemia as well as other types of megaloblastic anemias.

The increases in numbers of red blood cells observed in the test animals at each of the 4 levels of vit. B_{12} for periods of 2 and 4 days are shown in Fig. 2. At 4 days particularly it may be noted that the increase in red cell counts was directly proportional to the concentration of vit. B_{12} administered. These results suggest that an assay procedure for the determination of vit. B_{12} could be developed in terms of the increase in red blood cell counts at different dosages of vit. B_{12}. Experiments toward this end are in progress.

Summary. The number of red blood cells per unit volume, but not hemoglobin concentration, was increased by crystalline vit. B_{12} injected intraperitoneally into mice rendered anemic by the administration of phenylhydrazine hydrochloride. The mice were maintained prior to and during the experiment on a chemically-defined diet probably containing a relatively small amount of vit. B_{12} but adequate quantities of other hematopoietic substances. The increase in red blood cells, particularly during the first 4 days of severe anemia, was found to be directly proportional to the amount of vit. B_{12} administered. These results suggested the possible development of an assay procedure for the determination of vit. B_{12}.

Received August 21, 1950. P.S.E.B.M., 1950, v75.

Some Spectrophotometric Observations on Invertebrate Nerves and their Extracts.* (18333)

A. M. SHANES,† AND T. J. DEKORNFELD.

From the School of Medicine, Georgetown University, Washington, D. C. and the Marine Biological Laboratory, Woods Hole, Mass.

Recent experimental results have led to the suggestion that metabolic reactions may be intimately associated with the subliminal and threshold response of nerve. Optical methods, such as employed by Keilin(1), Urban and Peugnet(2) and Arvanitaki and Chalazonitis(3), appear to offer the means of fol-

* Supported in part by a research grant from the Division of Research Grants and Fellowships of the National Institutes of Health, United States Public Health Service.

† Present address: Experimental Biology and Medicine Institute, National Institutes of Health, Bethesda, Md.

1. Keilin, D., *Proc. Roy. Soc.*, Ser. B., 1928, v98. 312.

2. Urban, F. and Peugnet, H., *Proc. Roy. Soc.*, Ser. B., 1938, v125, 93.

lowing such reactions at rates comparable to those of the bioelectrical phenomena.

In the present study the possibility of detecting specific enzyme systems spectrophotometrically has been explored for intact nerves of *Libinia emarginata* and *Loligo pealii*. Observations have also been made on extracts from these nerves both in visible and ultraviolet light.

Methods. The leg nerves of Libinia, obtained by the method of Shanes and Hopkins(4), and the fin nerves of Loligo were employed. All nerves were ligated at both ends and suspended in sea water for 5 to 15 minutes before being mounted. They were then placed in standard, 10 mm corex glass cells, where they were maintained in position by the gentle pressure of a perforated metal plate. Absorption data were obtained with the Beckman spectrophotometer. A modification was made which consisted of the insertion of brass tubes into one side wall of the cell compartment. These tubes were connected by rubber tubing to a specially designed metal cap which fitted snugly over the experimental absorption cell. A metal tube extended from the cap to about 2 mm from the bottom of the glass cell. Humidified oxygen or specially purified "Linde" (99.9%) nitrogen entered by way of this tube and left by way of an exit tube at the top of the cap. During the course of the experiments a continual, gentle bubbling of gas was maintained through the cell. The control cell carried an identical perforated plate and contained the same medium. The calibration of the spectrophotometer was checked for visible light with the major sodium and potassium emission lines and for the ultraviolet with desoxyribonucleic acid. A typical experimental series consisted of 3 sets of measurements of the nerve absorption over the wavelength range of 1000 to 400 mμ, the first and third set being in oxygenated, the second in nitrogenated sea water. After the completion of each set of determinations the contents of the experimental cells were usually decanted and stored in the refrigerator at 4°C overnight for examination the following day. At the end of the series the nerves were placed into 5 cc of distilled water and refrigerated. A number of aqueous extracts were prepared with macerated nerves placed into 3 to 5 cc of distilled water, for periods of time varying from 30 minutes to 24 hours. The longer extractions were carried out at 4°C. The extracts and the sea water which had been in contact with the nerves were also examined in ultraviolet light over the wavelength range of 300 to 240 mμ. The dialysis of the nerve extracts was performed by placing 10 cc of extract into a dialyzing bag, in turn suspended in a beaker containing 10 cc of distilled water and mounted on a shaker table. Samples were taken after one hour and 4 hours both from the bag and from the dialyzate. After 4 hours the bag was kept in running water, the residue being tested after 24 and 48 hours. For comparative purposes some observations have also been made on Loligo axoplasm, Libinia muscle extracts, and Libinia blood. All experiments were performed at room temperature—approximately 20° to 24°C. It was noted that the temperature of the contents of the cells increased approximately 4°C during the course of the determinations lasting 25 to 50 minutes.

