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Effects of Pituitary and Adrenal Hormones on the Numbers of Thoracic Duct Lymphocytes

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A CONSIDERABLE body of evidence is at hand to indicate that the level of circulating blood lymphocytes is inversely related to the level of adrenal cortical secretions. The evidence leading to this generalization has recently been reviewed by Dougherty and White,[1] Valentine, Craddock and Lawrence,[2] and Yoffey.[3]

It has been of interest to determine whether or not the level of lymphocytes entering the blood stream through the thoracic duct is related in a similar manner to the level of adrenal cortical secretions. Reinhardt and Li[4] reported, in a preliminary note, that the administration of adrenocorticotropic hormone (ACTH) produced a rapid and persistent fall in the number of thoracic duct lymphocytes in the rat. Yoffey, Reiss and Baxter[5] also presented evidence that a similar result was effected by the administration of ACTH to cats. Hungerford and Reinhardt[6] reported that within two or three hours after adrenalectomy there was a significant rise in the number of thoracic duct lymphocytes in rats. These reports suggested that the lymphocytes in thoracic duct lymph were influenced by the adrenal cortical secretions in a manner similar to that observed in studies on blood lymphocyte levels. On the other hand, Valentine, Craddock and Lawrence[2] have reported that a commercial aqueous adrenal cortical extract was without effect on the number of thoracic duct lymphocytes when administered to normal and adrenalectomized cats.

Two methods have been employed for ascertaining quantitative changes in the number of thoracic duct lymphocytes. One method utilizes relatively long term (6 to 10 hours) lymph collection experiments, each animal serving as its own control, initial control levels being compared with values obtained subsequent to the administration of various types of hormones. The second method, using large groups of standard animals of known age, sex, and nutritional status, allows the administration of various hormones before lymph collections are

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begun, the values for lymph flow and lymphocyte levels being determined for a relatively shorter period of time, (1½ hours), thus avoiding the alterations in lymph flow and cell count which are known to occur after long term anesthetization and lymph collection. This latter method was chosen for the present survey of the influence on thoracic duct lymphocytes of: anterior pituitary growth hormone (GH), and adrenocorticotropic hormone (ACTH protein or peptide mixtures); posterior pituitary hormones (commercial Pitressin and Pitocin); adrenal cortical hormones (commercial Adrenal Cortex Extract, cortisone acetate, desoxycorticosterone glucoside), and adrenal medullary hormone (commercial Adrenalin Hydrochloride).

Experimental Methods

In order to determine the most satisfactory conditions for carrying out experiments concerning the influence of endocrine substances on thoracic duct lymph, comparative studies of age, anesthetic agent and site of lymph collection in the rat were made. These experiments, reported elsewhere,[7] provided evidence leading to the choice of the following experimental conditions.

Male rats of the Long-Evans strain, 60 days of age were employed. The normal animals were maintained on the regular stock diet (XIV), supplemented with fresh lettuce twice weekly. Adrenalectomized rats received a similar regimen with the exception that they were given a 1 per cent sodium chloride solution to drink. Rats hypophysectomized twenty days before use were given an enriched wet diet (I) in addition to the regular regimen for normal rats. Each paired-fed sham-operated rat received the enriched diet in the amount consumed by the corresponding hypophysectomized rat.

Hypophysectomy was carried out using the parapharyngeal approach under ether anesthesia. The sella turcica was carefully examined at autopsy for possible pituitary remnants. Bilateral adrenalectomy was carried out using a dorsal approach under ether anesthesia. The operative sites were carefully examined at autopsy for adrenal cortical remnants. Incompletely operated animals were discarded.

Lymph was collected from the jugular lymph sac in the neck using an operative approach described by Reinhardt.[8] A small drop of heparin (Upjohn) was added to each sample to prevent clotting. The animals were maintained under sodium pentobarbital anesthesia during the period of lymph collection. A 2 per cent aqueous solution of sodium pentobarbital was injected intraperitoneally in the amount of 7 mg. per 100 Gm. of body weight. Smaller amounts were subsequently injected when necessary to maintain a uniform anesthesia. Lymph was collected for a 90 minute period from each rat. The quantity of lymph was measured in a tuberculin syringe, and a white blood cell count was made on each sample. Care was taken to shake each sample of lymph thoroughly before filling the diluting pipets. The diluent was a two per cent acetic acid solution with added methylene blue. The counts were made on a Spencer Bright Line hemocytometer.

