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RESPONSE OF SOME HYDROLASES IN THYMUS AND LYMPHOSARCOMA OF RATS TO INJECTION OF ADRENAL STEROID HORMONES‡

It is a pleasure to contribute to an issue of the *Yale Journal of Biology and Medicine* dedicated to Professor C. N. Hugh Long, mentor and colleague from 1936-1948, a continuing and warm friend, who provided significant catalytic influence for my entry into Experimental Endocrinology. A.W.

The acute degenerative alterations produced in lymphoid tissue of several species following a single injection of glycogenic adrenal cortical steroids, and the concomittant lymphopenia, were described in detail by Dougherty and White.[1,2] Subsequently, numerous investigators have been concerned with the biochemical effects of these steroid hormones on lymphoid tissue in efforts to elucidate the mode of action of corticosteroids.[c.f. 3–13]

The changes induced by adrenal steroids in lymphocytes, initially termed lymphocytolysis[2] and subsequently more precisely described by the term lymphocytokaryorrhexis,[13] with the resultant decrease in size of lymphoid organs, pointed to a possible relationship of these morphological alterations to the activity of certain hydrolases. De Duve and his colleagues[14,15] have suggested that lysosomal hydrolases have a significant role in degenerative changes seen in a number of tissues. This led to our study of a possible correlation between the alterations in morphology and thymic size and the increased release of a group of thymic hydrolases in rats injected with cortisol. It has also been reported that the administration of a corticosteroid increased RNase activity in rat thymus[16] and in a corticosteroid sensitive transplantable lymphosarcoma in mice.[17,18] In contrast, a steroid insensitive lymphosarcoma did not show elevated RNase activity after hormone injection.[17,18] It was suggested that RNase in lymphoid tissue may be of major importance in the glucocorticoid induced involution of these tissues. The present communication reports data obtained in an extension of our previous preliminary studies of rat thymic tissue[19] and also includes certain comparative studies with a transplantable lymphosarcoma.

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‡ This investigation was supported by grants from the National Science Foundation (GB-2211), The American Cancer Society (P-68-G and P-68-H), and The National Cancer Institute, National Institutes of Health, U. S. Public Health Service (CA-07470).

MATERIALS AND METHODS

Animals and treatment: Male rats of the Sprague-Dawley strain, weighing 150 to 210 g., were used in these studies. All animals received Rockland rat diet and water *ad libitum*. The rat transplantable lymphosarcoma used in these experiments was the Murphy-Sturm tumor, kindly supplied by Dr. K. Sigura of the Sloan-Kettering Institute for Cancer Research, and carried in our laboratory in Sprague-Dawley rats.

Rats were injected either subcutaneously or intraperitoneally with steroid, finely dispersed by grinding, in a 0.9% NaCl suspension. Animals were sacrificed by decapitation, the thymus rapidly excised, weighed and homogenized in cold 0.15 M KCl containing 10^{-3}M $MgCl_2$. The homogenate was centrifuged at 700 $\times$ *g* for 5 minutes in an International refrigerated centrifuge and the supernatant fluid was used for enzyme assay. In some cases homogenates were first centrifuged as above and the supernatant fluid then centrifuged at 15,000 $\times$ *g* for 30 minutes. The supernatant fluid from the last centrifugation was used for assay of "soluble" enzymic activity.

In other experiments, the thymus or tumor tissue was homogenized in the cold with the aid of a Teflon pestle fitted into a smooth walled test tube, using one gram of tissue with 10 volumes of cold 0.25 M sucrose-0.1 M $CaCl_2$. The homogenates were filtered through gauze and the filtrate used for enzyme assay.

Cannulation of mesenteric lymphatics was performed under ether anesthesia.* The rats were allowed to recover and placed in a cage that restrained movement. Lymph was collected from the catheter into 4% sodium citrate-0.9% NaCl over a period of 22 hr.; cortisol or 0.9% NaCl was injected intraperitoneally and lymph collection continued for a subsequent 6 hr. and 45 min. period and the animals then sacrificed. The lymph samples were centrifuged, the supernatant fluid decanted and saved. The cells were washed twice with 0.9% NaCl. Microscopic examination revealed that the cells were almost all small lymphocytes, although a macrophage was seen occasionally. The lymph cells were then homogenized in one volume of 0.25 M sucrose-0.1M sodium citrate.