Results. Visible light. As may be seen in Fig. 1, a series of peaks in intact nerves lies between 568 and 538 mμ. They are more clearly defined in the absence of oxygen and occur within a range of ± 2 mμ at 542, 550 and 556 mμ. Sodium hydrosulfite accentuates these peaks to a somewhat more marked extent than nitrogen under the conditions employed, and makes an additional peak evident at 568 mμ. Some recovery was noted in the hydrosulfite treated nerves when they were washed in sea water or oxygenated. It seems likely that the 550 mμ peak is that of cytochrome c. Further experiments are necessary, however, for the positive identification of this and the other peaks observed. Absorption curves obtained for one sample of Loligo axoplasm demonstrated similar peaks only in nitrogen. Examination of the aqueous

3. Arvanitaki, A. and Chalazonitis, N., *Arch. Int. de Physiol.*, 1947, v54, 441.

4. Shanes, A. M., and Hopkins, H. S., *J. Neurophysiol.*, 1948, v11, 331.

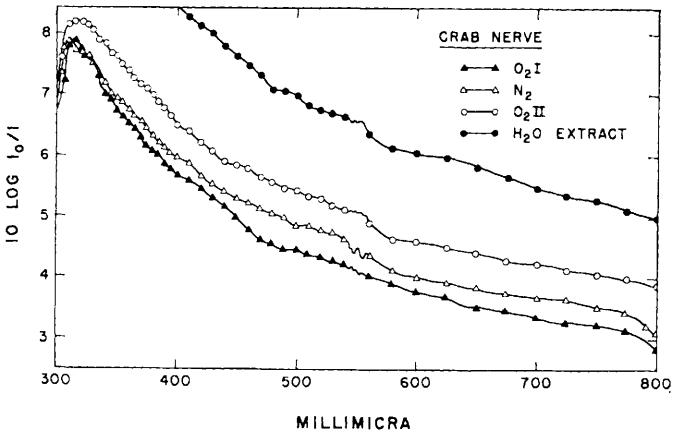

FIG. 1.

Absorption curves for one Libinia nerve subjected to oxygen, then nitrogen, and finally oxygen again, and for its 16 hr aqueous extract. The apparent peak below 400 mμ is an instrumental artifact.

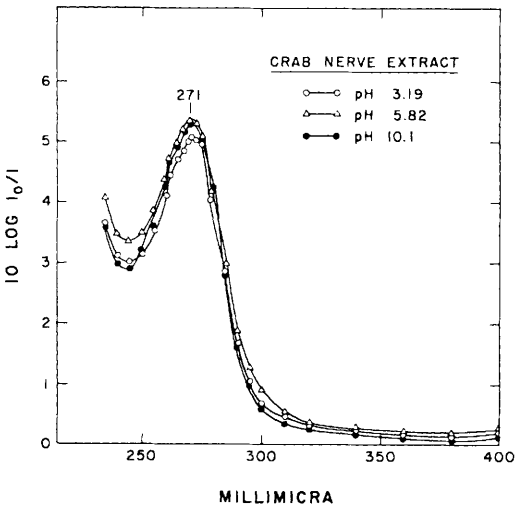

FIG. 2.

Typical absorption curves obtained with the same nerve extract at the indicated pH values. 7.5 normal acid and 2.5 normal alkali had no greater effect on the peak.

extracts and of the sea water which had been in contact with the nerves indicated that the peaks were present to a slight degree.

Ultraviolet light. Sea water which had been in contact with the nerves, as well as the aqueous extracts, gave a single, sharp peak which consistently occurred within one mμ of 271 mμ (Fig. 2). Fifteen cc distilled water, after one hour contact with 100 mg nerve tissue, gave a maximum peak density of approximately 0.5 in the standard 10 mm quartz cell. This peak was insensitive to pH changes ranging from 3.2 to 10.1. The same absorption maximum was obtained in Loligo fin nerve extracts and for the protoplasm of the giant axon of Loligo. The common occurence of this peak suggested the examination of other tissues. It was found that Libinia muscle extracts and Libinia blood gave distinctly different maxima; the former, which is little altered by pH, gave a maximum at 267 mμ, and the latter at 278 mμ.

The nature of the substance responsible for the 271 mμ peak can not be specified as yet. It is not a protein since it gives a negative Biuret test, is not precipitated by perchloric acid, and is readily dialyzable. It is highly polar, for it is not extractable from aqueous solution by ether or amyl alcohol.

