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.

by treating with cysteine. The results are given in Table 1, and again suggest that oxidation has a much

TABLE 1

Treatment	Per cent. of initial activity measured at the same pH		
	Myosin 1	Myosin 2	
	Measured at pH 9.2	Measured at pH 9.0	Measured at pH 6.3
0.0075 per cent. H_2O_2 for 15 min. at 22° C.	5	14	49
H_2O_2 as above followed by 0.02 M cysteine for 15 min. at 22° C.	32	49	58

greater effect upon the activity measured in the more alkaline range. When oxidation has taken place, restoration of the activity by reduction takes place to only a small extent for the acid range, but to a substantial extent for the alkaline range.

One might be tempted to look upon the differential effect of oxidation and reduction as evidence for two types of enzyme in the myosin preparations. However, we have been unable to obtain any evidence for fractionation by precipitation at high or low salt concentrations. The failure of attempts to separate the activity from myosin has also been reported by Bailey.[6] The possibility that a different type of functional group in the same molecule or micelle may be primarily responsible for enzymatic activity in each of the pH ranges also suggests itself. Most studies of this enzyme have been made in the pH ranges around 8.5 to 9.5. As the pH is reduced the activity declines and then rises again in the region between 7.5 and 6.5. Although the activity is only about half as great as that at the alkaline optimum, it is this pH which may be expected to be found in the intact muscle. The present studies indicate that the behavior of the enzyme may differ qualitatively as well as quantitatively in the two pH ranges. Whatever the ultimate explanation may be, these experiments do indicate the need for particular attention to pH effects in considering the *in vivo* action of muscle ATP-ase.

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STUDIES ON THE PERIPHERAL BLOOD IN PATIENTS WITH THERMAL BURNS. 1. THROMBOCYTOPENIA[1, 2, 3]

THE blood platelets, counted by a direct method,[4] were followed on 13 patients with thermal burns.

The patients ranged in age from 4 to 81 years; 5 were males and 8 females. The total area of body surface burned varied from 10 to 75 per cent. and in 9 of the 13 patients was 25 per cent. or more. Third-degree burn was present in all. In 9 cases 20 per cent. or more of the body surface was involved with a third-degree burn.

Surface treatment in most cases was petroleum jelly dressings, often with pressure dressings or plaster casts. One patient was treated with tannic acid and silver nitrate, and one with "triple dye."

Shock was present in 6 cases; in 2 it was slight and in 4 moderate. All the patients received citrated plasma. The administration of large amounts of citrated plasma to normal subjects did not cause a thrombocytopenia.

Hemoglobinemia was present for varying periods of time in all the 13 patients.

For purposes of analysis, platelet counts below 150,000 per cu. mm were considered decreased. In 10 patients the initial platelet count a few hours after burn was above this level. In 3 cases the first platelet count, obtained 9, 38 and 72 hours after the burn, was already below 150,000 per cu. mm.

No decrease in platelets was observed in one patient, who died 7 hours after the burn. He was a 75-year-old man with 15 per cent. of his body surface involved, of which 5 per cent. was a third-degree burn.

In 12 cases, a decreased platelet count was observed 7 to 57 hours after the burn. The lowest counts obtained occurred within 23 to 96 hours after injury and ranged from 9,000 to 96,000 per cu. mm. Six of these patients died within 4 days after the injury, and in these cases the thrombocytopenia persisted until death. In the remaining six, the platelets returned to a normal level in from 4 to 9 days. However, death occurred subsequently in all but one case. This patient is living and apparently completely recovered, fifteen months after injury.

Purpura was observed in 2 patients. In one case petechiae and ecchymoses were widespread.

The cause of the thrombocytopenia and its possible role in the complication of burns is at present under investigation.

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[6] Kenneth Bailey, *Biochem. Jour.*, 36: 121, 1942.

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[2] The work described in this paper was done in part under a contract recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Harvard University.

[3] Read before the meeting of the Burns Committee, Washington, D. C., December 21, 1943.

[4] F. J. Pohle, *Am. Jour. Med. Sci.*, 197: 40–47, 1939.