Chemicals used and sources: The following chemicals were purchased from Sigma Chemical Co., St. Louis, Mo.: phenolphthalein glucuronide, p-nitrocatechol sulfate, progesterone, bovine hemoglobin, and yeast RNA. Deoxycorticosterone and cortisol were purchased from Steraloids, Inc. Pawling, N. Y. Calf thymus DNA was purchased from Calbiochem, Los Angeles, Calif.; 9α-fluoro-Δ^1-cortisol was a generous gift of Merck, Sharp and Dohme Co. Inc., Rahway, N. Y.

Methods of enzyme assay: β-glucuronidase, acid phosphatase, and cathepsin activities were measured as described by Gianetto and DeDuve,[20] arylsulfatase in acetate buffer (pH 5.0) by the procedure described by Roy,[21] and DNase II, acid RNase and alkaline RNase as described by Wiernick and MacLeod.[16] No attempt was made to distinguish among the several proteinases which have been delineated in rat lymphoid tissue.[22]

Other procedures: Total protein was determined either by the method of Lowry, *et al.*[23] or of Gornall, *et al.*,[24] using human serum albumin as a standard. Histochemical examination of thymic sections for localization of acid phosphatase activity was carried out as described by Gomori.[25]**

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* We are indebted to Dr. Paul S. Roheim for the operative procedures making possible lymph collection in these rats.

** The technique of Gomori[25] was modified in that frozen sections of thymus and β-glycerol phosphate as substrate were used. Drs. Alex B. Novikoff and Sidney L. Goldfischer kindly conducted these histochemical techniques and generously provided the photographs of thymic sections.

RESULTS

In the present communication, the particulate bound hydrolases released into the 700 × *g* supernatant fraction are designated as "free" enzyme. The hydrolases measured in the 700 × *g* supernatant fraction were not sedimented by further centrifugation at 15,000 × *g* for 30 minutes. "Total" activity was obtained by treating the 700 × *g* supernatant fraction with Triton X-100 (final concentration 0.1 per cent); all lysosomal enzymes are released.[14]

The results given in Table 1 show the acute effect of cortisol injection on the activity of several thymic hydrolases. Animals in these experiments received 5 mg. of steroid per 100 g. body weight and were sacrificed as early as one hour after injection. No appreciable histological changes[2] were seen in lymphoid organs at earlier times following steroid injection. "Free" activity of both β-glucuronidase and DNase II was significantly elevated at two hours after steroid treatment. We have previously reported that elevated DNase II activity was seen only in the 700 × *g* supernatant fraction, whereas little change had occurred in this activity in the nuclear fraction.[10] Acid phosphatase activity was elevated slightly at three hours after steroid treatment.* Acid and alkaline RNase activity was not affected at the shorter time of exposure to either cortisol or the more thymolytically potent 9α-fluoro-Δ^1-cortisol. Administration of 9α-fluoro-Δ^1-cortisol to rats elevated the activity of both β-glucuronidase and DNase II, whereas injection of progesterone had no significant influence on the activity of either enzyme. In results not presented here, the administration of deoxycorticosterone was also without significant effect on the activity of either "free" or "total" β-glucuronidase.

In the experiments represented by the data in Table 2, rats were injected with a single dose of cortisol at either three hours or 20 hours prior to sacrifice. Both "free" and "total" activity of β-glucuronidase, DNase II, acid phosphatase, acid RNase and alkaline RNase of thymus were assayed. At three hours after steroid, only the "free" activity of β-glucuronidase, DNase II and acid phosphatase was elevated. In contrast, when animals were sacrificed at 20 hours following cortisol injection, a marked elevation was seen not only in the "free" activity, but also in the "total" activity of all enzymes studied. The increase in activity of these hydrolases occurred at a time when both the weight and total protein content of the thymus had decreased.

