the biologic test as a basis of comparison; clinical histories can be later used to further assess accuracy.

Included in the series of urines studied were specimens from 3 patients with hydatidiform moles, 1 with chorionepithelioma and 3 with testicular tumors. These specimens were tested either by the rat or frog test, and also by the Aschheim-Zondek method. The serological results with these specimens coincided with the biological assays.

Summary. 1. Seventeen rabbits were immunized with "APL," which is a combined extract of human placental tissue and pregnancy urine. Seven rabbits (6 females) produced detectable antibodies. 2. Two hundred eighty-five urines submitted for pregnancy tests were tested for the presence of CG by biological and serological methods. 3. Native rabbit CG antiserum tends to give many cross-reactions. Therefore, it is necessary to absorb the serum and test it for potency and specificity prior to use in testing urines for the presence of increased amounts of CG. 4. It is suggested that it may be possible to use this serologic method for the detection of pregnancy; the development of an antiserum of somewhat greater specificity would be advantageous.

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Relation of the Adrenal to Glycogen Content and Respiration of Lymphoid Organs. (18264)

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The effects produced by adrenal cortical factors and by epinephrine upon the storage, breakdown and utilization of carbohydrate have been described at length(1-5). Although studies have been made of the influence of the adrenal gland upon the quantities of carbohydrate substances, principally glycogen, in the liver, muscle and certain other organs(6-8), no attention has been paid to the part played by this gland in the carbohydrate metabolism of lymphoid tissue. This is surprising in view of recent work which has shown the importance of the lymphoid organs in a variety of defense reactions within the body and which has stressed the influence of adrenal cortical factors upon this function (9-11).

A knowledge of carbohydrate changes within the lymphoid organs, induced by alterations in adrenal hormone levels, might help to elucidate further the role of this endocrine gland in defense mechanisms. This report describes experiments dealing with the chronic effects of adrenalectomy and cortical extract or epinephrine administration upon the glycogen content of lymphoid organs, the liver glycogen and the respiratory activity of these tissues.

Materials and methods. 1. *Animals.* Female rats of a hardy, closely inbred strain, weighing 140-170 g at the time of operation, were used. The 36 adrenalectomized rats employed were divided into three groups, the first of which consisted of 10 rats, to

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TABLE I. Mean % Glycogen (± Average Deviation).

Wks after operation	No. of rats	Final body wt (g)	Lymph node	Thymus	Spleen	Liver
			I(A) . . . SHAM-ADRENX.			
1	2	164	.192 ± .008	.303 ± .065	.150 ± .049	1.900 ± .180
2	2	176	.464 ± .272	.188 ± .102	.128 ± .015	2.043 ± 1.280
3	2	205	.212 ± .140	.110 ± .092	.078 ± .014	2.350 ± .550
4	2	164	.241 ± .082	.170 ± .095	.156 ± .042	1.720 ± .260
			I(B) . . . ADRENX.			
1	2	176	.132 ± .029	.116 ± .009	.125 ± .002	2.795 ± .215
2	4	189	.076 ± .038	.036 ± .011	.082 ± .017	.312 ± .213
3	4	181	.099 ± .067	.071 ± .032	.070 ± .012	.489 ± .422
4	2	186	.083 ± .013	.094 ± .013	.048 ± .019	.275 ± .083
			I(C) . . . ADRENX. + cortical ext.			
1	2	170	.143 ± .101	.101 ± .069	.177 ± .091	5.530 ± .760
2	4	157	.180 ± .029	.100 ± .022	.134 ± .029	1.799 ± .432
3	2	184	.222 ± .054	.104 ± .037	.106 ± .025	2.760 ± .720
4	2	174	.066 ± .045	.084 ± .022	.034 ± .003	1.395 ± .765
			I(D) . . . ADRENX. + epinephrine			
1	4	170	.082 ± .062	.072 ± .043	.097 ± .025	1.018 ± .388
2	3	169	.657 ± .365	.164 ± .110	.108 ± .074	3.857 ± .443
3	4	168	.976 ± .126	1.016 ± .673	.149 ± .049	3.596 ± .443
4	3	158	.088 ± .025	.061 ± .022	.070 ± .020	1.798 ± .371

