hyperthyroid, every one of these tissues has a P in excess of 0.1, indicating a very low level of confidence in any differences present. Examination of the entire series shows that several tissues exhibited increases of 7 to 10% during thyroxine injection; it is quite possible that accumulation of many more data for each one would eventually produce statistically valid differences. The group of tissues would still, however, stand in contrast to the others with much more marked effects. The conclusion seems valid that brain, lymphoid tissue, gonads and accessory reproductive structures are not responsive to thyroid hormonal control of metabolism, in contrast to several kinds of muscle and glands producing digestive secretions.

Preliminary results obtained on the distribution of radioactive thyroxine‡ following a single injection indicate that the lack of metabolic stimulation by the hormone, where this situation prevailed, cannot be attributed to a lack of exposure of these tissues to the hormone. With an impressive list accumulating of tissues not responding to thyroxine, the question of mechanism of action of this material becomes ever more intriguing. If the primary effect is one of uncoupling of oxidative and phosphorylative reactions or a direct involvement of the hormone as a member of an oxidation-reduction system(5), marked variations must occur in how these processes respond in different tissues.

Summary. Salivary gland and pancreas have been added to the list of tissues whose energy metabolism is controlled by the thyroid gland. In contrast, lymph nodes, thymus, ovaries, uterus, prostate and seminal vesicles have metabolic rates unaltered (within 10%) by thyroid status.

‡ Radioactive thyroxine synthesized by S. C. Wang, T. Winnick, S. Notrica, and R. J. Winzler from I^{131} allocated by the Atomic Energy Commission.

1. Barker, S. B., and Klitgaard, H. M., *Am. J. Physiol.*, 1952, v170, 81.

2. Umbreit, W. W., Burris, R. H., and Stauffer, J. F., *Manometric Techniques and Tissue Metabolism,* Burgess Pub. Co., Minneapolis, 1949, pp. 110-111.

3. Nelson, W. O., and Wheeler, H. E., *Fed. Proc.,* 1948, v7, 85.

4. Langham, W., and Gustavson, R. G., *Am. J. Physiol.*, 1947, v150, 760.

5. Barker, S. B., *Physiol. Rev.*, 1951, v31, 205.

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Effect of Cortisone on Diphtheria Intoxication and the Schick Test in Guinea Pigs.* (20397)

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A study of the effect of cortisone on diphtheria intoxication in guinea pigs was carried out as part of an attempt to assess the value of cortisone in infections. Diphtheria toxin, rather than *C. diphtheriae,* was used in order to exclude complications which might have resulted from the interference of cortisone with host resistance to invading microorganisms(1-4).

The observation that ACTH appeared to alleviate the effects of the neurotoxin elaborated by the black widow spider(5) suggested that cortisone might be of benefit in diphtheria intoxication.

Material and methods. Male and female guinea pigs weighing approximately 300 g were used. They were kept in separate cages, fed cabbage and rabbit pellets, and offered both tap water and 1% KCl solution *ad lib.* The last was used to prevent cortisone-induced hypokalemia. Animals were inspected daily. They were weighed initially and at the time of death or at the end of 10 days in the event of

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TABLE I. Effect of Cortisone on the Schick Test.

	No. of animals		Time of max reaction (days)	Area of max reaction (cm^2)	Healing time (days)
Controls	10	Avg	3.2	4.0	10.1
		Range	2–5	1–12	5–14
Cortisone-treated	7	Avg	2.4	5.2	12.7
		Range	1–3	0.25–8	5–20

TABLE II. Effect of Cortisone on Guinea Pigs Given 0.5 M.L.D. Diphtheria Toxin.

	No. of animals	No. of deaths	Avg initial wt (g)	Avg final wt (g)	Avg survival time of animals (days)
Controls	35	29	308	256	4.3
Cortisone-treated	35	27	307	248	5.0

their survival. All injections other than Schick tests were given subcutaneously in the lumbar region. There were 3 experimental groups of guinea pigs, each consisting of concurrently observed cortisone-treated and control animals. The first group was Schick tested with 0.02 M.L.D. of diphtheria toxin in 0.1 cc of normal saline in the plucked skin of the anterior abdominal wall. The second group was given lethal or near lethal doses of diphtheria toxin, some animals receiving 14 M.L.D., others 0.5 M.L.D. The last group of animals was injected with 14 M.L.D. diphtheria toxin and 10 units of diphtheria antitoxin at separate sites. Part of this group received antitoxin within one minute of toxin; the others were given antitoxin 4 hours after toxin injection. Cortisone acetate was administered in single daily doses of 5 mg for 2 days prior to and 4 days after injection of diphtheria toxin. Autopsies were performed on all animals. Hematoxylin and eosin sections of skin, heart, liver, spleen, and adrenals were taken in alternate animals.