* For the data in the tables, increases in enzymic activity of greater than 15 per cent are statistically significant for measurements involving three or more individual experiments.

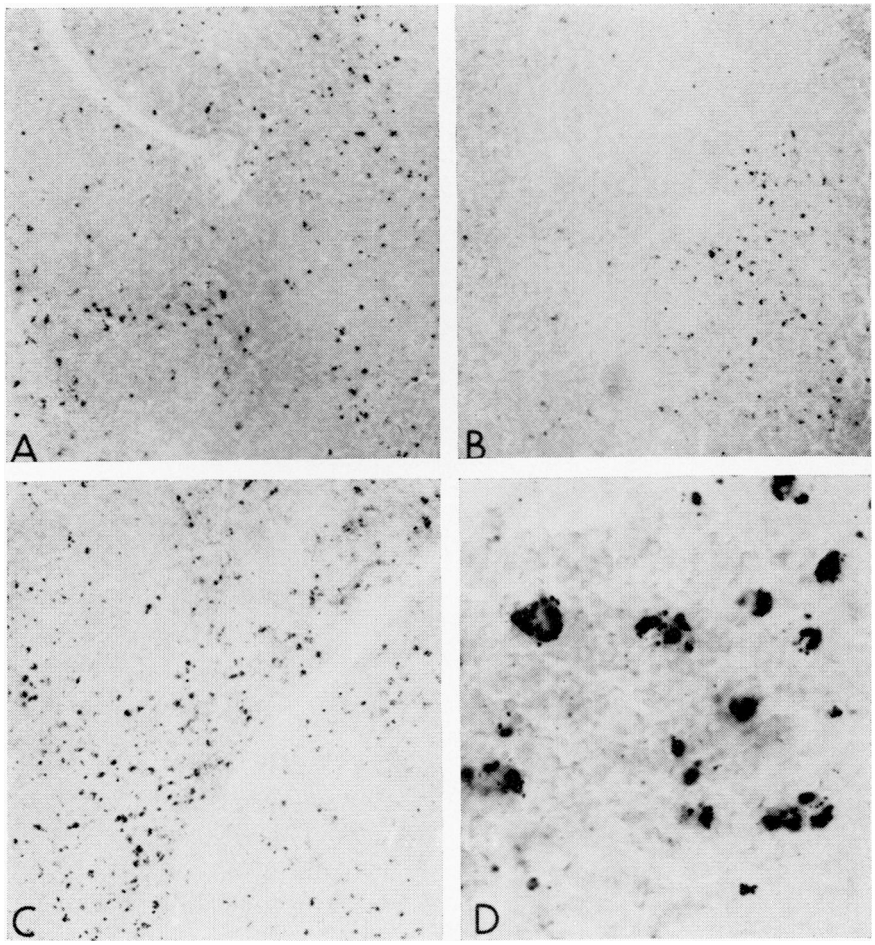

FIG. 1. **A.** Thymus from control rat injected with 0.9% NaCl. Tissue incubated for acid phosphatase activity for 20 min. at 37° C. ×100. Note darkly-stained macrophages. **B.** Thymus from rat injected with 5 mg. cortisol/100 g. body weight 3 hr. prior to sacrifice and incubated simultaneously as the section in A. ×100. No significant increase in acid phosphatase staining cells is evident. **C.** Thymus from cortisol-injected rat sacrificed at 20 hr. following steroid administration. ×100. The increase in acid phosphatase staining cells is apparent. **D.** Thymus from rat injected with cortisol 20 hr. prior to sacrifice and tissue incubated for acid phosphatase for 45 min. at 37° C. ×550. The macrophages are almost totally black in appearance after this longer incubation period. Small granules appear to be present within the cytoplasm of some lymphocytes.

