and eighth day after inoculation; otherwise there has been no definite clinical evidence of rabies. Most of the embryos appeared vigorous and it is believed that they would have hatched if they had not been sacrificed.

Microscopic examination of brains and cords of embryos killed on the sixth or seventh day revealed an enormous number of Negri bodies and acute neuronal necrosis. Evidence of inflammation was never very impressive, but there was some phagocytosis of necrotic ganglion cells in the cord.

Sixth passage virus was inoculated in 0.03 cc volume in the brain, in the thigh, on the chorio-allantoic membrane, in the eye and in the amnion of separate embryos. Six days following inoculation two embryos inoculated by each route were killed and fixed for study. Histological examination of embryos inoculated intracerebrally and intra-ocularly showed Negri bodies throughout the central nervous system, in the neurones of the retina and in some peripheral ganglia. Negri bodies were found only in embryos inoculated in the brain or in the eye. Furthermore, two embryos inoculated on the chorio-allantoic membrane were allowed to hatch, and neither one showed any clinical evidence of rabies during a ten-day period of observation.[2]

Experiments are being performed at present to determine the virus content of various organs and tissues of embryos inoculated by various routes, the biological changes which may have taken place in the virus following adaptation to this new host and the histological evidence of change in tropism of the virus.

The work at present indicates that the chick embryo brain is an ideal medium for the propagation of rabies virus. This method of virus culture has the advantage over *in vitro* methods in that one is able to study the pathological changes induced in the embryos by the multiplication of virus. We feel that chick embryo brain provides a richer source of rabies virus than has been obtainable in the past and that for this reason alone it may be of value in the preparation of a vaccine.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

USE OF LUMINOUS PAINT FOR OBSERVATION OF ANIMAL MOVEMENTS IN THE DARK

THE behavior of animals in the dark is often a matter of interest, but one which is difficult to study. It might be of value, therefore, to report a technique involving the use of luminous paint. This method was developed as a means for observing the swimming position of the brine shrimp, *Artemia,* when placed in total darkness.

An adult *Artemia* is laid out on a glass slide and carefully dried with filter paper. A drop of "Durofix"[1] is then placed in a watch glass, diluted with acetone, and mixed with luminous paint powder. The mixture is transferred to the surface of the animal on the tip of a suitable needle. After about ten minutes small traces of moisture are applied to those parts of the animal's body which are not too near the paint. Finally more moisture is added and the slide is carefully lowered into a jar of water. Practice is required to determine the best mixture for good adhesion. In successful cases the paint remains attached until the next moult and the animals live for many days.

The blobs of paint affixed to *Artemia* have been usually half a millimeter or less in diameter. Tests have shown that only the non-radio-active paints are satisfactory for such small spots. A paint supplied by Harrington Brothers, of London, gives a glow clearly visible to dark-accustomed eyes for fifteen minutes after daylight activation. Larger spots glow for a longer time.

With animals not so small as *Artemia* radio-active paints would be more useful. The glow which they give is continuous, but unfortunately less bright than can be obtained by daylight activation. The United States Radium Corporation of New York supply a paint which I have tested in dots of various sizes, prepared in the manner outlined above. The smallest spots glowed too faintly to be seen. Larger spots, one millimeter or more in diameter, gave good results.

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QUANTITATIVE TRANSFER OF AQUEOUS SOLUTIONS

IN the study of cell metabolism employing either the Barcroft-Warburg respirometer or the reduction method of Tünberg, it is often essential for the accuracy of the determination that the total volume of "test solution" contained in the side-arm of the vessel be transferred quantitatively to the cell suspension. In order to eliminate the factor of drainage a non-wettable surface is essential. For this purpose a coating of ferric stearate has proven of great utility, far sur-

[1] A waterproof, transparent adhesive, manufactured by the Rawlplug Co., Ltd., London.

[2] I. J. Kligler and H. Bernkopf, *Proceedings of the Soc. for Exper. Biol. and Med.*, Vol. 39, No. 1, p. 212, October, 1938.

passing paraffin, due to its negligible volume, ease of application and absence of contaminated areas on which a portion of the solution may remain. It is applied by filling the vessel with a one-quarter saturated solution of ferric stearate in benzene, draining, and allowing the solvent to evaporate. This leaves a very thin coating of ferric stearate. The hydrophobic surface so formed is not attacked by thirty minutes' exposure to 0.1: N HCl, 0.1 N NaOH, saturated NaCl, petroleum ether, chloroform or ether. Further it does not adsorb methylene blue as does glass, nor interfere either in respiration or dye reduction in any of the systems so far studied. Ferric stearate may also advantageously replace paraffin in coating micro-capillary pipettes, as employed by Wigglesworth[1] in the micro-estimation of chloride. The sample of ferric stearate employed was prepared by mixing ferric chloride with a warm, concentrated aqueous solution of sodium stearate, followed by filtration and washing (*c.f.* Langmuir and Schaefer[2]).

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AMPHIBIAN GAMETES AS BIOLOGICAL TEST MATERIAL

BIOLOGICAL material suitable for testing physical or chemical variables has not been abundant, dependable nor constantly available. Through the discovery that hibernating frogs can be stimulated by the anterior pituitary hormone to release their gametes, there is now available material which may be the answer to the experimental biologist's needs. Between September and March female frogs can be induced to provide upwards of 2,000 eggs (each) at the identical stage of maturation and 24 hours after pituitary stimulation. The eggs may be stripped from the female as needed, in lots of from 50 to 100, or in case of experiments where quantitative data are desired, entire uteri may be tied off as sacks full of eggs and removed from the body. The eggs from one uterus may be used for control as against the eggs of the other uterus, which are subjected to the experimental variables. The frog testes may either be dissected in Holtfreter's modification of amphibian Ringer's (diluted to 10 per cent.) or the male may be similarly stimulated by hormone treatment to release the spermatozoa into its seminal vesicles. Uniform and concentrated suspensions of spermatozoa may be kept for many hours without loss of inseminating powers. This period is shortened with dilution and high temperatures and may be extended if the suspensions are kept at refrigerator temperatures.

[1] V. B. Wigglesworth, *Biochem. Jour.*, 31: 1719, 1937.
[2] I. Langmuir and V. Schaefer, *Jour. Am. Chem. Soc.*, 59: 2400, 1937.

In some recent investigations with both low and high voltage x-radiation, embryos from radiated gametes have shown consistent and quite uniform results. With carefully controlled x-radiation of either sperm or eggs, many of the earlier predictions of Hertwig and of Bardeen have been confirmed. There are, however, many new and biologically significant aspects of this radiation problem, which have been revealed by our modern precision equipment and this newly available biological material. It has been impossible, for instance, to render immotile frog spermatozoa with high voltage radiation even up to 120,000 r., although some abnormal embryos appear when the spermatozoa receive as little as 25 r. Early cleavage of eggs fertilized by radiated sperm is perfectly normal in both rate and pattern. There is, however, some evidence that near 10,000 r. the sperm nucleus is sufficiently damaged as to prevent neurulation, but eggs inseminated with spermatozoa which have been exposed to upwards of 30,000 r. will result in quite normal-appearing tadpoles, which may, however, be haploids. Both frog's sperm and eggs are being used to test, from a biological point of view, the qualitative difference between the soft and the hard x-rays.

The details of these radiation experiments will be reported elsewhere, but it is the purpose of this note to call attention to this extremely abundant and dependable biological test material which can be used along the lines of genetics, cytology, cell physiology and embryology.

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