Kansas State College; *President-elect,* Dr. John W. Breukelman, Kansas State Teachers College, Emporia; *Vice-president,* Dr. Claude W. Hibbard, University of Kansas; *Secretary,* Dr. Donald J. Ameel, Kansas State College; *Treasurer,* Dr. F. W. Albertson, Fort Hays Kansas State College; additional executive council members, Dr. Harvey A. Zinszer, Fort Hays Kansas State College; Miss Edith P. Beach, Lawrence High School; Dr. Paul G. Murphy, Kansas State Teachers College, Pittsburg; and Dr. Philip S. Riggs, Washburn Municipal University. Dr. Robert Taft, of the University of Kansas, was reelected editor of the *Transactions* for a period of three years. Dr. Stuart M. Pady, of Ottawa University, was elected associate editor for a term of three years. Dr. John C. Frazier, of Kansas State College, was elected for a three-year term as representative to the American Association for the Advancement of Science. Dr. M. J. Harbaugh, Kansas State College, was elected academy librarian.

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SPECIAL ARTICLES

INTERFERENCE BETWEEN ST. LOUIS ENCEPHALITIS VIRUS AND EQUINE ENCEPHALOMYELITIS VIRUS (WESTERN TYPE) IN THE CHICK EMBRYO

CERTAIN viruses, when injected into experimental animals, have been shown to influence the course of disease produced by a virus subsequently injected.[1] It has recently been found that this so-called "interference phenomenon" may occur even when the viruses are injected simultaneously.[2] This interference has usually been observed only when closely related viruses were being studied. Data obtained from the experiments reported here indicate that the "interference tween such unrelated viruses as St. Louis encephalitis and equine encephalomyelitis.

EXPERIMENTAL

Ten-day old chick embryos were inoculated with 0.2 cc of a 10 per cent. suspension of either: (1) mouse brain infected with St. Louis encephalitis virus; (2) mouse brain infected with St. Louis encephalitis virus which had been heated at 56° C for 30 minutes, or (3) normal mouse brain. Normal rabbit serum which had been inactivated at 56° C for 30 minutes was used in preparing the above suspensions. The embryos were incubated at 36 to 37° C for 48 hours and then inoculated with 0.2 cc of a 1 to 1,000 suspension of chick embryo infected with equine encephalomyelitis virus (Western type). All injections were made with a ¾-inch 23-gauge needle through the air-space

TABLE 1

TITER OF VIRUS IN ALLANTOIC FLUIDS AND EMBRYO SUSPENSIONS

First inoculum	Second inoculum	Material titrated	Dilutions tested 10^{-1}	10^{-3}	10^{-5}	10^{-7}	10^{-9}	LD 50 titer log of dilution
N. M. B. 5/24/43	W. E. E. 5/26/43	Allantoic fluid	4/4	N. T.	4/4	2/4	0/4*	7.0
		Embryo Suspensions	3/3	3/3	3/3	3/3	0/3	8.0
S. L. E. 5/24/43	W. E. E. 5/26/43	Allantoic fluid	4/4	N. T.	0/4	0/4	0/4	$>1.0<5.0$
		Embryo Suspensions	3/3	3/3	0/3	0/3	0/3	4.0
N. M. B. 6/5/43	W. E. E. 6/7/43	Allantoic fluid	3/3	3/3	1/3	2/3	0/3	6.0
		Embryo Suspensions	3/3	3/3	3/3	3/3	0/3	8.0
S. L. E. (Heated) 6/5/43	W. E. E. 6/7/43	Allantoic fluid	3/3	3/3	3/3	0/3	1/3	6.4
		Embryo Suspensions	3/3	3/3	3/3	3/3	1/3	8.4
S. L. E. 6/5/43	W. E. E. 6/7/43	Allantoic fluid	3/3	1/3	0/3	0/3	0/3	2.4
		Embryo Suspensions	3/3	3/3	0/3	0/3	0/3	4.0

* Numerator = number of deaths; denominator = number of mice tested.
N. M. B. = normal mouse brain.
S. L. E. = St. Louis encephalitis virus (Strain No. 3).
W. E. E. = Equine encephalomyelitis virus (Western type).
N. T. = Not tested.

phenomenon" is not limited to those viruses which are closely related, but that interference may occur be-

[1] M. Hoskins, *Amer. Jour. Trop. Med.,* 15: 675, 1935; G. M. Findlay and F. O. MacCallum, *Brit. Jour. Exp. Path.,* 44: 405, 1937.

[2] W. Henle and G. Henle, SCIENCE, 98: 87, 1943.

end of the egg. After receiving the second injection the embryos were incubated an additional 20 to 22 hours.

