Hormonal Control of Lymphocyte Reaction

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The immunological role of lymphocytes and lymphoid organs has attracted great attention in recent year. Although not emphasized recently, it has been shown by several authors that lymphocytes serve as trephocytes for other cells (Kellsall and Crabb 1958, Loutit 1962). It is conceivable that the trophic function is closely related to the lysis of lymphocytes, mediated by the action of adrenocortical and gonadal steroid hormones. The products resulting from lymphocytolysis, such as histone, nonhistone protein, RNA, DNA and various kind of amino acids, are reutilized by other cells as trephones. Some of these products are also available in the processes essential to the achievement of immunologic reactions.

Considering such trephocytic concept, this paper reports the regulatory influence of the adrenals and the gonads on lymphocytes and lymphoid organs.

The following experiments were performed on Wistar strain rats and C_{57}BL, Strong A and CF#1 strain mice. Male and female animals of various age groups were used: a) Rats and mice were adrenalectomized, b) mice were subjected to gonadectomy, c) mice either adrenalectomized or gonadectomized were injected with cortisone, d) gonadectomized mice were injected with gonadal steroid hormones, and e) mice were fed a protein-free diet. In all groups, the relative weight and total number of nucleated cells of the thymus, the mesenteric lymph nodes and the spleen were estimated by the method previously described (Awaya 1962). The content of Feulgen-DNA and fast green-histone in lymphocytes was determined by microspectrophotometry (Tomonaga et al. 1970, 1971, Alfert and Geshwind 1953, Deitch 1966, Black and Ansley 1965, Seno and Utsumi 1965). Light and electron microscopic observations were also made on lymphoid organs, in particular on lymphocytes.

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1. LYMPHOID HYPERPLASIA IN ANIMALS SUBJECTED TO ADRENALECTOMY AND GONADECTOMY.

There are many investigations concerning the effect of the adrenals and the gonads on lymphoid organs. The general agreement appears to be that removal of the adrenals and the gonads results in lymphoid hyperplasia whereas the administration of adrenocortical and gonadal hormones causes a lymphoid hypoplasia. However, the details of the reported results have not necessarily been in accord (Dougherty 1952, Money et al. 1952, Weaver 1955, Santisteban 1960, Inada 1960, Dorfman and Dorfman 1961, Browning et al. 1961, Ito and Hoshino 1962, 1963, Imai 1965, Kijima 1967). Our data on this subject will be described in sections 1 and 2. It is felt that the application of quantitative techniques to this problem will help clarify some of the existing discrepancies in the literature.

Adrenalectomy in Wistar rats (Tables 1, 2).

Young rats, 1 and 2 months of age, and senile rats, 17 months of age, were subjected to bilateral adrenalectomy and given 1% saline as drinking water. The young group was examined 30 days and the senile group 12 to 15 days after surgery. Only 18 of the 80 adrenalectomized animals survived throughout the period studied. Marked hyperplasia of the thymus, the mesenteric lymph nodes and the spleen was seen in young male rats adrenalectomized at 1 month of age (Table 1). In normal control animals the thymus is involuting at this stage (Awaya and Oda 1965). Similar hyperplasia of the mesenteric lymph nodes was observed in young rats of both sexes subjected to adrenalectomy at 2 months of age (Table 1). However, hyperplasia of the thymus was very slight in animals of this group and thus differed markedly from that seen in rats adrenalectomized at 1 month of age. This may be related to the loss of weight of lymph nodes following cortisone administration but not following the administration of sex hormones (Weaver 1955). Sexual maturity may effect an elevation of thymolytic action of sex hormones in rats at this age.

Marked regeneration of the thymus and the mesenteric lymph nodes was observed in the senile female rats subjected to adrenalectomy at 17 months of age (Table 2). Quantitative and histological findings of the thymus were much the same as those of normal animals 6 to 8 months of age (Figs. 1, 2). A slight hyperplasia of the spleen was observed. These findings may be related to the hypofunction of sex hormones due to aging.

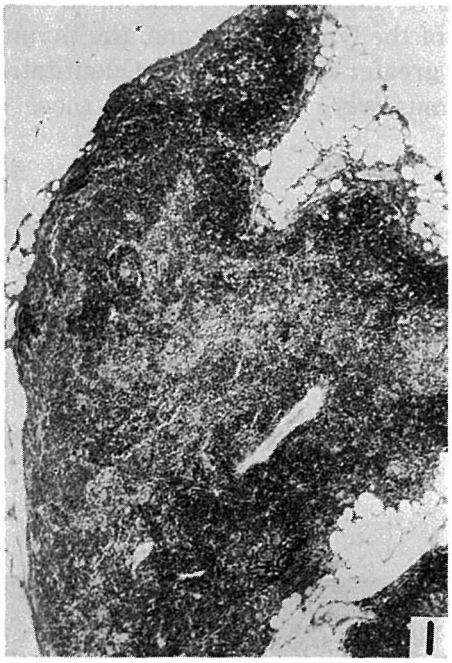

Fig. 1.

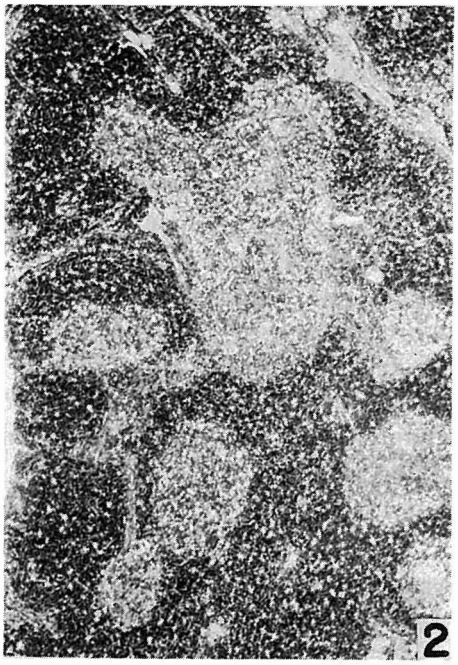

Fig. 2.