TABLE 1. ACUTE CHANGES IN "FREE" HYDROLASE ACTIVITY IN RAT THYMIC TISSUE IN RESPONSE TO STEROID INJECTION

	*KCl Homogenates**									
Type of treatment	*β-Glucuron-idase***	*Per cent change*	*DNase II*	*Per cent change*	*Acid Phos-phatase*	*Per cent change*	*Acid RNase*	*Per cent change*	*Alkaline RNase*	*Per cent change*
Saline	133.8(3)***	—	54.9(2)	—	1134(2)	—	—	—	—	—
Cortisol,† 1 hr.	137.4(3)	+2	56.3(2)	+2	1092(2)	−4	—	—	—	—
Saline	139.2(5)	—	48.0(3)	—	1110(2)	—	—	—	—	—
Cortisol, 2 hr.	158.4(5)	+14	64.5(3)	+34	1098(2)	−1	—	—	—	—
Saline	151.8(5)	—	48.2(12)	—	996(5)	—	21.6(3)	—	2.57(3)	—
Cortisol, 3 hr.	184.2(5)	+21	72.9(12)	+51	1134(5)	+14	23.2(3)	+7	2.35(3)	−9
Saline	150.0(1)	—	48.5(2)	—	—	—	—	—	—	—
9 α-Fluoro-Δ^1-cortisol, 3 hr.	178.8(1)	+19	75.5(2)	+56	—	—	—	—	—	—
Saline	146.4(3)	—	48.5(2)	—	—	—	—	—	—	—
Progesterone, 3 hr.	148.8(3)	+1	53.0(2)	+9	—	—	—	—	—	—
				H_2O Homogenates‡						
Saline	—	—	47.5(2)	—	—	—	—	—	—	—
Cortisol, 3 hr.	—	—	58.4(2)	+23	—	—	—	—	—	—
Saline	—	—	—	—	—	—	27.6(1)	—	3.93(1)	—
Cortisol, 4 hr.	—	—	—	—	—	—	24.1(1)	−13	3.36(1)	−14
9 α-Fluoro-Δ^1-cortisol, 4 hr.	—	—	—	—	—	—	29.2(1)	+6	3.85(1)	−2

* See METHODS.

**β-Glucuronidase activity = mμ moles phenolphthalein liberated/mg. protein/60 min.; DNase II activity = μg DNA degraded/mg.protein/60 min.; acid phosphatase activity = mμ moles inorganic phosphate released/mg.protein/60 min.; acid and alkaline RNase activity = change in optical density at 260 mμ/mg. protein/60 min.

*** Number of individual experiments in parentheses.

† 5 mg. steroid/100 g. body weight injected intraperitoneally; time indicated is that elapsing between steroid injection and sacrifice of animals. Control rats received saline injections.

‡ Single rat thymi homogenized in 5 ml. of cold distilled H_2O; homogenates frozen and thawed three times and then centrifuged at 2°C. at 1000 × *g* for 10 min. Supernatant fluid used for assay.

TABLE 2. ALTERATIONS IN "FREE" AND "TOTAL" HYDROLASE ACTIVITY IN 700 × *g* SUPERNATANT FRACTION OF KCl HOMOGENATES OF RAT THYMUS IN RESPONSE TO A SINGLE INJECTION OF CORTISOL*

Type of treatment	*DNase II***				*β-Glucuronidase*			
	Free activity	*Per cent change*	*Total activity*	*Per cent change*	*Free activity*	*Per cent change*	*Total activity*	*Per cent change*
Saline	46.8(6)†	—	115.8(3)	—	155.4(3)	—	1504(2)	—
Cortisol, 3 hr.	61.1(6)	+30	112.2(3)	−3	186.0(3)	+20	1527(2)	+1
Saline	62.8(2)	—	116.5(2)	—	151.2(2)	—	1470(2)	—
Cortisol, 20 hr.	112.2(2)	+78	247.0(2)	+112	271.8(2)	+80	2412(2)	+64
	Acid phosphatase				*Acid RNase*			
Saline	996(2)		2120(2)	—	21.6(3)	—	28.3(3)	—
Cortisol, 3 hr.	1134(2)	+14	1960(2)	−8	23.2(3)	+7	28.8(3)	+2
Saline	978(2)	—	1950(2)	—	26.6(3)	—	—	—
Cortisol, 20 hr.	1812(2)	+85	2930(2)	+50	38.1(3)	+43	—	—
	Alkaline RNase							
Saline	2.57(3)	—	2.66(3)	—				
Cortisol, 3 hr.	2.35(3)	−9	2.43(3)	−9				
Saline	3.70(3)	—	—	—				
Cortisol, 20 hr.	7.80(3)	+110	—	—				

