lated by Francis, Quilligan and Minuse(20) poses another problem as regards the current vaccine. Whether these will eventually be included as immunizing agents remains to be determined, but at the moment the question is essentially a theoretical one since the new strains are difficult to grow by the methods commonly employed for commercial production of influenza virus.

While the selection of strains of influenza virus for use in a vaccine cannot be based on *in vitro* studies alone, the agglutination-inhibition test might be expected to assist materially in the choice of new strains for detailed examinations. The marked strain specificity of chicken antisera when employed in the influenza agglutination-inhibition test appears to provide a simple and effective means for the rapid preliminary antigenic survey of a large number of strains.

20. Francis, T., Jr., Quilligan, J. J., Jr., and Minuse, E., *Science,* 1950, v112, 495.

Summary. Influenza A viruses, as revealed by agglutination-inhibition tests with rooster antisera, comprised a graded series of antigenic forms in which precise subgrouping was not possible by the present method. The B strains examined fell into 3 subgroups. The reappearance from time to time of strains closely related to an earlier prototype, necessitates the selection of a group of strains for a polyvalent vaccine which possess broad immunogenic activity. Certain possible deficiencies in the present formula vaccine were mentioned. The recent outbreak of influenza among Eskimos in remote Victoria Island, Canada, in which there was mortality and morbidity approaching the pandemic form of disease, was apparently caused by 2 different viruses which resembled the well-studied PR8 and FM1 strains of influenza A.

The technical assistance of SFC F. Hawk and SFC W. O. Blair is gratefully acknowledged.

Received November 20, 1950. P.S.E.B.M., 1950, v75.

Enhancing Effect of Cortisone upon Poliomyelitis Infection (Strain MEF1) in Hamsters and Mice. (18362)

GREGORY SHWARTZMAN.

From the Division of Bacteriology, Laboratories of the Mount Sinai Hospital, New York City.

It was recently shown that ACTH and cortisone were capable of inhibiting the phenomenon of local tissue reactivity. The substances failed to suppress the state of reactivity following the preparatory injection of the bacterial toxin but conferred protection against the effect of the provocative intravenous injection of the toxin(1,2). These findings were confirmed by Thomas and Mogabgab. In extending the studies they found that when the preparatory intradermal injection of the toxin was accompanied by an intramuscular injection of ACTH or cortisone, there appeared a petechial reaction at the site of the toxin injection in the absence of the provocative intravenous injection of the toxin. In rabbits receiving no cortisone or ACTH the intradermal injection of toxin failed to produce any hemorrhagic reaction unless followed by a provocative injection of the toxin. The lesions described differed sharply in appearance from the typical reaction of the phenomenon of local tissue reactivity(3). Since the observations suggested that ACTH and cortisone may be capable of enhancing the susceptibility of tissues to the primary effect of bacterial toxins, they were led to determine the effect of the hormones upon bacterial infections. In experiments in this direction Mogabgab and Thomas

1. Soffer, L. J., Shwartzman, G., Schneierson, S. S., and Gabrilove, J. L., *Science,* 1950, v111, 303.

2. Shwartzman, G., Schneierson, S. S., and Soffer, L. J., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 175.

3. Thomas, L., and Mogabgab, W. J., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 829.

observed a marked enhancement of blood and tissue infection by *Streptococcus hemolyticus* in rabbits following injection of cortisone(4). The appearance of a bacteremia during crisis in a case of pneumonia treated with ACTH by Finland, Kass and Ingbar is also of interest in this connection(5).*

Taking into consideration the above findings and the fact that pregnant women in whom there is a greatly enhanced adrenocortical function(6), show an increased incidence of poliomyelitis(7), the author of this paper carried out the following studies on the effect of ACTH and cortisone upon experimental poliomyelitis infection.

Experimental. Strain MEF1 kindly supplied by Dr. Peter K. Olitsky was employed in these studies. The virus was maintained in this laboratory by serial intracerebral mouse passages. In the course of the work the identity of the virus was checked by neutralization tests with anti-Lansing monkey serum, received from the Lederle Laboratories through the courtesy of Dr. Hilary Koprowski. Swiss male mice weighing 10-14 g and male hamsters weighing 22-27 g were used in the experiments described. Only mouse brain passages were employed for inoculation of mice and hamsters. The intracerebral inoculation dose for both animal species was 0.05 ml of the brain emulsion, suitably diluted with saline. All surviving animals were examined daily for a period of one month. In a large control series carried out previously in this laboratory in connection with other studies, the hamsters were found quite resistant to poliomyelitis infection. Intracerebral inoculation of MEF1 strain gave rise to a mild disease of irregular onset, low incidence, and very low mortality rate. Thus, out of 61 hamsters 10 became paralyzed 4-19 days following inoculation with MEF1 mouse brain emulsion diluted 1 : 20. Three paralyzed hamsters died several days later, while 7 survived during the entire period of observation. The remaining 51 hamsters had no symptoms and showed normal weight gain. The above observations confirm the previous studies of Stebbins and Lensen(8).