which was administered adrenal cortical extract* in dosages of 1-2 cc daily, starting on the day of operation. The second group of 14 adrenalectomized rats received epinephrine (adrenalin hydrochloride, Parke, Davis and Co.) in dosages of 200 μg daily, beginning with the day of operation. Half of this group received 1 cc of 1-10,000 epinephrine twice a day, while the remaining animals received 1 cc of 1-5,000 epinephrine once a day. The third group of 12 adrenalectomized rats received no hormone. Eight animals subjected to sham-adrenalectomy received no hormonal treatment. All adrenalectomized animals were maintained on 1% saline given as drinking water.

2. *Glycogen determinations.* The method used was essentially that of Good, Kramer and Somogyi(12) and Shaffer and Somogyi (13). The animals in the 4 groups were killed in the forenoon, at 1, 2, 3 and 4 week intervals after the operation. Determinations were made upon the liver, spleen, thymus and pooled lymph nodes from the iliac, mesenteric and axillary regions. The rats were anesthetized lightly with ether, rapidly exsanguinated by cardiac puncture, and the organs removed. Especial attention was paid to ensuring rapid introduction and dissolution of the tissues in the 30% potassium hydroxide. Wet weights of the organs were then determined. The liver was the first tissue to be excised, and the right lobe was used consistently for the determinations. Except for thin slices which were utilized in the respiration studies, the complete spleens, thymuses and pooled lymph nodes were used for glycogen determinations.

3. *Respiration studies.* The reduction of 2,3,5 triphenyl tetrazolium chloride(14) was utilized to ascertain the respiratory activity of the various tissues. A ratio between the colorimeter reading and the weight in mg of dry tissue, served as an index of relative respiration.

Results. 1. *Glycogen content of liver and lymphoid organs.* Table I A, B, C and D summarize the data for the weekly effects of adrenalectomy and replacement therapy upon the glycogen content of lymphoid tissues and liver. Table IA indicates that the liver

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and splenic glycogen concentrations in the sham-adrenalectomized rats are fairly constant over the 4-week period; thymic and lymph node glycogen shows some fluctuation from week to week, but it is doubtful that these variations are significant. In the adrenalectomized animals (Table IB), the glycogen levels in the lymphoid organs and liver drop sharply after the first week and remain low during the ensuing 3 weeks. Liver glycogen values in the adrenalectomized animals treated with adrenal cortical extract (Table IC) are significantly above those in the sham-adrenalectomized rats at the first week. Thereafter, they descend to levels approximating those of the controls. In the adrenalectomized group given cortical extract, splenic, lymph node and thymic glycogen values are maintained at control levels until about the 3rd week, after which time they tend to fall abruptly. Adrenalectomized rats treated with epinephrine (Table ID) show a rise in liver, splenic, thymic and lymph node glycogen, maximal levels being attained by the 2nd or 3rd week. For the thymus and lymph nodes, this represents an increase in glycogen values above those in the sham-operated animal. At the 4th week, the glycogen in these organs, with the exception of the liver, is depleted to levels characteristic of the adrenalectomized rat. Fig. 1 illustrates the combined data for the 4-week period. The histograms indicate the depletion of the glycogen levels in the lymphoid tissues and liver following adrenalectomy. The greater effectiveness of epinephrine over the cortical extract, in the dosages employed, is also apparent from this figure.

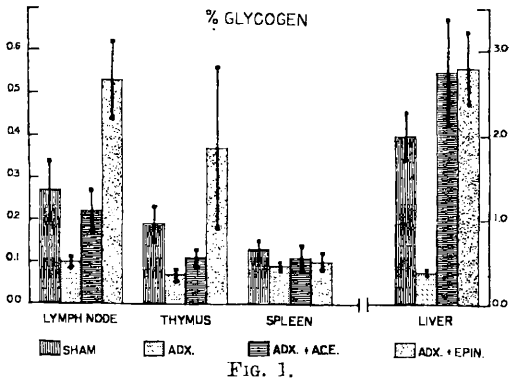

FIG. 1.