* Cortisol, 5 mg./100 g. body weight, injected at either 3 hr. or 20 hr. prior to sacrifice of animals. Control rats received saline injections.

** Units of activity as in Table 1.

† Number of individual experiments in parentheses.

The results shown in Figure 1 support the suggestion that a marked increase in both "free" and "total" hydrolase activity at 20 hours after steroid injection was a consequence of involutionary changes in thymic tissue with an influx of phagocytic cells. Histochemical localization of acid phosphatase in thymic sections from these control and cortisol injected rats revealed differences in both the distribution and quantity of this enzyme. The enzyme in control tissue appears to be localized in a few macrophages; the number of these cells is not greatly increased in thymic tissue of rats injected with steroid three hours prior to sacrifice.

In a further effort to ascertain whether increased hydrolase activity in lymphoid tissue exposed to a corticosteroid is causally related to lymphoid

tissue involution, the data presented in Table 3 were obtained. The experiments were based on the assumption that cells in lymph would be primarily lymphocytes, and that analyses of these cells for hydrolase activity would not be influenced by the presence of other cell types, notably macrophages. Indeed, histological examination of smears of lymph from control and cortisol injected rats revealed almost entirely mature, small lymphocytes. The data in Table 3 show that the β-glucuronidase and acid phosphatase activity of lymph cells collected from control rats is significantly lower than that in thymic tissue of the same animals. Administration of very large doses of cortisol for a longer period of time did not alter the β-glucuronidase and acid phosphatase activity in lymph cells collected at a time when marked thymic involution was occurring. In contrast, hydrolase activity in the thymic tissue of the cannulated rats was markedly elevated in response to steroid injection. Also, the failure to detect hydrolase activity in the cell-free lymph suggests either that cellular destruction in lymphoid tissue has not led to significant loss of enzymes to the circulating lymph or that these enzymes are present in very low concentrations. The data in this table support the suggestion that the increase in activity of certain hydrolases in thymus exposed to an active corticosteroid is a result of the influx into this tissue of cell types other than lymphocytes in response to an initiation of lymphocytokaryorrhexis.

The data in Table 4 show the response to cortisol of three hydrolases of rat thymus and lymphosarcoma. The thymus of tumor bearing rats exhibited a significantly higher level of hydrolase activity as compared to the thymus of non-tumor bearing animals. The activity of these enzymes in the thymus of tumor bearing rats was at the level seen in the same tissue from non-tumor bearing animals that had been injected with a large dose of cortisol. This may be a reflection of a nonspecific stressful action of the tumor on pituitary-adrenal cortical secretory activity, since stress augments the level of functioning of this endocrine mechanism.[26,27] The tumor tissue itself also appears to have hydrolase activity somewhat higher than that in the thymus of non-tumor bearing animals treated with cortisol. It is also apparent that the β-glucuronidase level in tumor tissue responded to involuting doses of steroid in a manner similar to thymus. However, in contrast to the data obtained from non-tumor bearing rats, cathepsin and arysulfatase activities in thymus and lymphosarcoma of tumor bearing animals did not respond to cortisol treatment. The reason for this discrepancy is not clear; the high values for arysulfatase in the thymus from control rats bearing a lymphosarcoma may represent maximal response to endogenous steroid, although this does not necessarily appear to be the case for the other two enzymes studied.