The experimental groups and the accompanying control series of animals recorded in Table I were inoculated with the same brain emulsions. Several experimental hamsters and mice were killed at various stages of the disease. The brain, liver, spleen, and blood were cultured on a variety of aerobic and anaerobic media, and the brain emulsions diluted 1:100 were inoculated intracerebrally into mice in order to ascertain the presence of poliomyelitis virus. With the exception of a few surface contaminations of brain and liver (*i.e., Staphylococcus albus* and *E. coli*), the sacrificed animals showed no evidence of bacterial infection. The emulsions of the brains removed from the experimental animals produced typical poliomyelitis in mice. ACTH and cortisone were administered intramuscularly, each dose being 5 mg. When 3 injections were made, the intervals of time were 4 and 19 hours. The results of the studies were as follows:

In the first series of experiments 10 mice receiving one injection of ACTH and two injections of cortisone and 10 untreated mice were each inoculated intraperitoneally with 0.5 ml of MEF1 mouse brain emulsion diluted

4. Mogabgab, W. I., and Thomas, L., personal communication subsequently presented before the Central Soc. for Clinic. Res. 23rd Ann. Meet., Chicago, Nov. 3 and 4, 1950.

5. Finland, M., Kass, E. H., and Ingbar, S. H., *Proc. of the First Clinical ACTH Conference,* John R. Mote, Editor. The Blakiston Co., publishers, 1950, 529.

* Following submission of this paper for publication reports dealing with enhancement of tuberculous(9,10) and syphilitic(11) experimental lesions by cortisone came to the attention of the author.

6. Venning, E. H., *Endocrinology,* 1946, v39, 203.

7. McGoogan, L. S., *Am. J. Obstet. Gynec.,* 1932, v24, 215; Aycock, W. L., *New Engl. J. Med.,* 1941, v225, 405; *Idem, Ibid.,* 1946, v235, 160, and others.

8. Stebbins, M. R., and Lensen, S. G., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 272.

9. Spain, D. M., and Molomut, N., *Am. Rev. Tuberc.,* 1950, v62, 337.

10. Michael, M., Cummings, M. M., and Bloom, W. L., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 613.

11. Turner, Th. B., and Hollander, D. H., *Bull. Johns Hopkins Hosp.,* 1950, v37, 505.

TABLE I. Effect of ACTH and Cortisone Upon Experimental Poliomyelitis.

Group No.	Animal species	Total No. of animals	Treatment	Intracerebral inoc. of MEF1: Dose, dil.	Intracerebral inoc. of MEF1: Time interval between treatment and inoc.	Deaths without paralysis: No.	Deaths without paralysis: Days after inoc.	Animals paralyzed: No.	Animals paralyzed: Days after inoc.	Deaths following paralysis: No.	Deaths following paralysis: Days after inoc.	No. survivors with paralysis	No. survivors showing no symptoms
1	Mouse	9	—	1:100	—	—	—	7	8-12	6	9-11	1	2
2	"	9	ACTH 1x Cortisone 2x	1:100	2 hr after ACTH	—	—	9	2-4	7	3-8	2	—
3	Hamster	21	—	1:20	—	—	—	5	4-6	2	6-7	3	16
4	"	21	ACTH 1x Cortisone 2x	1:20	2 hr after ACTH	5	3-4	16	4-7	16	5-8	—	—
5	"	10	Cortisone 3x	1:20	2 hr after 1st injection Cortisone	—	—	10	2-5	10	4-6	—	—
6	"	10	Cortisone 1x	1:20	Simultaneously with Cortisone	—	—	10	2-6	10	3-8	—	—
7	"	10	ACTH 3x	1:20	2 hr after 1st inj. ACTH	—	—	4	3-6	1	6	3	6
8	"	10	—	1:100	—	—	—	3	7	—	—	3	7
9	"	10	ACTH 1x Cortisone 2x	1:100	2 hr after ACTH	—	—	10	2-6	10	4-8	—	—

1:10. In the treated group, the inoculation of the virus was made 2 hours after the injection of ACTH. Two mice of the treated group died 8 days following virus inoculation, having shown no symptoms of poliomyelitis, while 8 mice of the treated group and all 10 mice of the untreated group remained well during the entire period of observation. Thus, the hormones failed to induce in mice susceptibility to parenteral inoculation of MEF1 strain of poliomyelitis virus.

The results of the second series of experiments in which all the virus inoculations were made intracerebrally are summarized in Table I.

