

The same picture found in experimentally-produced cases of parasitism was noted in a naturally infected Brahma heifer about 1½ years old. This animal was brought to the laboratory in a much weakened condition, greatly emaciated, and passing very small quantities of liquid fecal material with a very fetid odor. Fecal examinations during the entire time of observations revealed a very high number of parasite eggs. This animal showed the characteristic drop in the number of red cells to 5.25 million, with a phenomenal rise in the white cells to 31,000 correlated with an increase of 35% in the neutrophils, which reached its maximum 24 hours before death.

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#### **Cultivation of Certain Viruses Using Yolk of Chick Embryo as Route of Injection.**

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Cox<sup>1</sup> recently reported the use of the yolk sac of the developing chick embryo as a medium for growing rickettsiae of Rocky Mountain spotted fever and typhus fever. Success was also attained in the cultivation of Eastern and Western strains of equine encephalomyelitis by the same worker (personal communication May 2, 1939).

This preliminary report confirms the results of Cox with the Eastern and Western strains of equine encephalomyelitis virus and in addition summarizes the results obtained with the virus of St. Louis encephalitis and 3 strains of poliomyelitis virus recovered locally.

In essentials the method employed is the same as that described by Cox. Fertile eggs were incubated at 39°C until injected and then at 37.5°C for the cultivation studies. Injections of the inoculum in doses of 0.1 to 0.5 cc were made directly into the yolk through a small opening in the air sac end of the egg which would just allow the passage of a 21 gauge needle. The opening was then closed with melted paraffin. Three to 4 eggs were used for the same inoculum in each passage and infectivity tests of the embryonic tissues, ex-

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<sup>1</sup> Cox, H. R., *Public Health Reports*, 1938, **53**, 2241.

cept when titrating for end point infectivity, were made from 10% ground suspensions. Intracerebral doses of 1.0 cc were given to Rhesus monkeys for testing infectivity in the experiments with poliomyelitis virus and 0.03 cc given to white mice for the other viruses. One monkey as compared with 2 to 4 mice was used for each suspension as required.

Three series of egg passages, represented by different doses of original infective mouse brain inoculum—0.1, 0.2, and 0.5 cc were maintained for each of the following viruses: Eastern equine encephalomyelitis, Western equine encephalomyelitis and St. Louis encephalitis. Six-day eggs were used and the embryonic tissues were harvested and pooled for infectivity tests and egg passage 3 days after injection. Embryo tissue of the second egg passage of Eastern equine encephalomyelitis virus was infectious in mice in dilution of 1-100,000. The 1-500,000 dilution was negative in mice but when transferred to the third egg passage the pooled embryonic tissues of this passage produced fatal infection in mice. Yolk sac tissue was infectious in mice in the dilution of 1-10,000 but when transferred to the third egg passage the infectivity was lost. The Western equine encephalomyelitis virus has not been titrated from egg passage as yet but the embryonic tissues were infectious in the 0.1 cc and 0.2 cc dose series through the fourth passage in the usual mouse dose. The St. Louis encephalitis virus has maintained constant infectivity in the 0.1 cc dose series through the ninth passage and the 0.2 cc dose series through the seventh passage. This virus has not been titrated to end-point infectivity but was found infectious in the eighth passage in dilution of 1-1000. In all cases the embryos were dead when harvested on the third day and it is suggested in future work that the tissues be taken earlier. It is of interest to note that in the 0.5 cc dose series of original inoculum the infectivity was lost in the second or third passage in each case. This may possibly have resulted from very early death of the embryo by use of the large dose.

The strains of poliomyelitis virus selected were the McS, which produces experimental poliomyelitis of moderate severity; the BK, which produces a more or less mild degree of severity, and the ST, which produces a severe form of the disease. Each of these strains has been carried in 12 or more monkey passages and reported in previous work.<sup>2, 3</sup> Four series of fertile eggs incubated 7, 8, 9, and 10 days respectively were injected with 0.5 cc of a 10% suspension of McS monkey cord (fresh, not glycerolated). Additional eggs in

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<sup>2</sup> Stimpert, F. D., and Kessel, J. F., *Am. J. Hyg.*, 1939, **29**, 57.

<sup>3</sup> Kessel, J. F., Stimpert, F. D., and Fisk, R. T., *Am. J. Hyg.*, 1939, **29**, 45.

each series were also injected with 1% and 0.1% suspensions of the same virus material. One series of 12-day eggs was injected with 0.2 cc doses of a 10% suspension of glycerolated BK virus cord pool. Two series, 9- and 12-day eggs were injected with 0.3 cc of a 10% suspension of glycerolated St virus cord pool. Three or more eggs were injected in each series and after 3 to 4 days' incubation the embryonic nervous tissue and the yolk sacs were recovered separately from each egg, pooled, ground and injected into monkeys to determine infectivity. All results were negative in the 22 monkeys employed. Control animals given the virus suspensions used in the egg injections showed typical infection.

## 10721

### A Spectrophotometric Method for the Study of Fat Transport and Phosphorylation\*

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The study of fat transport has been facilitated by the use of labelled molecules whose fate in the animal body could be followed. The tracers thus applied to fat transport studies have been: iodized fatty acids,<sup>1</sup> elaidic acid,<sup>2</sup> deuterium,<sup>3</sup> and the radioactive isotope of phosphorus.<sup>4</sup> Miller and Burr<sup>5</sup> have followed the transport and distribution of tung oil in rats by utilizing the characteristic absorption band of eleostearic acid which makes up more than 90% of tung oil. The characteristic absorption spectra of eleostearic acid, due to its 3 conjugated double bonds, makes possible the distinction of this acid from normal body fats by spectroscopic analysis. However, tung oil offers two disadvantages. First, it is poorly tolerated by animals. And second, one double bond may be selectively destroyed

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<sup>1</sup> Artom, C., *Arch. intern. physiol.*, 1933, **36**, 101.

<sup>2</sup> Sinclair, R. G., *J. Biol. Chem.*, 1935, **111**, 515.

<sup>3</sup> Schoenheimer, R., and Rittenberg, D., *J. Biol. Chem.*, 1935, **111**, 163.

<sup>4</sup> Hevesy, G., *Nature*, 1936, **136**, 754.

<sup>5</sup> Miller, E. S., and Burr, G. O., *Proc. Soc. Exp. Biol. and Med.*, 1937, **36**, 726.