Fig. 1. Thymus of normal female rat of the Wistar strain 17 months of age. Note the atrophic parenchyma showing advanced age involution. Rat No. 17M-2. ×50.

Fig. 2. Thymus of a female rat of the Wistar strain 15 days after bilateral adrenalectomy at 17 months of age. Note the remarkable regeneration and hyperplasia of the cortical area. Rat No. RAD-5. ×50.

Adrenalectomy in mice (Tables 3, 4, 5).

Young mice of the CF#1 strain, 21 and 30 days of age, were bilaterally adrenalectomized and given 1% saline as drinking water. They were examined 7 and 14 days after the surgery (Tabels 3, 4). A moderate increase in the relative number of nucleated cells in all the lympoid organs was observed in both age groups. The degree of hyperplasia was similar in both groups. However, hyperplasia of the thymus was more marked 7 days after adrenalectomy while hyperplasia of the mesenteric lymph nodes and spleen was observed 14 days after the surgery. This indicates that adrenalectomy first causes thymic enlargement and is then followed by hyperplasia of other lymphoid organs. No difference in the degree of hyperplasia was found according to the age at surgery and the sex of the animals.

Ten female and 10 male senile mice of the C_{57}BL and the Strong A strains, aged 14 to 22 months, were subjected to bilateral adrenalectomy.

Only 5 mice, all female, survived for more than 14 days (Table 5). In these mice, there was extensive hyperplasia of the lymphoid organs, the thymus in particular. Hyperplasia tended to be greater in C_{57}BL mice. Quantitative and histological findings in the thymus were comparable to those of normal mice 6 to 7 months of age.

Gonadectomy in mice (Tables 6, 7, 8).

Young mice of the CF♯1 and C_{57}BL strains, 7 and 21 days of age, were gonadectomized and examined after 38 and 24 days, respectively, i.e. at 45 days of age (Tables 6, 7). The degree of thymic enlargement was greater in orchiectomized mice suggesting that hormonal control of the thymus by the gonads is stronger in males. This effect was more pronounced in mice of the CF♯1 strain and occurred in both age groups. In C_{57}BL mice, thymus hyperplasia was more prominent in mice gonadectomized at 7 days of age.

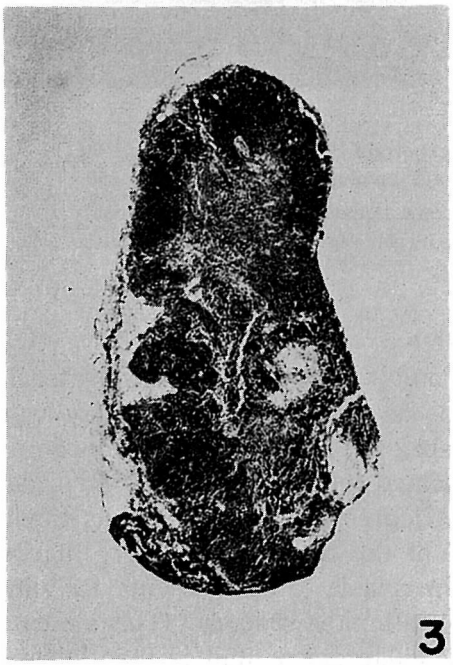

Fig. 3.

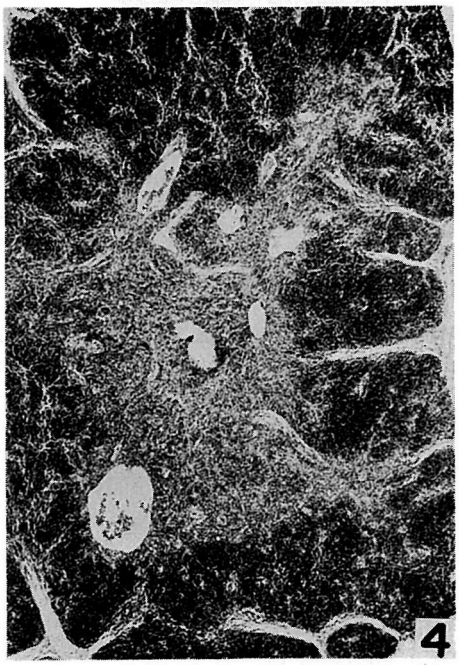

Fig. 4.

Fig. 3. Thymus of a female C_{57}BL mouse 18 months of age. Note the atrophic parenchyma showing advanced age involution. Mouse No. Ov 4. ×50.

Fig. 4. Thymus of a female C_{57}BL mouse 30 days after adrenalectomy at 18 months of age. Note the remarkable regeneration and hyperplasia of the cortical area. Mouse No. Ov 13. ×50.

Senile C_{57}BL mice, 18 months of age, were gonadectomized and examined 14 and 30 days after the surgery (Table 8). These animals showed marked hyperplasia of the thymus, which again was more prominent in orchiectomized mice. Histologically there was evidence of cortical hypertrophy and a well-demarcated corticomedullary border (Figs. 3, 4). Hyperplasia of the mesenteric lymph nodes and the spleen was not so pronounced. In the mesenteric lymph nodes, accumulations of lymphocytes were occasionally seen arround the postcapillary venules in the paracortical area and secondary nodules were present in the medulla.

Five senile male mice of the C_{57}BL strain, 30 months of age, were subjected to adrenalectomy and gonadectomy. In the 2 mice which survived for 21 days, the degree of hyperplasia of lymphoid organs was more marked and was greatest in the thymus.

A slight increase in the Feulgen-DNA content of large lymphocytes was observed in the thymus and mesenteric lymph nodes of young mice subjected to orchiectomy (Figs. 5, 6). This reflects the hyperplasia of lymphoid organs mentioned above.