Results. I. *Effect of cortisone on the Schick test.* The data summarized in Table I show that the time of appearance and the extent of skin lesions resulting from Schick testing were not significantly different in cortisone-treated and control animals. Sloughs and eschars formed at the sites of toxin injection in both and healed in approximately equal time.

II. *Effect of cortisone on guinea pigs given diphtheria toxin.* A. *14 M.L.D.* Large doses of diphtheria toxin were first used. Two control animals and 6 treated with cortisone died within 36 hours. At autopsy the outstanding findings were petechial hemorrhages in the viscera, with marked hemorrhages in the zona fasciculata of the adrenals. These findings were similar in both cortisone treated and control animals.

B. *0.5 M.L.D.*† When it became evident that cortisone was ineffective against a lethal dose of diphtheria toxin, it was decided to ascertain whether cortisone offered any protection against a near lethal dose, 0.5 M.L.D. The results are summarized in Table II.

From Table II it is evident that cortisone did not prevent death or influence survival time and weight loss in pigs given 0.5 M.L.D. diphtheria toxin. Skin lesions at the site of toxin injection developed rarely and were of equal extent in the 2 groups. Autopsies again showed petechial hemorrhages in visceral organs, with either gross or microscopic hemorrhages in the adrenals, particularly marked in the zona fasciculata. Cortisone did not influence the inflammatory response or the amount of hemorrhage in the sections studied (Fig. 1).

C. *Effect of mixing cortisone and diphtheria toxin in vitro prior to injection into guinea pigs.* In 3 animals 0.5 M.L.D. diphtheria toxin was mixed with 5 mg cortisone in the same syringe prior to injection to determine whether cortisone had any neutralizing

† 0.5 M.L.D. according to manufacturer's standardization.