The total number of lymphocytes in each sample was calculated by multiplying the volume of lymph by the number of lymphocytes per cu. mm. The 90 minute totals for lymph flow and total cells were calculated and converted to twenty-four hour values. In addition, these twenty-four hour calculations were converted to values relative to 100 Gm. of body weight. Differences of mean values were tested for statistical significance by calculation of standard error of the means and evaluation of "t" values.[9] A difference between means was considered highly significant if p was $< .01$, and of probable significance if p was $< .05$.

The ACTH preparations employed were isolated from sheep pituitary glands.[10] Two of the ACTH preparations were in the form of peptide mixtures. One peptide mixture was prepared by digesting an ACTH protein with pepsin[11] and is referred to as ACTH pepsin peptide mixture. The other peptide preparation was made by hydrolyzing an ACTH protein with 1 N hydrochloric acid[12] and is referred to as ACTH acid peptide mixture. All ACTH preparations were assayed for biological activity either by the adrenal weight

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maintenance method[13] or by the adrenal ascorbic acid-depletion method.[14] Some of the ACTH preparations were also assayed for antidiuretic activity employing a method described by Birnie, et al.[15] All of the above ACTH preparations possessed demonstrable antidiuretic activity, excepting the ACTH acid peptide mixture.[23]

Growth hormone isolated from beef anterior pituitary glands[16] was employed. This preparation was assayed according to the method of Marx, Simpson and Evans,[17] and it was found that 0.01 mg. administered daily to hypophysectomized rats for 10 days produced a 10 Gm. increment in body weight.

Pitressin (20 pressor units per cc.), Pitocin (10 international units per cc.) and Adrenalin hydrochloride (1:1000 solution) were commercial products of the Parke, Davis Company.

Adrenal Cortex Extract was an aqueous commercial preparation made by the Upjohn Company.[18]

TABLE 1—*Rate of Flow and Total Cell Content of Thoracic Duct Lymph in Normal Rats and after Removal of the Pituitary or Adrenal Glands in 60 Day Old Male Rats*

Type Animal	Day P. O.	No. of Rats	Body Wt.	WBC	per 24 hrs.		per 100 Gm/24 hrs.	
					Lymph Flow	Total Cells	Lymph Flow	Total Cells
Normal	—	28	252	31500	21.0 (0.8)	642 (38)	8.4 (0.3)	252 (13)
Hypophysectomized	1 and 2	10	230	52600	17.6 (0.9)	917 (60)	7.7 (0.4)	400 (27)
Hypophysectomized	20	8	130	45000	11.1 (1.3)	494 (67)	8.6 (0.9)	375 (47)
Hypophysectomized*	20	9	131	60300	5.9 (0.6)	374 (79)	4.5 (0.4)	285 (56)
Sham-operated*	20	6	158	36050	6.7 (0.8)	227 (25)	4.3 (0.6)	144 (16)
Adrenalectomized	20	12	225	36900	20.9 (1.4)	777 (98)	9.3 (0.6)	343 (41)

Mean values with standard errors of the means in parentheses.

Body weight expressed in grams; WBC per mm.^{3}; lymph flow in cc.; and total cells in millions.

* Denotes groups undergoing paired-feeding technic while all other groups were on standard regimen.

The Cortisone used was a commercial product of Merck and Company. This was a synthetic compound (11-dehydro-17-hydroxycorticosterone-21-acetate), in crystalline suspension.

The desoxycorticosterone glucoside (with vehicle) was a synthetic product of the Ciba Company.

RESULTS

Effect of Hypophysectomy (table 1)

Lymph was collected from hypophysectomized rats under three different conditions: (1) lymph was collected from rats in the 60 day old group which had been hypophysectomized twenty-four or forty-eight hours previously; (2) lymph was collected from rats hypophysectomized at 40 days of age, but studied at 60

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days of age; (3) for purposes of studying the effects of paired-feeding, lymph was collected from rats hypophysectomized at 40 days of age, but used at 60 days of age. These rats, however, were housed one to a cage on wire screen bottoms, and fed enriched wet diet, so that a sham-operated, paired-fed group could be obtained for study. These groups of hypophysectomized and control rats are referred to as the paired-fed groups.