2. LYMPHOID HYPOPLASIA IN MICE RECEIVING STEROID HORMONES.

Non-operated mice receiving cortisone acetate (Tables 9, 10).

Normal female and male mice of the CF#1 strain, 34 days of age, were injected intramuscularly with a single dose of 10 mg per 100 g body weight of cortisone acetate and examined 24 hours later. A marked decrease in the relative weight and in the number of nucleated cells was observed in the thymus, the mesenteric lymph nodes and the spleen. In contrast, the weight of liver was increased. Histological examination revealed a striking disintegration of cortical lymphocytes in the thymus, but no destructive changes were seen in the lymph nodes or in the spleen.

Adrenalectomized mice receiving cortisone acetate (Tables 11, 12).

CF#1 male mice adrenalectomized at 28 days of age were injected intramuscularly with a single dose of 10 mg per 100 g body weight of cortisone acetate 6 days after the surgery and examined 24 hours later. There was pronounced involution of the thymus and the other lymphoid organs but an increase in the weight of the liver. Histologically, thymic atrophy was the main finding. Groups of pyknotic and disintegrating cortical lymphocytes were scattered throughout the thymic cortex.

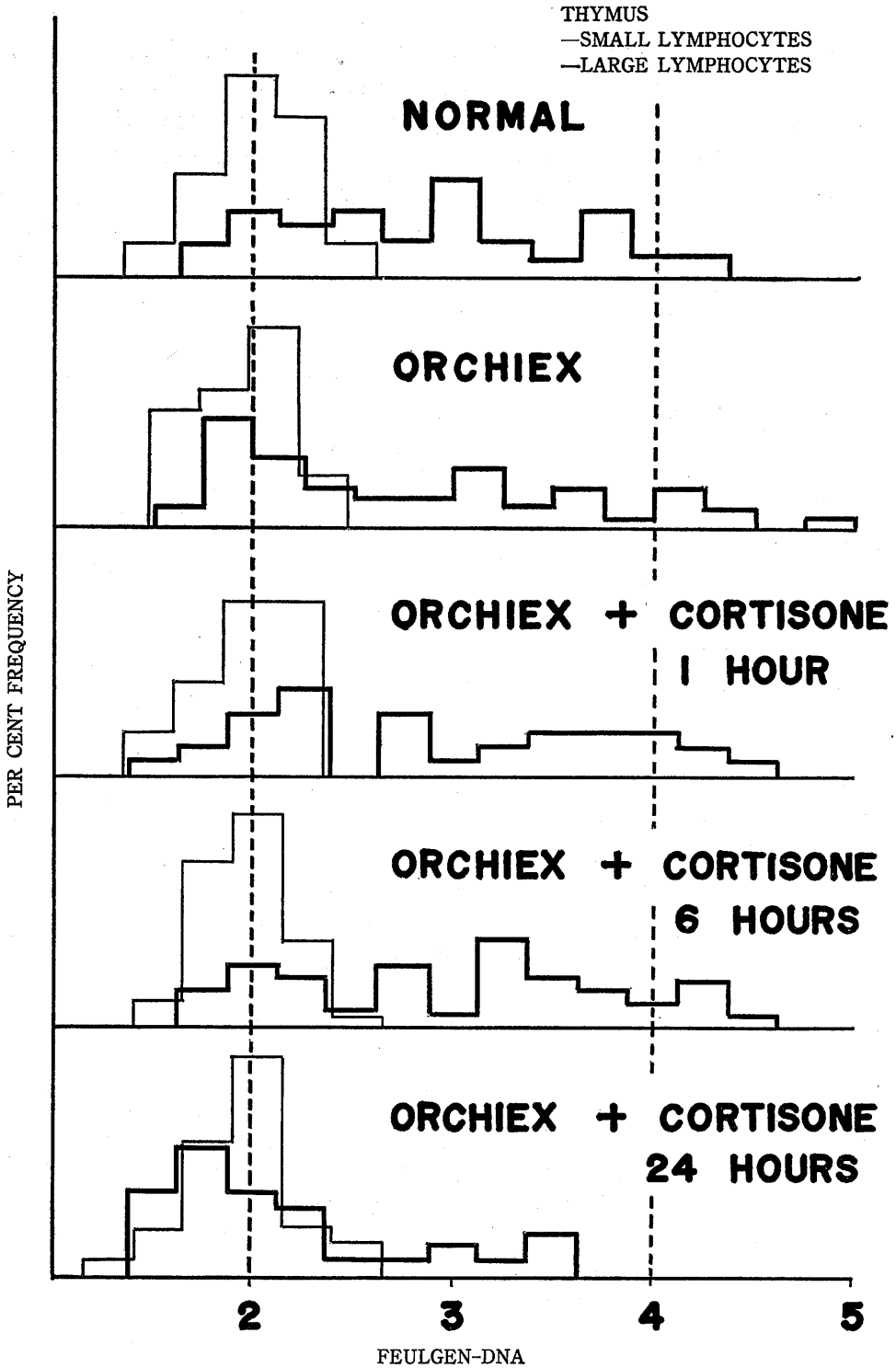
THYMUS
—SMALL LYMPHOCYTES
—LARGE LYMPHOCYTES
NORMAL
ORCHIEX
ORCHIEX + CORTISONE
1 HOUR
ORCHIEX + CORTISONE
6 HOURS
ORCHIEX + CORTISONE
24 HOURS
PER CENT FREQUENCY
2
3
4
5
FEULGEN-DNA