Mean % glycogen (combined 4 weekly values) of lymphoid organs and liver as affected by the procedures indicated in graph. Vertical lines represent values of twice the standard error of the mean.

TABLE II. Mean Thymic and Splenic Weights (± Standard Errors).

Treatment	No. of rats	Final body wt (g)	Thymic wt (mg)	Splenic wt (mg)
Sham-Adrenx.	8	177	226 ± 46	623 ± 79
Adrenx.	12	183	346 ± 15	552 ± 111
Adrenx. and Cort. Ext.	8	171	175 ± 23	566 ± 26
Adrenx. and Epineph.	13	166	176 ± 23	495 ± 33

2. *Thymic and splenic weights.* Adrenalectomy results in an increase in thymic weight, which attains a maximum at approximately two weeks following the operation. The weights then fall off, approximating the values in the sham-adrenalectomized rats by the 4th week. Both cortical hormone and epinephrine tend to prevent the thymic hypertrophy, the maximal inhibitory influence being observed at approximately 2 weeks subsequent to adrenalectomy. Thereafter, these hormonal effects are no longer in evidence. Splenic weights were not significantly influenced by adrenalectomy nor by the hormonal treatments employed. The combined data for the 4 weekly periods are presented in Table II.

3. *Respiration studies.* The tissues of the adrenalectomized rats show a greater ability to reduce tetrazolium than those from the sham-operated animals. This effect is seen most markedly during the 1st and 2nd week following adrenal removal. By the 3rd or 4th week, the respiratory activity has fallen to the level seen in the sham-operated rats. Both adrenal cortical extract and epinephrine prevent the rise in respiration noted in the adrenalectomized animal. Here again, the most marked effects are observed during the 1st or 2nd week of treatment. By the 3rd or 4th week, the depressing influence of the hormones upon tetrazolium reduction by the various tissues is no longer seen. Fig. 2 presents the combined data for the 4 weekly periods.

Discussion. As far as the authors are

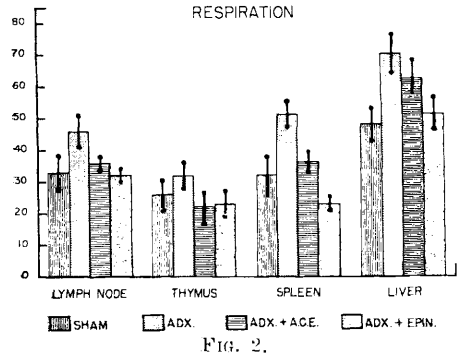

FIG. 2.

Mean respiration—tetrazolium reduction (combined 4 weekly values) of lymphoid organs and liver as affected by the procedures indicated in graph. Vertical lines represent values of twice the standard error of the mean.

aware, these studies are the first to show a relation between the adrenal gland and carbohydrate factors in lymphoid tissues. It is quite apparent that the glycogen content of the combined lymphoid organs is considerable, and that adrenalectomy depletes the glycogen levels of lymphoid tissue as well as that of liver. Both adrenal cortical extract and the medullary hormone restore lymphoid and liver glycogen towards normal in the adrenalectomized rat, this effect being noted particularly at the 2nd or 3rd week of treatment. Although Wagner(15) claims that glycogen is not present in the peripheral lymphocyte, Smith and Thomas(16) have demonstrated its presence in the thymus where it is confined to the small lymphocytes. Thus it is possible that the adrenal hormones affect lymphoid organ glycogen through an action on the contained lymphocytes. The present experiments indicate that the hypertrophy of the lymphoid organs (*e.g.* thymus) following adrenalectomy is accompanied by a decrease in the concentration of glycogen within them, while the partial dissolution of these tissues, caused by the adrenal hormones, occurs concomitantly with an increase in their glycogen concentration. A study is in order, correlating the cytological alterations (*e.g.* ratio of small to large lymphocytes) in the lymphoid organs, to their glycogen content in both the acute and chronic experiment entailing hormone administration.