1. Lymph collected from rats one and two days after hypophysectomy had a total cell content which was significantly higher ($p < .01$) than in the normal intact rats, both on an absolute basis and when referred to body weight. This increased total cell content was noted in spite of a lowered lymph flow in the hypophysectomized group which was not, however, significantly different relative to body weight, but was significantly lower ($p < .01$) on an absolute basis.

2. In animals hypophysectomized at 40 days of age, and in which lymph was collected 20 days postoperatively, the total cell content of the lymph was significantly higher ($p < .01$) relative to body weight than in normal rats of 60 days of age. Lymph flow, however, was unaltered when related to body weight. The absolute values for lymph flow and total cell content, on the other hand, were significantly lower ($p < .01$) when compared with the normal 60 day old group, because of the marked disparity in body weights of the two groups.

3. In rats hypophysectomized at 40 days of age and paired-fed with a sham-operated group until 60 days of age, a marked reduction in lymph flow was observed ($p < .01$) when compared with the normal 60 day old group. The sham-operated, paired-fed control group also showed a reduction ($p < .01$) in lymph flow of the same magnitude as in the hypophysectomized group. The total cell content of the lymph of the hypophysectomized paired-fed group was significantly higher ($p < .01$), however, when compared with the sham-operated, paired-fed control group.

In conclusion, it can be said that the total cell content of thoracic duct lymph is increased following hypophysectomy. This becomes apparent within twenty-four hours after operation and is still observed three weeks later. It should be noted, moreover, that the lymphocyte content of the thoracic duct lymph of hypophysectomized animals is higher than in paired-fed animals given the amount of food actually consumed by the hypophysectomized animals.

Effect of Adrenalectomy (table 1)

Lymph was collected from a group of rats at 60 days of age which had been adrenalectomized at 40 days of age. Thoracic duct lymph flow was unaltered in this group on an absolute basis and relative to body weight when compared with the normal group of rats. Total cell content of the lymph was relatively higher, the difference proving to be of probable significance ($p < .05$). A similar trend was observed in subsequent treated adrenalectomized groups, so it would appear that this moderate elevation of the total cell content of lymph is a consistent finding.

Effect of a Single Injection of ACTH (table 2)

Several different ACTH preparations were injected intraperitoneally into normal, hypophysectomized and adrenalectomized rats. The ACTH was pre-

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pared either as a protein, pepsin peptide mixture or as an acid peptide mixture. Lymph collection was started at a specified time interval after injection of the hormone preparations.

TABLE 2—*Rate of Flow and Total Cell Content of Thoracic Duct Lymph after a Single Injection of ACTH Protein or Peptide into Normal, Hypophysectomized and Adrenalectomized 60 Day Old Male Rats*

Injection Material	No. of Rats	Body Wt.	WBC	per 24 hrs.		per 100 Gm/24 hrs.	
				Lymph Flow	Total Cells	Lymph Flow	Total Cells
Normal Rats							
None	28	252	31500	21.0	642	8.4	252
				(0.8)	(38)	(0.3)	(13)
0.9% NaCl, 0.5 cc.	10	240	22600	23.8	521	9.9	215
				(2.3)	(47)	(0.9)	(13)
ACTH protein, 3.0 mg.	13	240	21600	14.8	317	6.2	134
				(0.7)	(26)	(0.3)	(12)
ACTH pepsin peptide, 1.5 mg.	10	238	19200	17.4	322	7.3	135
				(1.3)	(34)	(0.5)	(14)
ACTH acid peptide, 1.5 mg.	14	233	28900	20.7	614	8.9	262
				(1.3)	(74)	(0.5)	(30)
ACTH protein*, 1.0 mg.	9	257	36700	17.0	630	6.7	247
				(0.7)	(65)	(0.3)	(26)
Adrenalectomized Rats							
None	12	225	36900	20.9	777	9.3	343
				(1.4)	(98)	(0.6)	(41)
ACTH protein, 3.0 mg.	10	182	32800	20.7	654	11.2	357
				(2.2)	(55)	(1.0)	(27)
Hypophysectomized Rats							
None	10	230	52600	17.6	917	7.7	400
				(0.9)	(60)	(0.4)	(27)
ACTH protein, 2.5 mg.	8	214	42700	9.9	426	4.6	197
				(0.6)	(41)	(0.3)	(17)
ACTH pepsin peptide, 1.5 mg.	8	240	47300	13.7	651	5.9	258
				(1.0)	(56)	(0.4)	(26)

Mean values presented with standard errors of the means in parentheses.