Fig. 5

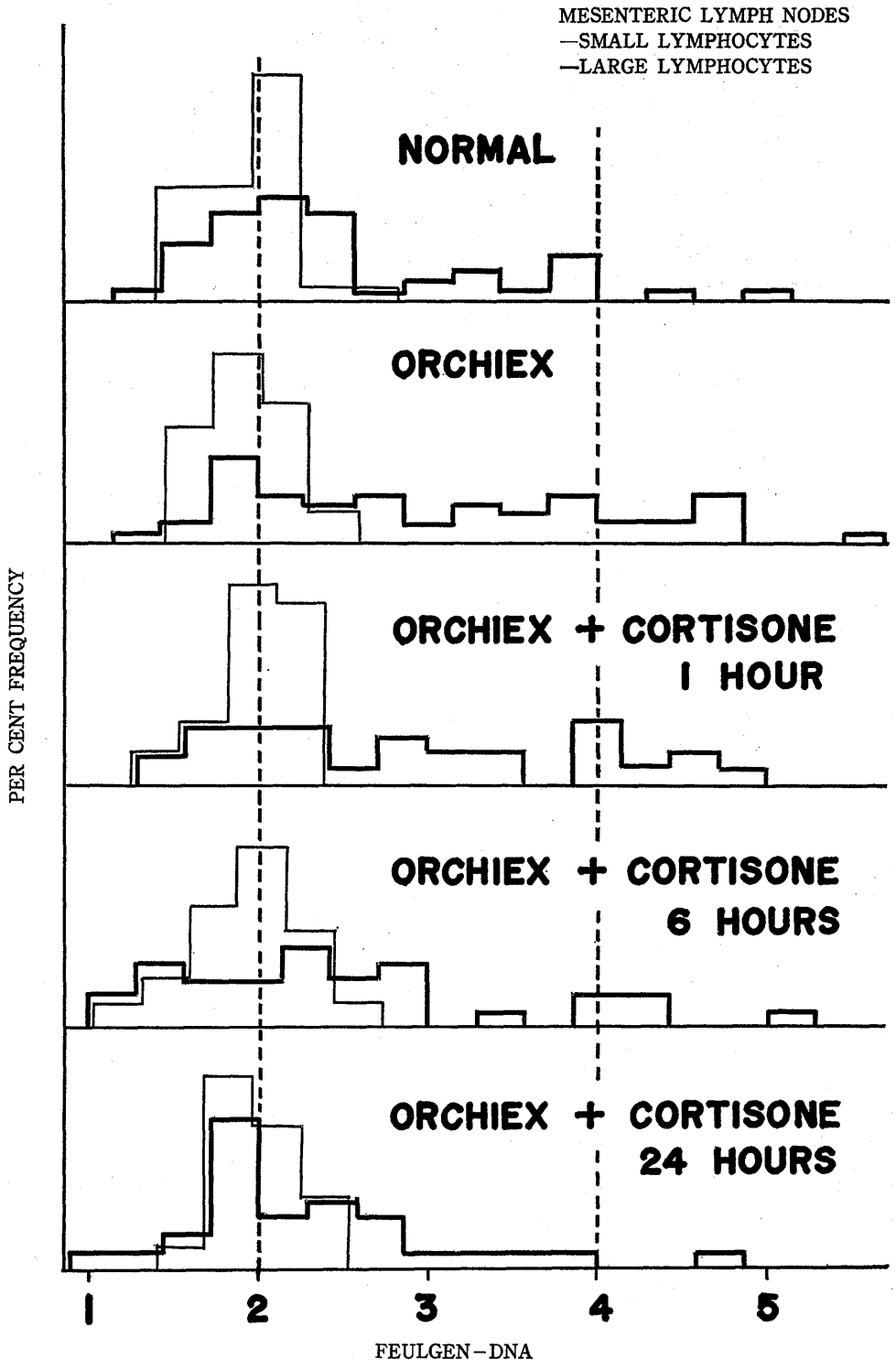
MESENTERIC LYMPH NODES
—SMALL LYMPHOCYTES
—LARGE LYMPHOCYTES
NORMAL
ORCHIEX
ORCHIEX + CORTISONE
1 HOUR
ORCHIEX + CORTISONE
6 HOURS
ORCHIEX + CORTISONE
24 HOURS
PER CENT FREQUENCY
1
2
3
4
5
FEULGEN–DNA

Fig. 6

Fig. 5. Histogram of the Feulgen-DNA content in thymic lymphocytes in CF #1 male mice subjected to gonadectomy and cortisone treatment.
Normal: Normal male mice at 35 days of age.
Orchiex: Male mice 14 days after gonadectomy at 21 days of age.
Orchiex + Cortisone: Male mice which were subjected to gonadectomy at 21 days of age and injected intramuscularly with a single dose of 10 mg per 100g body weight of cortisone acetate 13 days after gonadectomy. These mice were examined 1, 6 and 24 hours after the injection.

Fig. 6. Histogram of the Feulgen-DNA content in lymphocytes of the mesenteric lymph nodes in CF #1 male mice subjected to gonadectomy and cortisone treatment. Explanation of the group is the same as shown in Fig. 5.

Gonadectomized mice receiving cortisone acetate (Tables 13, 14).

CF#1 female and male mice gonadectomized at 21 days of age were injected intramuscularly with the same dose of cortisone acetate 13 days after the surgery and examined 24 hours after the injection. As in the previous group, the administration of this steroid led to marked hypoplasia of all the lymphoid organs which was most pronounced in the thymus. An increase in liver weight was noted in males but not in females.

The Feulgen-DNA content of thymic and lymph node lymphocytes in these animals decreased markedly 6 to 24 hours after the injection of cortisone (Figs. 5, 6). This is consistent with the lymphoid hypoplasia observed in this group.

Morphology of cortisone-sensitive lymphocytes.