The dialysis experiments demonstrated the complete removal of the unknown in 24 hours. After this period the residue had a peak between 265 and 268 mμ, while after 48 hours the peak was found to have shifted to 275 mμ.

Summary. 1) A method is presented for the spectrophotometric observation of intact, invertebrate nerves and their extracts in the presence or absence of oxygen. 2) Absorption maxima were observed for intact nerves between 538 and 568 mμ, and at 271 mμ for their aqueous extracts.

The authors are indebted to Dr. M. A. Jakus and Dr. A. J. Hodge of the Massachusetts Institute of Technology for the Loligo axoplasm; to Mr. W. S. Vincent of the University of Pennsylvania for the DNA; to Dr. Alan Mehler of the University of Chicago for his many helpful suggestions, and to Mr. Olivier Heroux of Laval University, Quebec City, P. Q., Canada, for his generous assistance, particularly in the dialysis experiments.

Received October 9, 1950. P.S.E.B.M., 1950, v75.

Concentration of Streptomycin in Brain and Other Tissues of Cats After Acute and Chronic Intoxication.* (18334)

JOSEPH E. HAWKINS, Jr., GEORGE E. BOXER, AND VIOLA C. JELINEK.

From the Merck Institute for Therapeutic Research and the Research Laboratories of Merck and Co., Rahway, N. J.

The disturbance of equilibrium which can be produced in the cat by the parenteral administration of suitably large doses of streptomycin develops only after several days of treatment(1). The time of onset of the characteristic ataxia, accompanied by loss of nystagmus in response to rotation, varies inversely with the size of the dose employed (Fig. 1). The manner in which this cumulative toxic effect is produced is still unknown. The experiments to be described were made to test one possible explanation, namely that chronic streptomycin intoxication is associated with an accumulation of the drug in the cerebrospinal fluid and in the central nervous system. The negative results, demonstrating that such an accumulation does not occur, are in accord with histological(2,3) and electrophysiological(4,5) studies which have been published since this work was done, indicating that the primary site of the toxic action of streptomycin is not in the brain stem but rather in the vestibular and cochlear end-organs. On the other hand the present experiments are of interest because they show that, unlike the brain, other organs such as the lungs and liver contain significant concentrations of the drug even 72 hours after a single dose.

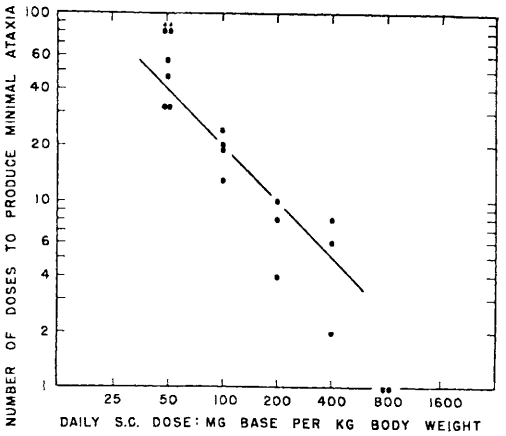

FIG. 1.

No. of doses of streptomycin required to produce minimal ataxia in cats, plotted as a function of the size of the daily dose. The straight line represents a *constant total dose* of 2 g per kg, *e.g.*, 200 mg/kg/day for 10 days. The data may be interpreted to mean that within the range 50 to 400 mg/kg an approximately constant total quantity of streptomycin is required to produce signs of vestibular disturbance. A single dose of 800 mg/kg is lethal. Streptomycin hydrochloride Merck, Lot No. 731. (From data of Hawkins and O'Shanny presented at the meeting of the Fed. Am. Soc. Exp. Biol., Atlantic City, March 1948).

Method. The fluorometric method for the determination of streptomycin in body fluids(6) and in tissues(7) was employed.

* Presented at the meeting of the Federation of American Societies for Experimental Biology, Detroit, April 1949.

1. Hawkins, J. E., Jr., *Fed. Proc.*, 1947, v6, 125.

2. Caussé, R., Gondet, I., and Vallancien, B., *Compt. rend. Soc. Biol.*, 1949, v143, 619.

3. Berg, K., *Ann. Otol.*, 1949, v58, 448.

4. Vanderhaeghe, H., *Arch. Int. Pharm.*, 1949, v79, 269.

5. Hawkins, J. E., Jr., *J. Pharmacol.*, 1950, v100, 38.

6. Boxer, G. E., and Jelinek, V. C., *J. Biol. Chem.*, 1947, v170, 491.