TABLE 3. RESPONSE OF HYDROLASE ACTIVITY OF RAT THYMIC AND MESENTERIC LYMPH CELL HOMOGENATES TO CORTISOL INJECTION*

Type of treatment and tissue used	*Arylsulfatase***				*β-Glucuronidase*				*Acid phosphatase*	
	Free activity	*Per cent change*	*Total activity*	*Per cent change*	*Free activity*	*Per cent change*	*Total activity*	*Per cent change*	*Total activity*	*Per cent change*
Saline, thymus (20)†	7.9	—	15.3	—	73.9	—	175.0	—	1940	—
Cortisol, thymus (cannulated rats) (3)	86.1	+990	123.3	+710	431.0	+483	651.0	+272	4280	+121
Control, lymph cells (3)	0	—	0	—	59.8	—	127.0	—	390	—
Cortisol, lymph cells (3)	0	—	0	—	39.6	−34	133.0	+5	330	−15
Control, lymph cell supernatant fluid (3)	0	—	0	—	0	—	0	—	0	—
Cortisol, lymph cell supernatant fluid (3)	0	—	0	—	0	—	0	—	0	—

* Animals injected with cortisol received 15 mg. steroid/100 g. body weight at 6 hr. and 45 min. prior to sacrifice. Lymph collected during the prior 22 hr. served as control for analysis.

** Arylsulfatase activity = μg nitrocatechol liberated/mg. protein/60 min. Other units as in Table 1.

† Number of experiments in parentheses.

TABLE.4. ALTERATIONS IN HYDROLASE ACTIVITY IN THYMIC AND LYMPHOSARCOMA HOMOGENATES OF TUMOR BEARING RATS IN RESPONSE TO CORTISOL INJECTION*

Type of treatment and tissue used	*Cathepsin***		*β-Glucuronidase*				*Arylsulfatase*			
	Total activity	*Per cent change*	*Free activity*	*Per cent change*	*Total activity*	*Per cent change*	*Free activity*	*Per cent change*	*activity Total*	*Per cent change*
Saline, thymus (non-tumor bearing)	2.58±0.2†(20)‡	—	73.9± 3.8(20)	—	175.0± 5.0(20)	—	7.9±1.0(20)	—	15.3±2.5(20)	—
Cortisol, thymus (non-tumor bearing)	3.41±0.1(12)	+32	191.7±22.2(7)	+160	346.0±38.0(12)	+98	19.1±2.8(12)	+142	32.9±4.4(12)	+115
Saline, thymus (tumor bearing)	4.44±0.2(10)	—	209.0±19.8(10)	—	443.0±25.8(10)	—	18.2±2.9(10)	—	36.6±3.8(10)	—
Cortisol, thymus (tumor bearing)	4.80±0.3(5)	+8	406.0±22.3(5)	+94	780.0±28.6(5)	+76	18.6±2.7(5)	+2	28.1±3.0(5)	−23
Saline, lymphosarcoma	6.01±0.3(10)	—	245.0±17.3(10)	—	522.0±28.3(10)	—	12.7±1.9(10)	—	21.3±2.8(10)	—
Cortisol, lymphosarcoma	6.34±0.2(7)	+5	329.0±19.2(7)	+34	635.0±28.9(7)	+22	11.1±1.4(7)	−13	19.1±2.1(7)	−10

* Cortisol treated rats received two subcutaneous injections of steroid, each 10 mg./100 g. body weight, at 20 hr. and at 4 hr. prior to sacrifice; control rats received saline injections.

** Cathepsin activity = μmoles tyrosine liberated/mg. protein/60 min.; other units as in Table 3.

† All values in this table are means ± the standard errors of the means.

‡ Number of individual experiments in parentheses.

