York City, who supplied the dioxolanes; the E. I. du Pont de Nemours Company of Wilmington, Del., for the dihydropyran and tetrahydropyran; and to Dr. Robert Stickler of the Department of Surgery, State University of Iowa, who courteously prepared and examined microscopic sections of the tissues.

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Anatomic Changes Produced in Mice Treated with Excessive Doses of Cortisone. (17648)

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Hench, Kendall, Slocumb, Polley, Barnes and Smith(1,2) reported remarkable effects of Cortisone in patients with rheumatoid arthritis and rheumatic fever. These findings suggested the study of effects of large doses of Cortisone in animals. Accordingly, a dose equivalent to 50 times the amount used for humans was administered subcutaneously to mice. The limited quantity of Cortisone available sharply restricted our dose range and the number of animals treated. As additional amounts of Cortisone become available, we plan to use varying doses and different species.

Method. Two groups of mice were used. A) Nineteen (male and female) immature mice (21 to 30 days old) received daily subcutaneous injections of 1.25 mg Cortisone, suspended in 0.1 cc saline, for 6 successive days, unless they died or were sacrificed earlier as indicated below. Twenty comparable controls received 0.1 cc saline only on the same days by the same route. B) Seventeen mature (male and female) mice weighing 19-26 g received daily subcutaneous injections of 2.5 mg Cortisone suspended in 0.1 cc saline. They were thus injected for 9 days, unless they died or were sacrificed earlier as indicated below. Twenty comparable controls received 0.1 cc saline only for the same time by the same route. The mice were kept in an air conditioned room the temperature of which ranged from 79-85°F. They received water and Rockland Farm pellets *ad lib.*

Results. Of the 19 immature (21 to 30 day old) mice, one treated mouse with its corresponding control was sacrificed on the 2nd, 3rd, 4th, 7th, 9th, 10th, and 11th day after initiation of treatment. One died on the 5th day, one on the 6th day, five on the 8th day, 2 on the 9th day and one each on the 11th, 12th and 15th day. Of the 17 mature mice, one treated mouse and its corresponding control, was sacrificed on the 3rd, 9th and 17th day, after initiation of treatment, and one mouse died on the 5th, 7th, 9th, 16th, 17th and 19th day. Three died on the 8th, day, 3 on the 11th day, and 2 on the 13th day.

Some of the treated animals gained weight for one to 2 days and then lost weight; others lost weight from the outset. Those animals which died showed a precipitous weight loss during their last days. The average weight change of the immature group is shown in Fig. 1. This shows the loss of weight in the treated animals in contrast to the controls which gained weight. The results were essentially alike in the mature mice. Loss of body weight following Cortisone administration was reported by Kendall(3) and Kocha-

* From the Joseph and Helen Ycamans Levy Foundation, established in memory of their daughter, Miriam Levy Finn, and the laboratories of Beth Israel Hospital, New York City.

1. Hench, P. S., Kendall, E. C., Slocumb, C. H., and Polley, H. F., *Proc. Staff Meet. of the Mayo Clinic,* 1949, v24, 181.

2. Hench, P. S., Slocumb, C. H., Barnes, A. R., Smith, H. L., Polley, H. F., and Kendall, E. C., *Proc. Staff Meet. of the Mayo Clinic,* 1949, v24, 277.

3. Kendall, E. C., *Conference on Metabolic Aspects of Convalescence Including Bone and Wound Healing,* 10th Meeting, 1945, p. 87.

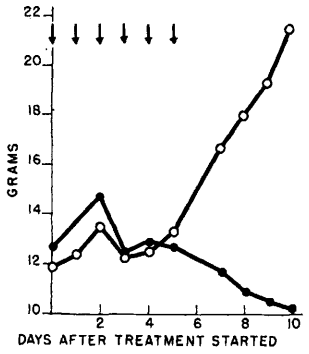

FIG. 1.
Weight Chart of Immature (21-30-day-old) Mice.
● Cortisone treated.
○ Control.
↓ Injection of 1.25 mg Cortisone or 0.1 cc saline.

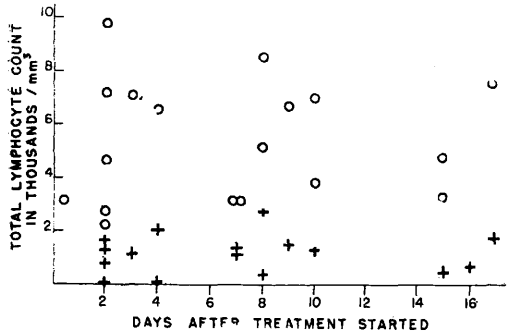

FIG. 2.
Total Lymphocytes in Peripheral Blood of Mice.
+ Cortisone treated.
○ Control.
Each point indicates a single determination on one mouse.

kian(4).