As mentioned above, in all the groups, cortisone induced the most marked lymphoid hypoplasia in the thymus which is mainly due to the disintegration of cortical lymphycytes. In normal mice, 3 types of cortical lymphocytes of the thymus are identified according to chromatin characteristics: Lymphocytes with centrally condensed chromatin, lymphocytes with more dispersed chromatin and lymphocytes with less chromatin. These 3 types are most clearly discernible by electron microscopic observations (Fig. 7). Differential counts were done on toluidine blue-stained semi-thin sections of the block prepared for electron microscopic observation. Results obtained showed that the cortisone administration caused a selective decrease in the number of lymphocytes with centrally condensed chromatin (Fig. 8) and suggests that this type of lymphocyte may be more cortisone-sensitive. Further investigations on their characteristics are now in progress.

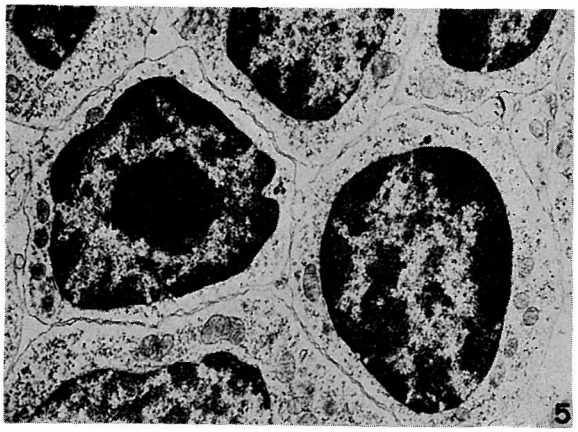

Fig. 7. Electron micrograph of thymic lymphocytes in a CF #1 male mouse. Three types of lymphocytes can be distinguished according to chromatin characterstics.
Left: Lymphocyte with centrally condensed chromatin.
Right: Lymphocyte with more dispersed chromatin.
Upper central: Lymphocyte with less chromatin.

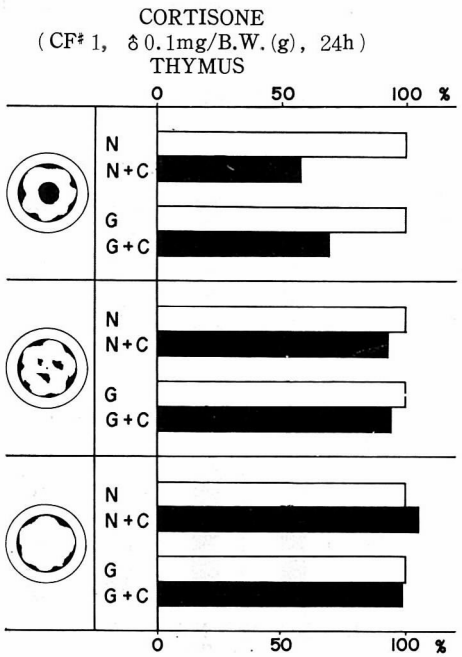

Fig. 8. Per cent change in the differential counts of the three types of thymic lymphocytes in normal and gonadectomized mice of CF #1 strain 24 hours after the injection of cortisone.
N: Normal male mice at 35 days of age.
N+C: Male mice injected with cortisone acetate at 34 days of age.
G: Male mice 14 days after gonadectomy at 21 days of age.
G+C: Male mice gonadectomized at 21 days of age and injected intramuscularly with a single dose of 10 mg per 100 g body weight of cortisone acetate 13 days after gonadectomy. Twenty four hours later the mice were examined.

Gonadectomized mice receiving gonadal steroid hormones (Tables 15, 16).

CF♯1 female mice ovariectomized at 21 days of age were injected intraperitoneally with 2.5 mg per 100 g body weight of estradiol benzoate on 3 consecutive days, beginning with the 11th day after surgery. Twenty four hours after the last injection, there was marked thymic involution, slight splenic involution, but the lymph nodes appeared unaffected. The liver weight was inceased in this group (Table 15).

CF♯1 male mice orchiectomized at 21 days were injected intraperitoneally with 5 mg per 100 g body weight of testosterone propionate on 5 consecutive days, beginning with the 9th day after surgery, Twenty four hours after the last injection, there was fairly marked thymic involution and slight involution in the lymph nodes, but the liver weight was unchanged (Table 15).

Effect of glutamate (Fig. 9).

Five-week-old female CF♯1 mice received a large dose of glutamate which is known to be a product of cortisone-induced hepatic transamination.

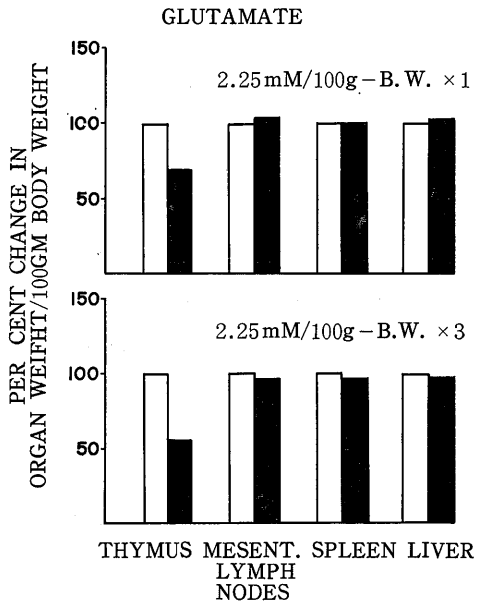

Fig. 9. Effect of glutamate injection on the weight of lymphoid organs and the liver per 100 g body weight in 5-weeks-old female mice of the CF ♯1 strain. Note the marked decrease in thymus weight 24 hours after the administration of glutamate (2.25 mM per 100 g body weight: a single injection and a daily injection for 3 days).

One group were injected intraperitoneally with a single dose of 2.25 mM per 100 g body weight and the other group with the same dose for 3 consecutive days. Twenty four hours after the injection, the weights of lymphoid organs and of the liver were estimated. As shown in Fig. 9, the weight of the thymus was markedly decreased. A similar effect had been noted in cortisone injected mice (see Table 9). Thus the results of this study and others (Feigelson and Feigelson 1966), suggest the possibility that the effect of cortisone on lymphoid organs may be mediated by the liver.

