acids, purines, and pyrimidines in malignant melanoma S-91 have been determined at various stages of its development. The lower values for uracil and thymine found in the necrotic portion of the tumor as compared with those found in the non-necrotic portion point also to a drop of both RNA and DNA in the necrotic portion.

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ACTH and Cortisone Aggravation or Suppression of the Febrile Response of Rabbits to Bacterial Endotoxin. (19182)

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Recent reports(1,2) indicate that ACTH and cortisone reduce the febrile response of rabbits to typhoid vaccine and, furthermore, cortisone has been shown to markedly reduce the temperature of patients with typhoid fever(3). Since the somatic antigens of Salmonella and Shigella organisms account for practically all of the toxicity of these bacteria, and may be responsible for the febrile reactions accompanying infections produced by these organisms(4-6), it was decided to investigate the effects of ACTH and cortisone on the response of rabbits to intravenous injections of a bacterial endotoxin, the purified somatic antigen of *Shigella dysenteriae* (*Shiga*)(7). Since some recent experiments suggested that ACTH and cortisone had no effect on the febrile response of rabbits to crude endotoxin prepared from *S. typhosa*(8) in contrast to the results reported by the other investigators(1,2), special attention was paid in these studies to an analysis of the time-dose relationship of the bacterial endotoxin and the ACTH or cortisone since the variations in effect produced could be due to differences in dosage and time of injection of the hormones.

Method. Young adult, male, albino rabbits weighing from 2.5 to 3 kg were given preparatory treatment with ACTH and cortisone* in groups of 2 animals each. One

* The ACTH was supplied through the kindness of Armour and Co. and the cortisone by Merck and Co.

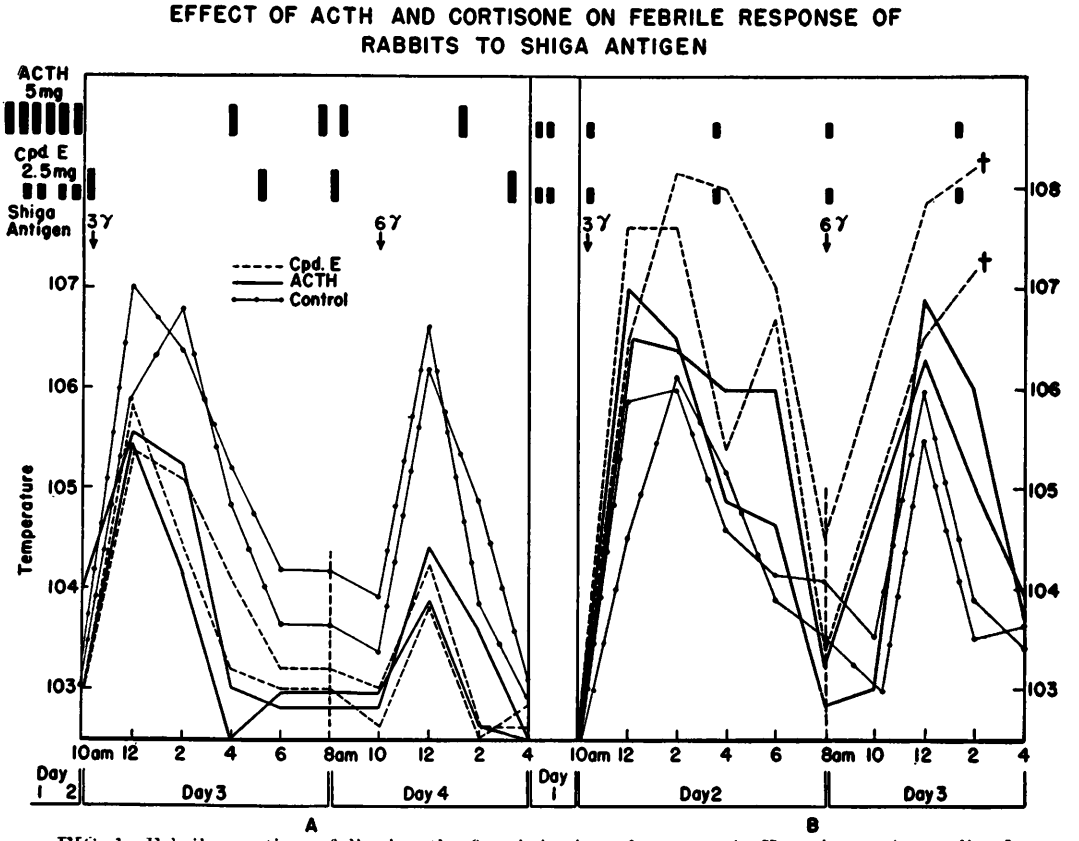

FIG. 1. Febrile reactions, following the first injection of pyrogen in Experiment A, are listed in terms of fever units.

