the spread of an hemoglobin spot in the skin of the rat, under standard conditions of treatment with various hormones or hormone combinations.

Material and methods. Seventy-five piebald, female rats of an average initial body weight of 150 g were divided into 11 groups. The animals of the first group were used as controls. The rats of the other groups received separate daily injections of 5 mg of desoxycorticosterone acetate (DCA), 10 mg of methyl testosterone, 0.5 mg of thyroxine, 5 mg of cortisone, 3 mg of somatotrophic hormone (growth hormone or STH), 40 mg of lyophilized anterior pituitary (LAP) and the following combinations at the same dosage level: LAP and DCA, STH and cortisone, methyl testosterone and cortisone, thyroxine and cortisone. After 10 days of treatment 0.1 cc of a 15% solution of hemoglobin was injected into the posterior region of the back, which area had been depilated the previous day. The spot was measured two and one half hours later. The area was calculated by the following formula:

$$S = \pi \frac{\text{larger diameter x smaller diameter}}{4}$$

Results. As seen in Fig. 1, cortisone significantly inhibits the spread while DCA, STH, LAP and thyroxine enhance it. Testosterone has no notable action. In the association of cortisone with STH and thyroxine, the spread inhibiting effect of cortisone prevails over the enhancing action of these compounds. This property is less pronounced when cortisone is combined with LAP. The enhancement observed following concurrent treatment with DCA and LAP represents the sum of their separate actions.

Summary and conclusions. The spread of an hemoglobin spot was studied under various hormonal conditions. At the dose level utilized, cortisone antagonizes the enhancement of the spread normally occasioned by STH, thyroxine and LAP. Our results confirm the antagonism between DCA and cortisone first suggested by Selye(8) concerning their action on vessels and inflammation. LAP, STH and thyroxine in this respect have a DCA-like action opposite to the effect of cortisone.

8. Selye, H., Stress, Acta Inc. Publ., Montreal, 1950.

Received February 14, 1951. P.S.E.B.M., 1951, v76.

Failure of ACTH to Protect Against Acutely Lethal Toxins of Influenza Virus and Rickettsiae.* (18557)

EDWARD H. KASS,† FRANKLIN A. NEVA,‡ AND MAXWELL FINLAND.

From the Thorndike Memorial Laboratory, Boston City Hospital, Department of Medicine, Harvard Medical School and Department of Microbiology, Harvard School of Public Health.

The capacity of adrenocorticotropic hormone (ACTH) to alter the clinical course of certain infectious diseases(1-3) suggests the need for investigation of the mechanisms involved in these changes. One of the possible explanations of the effect of ACTH in clinical infections is that the adrenal cortical hormones aid in the detoxification of the noxious products of the infectious agent. In order to test this hypothesis, the effect of ACTH

* Aided by grants from the U. S. Public Health Service.

† Senior Fellow in Virus Diseases, National Research Council.

‡ Fellow in Poliomyelitis, National Research Council.

1. Kass, E. H., Ingbar, S. H., and Finland, M., *Ann. Int. Med.*, 1950, v33, 1081.

2. Freeman, S., Fershing, J., Wang, C. C., and Smith, L. C., Proc. First Clinical ACTH Conference, Blakiston Co., 1950, p. 509.

3. Tompsett, R., LeMaistre, C., Muschenheim, C., and McDermott, W., *J. Clin. Invest.*, (Abst.), 1950, v29, 849.

on the acute toxic action of concentrated suspensions of influenza virus and of rickettsiae has been investigated using doses of the hormone which have been demonstrated to produce prolonged eosinopenia in mice(4) and rats(5).

Materials and methods. ACTH§ was diluted in saline and 0.5 ml containing 2.5 mg was injected intraperitoneally into mice every 12 hours(4). The rats received 2 mg subcutaneously every 6 hours. The PR8 strain of influenza virus was used throughout; the method of obtaining and administering the virus has been described(6). Infected allantoic fluids were concentrated by adsorption on and elution from chicken erythrocytes and were injected intravenously into mice or rats. Concentrations of virus were such that 1.5 ml per 100 g of body weight killed about 70% of the mice within 24 hours and uniformly killed rats in 9-12 hours. In the strain of rats used, the time of survival after intravenous injection of the virus was roughly inversely proportional to the dose; twice the dose employed was fatal in 4-6 hours while rats receiving half that amount survived up to 24 hours and still smaller doses permitted increasing rates of survival(5). The rickettsiae used were the Wilmington strain of murine typhus *(R. mooseri)* and the Breinl strain of epidemic typhus *(R. prowazeki)*. Infected yolk sacs were pooled and diluted in a buffered salt solution containing sucrose and glutamate (7). The toxicity of the rickettsial suspensions was determined by injecting 0.25 ml of the test suspension into the tail veins of lightly anesthetized mice. Four to 6 mice were used for each dilution and mice dying within 24 hours were considered to have succumbed to the acute toxic effect of the rickettsiae. Half-logarithmic dilutions were titrated in this manner and the LD_{50} of a given preparation of rickettsiae was calculated by the method of Reed and Muench(8). Experience with this method of testing acute toxicity of rickettsiae in mice has shown that differences in the LD_{50} greater than 3-fold (approximately 0.5 log dilutions) may be considered significant(9). The infectious agents were stored at −70°C between tests. The mice were white Swiss mice of the Tumblebrook Farm strain weighing 10-14 g for the rickettsial experiments and 15-18 g for the influenza virus experiments. The rats were males of the Sprague-Dawley strain weighing 110-125 g. Immune animals received sublethal doses of the influenza virus 2-4 weeks before they were challenged.