3. ARE LYMPHOCYTES FOLLOWING ADRENALECTOMY OR GONADECTOMY STEROID HORMONE-SENSITIVE AND LESS REACTIVE TO ANTIGEN?

Adrenalectomy or gonadectomy caused, to a greater or lesser degree, an increase in the number of nucleated cells in the lymphoid organs. After steroid hormone administration such an increase was followed by a marked reduction especially in the thymus (Tables 12, 14, 16). Lymphoid hypoplasia after steroid hormone treatment was more pronounced in adrenalectomized or gonadectomized mice than in unoperated mice. This suggests that lymphocytes which proliferate following adrenalectomy or gonadectomy may be more sensitive to steroid hormones.

The reactivity of lymphocytes to antigen was studied in regional lymph nodes of gonadectomized mice. In one group of CF#1 strain mice, gonadectomized at 21 days of age, 0.1 ml of typhoid-paratyphoid vaccine emulsified in an equal quantity of complete Freund's adjuvant was injected into the hind foot pads 7 days after of gonadectomy. Seven days later, the popliteal lymph nodes draining the site of injection were examined. In mice of this group, the degree of increase in the number of nucleated cells in the lymph nodes was less than that in the non-operated group (Fig. 10). Thus, lymphocytes proliferating following gonadectomy seem to be less reactive to antigen stimulus. In another group of the same strain mice, which was subjected to antigen stimulation 14 days after gonadectomy, proliferation of nucleated cells in the regional popliteal lymph nodes was more marked. These findings suggest that the time elapsed after gonadectomy may determine the degree of reactivity of lymphocytes to anitgen stimulation.

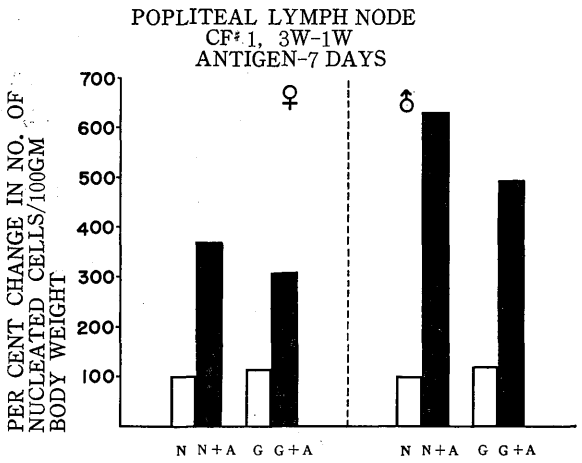

Fig. 10. Per cent change in the number of nucleated cells per 100 g body weight in the regional popliteal lymph nodes of normal and gonadectomized mice of the CF ♯1 strain 7 days after the injection of antigen.
N: Normal female and male mice 35 days of age.
N+A: Female and male mice injected with antigen at 28 days of age and examined 7 days later.
G: Female and male mice gonadectomized at 21 days of age and examined 14 days later.
G+A: Female and male mice gonadectomized at 21 days of age and injected with antigen 7 days after the gonadectomy. The mice were examined 7 days later.
Antigen: 0.1 ml of typhoid-paratyphoid vaccine in an equal quantity of complete Freund's adjuvant. Details of the experimental procedures are described in the text.

4. LYMPHOID HYPOPLASIA IN PROTEIN DEFICIENT MICE IS ASSOCIATED WITH THE PRESENCE OF INTACT ADRENALS AND GONADS.

CF♯1 strain female mice were fed a protein free diet from 21 to 35 days of age. The content of the protein free diet is shown in Text-table 1. In these animals, the weight and the total number of nucleated cells of lymphoid organs per 100 g body weight was much reduced (Fig. 11, Table 17). However, as shown in Fig. 11, the relative weight of the adrenals and of the gonads increased and no histological changes were seen in these organs. These findings suggest that lymphocytes or at least some of them, may act as trephocytes for other cells in protein deficiency. This may depend on the presence of intact adrenals and gonads.

Text-table 1. Regular and protein free diets.*

	Carbo-hydrate	Fat	Protein	Vitamin mixture	Salt mixture	Cellulose	Kilocal./100 gm of diets
Regular	53	6	25	2	6	8	366
Protein free	78	6	—	2	6	8	366

*Obtained from Oriental Yeast Mfg. Ltd., Tokyo.

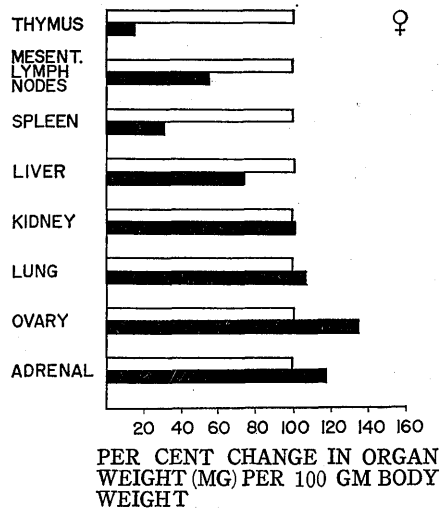

Fig. 11. Percent change in the weight of lymphoid organs and other organs per 100 g body weight in CF #1 female mice fed a protein free diet from 21 to 35 days of age.
White bar: Regular diet.
Black bar: Protein free diet.

In another group, the same strain mice, fed a protein free diet from 21–35 days of age, were injected subcutaneously with antigen as described in section 3. The regional inguinal lymph nodes were examined 3 and 7 days later. Antigen reactivity of the lymphocytes of this group seemed to be weak and cytochemical changes of nuclear histone in antigen-stimulated lymphocytes, which will be described below, were not observed.