The mice recorded in Table I were inoculated with MEF1 brain emulsion diluted 1:100 in order to obtain a delayed infection. The control group (Group 1) developed the disease 8-12 days following inoculation. There was observed in mice treated with ACTH and cortisone (Group 2) a marked shortening of the incubation period, paralysis appearing 2-4 days following inoculation with a correspondingly earlier mortality. Thus, the experiments indicate a marked acceleration of the infection in a susceptible host upon combined administration of ACTH and cortisone. The results assume, however, a much greater significance when viewed in the light of the following findings in hamsters. The administration of the hormones induced a prompt and violent infection. Within a day or two after inoculation the treated hamsters appeared very weak and drowsy. They showed swollen eyelids, conjunctivitis, ruffled fur, and hunched backs. The paralysis often occurred 2 days following inoculation, and was quite extensive involving all extremities of some animals. The disease was uniformly fatal. As may be seen from Table I, all 51 hamsters of groups 4, 5, 6, and 9 treated with ACTH and cortisone or cortisone alone died 3-8 days following the virus inoculation. While 5 died having shown no paralysis, the remaining 46 hamsters developed paralysis 1-3 days prior to death. When these results are compared with the controls recorded in Table I (Group 3 and 8) and the control series mentioned in the text, it becomes obvious that the hormones are capable of inducing an extraordinary enhancement of susceptibility to the experimental poliomyelitis infection in the hamster. The hormonal effect transforms a

mild infection into a rapidly progressing, violent and uniformly fatal disease, and eliminates completely the natural refractoriness of the animal.

In the initial experiment both ACTH and cortisone were used. On further study it was found that cortisone alone was necessary for the effect, a single intramuscular injection of cortisone administered simultaneously with the intracranial inoculation of the virus sufficing for the production of an equally marked enhancement of susceptibility to the infection, (Groups 5 and 6). It may be noted, however, that hamsters which received 3 injections of ACTH alone in a total dose as large as 15 mg, (Group 7) failed to show enhanced susceptibility to the infection. These results suggest the possibility that in addition to cortisone, ACTH stimulates also the secretion of another factor which may be capable of reversing the enhancing effect of cortisone.

The following control series of experiments were done in order to ascertain that the results obtained were not due to enhancement by the treatment with the hormones of some latent animal virus, rather than to the enhancement of susceptibility to the poliomyelitis virus inoculated. The materials tested were obtained as follows:

1. Two mice received one injection of ACTH and two injections of cortisone at time intervals used in the experimental series. The brains were removed 2 hours after the second injection of cortisone; 2. brains of two normal mice, and 3. two mice were injected intracerebrally with 0.05 ml of broth diluted 1:10 with saline. The brains were removed 24 hours later.

Each brain emulsion diluted 1:10 with saline was inoculated intracerebrally into 10 untreated mice, 10 untreated hamsters, and 5 mice and 10 hamsters which received one injection of cortisone simultaneously with the inoculation. All animals remained well during the entire period of observation.

Summary and Conclusions. ACTH and cortisone in combination or cortisone alone produce a marked acceleration of poliomyelitis infection (strain MEF1) in mice and an extraordinary enhancement of susceptibility to this infection in hamsters giving rise to a violent and uniformly fatal disease. ACTH alone fails to produce this effect possibly due to elaboration of an unknown factor capable of reversing the enhancing effect of cortisone. The experiments seem to indicate the existence of a significant relation between adrenocortical function and susceptibility of mice and hamsters to experimental poliomyelitis.

Capable assistance of Miss Alice Fisher and Miss Doreen Hollings is gratefully acknowledged.

Received November 27, 1950. P.S.E.B.M., 1950, v75.

Effect of Succinate on Contractility and Phosphocreatine and Adenosine Triphosphate Content of the Dog Heart.* (18363)

ALBERT WOLLENBERGER AND SUMNER J. YAFFE.
(Introduced by Otto Krayer.)
From the Department of Pharmacology, Harvard Medical School, Boston.

The oxidation of succinate is catalyzed in cardiac muscle with great rapidity. Addition of succinate in substrate quantities to excised cardiac muscle causes a striking acceleration of oxygen consumption, both at high(1-3) and at low(4-5) oxygen tensions. On this

* This work was supported by a grant from the Life Insurance Medical Research Fund.

1. Angerer, C. A., and Gonzales, Q. J., *Fed. Proc.*, 1947, v6, 69.

2. Olson, R. E., Miller, O. N., Topper, Y. J., and Stare, F. J., *J. Biol. Chem.*, 1948, v175, 503.

3. Wollenberger, A., *J. Pharmacol. Exper. Ther.*, 1948, v94, 444.

4. Bernheim, F., and Bernheim, M. L. C., *Am. J. Physiol.*, 1944, v142, 195.

5. Furchtgott, R. F., and Shorr, E., *J. Biol. Chem.*, 1948, v175, 201.