Body weight expressed in grams; WBC per mm.^{3}; lymph flow in cc.; total cells in millions. Rats used 20 days after adrenalectomy; or 1 and 2 days after hypophysectomy. All injections made intraperitoneally.

* Denotes group injected 45 minutes prior to lymph collection while all other groups were injected 2 hours prior to lymph collection.

Two hours after a single injection of ACTH protein or pepsin peptide mixture into normal or hypophysectomized rats, the total cell content of lymph was significantly lowered ($p < .01$) on an absolute basis and relative to body weight, when compared with the normal untreated or saline injected control groups. The lymph flow was similarly significantly reduced ($p < .01$). The effect of these

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ACTH preparations in lowering the total cell content of lymph can be attributed to their effect in lowering both lymph flow and the number of lymphocytes per unit volume of lymph. If a shorter time interval (45 minutes) was allowed to elapse after injection of an ACTH protein into normal animals, no significant alteration of the total cell content of the lymph was observed, when compared with the control groups. These ACTH preparations exhibited no effect when administered to adrenalectomized rats, as compared with the control groups of adrenalectomized rats.

TABLE 3—*Rate of Flow and Total Cell Content of Thoracic Duct Lymph after Daily Injections of ACTH Protein into Normal and Hypophysectomized 60 Day Old Male Rats*

Daily Injections	No. of Rats	Body Wt.	WBC	per 24 hrs.		per 100 Gm/24 hrs.	
				Lymph Flow	Total Cells	Lymph Flow	Total Cells
Normal Rats							
None	28	252	31500	21.0	642	8.4	252
				(0.8)	(38)	(0.3)	(13)
ACTH, 0.5 mg./day for 10 days	9	222	37150	14.2	490	6.3	217
				(1.4)	(40)	(0.5)	(17)
Albumin, 0.5 mg./day for 10 days	9	258	39450	19.9	741	7.7	305
				(1.4)	(59)	(0.4)	(34)
Hypophysectomized Rats							
None	8	130	45000	11.1	494	8.6	375
				(1.3)	(67)	(0.9)	(47)
ACTH, 0.5 mg./day for 10 days	8	133	49850	10.2	492	7.8	373
				(0.7)	(40)	(0.7)	(32)
ACTH, 0.25 mg./day for 15 days*	7	135	44700	12.9	556	9.6	410
				(1.0)	(38)	(0.8)	(18)

Mean values given with standard errors of the means in parentheses.

Body weight expressed in grams; WBC per $mm.^3$; lymph flow in cc.; and total cells in millions. All injections made intraperitoneally in three divided doses. Rats hypophysectomized at 40 days of age.

* Denotes group aged 55 days at lymph collection while all other groups were 60 days old.

Lymph was collected from a group of normal rats two hours after a single intraperitoneal injection of an ACTH acid peptide mixture. Thoracic duct lymph flow and total cell content did not, however, differ from any of the normal control groups.

Effect of Prolonged Administration of ACTH (table 3)

Thoracic duct lymph was collected from normal rats, treated for ten days with ACTH protein in the amount of 0.5 mg. per day, divided in three doses. The lymph flow was significantly reduced ($p < .01$) on an absolute basis and relative to body weight, when compared with the untreated control group. The total cell content of the lymph was significantly lowered ($p < .01$) on an absolute

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basis when compared with the control groups. Relative to body weight, however, the total cell content of the lymph was unaltered when compared with the control groups.

A control group of normal rats given injections of crystalline beef serum albumin, under the same conditions, showed no significant effect on either lymph flow or total cell content.

Lymph was collected from a group of rats hypophysectomized at 40 days of age and injected daily after operation with an ACTH protein for ten days in the amount of 0.5 mg. in three divided doses (see table 3). Thoracic duct lymph flow and total cell content were not altered when compared with the untreated hypophysectomized group of rats.