5. TRANSIENT DECREASE IN FAST GREEN-HISTONE CONTENT IN LYMPHOCYTES FOLLOWING ANTIGEN STIMULATION AND ITS INHIBITION BY CORTISONE ADMINISTRATION.

CF#1 strain female mice, 2 months of age, were injected with 0.1 ml

of typhoid-paratyphoid vaccine emulsified in an equal quantity of complete Freund's adjuvant into the left hind foot pad. Some of these mice were simultaneously injected with cortisone acetate. Cortisone was administered as described already in section 2. Lymph nodes draining the site of antigen injection were examined. One-half, 1, 3 and 6 hours after the injection, smears from the nodes were made on a microscopic slide and stained for histone with fast green according to Alfert and Geschwind (1953). The content of fast green-stained material in individual small lymphocytes was measured microspectrophotometrically at 635 mμ.

A significant decrease in the relative content of fast green stainable histone of small lymphocytes in draining lymph nodes occurred 1/2 to 3 hours after the injection of antigen (Fig. 12). However, by six hours, the histone content had returned to control values. On the other hand, simultaneous injection of antigen and cortisone acetate caused no such decrease (Fig. 12).

This suggests that cortisone may inhibit antigen-induced changes in lymphocyte nuclear histone through which immunocompetent small lymphocytes may transform to large immunoblasts.

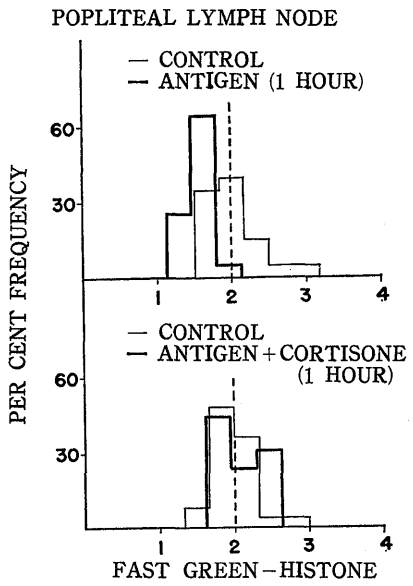

Fig. 12. Histogram of the fast green-histone content in small lymphocytes of the popliteal lymph nodes draining the site of injection 1 hour after the injection of antigen or after simultaneous injections of antigen and cortisone acetate. For experimental procedures: see text.

6. COMPARISON OF FAST GREEN-HISTONE CONTENTS OF LYMHOCYTES AND LIVER CELLS IN STEROID HORMONE-TREATED MICE.

It was shown in section 2 that the administration of cortisone acetate and estradiol benzoate caused an increase in the weight of the liver but a decrease in the weights of lymphoid organs in normal, adrenalectomized and gonadectomized mice (Tables 9, 11, 13, 15). In an attempt to obtain a cytochemical correlation, the degree of histone staining with fast green in smeared cells from the liver and the thymus was measured microspectrophotometrically in some mice of these experimental groups.

One to 3 hours after the injection of cortisone acetate, the fast green-histone content of liver cell nuclei was decreased by almost 10% in CF#1 female mice 35 days of age (Fig. 13). Similarly, an even greater but transient decrease in the histone content of liver cells was seen in mice of the same strain ovariectomized at 21 days of age and injected with cortisone acetate and estradiol benzoate 14 days after ovariectomy (Text-table 2). Such a decrease in the fast green-histone content was not observed in thymic lymphocytes (Fig. 13, Text-table 2).

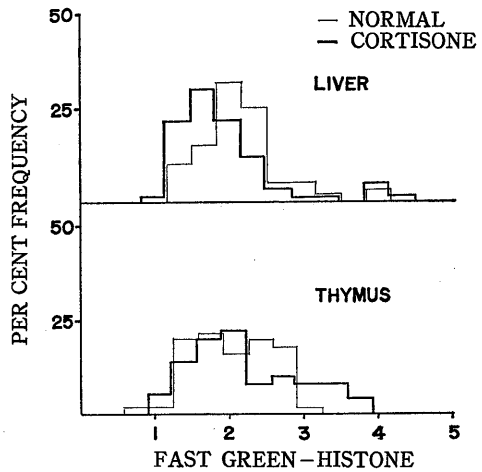

Fig. 13. Histogram of the fast green-histone content in the nuclei of liver cells and thymic lymphocytes of 5-weeks-old CF #1 mice 1 hour after the injection of cortisone acetate.
For experimental procedures: see text.

Text-table 2. Fast green-histone content of thymic lymphocytes and liver cells in female CF #1 mice gonadectomized and injected with steroid hormone. All figures are mean values 3 hours after the injection and are expressed as % changes of the mean value in mice subjeted to ovariectomy alone. Mean ± standard error. (n=50 cells)

Group*	Thymic lymphocytes	Liver cells
Gonadectomized	100	100
Gonadectomized + Steroid-injected		
Cotrisone	99.8±3.65	63.2±3.62
Estradiol (in water)	119.1±3.27	72.1±2.83
Testosterone	109.9±5.92	104.0±6.0

*Gonadectomized: Female mice 7 days after gonadectomy at 21 days of age.
Gonadectomized + Steroid-injected: Female mice gonadectomized at 21 days of age and injected with steroid hormones 7 days after the gonadectomy. These mice were injected intraperitoneally with a single dose of 10 mg of cortisone acetate, 7.5 mg of estradiol benzoate and 25 mg of testosterone propionate per 100 g body weight, respectively.

Distinct, but transient changes in nuclear histone have been reported in liver cells treated with cortisone and in lymphocytes responding to PHA or antigen: acetylation of histone (Allfrey 1968, Pogo et al. 1966), a transient decrease in fast green stainability of histone (Burton 1968, Black and Ansley 1965, 1965) and a decomposition in histone (Agrell and Molander 1969). Since it has been suggested that histones are involved in the regulation of gene expression at transcription level, the above changes of histones may reflect an early step in gene activation of these cells.