Experimental. ACTH was given to 30 mice

TABLE I. Effect of ACTH on Toxicity of Influenza A Virus to Mice.

Test substance inj.	No. of inj.* prior to challenge	No. dead / No. inj.
ACTH	3	10/10
	5	7/10
	7	10/10
Saline	3	7/10
	7	7/10
0 (immune)	—	0/6

Each animal received 1.5 ml of virus concentrate per 100 g body wt intrav.

* ACTH dosage was 2.5 mg in 0.5 ml intraper. every 12 hr until termination of experiment. Saline was given in 0.5 ml amounts every 12 hr.

TABLE II. Effect of ACTH on Toxicity of Influenza A Virus to Rats.

Test substance injected	Result				
ACTH*	9	9	9	9	10†
Saline	9	9	9	9	9
0 (immune)	S	S	S	S	S

Each animal received 1.5 ml of virus concentrate per 100 g of body wt intrav.

* ACTH dosage was 2 mg in 0.5 ml subcut. every 6 hr until death. Saline dosage was 0.5 ml subcut. every 6 hr.

†Hr after virus was inj. when death occurred.

S = Survived.

4. Kass, E. H., Lundgren, M. M., and Finland, M., *J. Lab. and Clin. Med.*, 1951, v37, 458.

5. Kass, E. H., Lundgren, M. M., and Finland, M., unpublished data.

§ Kindly supplied by Dr. John R. Mote of Armour Laboratories. Dosages are in terms of Armour LA-1A standard.

6. Kass, E. H., Lundgren, M. M., and Finland, M., Proc. Soc. Exp. Biol. and Med., 1950, v75, 520.

7. Bovarnick, M. R., Miller, J. C., and Snyder, J. C., *J. Bact.*, 1950, v59, 509.

8. Reed, L. J., and Muench, H., *Am. J. Hyg.*, 1938, v27, 493.

9. Murray, E. S., and Snyder, J. C., personal communication.

TABLE III. Effect of ACTH on Toxicity of Typhus Rickettsiae for Mice.

Rickettsial strain	Dilution of rickettsial suspension†	Results of challenge* in groups of mice prepared by			
		Saline‡	Bovine serum albumin‡	ACTH‡	No treatment
R. prowazeki (Breinl)	1.0	—	5/5	5/5	4/4
	1.5	—	4/4	5/5	4/4
	2.0	—	0/5	3/6	1/4
	2.5	—	0/4	0/4	0/4
	LD_{50}§		1.7	2.0	1.8
R. mooseri (Wilmington)	1.4	5/5	4/4	4/4	4/4
	1.9	5/5	3/5	6/6	5/5
	2.4	0/6	1/6	0/6	1/4
	2.9	0/4	0/4	0/4	0/4
	LD_{50}§	2.1	2.0	2.1	2.2

* Ratio: No. of mice died/total mice in group.

† Expressed as log of denominator of the dilution. Each mouse received intrav. 0.25 ml of stated dilution of yolk sac suspension of typhus rickettsiae.

‡ Mice received 3 doses intraper. at intervals of 12 hr in a volume of 0.5 ml for each dose; saline = 0.85% NaCl; bovine serum albumin = 5 mg per ml; ACTH = 5 mg per ml.

§ Expressed as the log of denominator of the dilution of the LD_{50}.

and groups of 10 of these mice were injected with influenza virus after the 3rd, 5th and 7th injections of hormone. Twenty control mice were given 0.5 ml of saline every 12 hours; half of them were given the virus after the 3rd, and the remainder after the 7th injection of saline. Table I shows that ACTH did not increase the survival rate of the mice dying of the acute toxic effects of influenza virus. Immune mice all survived the challenge.

Five rats were challenged with influenza virus after the second dose of ACTH had been given and 5 control animals which received the same volume of saline every 6 hours were challenged in a similar manner. Injections of ACTH and saline were continued until death of the animals had occurred. ACTH did not increase the survival time of the rats (Table II).

The rickettsiae were injected after the third dose of ACTH had been administered to the test mice. Control animals included groups which received injections of saline (0.5 ml) or bovine serum albumin (2.5 mg in 0.5 ml) every 12 hours. ACTH did not significantly affect the mortality due to rickettsial toxemia (Table III).