Lymph was collected from a group of rats hypophysectomized at 40 days of age and administered a "maintenance" dose of ACTH protein in the amount of

TABLE 4—*Rate of Flow and Total Cell Content of Thoracic Duct Lymph in Normal 60 Day Old Male Rats after a Single Injection of Pituitary Growth Hormone*

Injection Material	No. of Rats	Body Wt.	WBC	per 24 hrs.		per 100 Gm./ 24 hrs.	
				Lymph Flow	Total Cells	Lymph Flow	Total Cells
None	28	252	31500	21.0	642	8.4	252
				(0.8)	(38)	(0.3)	(13)
0.9% NaCl, 0.5 cc.	10	240	22600	23.8	521	9.9	215
				(2.3)	(47)	(0.9)	(13)
Growth Hormone, 2.5 mg.	12	252	24500	20.4	444	8.1	191
				(1.2)	(35)	(0.5)	(13)

Mean values presented with standard errors of the means in parentheses.

Body weight expressed in grams; WBC per $mm.^3$; lymph flow in cc.; and total cells in millions. Growth hormone injected intraperitoneally 1–3 hours before lymph collection was started, while NaCl solution was injected intraperitoneally 2 hours before lymph collection was started.

0.25 mg. per day divided into two doses for fifteen days. Lymph flow and total cell content of this group did not differ significantly when compared with the hypophysectomized control group.

Effect of a Single Injection of Growth Hormone (table 4)

Pituitary growth hormone was administered to a group of normal rats one to three hours before lymph collection was started. Thoracic duct lymph flow and total cell content were not significantly altered in this group, either on an absolute basis or when related to body weight, when compared with the untreated or saline injected control groups.

Effect of a Single Injection of Epinephrine (table 5)

Epinephrine hydrochloride was administered subcutaneously in the amount of 0.07 mg. per 100 Gm. body weight to normal, hypophysectomized and adrenalectomized rats. Lymph collection was started 30 minutes later.

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After epinephrine administration to normal rats, thoracic duct lymph flow was not changed, either on an absolute basis or relative to body weight, when compared with the untreated or with the saline treated control groups. The total cell

TABLE 5—*Rate of Flow and Total Cell Content of Thoracic Duct Lymph of 60 Day Old Normal, Hypophysectomized and Adrenalectomized Male Rats after a Single Injection of Epinephrine or of ACTH and Epinephrine*

Injection Material	No. of Rats	Body Wt.	WBC	per 24 hrs.		per 100 Gm./ 24 hrs.	
				Lymph Flow	Total Cells	Lymph Flow	Total Cells
Normal Rats							
None	28	252	31500	21.0	642	8.4	252
				(0.8)	(38)	(0.3)	(13)
0.9% NaCl, 0.2 cc.	10	250	28400	19.0	546	7.6	215
				(1.1)	(64)	(0.3)	(19)
Epinephrine, 0.07 mg./100 Gm. B. W.	15	241	15500	20.3	306	8.4	128
				(1.3)	(23)	(0.5)	(10)
ACTH protein 3.0 mg., 0.9% NaCl 0.2 cc.	10	236	19100	19.2	368	8.2	148
				(1.1)	(34)	(0.5)	(16)
ACTH protein 3.0 mg., Epinephrine 0.07 mg. per 100 Gm. B. W.	15	235	11600	17.1	200	7.2	84
				(0.8)	(17)	(0.3)	(8)
Hypophysectomized Rats							
None	10	230	52600	17.6	917	7.7	400
				(0.9)	(60)	(0.4)	(27)
Epinephrine 0 07 mg./100 Gm. B. W.	9	220	36600	12.3	437	5.6	201
				(0.9)	(47)	(0.4)	(23)
Adrenalectomized Rats							
None	12	225	36900	20.9	777	9.3	343
				(1.4)	(98)	(0.6)	(41)
Epinephrine 0.07 mg./100 Gm. B. W.	7	196	32500	18.5	594	9.5	302
				(1.3)	(37)	(0.6)	(16)

Mean values presented with standard errors of the means in parentheses.

Body weight expressed in grams; WBC per mm.^{3}; lymph flow in cc. and total cells in millions. Epinephrine and NaCl solutions injected subcutaneously 30 minutes before starting lymph collection and ACTH was injected intraperitoneally 2 hours before starting lymph collection. Animals were hypophysectomized 1 and 2 days before use but adrena'ectomized 20 days before use.

content of the lymph was, however, significantly reduced ($p < .01$), both absolutely and relative to body weight, when compared with the control groups.