DISCUSSION

The hydrolases studied in the present communication have been implicated as agents of importance for the involution of a variety of morphological structures.[14-18,28] In a preliminary publication,[19] we have reported that cortisol injection in rats resulted in a significant increase in the "free" and "total" specific activity of the thymic hydrolases studied, namely β-glucuronidase, arysulfatase, and cathepsin. The increase in the activity of these hydrolases was accompanied by a dramatic degenerative change in the thymus. It was important, therefore, to extend these studies in order to ascertain whether this increased hydrolase activity in thymus exposed to cortisol *in vivo* might have a causal significance for the thymolytic action of the hormone. When rats were treated with a smaller dose of cortisol at three hours prior to sacrifice, only the "free" activity of β-glucuronidase, DNase II, and acid phosphatase in the cytoplasmic fraction of thymic homogenates was elevated. Both acid and alkaline RNase levels were unchanged. These results are in contrast to the report by Wiernick and MacLeod[16] that the alkaline RNase activity of thymic homogenates prepared in cold distilled water and subjected to freezing and thawing showed significant increase at four hours after exposure of rats to 9α-fluoro-Δ^1-cortisol. However, our data are in agreement with those authors with regard to the elevation of DNase II resulting from exposure of lymphoid tissue to an active corticosteroid. Our previous data,[10] in addition, indicate that this increased DNase II activity is in the cytoplasm and not in the nucleus. An increase in both "free" and "total" specific activity of these hydrolases was seen only after longer exposure to cortisol. These changes induced by steroid are believed to be due to an increased release of hydrolases from the particulate fraction, and an apparent selective retention of these enzymes when extensive tissue protein loss is taking place in the thymus. This suggestion is supported by the lack of measurable activity in the cell-free lymph obtained from the cannulated rats injected with cortisol. The increase in the "total" specific activity appeared to be the consequence of an invasion of other cell types, notably macrophages, into the thymus.

The possible significance of the contributions by various cell types to measurements of hydrolase activity was examined carefully by Ambellan and Hollander.[18] These investigators measured RNase activity in a lymphoid tumor of mice treated *in vivo* with agents that caused tumor regression. Various cell types in the tumor were separated by glass bead chromatography so that it was possible to measure enzyme activity in "purified" tumor lymphocytes, uncontaminated by phagocytic cells. Agents that caused tumor regression, including effective adrenal corticosteroids, produced an increase in RNase activity in lymphocytes from tumor tissue. Drugs that

did not affect the tumor did not alter its RNase level. These authors suggested that RNase activity may play an important role in the drug-induced regression of the lymphosarcoma and that steroids might function via a selective activation of an endogenous RNase. However, in the studies reported here, acid and alkaline RNase measurements did not reveal significant alterations in the activity of these enzymes in thymic tissue in response to the injection of cortisol.

The lymph cells collected from cannulated mesenteric lymphatics of the rat provided a cell population of almost entirely mature small lymphocytes. These cells were ideal for the study of the possible relationship between elevated hydrolase activity in lymphoid tissue and the involutionary changes induced by a corticosteroid. The results of this study showed that the treatment of rats with a very large dose of cortisol did not alter the hydrolase activity in mesenteric lymph cells although the activity of these enzymes in the thymus of the same animals was markedly elevated. It is possible that lymph cells represent not only a more uniform population of lymphocytes, but also that these mature, non-proliferating cells are without cellular activities that might contribute to elevated hydrolase levels.

In rats bearing the Murphy-Sturm transplantable lymphosarcoma, hydrolase activity was significantly higher both in the tumor and in the thymus of tumor bearing rats than in the thymus from non-tumor bearing animals. It is not clear whether this may have been due to a possible nonspecific stress in animals bearing a tumor or to the presence of non-lymphoid cells in this transplantable lymphosarcoma. In any event, the β-glucuronidase level in the tumor could be elevated by injection of involuting doses of cortisol.