Diarrhea was occasionally encountered in the treated animals, but not in the controls.

The lymphocytes in the peripheral blood of the Cortisone treated animals fell below those of the controls and remained low for at least 17 days (Fig. 2). With the decrease in lymphocytes there was a corresponding increase in polymorphonuclear leucocytes†.

In one cage with 4 Cortisone treated immature mice, a leaking water bottle wet the cage and its contents for about 12 hours. The mice became wet, cold and clammy. Despite drying, removal to a warm room and special care, all 4 mice died within 48 hours. This was repeated with 4 non-treated immature mice; no ill effects were noted.

Post Mortem Findings. After several days, the treated mice at autopsy showed loss of body fat. The abdominal fat was decreased considerably and the hibernating fat bodies were smaller than in the controls. In some instances, foci of necrosis and calcification were found in the hibernating bodies. Kendall(3) found very little excess of body fat in Cortisone-treated mice. Table I gives the average weight and range of 11 organs in the Cortisone treated and in the control immature mice. It shows the striking decrease in weights of the thymus and spleen, the less striking decrease in the salivary glands, hibernating fat bodies, and adrenal, and the slight decrease in the pituitary.

The testes, seminal vesicles and prostate were also smaller than in the controls, and the ovaries appeared smaller.

A few of the histological findings are mentioned below. A detailed histologic report will follow. The thyroid was in the resting phase with an abundance or even an excess of deeply staining eosinophilic colloid. The liver was usually small, but occasionally contained necrotic foci, in which latter instances there was enlargement of the organ. The diminution in size of the adrenal was due almost entirely to narrowing of the cortex. The testes showed retarded development. None of these changes were observed in the controls. Similar changes were likewise found in the mature mice after Cortisone administration. However, the testes showed varying degrees of suppression of spermatogenesis with appearance of giant cells. The latter apparently were due to fusion of secondary spermatocytes.

Granulomatous nodules in the liver, kidney, and spleen (but not at the site of injection) were found in 8 treated animals, and not in the controls. From these, *Corynebacterium pseudotuberculosis murium* was cultured.

Discussion. Selye(5) has written extensively on the systemic reaction produced after administering various drugs or subjecting animals to physical stress such as excess exercise or

4. Kochakian, C. D., *ibid.*, 6th Meeting, 1944, p. 13.

† This will be reported in detail with Drs. H. Quitner, L. Sussman, and N. Wald.

TABLE I.

Comparison of Body and Organ Weights in Immature Mice Treated with Cortisone and in Saline Controls. The average of the body weight and 11 organs are given for both Cortisone treated and control mice. It shows the striking changes in some organs while others are comparatively unaffected.

	Controls (5 mice)		Cortisone-treated (9 mice)	
	Range, mg	Avg, mg	Range, mg	Avg, mg
Pituitary	0.85-1.35	1.15	0.60-1.83	1.03
Salivary Gland	132-212	168	40-154	102
Thymus	26.2-73	48.1	0.9-17.1	8.05
Heart	82-100	89	65-94	81
Spleen	142-185	164	32-78	51
Pancreas	70-95	79	28-100	67
Liver	905-1929	1180	480-1246	878
Hibernating Gland (interscapular)	73-175	128	18-220	59
Hibernating Gland (prescapular)	44-92	71	18-61	34
Adrenal	1.80-2.90	2.34	1.10-1.88	1.56
Kidneys	247-360	289	222-338	274
Body wt beginning of exper.	9-14 g	12.0 g	8.5-13 g	11.3 g
Body wt death of animal	12.5-18 g	14.8 g	7.0-13.0 g	10.6 g

exposure to cold. From our observations, it is apparent that large doses of Cortisone produced anatomic changes which in many respects were similar to those described by Selye after administration of "alarm producing agents". The assumption is that these "alarm producing agents" cause the liberation of pituitary adrenocorticotropic hormone; this, in turn, increases the activity of the adrenal cortex, causing hypertrophy with concomitant liberation of adrenal "corticoids". But in the Cortisone-treated animals, there was an atrophy of the adrenal cortex. This suggests that Cortisone may be one of the hormones responsible for, and capable of eliciting directly the "alarm reaction" without the intermediary of the pituitary or of the adrenal cortex. The administration of an overwhelming amount of this hormone in the body eliminated the need for any further cortical substance, therefore obviating the necessity for an enlargement of the adrenals. The atrophy of the adrenal cortex may also be explained on the basis of compensatory atrophy of the adrenals similar to that found by Selye with desoxycorticosterone(5).

The slight diminution in size of the pituitary in the treated mice suggests the possibility of an inhibiting effect of large doses of Cortisone upon this organ, with consequent diminution in ACTH production. Such diminution of the ACTH may also play a role in decreasing the size of the adrenal(5).