The allantoic fluid and embryos from eggs which had received identical inocula were harvested and pooled. The embryos were homogenized in a high-speed mechanical blender with sufficient buffered salt solution (pH 7.4) to make a 10 per cent. suspension. After centrifugation at 2,000 R. P. M. for 10 minutes the allantoic fluid and embryo suspensions were titrated intracerebrally in 3- to 4-week-old Swiss mice. The intracerebral titer of the virus recovered was determined by the method of Reed and Muench on the basis of 100-fold dilutions.

RESULTS

It was found (Table 1) that equine encephalomyelitis virus could be readily propagated in embryos which had been previously inoculated either with normal mouse brain or with heated St. Louis encephalitis virus. The amount of virus present in the allantoic fluid and in suspensions of the whole embryos was approximately that which is recovered when equine encephalomyelitis virus is grown in normal embryonated eggs. On the other hand, equine encephalomyelitis virus grew only to a limited degree, if at all, when injected into embryos in which St. Louis encephalitis virus was being propagated. A comparison of the titers of the virus recovered from the three groups indicated that the eggs which contained normal mouse brain and heated St. Louis encephalitis virus when injected with equine encephalomyelitis virus yielded about 10,000 times as much virus as those which had previously been infected with the encephalitis virus and later with the encephalomyelitis virus.

Because it has been observed[3] that in the developing chick embryo St. Louis encephalitis virus reaches a concentration equal to that found in the above eggs infected with the two viruses it is suggested that the growth of equine encephalomyelitis virus could have been completely inhibited, since all the virus recovered could have been that of St. Louis encephalitis.

Although the St. Louis encephalitis virus which was heated at 56° C for 30 minutes was not completely inactivated (2 of 6 mice injected intracerebrally with this material developed a fatal encephalitis) it did not inhibit the growth of equine encephalomyelitis virus. The probable explanation for this finding is that the amount of virus present in the heated material was insufficient to initiate infection in the chick embryo and therefore did not interfere with the growth of the equine encephalomyelitis virus which was subsequently injected.

Further studies along this line have revealed that, in the developing chick, influenza virus (PR8 strain) is able to interfere with or even completely inhibit the growth of equine encephalomyelitis virus. The degree of inhibition is dependent upon the time transpiring between the injection of the influenza virus and the subsequently inoculated equine encephalomyelitis virus. A more detailed report of these findings will appear elsewhere.

[3] C. E. Duffy (unpublished data).

SUMMARY

Interference between two unrelated viruses is reported. Equine encephalomyelitis virus grows only to a limited degree, if at all, when injected into chick embryos in which St. Louis encephalitis virus is being propagated.

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THE OXIDATION AND REDUCTION OF MUSCLE ADENOSINETRIPHOSPHATASE[1]

ALTHOUGH it has been reported that cysteine does not influence the activity of the adenosinetriphosphatase (ATP-ase) of muscle,[2] Barron and Singer[3] have reported that -SH groups may be important for the activity of this enzyme. Our interest in the problem arose from the observation that the ATP-ase activity of rat muscle myosin may show a greater decrease during storage when activity measurements are made at a pH in the neighborhood of 9 than when the measurements are made at pH 6 to 7. Since this effect could not be produced by heat inactivation, it seemed possible that it might be related to the oxidation of the free -SH groups which exist in native myosin.[4] An aged myosin preparation which had shown a greater loss of activity in the alkaline range than in the acid range was treated with 0.02 M cysteine for 20 minutes at 20° C. When the activity was again measured,[5] it was found that the activity at pH 6.8 had decreased 6 per cent., while the activity at pH 9.2 had increased 20 per cent.

In order to show that the ATP-ase activity of myosin is altered by oxidation and that the effects are dependent upon the pH of the activity measurements, myosin was treated with H_2O_2 at pH 7 and then an attempt was made to reverse the effect of peroxide

[1] Aided by a grant from the Rockefeller Foundation.

[2] Morris Ziff, *Proc. Soc. Exp. Biol. and Med.*, 51: 249, 1942. Since this manuscript was submitted for publication, a second paper by Morris Ziff, *Jour. Biol. Chem.*, 153: 25, 1944, has appeared. Our findings regarding the effects of oxidation and reduction on the alkaline activity are confirmed and extended.

[3] E. S. Guzman Barron and T. P. Singer, SCIENCE, 97: 356, 1943.

[4] Jesse P. Greenstein and John T. Edsall, *Jour. Biol. Chem.*, 133: 397, 1940.

[5] John W. Mehl and Edwin L. Sexton, *Proc. Soc. Exp. Biol. and Med.*, 52: 38, 1943.