After epinephrine administration to hypophysectomized rats (one and two days postoperative), lymph flow and total cell content were significantly reduced ($p < .01$), both absolutely and relative to body weight, when compared with the control groups.

After injecting epinephrine into adrenalectomized rats, lymph flow and total

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cell content were *not* altered, when compared with the untreated adrenalectomized control group.

Effect of a Single Injection of ACTH Protein and Epinephrine (table 5)

Under the conditions of the above experiments, both ACTH protein and epinephrine were effective in lowering the total lymphocyte content, when administered to normal rats. A group of normal rats was, consequently, injected with an ACTH protein and after a 90 minute interval, received an epinephrine injection. Lymph collection was started thirty minutes after the epinephrine injection (2 hours after the ACTH injection).

The total cell content of the lymph collected from this doubly treated group was strikingly and significantly reduced ($p < .01$) when compared with the normal control group. In addition, the total cell content was significantly lower ($p < .01$) than in the groups administered either epinephrine or ACTH alone, or when compared with a group given ACTH and saline (in place of epinephrine). The lymph flow of the group treated with both ACTH and epinephrine was also significantly lowered ($p < .01$), both on an absolute basis and when related to body weight.

Effect of a Single Injection of Pitressin or Pitocin (table 6)

Known contaminants of the ACTH preparations are posterior pituitary hormones.[23] Groups of rats were, therefore, injected with the commercial posterior pituitary preparations, Pitressin and Pitocin.

A large dose of Pitressin (varying from 2 to 20 units) was injected intraperitoneally into normal rats, and lymph was collected 2 hours later. Lymph flow and total cell content remained unaltered, both on an absolute basis and when related to body weight, when compared with the control groups. Injection of Pitressin into adrenalectomized rats was similarly without effect on lymph flow or total cell content when compared with the untreated adrenalectomized group. Administration of a smaller but biologically active dose of Pitressin (10 milliunits) to normal rats did not significantly alter the lymph flow or the total cell content, lymph collection being started two hours after the injection.

After injection of Pitocin (2 units) into normal rats, lymph flow and total content of the lymph were not significantly altered, on an absolute basis or relative to body weight, when compared with the control groups. Lymph collection was started forty-five minutes after administering the hormone.

Effect of a Single Injection of Adrenal Cortical Preparations (table 7)

In an attempt to duplicate the results obtained after injection of ACTH preparations and those obtained after epinephrine injection, several adrenal cortical preparations or steroids were administered to normal rats. Adrenal Cortex Extract (aqueous), cortisone acetate and desoxycorticosterone glucoside were injected intraperitoneally into different groups of rats and lymph collection was started from one to three hours later. Under the conditions of these experiments, thoracic duct lymph flow and total cell content were not significantly altered, either on an absolute basis or relative to body weight, after administration of any of the three preparations mentioned.

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TABLE 6—*Rate of Flow and Total Cell Content of Thoracic Duct Lymph in 60 Day Old Normal and Adrenalectomized Male Rats after a Single Injection of Pitocin or of Pitressin*

Injection Material	No. of Rats	Body Wt.	WBC	per 24 hrs.		per 100 Gm./ 24 hrs.	
				Lymph Flow	Total Cells	Lymph Flow	Total Cells
Normal Rats							
None	28	252	31500	21.0	642	8.4	252
				(0.8)	(38)	(0.3)	(13)
0.9% NaCl, 0.5 cc.	10	240	22600	23.8	521	9.9	215
				(2.3)	(47)	(0.9)	(13)
Pitressin 10 mu	11	230	24800	18.1	461	7.9	200
				(0.8)	(42)	(0.3)	(16)
Pitressin 2–20 units	15	236	30600	20.4	625	8.7	264
				(1.4)	(47)	(0.6)	(18)
Pitocin 2 units	8	241	29800	16.3	472	6.8	193
				(0.7)	(63)	(0.3)	(23)
Adrenalectomized Rats							
None	12	225	36900	20.9	777	9.3	343
				(1.4)	(98)	(0.6)	(41)
Pitressin 10 units	9	175	34600	22.2	762	12.4	423
				(3.3)	(118)	(1.6)	(57)

Mean values presented with standard errors of the means in parentheses.