The data presented in this communication indicate that the degree and manner of augmentation of the activity of certain hydrolases in lymphoid tissue are dependent upon the length of time rats are exposed to an active glucocorticoid. This time variable influences two parameters which have been studied, namely, a) the relative change in "free" as compared to "total" enzymic activity, and b) the morphological status of the thymic tissue of the steroid-treated rats. In the early period following cortisol injection, i.e., up to three hours, a slight but significant increase was observed only in the "free" activity of β-glucuronidase, DNase II, and acid phosphatase, but no alteration was seen in the level of "total" hydrolase activity. At this earlier time, no change was detected in the activity of acid or alkaline RNase. However these two enzymic levels, both "free" and "total," were elevated at 20 hours following steroid administration, as was the "total" enzymic activity of all other hydrolases studied (Table 2). Histo-

logical and histochemical (acid phosphatase) examination of thymic slices suggested that these later alterations in hydrolase levels in response to cortisol injection were a consequence of an influx into this tissue of significant numbers of cell types other than lymphocytes in response to the initiation of lymphocytokaryorrhexis[9] by some as yet unknown mechanism.

Previous studies from this laboratory[7,10,29,30] have provided evidence that two mechanisms may be involved in the more immediate effects of cortisol on lymphoid cells. One is the effect of the steroid on transport phenomena[10] and the other involves the action of steroid on a DNA dependent-RNA polymerase system.[30] The results presented in this communication support the previous observation that the acute inhibitory effect of cortisol on thymic RNA polymerase activity is not a consequence of elevated alkaline RNase in the nuclei or in the aggregate RNA polymerase preparation. The intact nuclei or the aggregate polymerase preparation from thymic tissue of either saline control or cortisol treated rats degraded RNA at the same rate.[29] Currently, we are investigating the possibility that incubation of thymocytes with cortisol *in vitro* may activate DNase II and other hydrolytic enzymes. Thymocytes prepared according to Makman, *et al.*[7] appear to be almost all small mature lymphocytes. Such preparations will eliminate the contribution by other cell types to measurements of the hydrolases of thymocytes.

SUMMARY

A study has been made of the effect of cortisol injection in rats on the activity of β-glucuronidase, DNase II, acid phosphatase, arylsulfatase, and cathepsin in lymphoid tissue homogenates. The response of certain of these enzymes to steroid was examined at intervals after hormone administration; comparisons of "free" and "total" enzymic activity have also been made. Experiments have also been conducted on the response to cortisol injection of thymic and lymphosarcoma hydrolase activity in tumor bearing rats, as well as in the lymph and lymph cells of non-tumor bearing rats.

Injection of 5 mg. cortisol/100 g. body weight into rats two hours prior to sacrifice resulted in an elevated level of activity of "free" β-glucuronidase and DNase II in the 700 × *g* fraction of homogenates of thymic tissue obtained from these animals. A shorter time of exposure to steroid *in vivo*, namely, one hour, did not alter these hydrolases. At three hours after steroid administration, acid phosphatase activity was also higher in homogenates of steroid injected animals than in homogenates from control rats given 0.9% saline. No alterations were seen in acid or alkaline RNase or in the "total" activity of all the hydrolases measured at this time. Injection of progesterone three hours prior to sacrifice of the rats did not alter any

of the enzymic levels. On the other hand, another potent thymolytic steroid, 9α-fluoro-Δ^1-cortisol, led to results similar to those obtained with cortisol administration.

Alterations in both "free" and "total" enzymic activity were seen for all hydrolases assayed in thymic homogenates prepared from rats exposed to cortisol *in vivo* for 20 hours (Table 2). The data were interpreted as being a consequence of the infiltration of significant numbers of phagocytic cells into thymic tissue undergoing involution rather than reflecting a causative basis for lymphocytolysis. This conclusion was supported by histochemical studies of thymic slices and by measurements of hydrolases in lymph and lymph cells obtained by cannulation of the mesenteric lymphatics of rats given large doses of cortisol.

The thymus of rats bearing the Murphy-Sturm transplantable lymphosarcoma, as well as the tumor itself, revealed levels of hydrolase activity that were markedly higher than those seen in the thymus of either normal rats or animals injected with cortisol. Nonetheless, administration of steroid to the tumor bearing rats produced a further elevation in the "free" and "total" activity of β-glucuronidase and in total cathepsin activity. However, the high arylsulfatase values seen in the thymus and lymphosarcoma of tumor bearing rats were not altered by steroid administration.

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