Discussion. Adrenalectomized animals are more susceptible than intact animals to many toxic substances and this increased susceptibility is alleviated in part or entirely by adequate doses of adrenal cortical extract(10). However, resistance to toxemia has been difficult to produce in intact animals despite the use of large doses of adrenal cortical extract, although there are conflicting observations on this point(10). There is evidence that neither ACTH nor cortisone inhibit bacterial or viral growth and there are indications that cortisone may actually aggravate infections in some instances(11-15). While the experiments reported in this paper were in progress, Jackson and Smadel(16) reported their failure to demonstrate any protective effect from ACTH or cortisone on the toxicity of *R. tsutsugamushi, R. prowazeki* and *Salmonella typhosa* in mice. However, the doses of hormone which they used were very much less

10. Perla, D. and Marmorston, J., Natural Resistance and Clinical Medicine, 1941, Little, Brown & Co., Boston, p. 475.

11. Kass, E. H., Ingbar, S. H., Lundgren, M. M., and Finland, M., *J. Lab. and Clin. Med.*, in press.

12. Mogabgab, W. J., and Thomas, L., *J. Lab. and Clin. Med.*, (Abst.), 1950, v36, 968.

13. Shwartzman, G., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 835.

14. Spain, D. W., and Molomut, N., *Am. Rev. Tuberc.*, 1950, v62, 337.

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

16. Jackson, E. B., and Smadel, J. E., *Bact. Proc.* (Soc. Am. Bact.), 1950, p. 92.

than those used in the present experiments and were probably inadequate for the production of sustained eosinopenia(4).

The failure of ACTH to reduce the acute toxic effect of influenza virus and of rickettsiae as well as the failure of the hormone to increase the survival rate of mice with pneumococcal and influenza viral infections(11,17,18) leaves unexplained the mechanism by which the dramatic changes in the clinical course of pneumococcal and viral pneumonias have been produced. It is possible that such changes represent relatively secondary phenomena reflecting such effects of ACTH as its antipyretic action(19). It appears unlikely that the clinical improvement which follows the administration of ACTH to patients with infectious diseases is due to stimulation of the capacity of the host to resist the acute toxic action of the infectious agent.

Adrenal cortical hormones are not essential to the metabolism of cells and it is conceivable, therefore, that the toxic agents tested in these experiments interfere with cellular metabolism at levels of enzymic activity which are not directly under regulation by adrenal cortical hormones.

Summary. ACTH did not reduce the acute toxic effect of influenza virus in mice or rats nor the similar action of the rickettsiae of murine and epidemic typhus in mice.

17. Glaser, R. J., Berry, J. W., Loeb, L. H., Wood, W. B., and Daughady, W. H., *J. Lab. and Clin. Med.*, (Abst.), 1950, v36, 826.

18. Loosli, C. G., Hull, R. B., Berlin, B. S., and Alexander, E. R., *J. Lab. and Clin. Med.*, 1951, v37, 464.

19. Kass, E. H., and Finland, M., *New England J. Med.*, 1950, v243, 693.

Received February 14, 1951. P.S.E.B.M., 1951, v76.

Development of Thyroid Neoplasms in the Rat Following a Single Injection of Radioactive Iodine.* (18558)

R. C. GOLDBERG† AND I. L. CHAIKOFF.

From the Division of Physiology, University of California School of Medicine, Berkeley.

In recent reports(1,2) we called attention to the presence of atypical epithelial cells in the thyroid glands of rats 8 months after they had been injected with single large doses of I^{131}. These cells resembled the so-called Hürthle cells found in both neoplastic and non-neoplastic thyroid glands of man. These cytological changes led us to extend our studies to later intervals.

The present report deals with 10 rats that were sacrificed 18 months after I^{131} treatment. Neoplastic changes were observed in the thyroids of two animals. In addition, Hürthle-like elements were prominent in all thyroid glands.

Experimental. Ten male rats of the Long-Evans strain, which had received a single intraperitoneal injection of 400 μc of I^{131} 18 months earlier, were anesthetized with ether and exsanguinated. The tracheas with attached thyroid glands were removed and fixed in 10% neutral formalin, embedded in paraffin, and sectioned serially at 10 μ. Representative sections of liver and kidney were sectioned at 6 μ. All tissues were stained with hematoxylin and eosin. Wilder's stain was employed for the demonstration of reticulum.

Thyroids of rats 1-8. Microscopic examination of the thyroid glands of 8 of the rats revealed the typical post-radiation picture of fibrosis, fibrosed and hyalinized vascular channels, and glandular atrophy. These changes were similar to those described in our earlier

* Aided by grants from the U. S. Public Health Service.

† Atomic Energy Commission Fellow.

1. Goldberg, R. C., Chaikoff, I. L., Lindsay, S., and Feller, D. D., *Endocrinology*, 1950, v46, 72.

2. Goldberg, R. C., and Chaikoff, I. L., *Endocrinology*, 1950, v46, 91.