The lymphopenia produced after the administration of Cortisone corresponds to the findings of White and Dougherty after the administration of ACTH(6). These authors believed the lymphopenia to be due to increased adrenal cortical secretion.

The diminished ability to withstand exposure to wetting may be explained on the basis of Selye's(5) observation that animals receiving repeated injections of desoxycorticosterone develop adrenal atrophy. Such animals "respond somewhat like adrenalectomized animals, and exhibit low general resistance and hypoglycemia during stress". The mechanism of the reaction may be similar to that in thiouracil treated rats, reported by Zarrow and Money(7), in which the hypothyroid rats developed adrenal atrophy.

The frequency of *Corynebacterium pseudotuberculosis murium* infection in the treated animals may depend upon a loss of immunity (8). With disappearance of the lymphocytes and their contained antibodies, there probably

5. Selye, Hans, *J. Clin. Endocrinology*, 1946, v6, 117; *Endocrinology*, 1937, v21, 169.

6. White, A., and Dougherty, T. F., PROC. SOC. EXP. BIOL. AND MED., 1944, v56, 26.

7. Zarrow, M. X., and Money, W. L., *Endocrinology*, 1949, v44, 345.

is depletion of immune bodies with resulting insufficient antibody for fixing the antigen.

Summary. 1. The administration to mice of Cortisone in doses approximately 50 times the dose for humans produced a striking lymphopenia, loss in body weight, atrophy of thymus and spleen, and diminution in size of adrenal cortex, salivary glands, pituitary, and hibernating fat bodies. A number of histologic changes are briefly described.

2. The anatomic findings correspond to those of the "alarm and adaptation" phenomena of Selye except for atrophy of the adrenal cortex in place of hypertrophy. Cortisone *per se* may produce some of the morphologic changes associated with the "alarm reaction."

3. Administration of these excessively large doses of Cortisone may produce exhaustion of protective mechanisms, including antibody formation.

We should like to express our appreciation to Drs. Philip Hench and James Carlisle for their interest in these investigations and for making available the Cortisone used in these studies.

8. Dougherty, T. F., Chase, J. H., and White, A., PROC. SOC. EXP. BIOL. AND MED., 1945, v58, 135.

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Weight Variations of Adrenalectomized Frog Muscles in Different Concentrations of Ringer's Solutions.* (17649)

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It has been shown that following adrenalectomy, body water tends to shift into certain tissues and cells (skeletal muscles of rats(1) and frogs(2), and red cells of dogs(3), cats(4) and rats(5)). According to Winter and Hartman(6), not confirmed by Ponder and Gaunt(7), excised skeletal muscles of adrenalectomized rats shift water in a given hypotonic solution at a greater rate than controls probably because of some alteration in the supposed plasma membranes of the myofibers. Some unrecognized variable, *viz.*, diet, water balance, etc., may be the basis for the observed discrepancy. We reinvestigated(8) the problem studied by the foregoing workers using their experimental technic on skeletal muscles of apparently more favorable material. The frog was selected for the following reasons: 1) nutrition is simplified, assuming that a plentiful supply of testicular fat at autopsy is indicative of at least a "basal" state; 2) imbibition of water is potentially continuous from an aqueous environment(9); 3) isolated muscles of the frog immersed in solutions at room temperature are more within their thermal biokinetic range than are muscles of poikilotherms in solutions from 15 to 20°C lower than their body temperature(10).

Methods. Male *Rana pipiens* (fall and win-

* This investigation was aided by a grant from the Comly-Coleman Research Fund of the Ohio State University.

1. Crismon, J. M., and Field, J., 2nd, *Am. J. Physiol.*, 1940, v130, 231.

2. Angerer, C. A., *Am. Physiol. Soc. Proc.* 1942, v1, 3.

3. Harrop, G. A., *Bull. Johns Hopkins Hosp.*, 1936, v59, 11.

4. Hegnauer, A. H., and Robinson, E. J., *J. Biol. Chem.*, 1936, v116, 769.

5. Gonzalez-Q., J., and Angerer, C. A., *Am. J. Physiol.*, 1947, v149, 502.

6. Winter, C. A., and Hartman, F. A., PROC. SOC. EXP. BIOL. AND MED., 1933, v31, 201.

7. Ponder, E., and Gaunt, R., PROC. SOC. EXP. BIOL. AND MED., 1934, v32, 202.

8. Angerer, C. A., and Angerer, H. H., *Am. J. Physiol.*, 1941, v133, 197.

9. Adolph, E. F., *Physiological Regulations*, p. 110, The Jaques Cattell Press, Lancaster, Pa., 1943.

10. Belehradek, J., *Temperature and Living Matter*, p. 136, Gebruder Borntraeger, Berlin, 1935.