Body weight expressed in grams; WBC per mm.^{3}; lymph flow in cc.; and total cells in millions. Pitressin and NaCl solutions were injected intraperitoneally 2 hours before lymph collection was started while Pitocin was injected intraperitoneally 45 minutes before lymph collection was started. Animals were adrenalectomized 20 days before use.

TABLE 7—*Rate of Flow and Total Cell Content of Thoracic Duct Lymph in Normal 60 Day Old Male Rats after a Single Injection of Aqueous Adrenal Cortical Extract (ACE), Cortisone Acetate, or Desoxycorticosterone Glucoside*

Injection Material	No. of Rats	Body Wt.	WBC	per 24 hrs.		per 100 Gm./ 24 hrs.	
				Lymph Flow	Total Cells	Lymph Flow	Total Cells
None	28	252	31500	21.0	642	8.4	252
				(0.8)	(38)	(0.3)	(13)
0.9% NaCl, 0.5 cc.	10	240	22600	23.8	521	9.9	215
				(2.3)	(47)	(0.9)	(13)
ACE (Upjohn) 1.0 cc.	11	242	25300	19.3	498	8.0	204
				(1.1)	(46)	(0.4)	(11)
Cortisone Acetate, 2.5 mg.	10	258	27900	21.1	567	8.2	218
				(1.2)	(55)	(0.4)	(17)
Desoxy. glucoside, 5.0 mg.	9	260	22300	21.5	485	8.3	186
				(1.7)	(52)	(0.6)	(11)

Mean values presented with standard errors of the means in parentheses.

Body weight expressed in grams; WBC per mm.^{3}; lymph flow in cc.; and total cells in millions. All injections given intraperitoneally 1–3 hours before lymph collection started.

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Discussion

Measurement of the number of lymphocytes delivered to the blood stream through the thoracic duct has been employed as a method of approaching the problem of hormonal regulation of the number of circulating blood lymphocytes. There are a number of possible explanations for the blood lymphopenic action of adrenal cortical hormones. Among these are: (1) increased removal of the lymphocytes from the blood stream; (2) decreased delivery of lymphocytes to the blood stream. It is recognized that several factors may operate at one time to produce the final result. Dougherty and White[20] have interpreted blood lymphopenia as resulting from decreased delivery of lymphocytes to the blood stream. This interpretation was based on morphological changes observed in the lymphoid tissues. Measurement of the number of lymphocytes in thoracic duct lymph should, then, reflect changes in the number of blood lymphocytes observed after altering the level of adrenal cortical secretions.

The number of circulating blood lymphocytes gradually increases following removal of the pituitary gland.[21] The present work indicates that the level of the thoracic duct lymphocytes is likewise increased following removal of the pituitary gland. This was observed within twenty-four hours after removal of the pituitary gland, and was still to be observed three weeks later. White and Dougherty[20] found that adrenalectomy was also followed by an increase in the number of circulating blood lymphocytes. The increased level of thoracic duct lymphocytes observed after adrenalectomy and maintenance on sodium chloride in the present investigation, was of probable significance, statistically, and was observed in all of the adrenalectomized groups.

Two hours after a single injection of ACTH protein or pepsin peptide mixture, a significantly lowered level of lymphocytes in thoracic duct lymph was observed in normal and hypophysectomized rats, but not in adrenalectomized rats. ACTH protein was ineffective in one group of normal rats when a shorter time interval was allowed to elapse after administering the hormone. Dougherty and White[1] found that the blood lymphocytes were most markedly reduced from 3 to 6 hours after administration of ACTH. Reinhardt and Li[4] and Yoffey, Reiss and Baxter[5] previously observed that ACTH administration lowered the number of lymphocytes in thoracic duct lymph. The present investigation confirms the work of these two reports. Another pathway for removal of blood lymphocytes which requires further investigation is suggested by the observations of Yoffey, Metcalf, Herdan and Nairn,[19] that the level of marrow lymphocytes is distinctly increased after adrenal cortical or ACTH administration.