The tetrazolium studies on tissue slices indicate that there is an increase in the respiration of lymphoid organs and liver after adrenalectomy. These results harmonize with the observation that peripheral oxidation of carbohydrate is accelerated in the adrenalectomized animal(17), but are at variance with the *in vitro* results of Tipton(18,19) who reported a decrease in the respiratory activity of liver tissue following adrenalectomy. The present observations that cortical extract induces a decrease in the respiration of liver and lymphoid tissues, are in accord with the concept that this hormone depresses carbohydrate oxidation within the organism(20, 21) and also with the finding that it inhibits the oxygen consumption of liver slices(22). Correlation of the respiration results to the glycogen levels demonstrates an inverse relation between these factors in the organs of the various groups examined. In this connection, Dempsey and Wislocki(23) have suggested, from their histochemical studies, that glycogen deposition is associated with a deficient circulation, or sluggish metabolism, or both.

The present work points to the possibility that carbohydrate components, as well as protein(10), within the lymphoid organs, may become available to the organism for use in stress, and moreover, that the adrenal hormones which cause release of these factors from the lymphoid tissues, induce, at the same time, a synthesis of glycogen within the remaining and regenerated cells.† Con-

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† Recent evidence indicates that the adrenal cortical hormones induce lymphoid dissolution primarily in the acute experiment (*e.g.* 6-12 hr following a single injection of hormone); with continued injections, lymphoid organ regeneration, and even hyperplasia may occur(11,24).

sidering the extent of the total lymphoid mass to be in the vicinity of 1-3% of the body weight, and the high lability and great regenerative capacity of lymphoid tissue, the turnover during stress of chemical factors contained within these organs may be greater, actually, than that occurring within the liver.

This investigation emphasizes the tendency of epinephrine to mimic, in the adrenalectomized rat, the action of adrenal cortical factors upon the various phenomena examined. The possibility of adrenal cortical rests in the adrenalectomized animals has been considered, but careful examination at autopsy, of the animals injected with epinephrine, only occasionally revealed the presence of small fragments of accessory tissue. It appears more likely that epinephrine, independently of the pituitary-adrenal cortical axis, may initiate, in the chronic type of experiment herein described, reactions which produce some of the end results found in animals treated with cortical factors. It is probable, however, that the mechanisms set into play by the two types of hormones are quite different. The effects of epinephrine upon carbohydrate and respiratory metabolism are complex, and appear to depend upon the dosage and duration of hormone treatment, and whether or not insulin is present(25). The extensive work of Ingle and his associates (5,26,27) has given proof of an extrahepatic function of epinephrine in the eviscerated animal.

Summary. Adrenalectomy in the female rat results in a lowering of the glycogen content of lymphoid tissues as well as liver. Respiration (tetrazolium reduction) of these organs is increased by adrenal removal. Chronic administration of adrenal cortical extract or epinephrine to adrenalectomized rats, restores the glycogen content and reduces the respiratory activity of the thymus, lymph nodes, spleen and liver. Similarly the hypertrophy of the thymus in the adrenalectomized animal is reduced by both hormones. Maximal effects are produced at the 2nd or 3rd week of treatment. By the 4th week, these effects of the hormones are no longer apparent. The demonstrated influence exerted by the adrenal gland upon lymphoid organ glycogen and respiration may be of importance in the general problem of resistance to stress.

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Nitrogenous Excretion in *Colpidium campylum.* (18265)

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As a waste product of protein metabolism, nitrogen is excreted as ammonia, urea, or uric acid. In the ciliate protozoa, the nitrogenous end products have not been identified with any degree of certainty. It has been reported that uric acid is excreted by ciliates (1,2). However, other investigators contend that most ciliates are ureotelic(3,5). There is, moreover, some evidence that ammonia

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