	Fever units	
	Exp. A	Exp. B
Control	15.8	12
Cortisone	4.1	22.3
ACTH	3.4	31.6

group (A) received 15 mg of ACTH in 3 doses or 5 mg of cortisone in 2 doses for 2 days prior to injection of antigen. On the 2 days of the injection of antigen, the rabbits received twice daily injections of 5 mg of ACTH or cortisone. Another group (B) received 5 mg of ACTH or 5 mg of cortisone in 2 divided doses on the day before treatment with antigen, and for the 2 days of the experiment. The ACTH was administered intramuscularly and the compound E subcutaneously. The endotoxin was prepared from *Sh. dysenteriae* (*Shiga*) by the method of phenol extraction as previously described(7), and was given intravenously in doses of 3 or 6 μg. Hourly rectal temperatures were taken until the temperatures returned to normal levels.

Results. The animals who received the larger doses of ACTH and cortisone (Exp. A) for at least 2 days prior to the injection of antigen showed a depression of the febrile response compared to that of the control animals. The inhibition was observed on the first and second days during which ACTH and cortisone were continued. In contrast to this was the aggravation of fever in the animals (Exp. B) treated with less intensive doses of ACTH and cortisone for 24 hours prior to the injection of toxin. The augmentation of fever was noted on both days of the endotoxin test injections. The cortisone-treated animals showed a more marked effect and these 2 rab-

bits died in hyperthermia following the second injection of the test antigen. The contrast in the effects obtained in these 2 experiments is noted in the chart of the febrile response in terms of fever units† on the first day of the 2 experiments for one animal of each pair treated.

The febrile reactions of control animals in Exp. B were somewhat less marked than those in Exp. A. This difference is probably due to variations in the room temperature which affect the body temperatures of the rabbits as indicated by the lower initial temperatures of all rabbits in Exp. B. Environmental temperature, however, affects experimental as well as control animals involved in the same experiment so that the influence of hormone treatment is not altered. These observations were confirmed in several similar experiments.

Discussion. The previously noted(1,2) antipyretic action of ACTH and cortisone for the endotoxins of enteric bacilli has been confirmed. In contrast to these previous reports of protective action, an aggravation of the febrile response is noted in the ACTH and cortisone-treated animals in whom the administration of these compounds was less intensive. It appears likely that if the proper time-dosage relationships had been chosen no significant effects might have been observed on the febrile response and the results would have corroborated the findings of Jackson and Smadel(8) with a crude typhoid endotoxin. The timing and quantity of ACTH or cortisone appears then to be an all-important factor in the determination of the effects of these materials on the febrile response of rabbits to these endotoxins. This appears to account for the discrepancies in the results previously reported.

In the light of the previous experiments demonstrating that repeated injections of bacterial endotoxins result in the development of a tolerance to their toxic effects which is not the result of antibody formation(4,5,7), the finding that ACTH or cortisone, in proper dosage, results in an increased resistance to the pyrogenic effects of endotoxins may indicate that a change in the functional state of the adrenal gland may be responsible, at least in part, for this tolerance.

Summary. (1) ACTH in divided doses of 15 mg per day and cortisone (5 mg per day) for 48 hours prior to the intravenous injection of a bacterial endotoxin (*Shiga dysenteriae*) and injection of 10 mg of either compound during the 2-day test period resulted in a significant lessening of the febrile response in the treated as compared to the control animals. (2) Rabbits given smaller doses of ACTH (5 mg per day) or cortisone (5 mg per day) for 24 hours prior to the injection of antigen, and continued through the test period, demonstrated an aggravated febrile and systemic response to the injected endotoxin as compared to the controls. (3) The significance of this time-dose relationship in the interpretation of experiments involving the use of ACTH and cortisone and bacterial endotoxins is discussed.

† A fever unit is a degree-hour of temperature rise over 104°F.

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