Administration of an ACTH acid peptide mixture in the present experiments was ineffective in altering the level of lymphocytes in thoracic duct lymph of normal rats. There are no published reports concerning the effect of this preparation on the level of circulating blood lymphocytes. This substance was found, however, to be very active when assayed by the ascorbic acid method. On the other hand, Reinhardt and Li[22] observed that this same preparation was much less active when assayed by the adrenal weight maintenance method, and furthermore, that the weight of the thymus was not greatly reduced after admin-

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istering this preparation. Possible explanations for this apparently anomalous behavior of an active ACTH preparation have been advanced by these authors.[22]

Most of the ACTH preparations used were contaminated with the antidiuretic substance of the posterior lobe of the pituitary gland.[23] Control experiments, however, showed that an effect similar to that noted after ACTH administration could not be obtained with Pitressin or Pitocin.

Prolonged administration of ACTH protein, for ten and fifteen days, to normal or hypophysectomized rats did not decrease the level of thoracic duct lymphocytes to the extent observed after a single injection of these hormones.

Epinephrine administration produced a marked decrease in the level of thoracic duct lymphocytes of normal and hypophysectomized rats but not in adrenalectomized rats. It is possible to explain this effect of epinephrine in lowering the number of lymphocytes in thoracic duct lymph as being the result of increased discharge of adrenal cortical hormone, since this effect was observed in hypophysectomized but not in adrenalectomized rats. Hungerford[21] has previously reported that the number of circulating blood lymphocytes was lowered after administering epinephrine at the same dose level to normal and hypophysectomized rats, but *not* in adrenalectomized rats. It remains to be determined whether or not this observed effect on blood and thoracic duct lymphocytes is the result of a direct action of epinephrine in discharging adrenal cortical hormones or whether its effect can be explained by an action on peripheral tissues.

The administration of both ACTH and epinephrine to normal rats lowered the level of thoracic duct lymphocytes even more than either one of these substances given separately. Since each of these substances was effective, this result may be merely an additive effect. It is possible, however, that this is a synergistic effect, which can be obtained only when the two substances are administered together and not obtained with increased amounts of either substance administered alone. It is noteworthy that aqueous Adrenal Cortex Extract, cortisone acetate and desoxycorticosterone glucoside were without a significant effect in altering the number of thoracic duct lymphocytes, or lymph flow. Valentine, Craddock and Lawrence[2] likewise found that an aqueous adrenal cortex extract was without effect in altering the number of thoracic duct lymphocytes in normal and adrenalectomized cats. While essentially the same findings were observed in the present experiments, the possibility must be considered that the correct dose level or time interval following administration of the preparation may not have been employed. The observation that cortical substances were not effective provokes the suggestion that the administration of ACTH causes the secretion of a substance from the adrenal cortex which is not present in the commercial adrenal cortical or synthetic steroid preparations as employed in these experiments. A question must also be raised as to a possible peripheral action of ACTH demonstrable only in the presence of increased levels of endogenous or exogenous cortical (or other steroid) hormones. This problem is a subject of current investigation.

Evidence is presented to substantiate the generalization that the administration of adrenocorticotropic hormone is effective in reducing the number of lymphocytes entering the blood stream by way of the thoracic duct. Since the administration of adrenal cortical hormones did not duplicate this effect, under

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the conditions of these experiments, the mechanism of action of ACTH on the adrenal gland in producing these effects remains to be elucidated.

Summary of Results

The endocrine influences of hypophysectomy, adrenalectomy, and the administration of pituitary and adrenal hormones on the level of thoracic duct lymphocytes have been studied in test animals under standardized conditions. The following effects have been noted:

1. Numbers of thoracic duct lymphocytes are elevated after either hypophysectomy or adrenalectomy in the rat.

2. After the administration of either ACTH protein or pepsin peptide mixtures to normal or hypophysectomized rats, the number of thoracic duct lymphocytes is significantly reduced. This effect was *not* noted in adrenalectomized rats. These effects were observed two hours after administration of the ACTH preparations. If a shorter time elapsed, no effect was noted with the ACTH protein, nor was any effect noted with an ACTH acid peptide mixture under the conditions employed.

3. Administration of epinephrine to normal or hypophysectomized rats, singly or in combination with ACTH protein (in which case its effect was potentiated), produced a significant reduction in the number of thoracic duct lymphocytes. This effect was not observed in the adrenalectomized rat, suggesting that epinephrine may act directly in producing this effect.

4. Growth hormone (beef anterior pituitary), Pitressin, Pitocin, Adrenal Cortex Extract, cortisone acetate, and desoxycorticosterone glucoside did not alter the levels of thoracic duct lymphocytes under the conditions of these experiments.

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