the staff, and we take pleasure in thanking them. Our thanks are especially due to Drs. A. J. Allen, M. B. Sampson and W. Danforth for the preparation of the radioactive sulfur and to Dr. E. F. Schroeder and Miss G. E. Woodward for the isolation and identification of the glutathione.

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CRYSTALLINE HORSE LIVER CATALASE

ONE of us (O. D. F.) observed that after dialysis for three weeks near the isoelectric point, a solution of horse liver catalase prepared by the method of Agner[1] deposited a small amount of active crystalline material. Not enough of this precipitate was obtained for accurate analysis.

Later, by modifying the method for preparing crystalline beef liver catalase[2] we obtained crystalline horse liver catalase in good yield. The crystals are in the form of very fine needles. After preliminary purification, including fractional precipitation with dioxane, crystallization was induced by slow addition of ammonium sulfate to the properly buffered catalase in a solution containing 3.0 per cent. of dioxane. Catalase crystals have been obtained also from preparations made by Agner's directions up to the point where the material is adsorbed by passing it through a column of calcium phosphate.

A sample of once-recrystallized horse liver catalase has been found to have a *Kat. f.* of 50 to 55 thousand, which is of the order of magnitude of that of the best preparation of Agner. We do not think that the material is yet entirely purified, since the content of iron is 0.2 per cent. instead of 0.09, which would correspond to the hemin iron. It is possible that our material was contaminated with a small amount of a protein high in iron which was observed during the preparation of the catalase crystals, and which is probably identical with the ferritin of Laufberger.[3]

The percentage of hemin, determined by colorimetry and by analysis for hemin iron, is about 0.9, which agrees with the claim of Stern and Wyckoff[4] that horse liver catalase contains about 0.1 per cent. of hematin.

Treatment of the once-recrystallized horse liver catalase with acetone and HCl split off the hemin which dissolved in the acetone. Evaporation of the acetone caused the hemin to precipitate, leaving a small part of the non-hemin iron in the supernatant liquid. Most of the non-hemin iron was in the protein residue.

Our analysis of the sample of once-recrystallized horse liver catalase showed that the copper content was practically negligible, contrary to the finding of Agner that the copper content of horse liver catalase is high enough to be of possible significance.

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INFECTION OF CHICKS AND CHICK EMBRYOS WITH RABIES[1]

IT is the purpose of this communication to present a preliminary report of observations made on chicks and chick embryos inoculated with rabies virus. These investigations were undertaken in order to study the lesions produced in this species and also to determine the changes which might take place in the virus following its adaptation to this unnatural host.

Rabid dog brain passed once through mice was the source of the virus used in this work. Histologically, this dog brain and the mouse-passage brain showed typical Negri bodies. The inoculum consisted of 0.03 cc of 10 per cent. brain emulsion.

Intracerebral inoculation of day-old chicks was followed by signs of rabies after 19 days on the first passage, and 4 passages did not reduce this period appreciably. There was considerable variation in the incubation period and also in the duration of the disease; some animals died within 2 days after the first signs of the disease, while others showed definite paralysis for 2 weeks before death. All these chicks went through a stage of excitement before the onset of flaccid paralysis.

At autopsy no gross lesions were demonstrable. Microscopic examination of brains and cords revealed many Negri bodies, some quite small, others huge. Non-specific changes in the form of acute necrosis of ganglion cells and massive perivascular accumulations of lymphocytes and large mononuclear phagocytes were present throughout the central nervous system.

Portions of brain from the 4th intracerebral passage in chicks were ground and emulsified and 0.03 cc were inoculated intracerebrally into 13-day-old chick embryos. The eggs were opened and the embryos were inoculated according to the technique which had been developed in this laboratory. After 4 days one embryo was sacrificed and its brain was inoculated into the brains of other embryos; subsequent passages have been made every 6 or 7 days, and the virus is now in its twelfth generation.

We have not allowed any chick embryos inoculated by the intracerebral route to hatch. Several embryos inoculated by this route have died between the sixth

1 Aided by grants from the John and Mary R. Markle Foundation.

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.