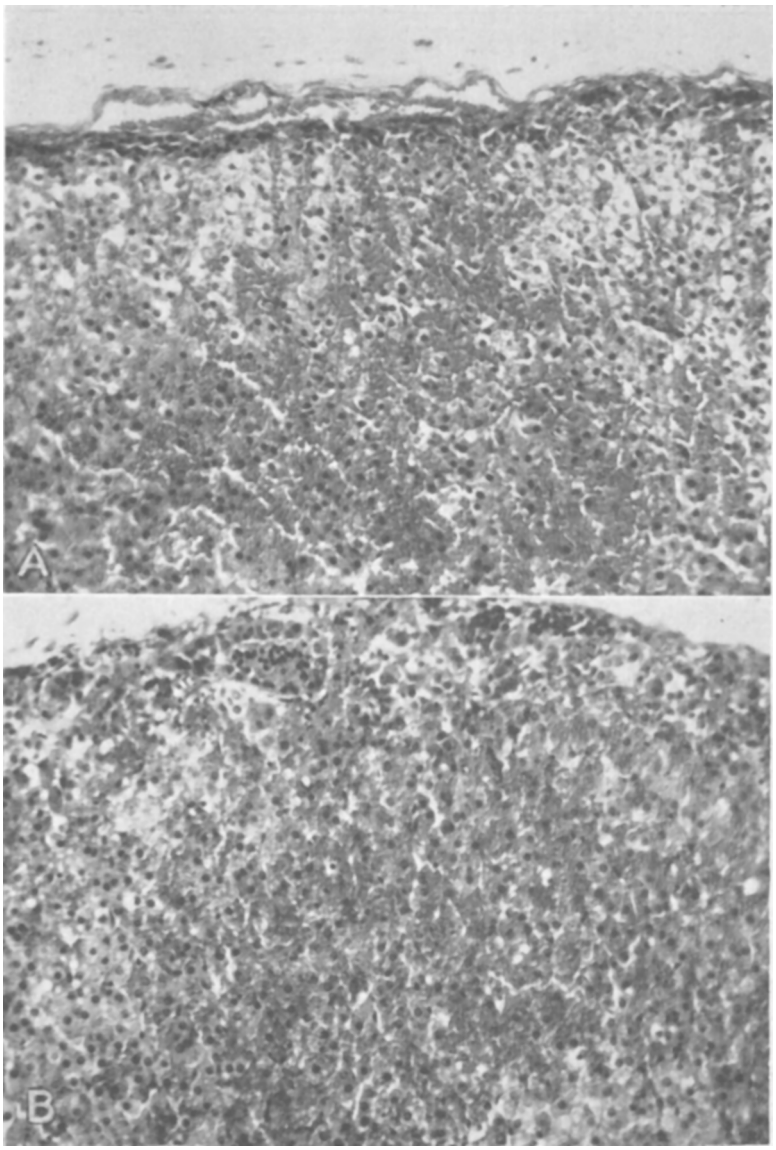

FIG. 1A. Adrenal gland of control guinea pig injected with 0.5 M.L.D. diphtheria toxin. Note hemorrhage and disruption of cord structure in zona fasciculata.

FIG. 1B. Adrenal gland of guinea pig injected with 0.5 M.L.D. diphtheria toxin and treated with cortisone. Note similarity to A.

effect, *in vitro,* on diphtheria toxin. All 3 animals died within 5 days of injection. Autopsy findings were similar to those in which toxin and cortisone were not mixed.

III. *Effect of cortisone on protective action of diphtheria antitoxin (10 units) against diphtheria toxin (14 M.L.D.).*

A. *Antitoxin injected simultaneously with toxin.* Diphtheria toxin and antitoxin were injected at separate sites within one minute of each other. Cortisone did not interfere with the life-saving property of diphtheria antitoxin. Sloughs and eschars formed at the site of toxin administration. Although these skin reactions appeared to be more extensive in cortisone-treated animals, the differences are not statistically significant (P lies between 0.1 and 0.2). Healing of skin lesions was incom-

TABLE III. Effect of Cortisone on Protective Action of Diphtheria Antitoxin (10 Units) Injected Simultaneously with Diphtheria Toxin (14 M.L.D.).

	No. of animals	No. of deaths	Time of death (days)	Mean and stand. dev. of max area of skin reaction (cm^2)
Controls	12	3	4, 7, 7	7.4 ± 5.8
Cortisone-treated	12	2	1, 4	12.5 ± 10.6

plete at the end of 3 weeks, at which time the animals were sacrificed. The results are summarized in Table III.

B. *Antitoxin injected 4 hours after toxin.* The previous experiment established that the protective action of diphtheria antitoxin, when administered within one minute of diphtheria toxin, was not adversely affected by cortisone. This experiment was designed to determine whether the fatal effect of a 4-hour delay in antitoxin administration(6) would be prevented by cortisone. Such a situation would simulate the delay in administration of antitoxin which usually obtains in human diphtheria infections.

Six controls and 6 cortisone-treated pigs all died within 36 hours of toxin injection. Skin lesions at the site of toxin administration did not develop in either group. Autopsy findings were similar in both cortisone-treated and control pigs.

Discussion. From this study it is apparent that cortisone in the dosage used did not significantly influence the effects of diphtheria toxin in guinea pigs. Tissue destruction, adrenal hemorrhages, and reactive inflammation were similar in cortisone-treated and control animals. In retrospect, if diphtheria toxin acts by interfering with internal oxidation in host cells, as suggested by Pappenheimer and Williams(7), one might not have expected cortisone to mitigate diphtheria intoxication. In accord with the results of the present study, Appel *et al.*(8) showed that Schick and Dick tests in humans were not affected by cortisone or ACTH. Spector and Mercer(9) concluded that ACTH did not prevent death in mice given tetanus toxin. The healing time of skin lesions produced by diphtheria toxin was not significantly altered by cortisone. In a study of the gross appearance and healing time of operative incisions in humans, Thompson(10) concluded that cortisone, in doses of 50 mg daily for one week, produced no noticeable effect. Recently, in spite of continued treatment with cortisone, healing of a traumatic fracture occurred normally on 2 occasions in a girl with disseminated lupus erythematosus, studied by the authors. This child had developed a marked Cushing's syndrome as a result of 23 months of cortisone therapy. There have been several reports, however, suggesting that cortisone does partially suppress fibroblastic activity(11-13).

Whereas diphtheria toxicity was not suppressed by cortisone, it seems important to emphasize that the protective action of diphtheria antitoxin, when injected soon after toxin, was not adversely affected by cortisone. These experiments strongly suggest, therefore, that antigen-antibody reaction, as exemplified by diphtheria toxin and antitoxin, is not influenced by cortisone.

Summary. 1. Cortisone did not affect the development of positive Schick reactions in non-immunized guinea pigs. 2. Cortisone did not prevent death, significantly increase survival time or prevent adrenal hemorrhages in guinea pigs given diphtheria toxin. 3. Cortisone did not interfere with the protective action of diphtheria antitoxin in guinea pigs given diphtheria toxin. 4. Cortisone had no direct neutralizing effect on diphtheria toxin *in vitro*.

1. Kligman, A. M., Baldridge, G. D., Rebell, G., and Pillsbury, D. M., *J. Lab. Clin. Med.*, 1951, v37, 651.

2. Kass, E. H., Ingbar, S. H., Lundgren, M. M., and Finland, M., *J. Lab. Clin. Med.*, 1951, v37, 780.

3. Mogabgab, W. J., and Thomas, J., *J. Lab. Clin. Med.*, 1950, v36, 968.

4. Spain, D. W., and Molomut, N., *Am. Rev. Tub.*, 1950, v62, 337.

5. Cluxton, H. E., Jr., 2nd Clin. ACTH Conf. (Mote), 1950, v2, 445.

6. Zinsser, H., Enders, J. F., and Fothergill, L. D.,

Imm. Prin. and App. in Med. and Pub. Health, 1939, Ed. 5, p. 504.

7. Pappenheimer, A. J., Jr., and Williams, C. M., *J. Gen. Physiol.,* 1952, v35, 727.

8. Appel, S. B., Fulton, L. A., and Orr, K. D., *U. S. Armed Forces Med. J.,* 1952, v3, 373.

9. Spector, S., and Mercer, R. D., *J. Inf. Dis,* 1951, v89, 224.

10. Thompson, I. M., personal communication, unpub. data.

11. Zoger, S., *Yale J. Biol. and Med.,* 1952, v25, 202.

12. Dougherty, T. F., and Schneebeli, G. L., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 854.

13. Ragan, C., Howes, E. L., Plotz, C. M., Meyer, K., and Blunt, J. W., PROC. SOC. EXP. BIOL. AND MED., 1949, v72, 718.

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Intrinsic Factor Studies I. Paper Electrophoresis of Mixture of Gastric Juice and Radioactive Vitamin B_{12}.* (20398)

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The simultaneous oral administration of normal human gastric juice and small amounts of vit. B_{12} to patients with pernicious anemia in relapse results in prompt hematopoiesis, while a similar amount of B_{12} without gastric juice is ineffective(1). The parenteral injection of microgram quantities of vit. B_{12} causes an excellent hematopoietic response in such patients(2). The simplest explanation of these observations is that the intrinsic factor activity of gastric juice promotes the intestinal absorption of the vitamin, either by means of a chemical binding of the B_{12} or by acting on the intestinal mucosa in such a manner as to facilitate the transport of the vitamin across the mucosa(3). Binding of vit. B_{12} has been studied most extensively by microbiological assay, and it has been shown that gastric juice combines with the vitamin in such a way as to render it unavailable to certain microbes(4). Several other substances, some known not to be active as intrinsic factor, combine with vit. B_{12} in a similar fashion (5,6).

This paper describes experiments designed to localize by paper electrophoresis the vit. B_{12} binding fraction in human gastric juice using radioactive vit. B_{12} (B_{12} Co^{60})‡(7) as a tracer.

Methods. a) Preparation of gastric juice. Gastric juice secreted after histamine injection was collected by a Levine tube from fasting normal subjects or patients with a peptic ulcer or an anxiety state. The juice in the stomach before histamine stimulation was not used. Only specimens showing an acid reaction to Toepfer's reagent were processed. After filtration through gauze to remove some of the mucus the juice was titrated with sodium hydroxide until neutral to brom thymol blue. After suction filtration through coarse sintered glass, the juice was stored at 4° or —10°C. Concentration of gastric juice was effected by dialysis against tap water and freeze-drying. In some experiments B_{12} Co^{60} was added before freeze-drying, and in others the powdered gastric juice was dissolved in an aqueous solution of the vitamin before application to the paper strip. b) *Paper electrophoresis.* A technic previously described(8) was used in these studies. Schleicher and Schuell No. 598 paper was found satisfactory for this work. Fifty microliters of the concentrated gastric juice-B_{12} Co^{60} mixture was applied to the paper, and after electrophoresis

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