Thus, our data in cortisone-treated mice demonstrating a decrease in the fast green-histone content of liver cells but unchanged levels in lymphocytes suggests that the site of action of cortisone in lymphocytes may not be at the histone level, but elsewhere.

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Table 1. Number ($\times 10^{-6}$) of nucleated cells per 100 g body weight in lymphoid organs of Wistar strain young rats 30 days after bilateral adrenalectomy. Mean ± standard error.

Age at adrenalectomy	Group and no. of animals		Thymus	Mesenteric lymph nodes	Spleen
1 month	♂ Adrenalectomized	2	1239.4	1109	884.3
	♂ Controls	5	576.6± 40.5	325.2±44.8	404.2±67.5
2 months	♀ Adrenalectomized	5	922.9±123.1	689.7±98.5	507.3±18.9
	♀ Controls	5	762.4±132.4	365.0±38.3	577.3±87.2
	♂ Adrenalectomized	5	619.4± 38.0	572.9±60.5	549.1±19.5
	♂ Controls	5	533.9± 76.6	316.3±43.1	485.1±64.5

Table 2. Number ($\times 10^{-6}$) of nucleated cells per 100 g body weight in lymphoid organs of Wistar strain senile female rats 12 to 15 days after bilateral adrenalectomy. Mean ± standard error.

Age at adrenalectomy	No. of animals		Thymus	Mesenteric lymph nodes	Spleen
17 months	Adrenalectomized	5	288.3±39.1	222.7±32.6	449.3±21.8
	Controls	6	42.9±10.5	80.1± 5.3	274.8±45.9

Table 3. Number ($\times 10^{-6}$) of nucleated cells per 100 g body weight in lymphoid organs of CF #1 strain mice 7 and 14 days following adrenalectomy at 21 days of age. Mean±standard error.

Days after adrenalectomy	Group and no. of animals		Thymus	Mesenteric lymph nodes	Spleen
7 days	♀ Adrenalectomized	5	1954.0±192.3	516.6± 89.6	1468.5±190.4
	♀ Controls (28 days of age)	5	1521.4±192.0	513.9±104.1	1151.4±164.2
	♂ Adrenalectomized	5	1659.8± 93.6	698.0±168.5	1806.5±364.1
	♂ Controls (28 days of age)	5	1216.2± 96.3	524.0± 89.0	1775.4±391.5
14 days	♀ Adrenalectomized	5	1342.3± 85.2	705.8±103.5	1426.7±239.0
	♀ Controls (35 days of age)	5	1194.3±180.1	534.5±152.7	1193.0±108.2
	♂ Adrenalectomized	5	1246.0±104.4	566.6±101.6	1789.4±247.9
	♂ Controls (35 days of age)	5	1081.9±212.2	444.0± 54.7	1561.9±136.5

Table 4. Number ($\times 10^{-6}$) of nucleated cells per 100 g body weight in lymphoid organs of CF #1 strain mice 7 and 14 days following adrenalectomy at 30 days of age. Mean ± standard error.

Days after adrenalectomy	Group and no. of animals		Thymus	Mesenteric lymph nodes	Spleen
7 days	♀	Adrenalectomized 5	1530.7±141.8	686.9± 54.4	1552.1±290.5
		Controls (37 days of age) 5	1075.1± 87.9	593.9± 76.0	2188.8±280.3
	♂	Adrenalectomized 5	1315.6±124.9	601.3±110.8	1833.7±230.3
		Controls (37 days of age) 5	901.8± 49.9	578.5± 40.5	2454.6±336.3
14 days	♀	Adrenalectnmized 8	1416.5±107.5	715.0± 75.7	1449.1±254.4
		Controls (45 days of age) 5	1121.1± 88.7	483.2± 32.3	1459.5±222.7
	♂	Adrenalectomized 7	837.2±103.0	699.2±101.2	1242.9±169.9
		Controls (45 days of age) 5	758.3± 58.5	603.9± 90.9	935.6± 84.2

Table 5. Organ weight (mg) and number ($\times 10^{-6}$) of nucleated cells per 100 g body weight in the thymus, the mesenteric lymph nodes and the spleen of senile mice subjected to adrenalectomy.

Strain and sex	Age at adrenalectomy	Time after adrenalectomy	Thymus		Mesenteric lymph nodes		Spleen	
			Weight	No. of nucleated cells	Weight	No. of nucleated cells	Weight	No. of nucleated cells
$C_{57}BL$ ♀	18 months	14 days	114.0	389.8	158.5	272.5	399.8	779.5
	19 〃	21 〃	93.8	320.9	776.0	1909.0	741.4	1430.9
	22 〃	21 〃	74.3	323.7	536.9	816.2	367.0	785.4
	Controls (18 months, Mean of 5 mice)		13.7	34.7	133.9	368.2	428.0	500.6
Strong A ♀	14 months	40 days	125.0	—	125.0	—	1166.7	—
	14 〃	60 〃	107.1	353.4	71.4	162.8	946.1	2112.2
	Controls (18 months, Mean of 5 mice)		69.3	202.0	121.0	204.1	334.0	785.2

Table 6. Number ($\times 10^{-6}$) of nucleated cells per 100 g body weight in lymphoid organs of CF #1 strain mice gonadectomized at 7 and 21 days of age and autopsied at 45 days of age. Mean ± standard error.

Age at gonadectomy	Sex and no. of animals	Thymus	Mesenteric lymph nodes	Spleen
7 days	♀ 5	1537.8±141.5	624.0±119.8	1065.7± 48.3
	♂ 6	1307.8±153.6	621.0± 59.9	1280.6±177.9
21 days	♀ 7	1455.7± 34.4	788.4± 41.4	1144.1± 60.1
	♂ 4	1382.5±228.3	576.6± 57.1	1260.9± 63.9
Controls (45 days of age)	♀ 5	1121.1± 88.7	483.2± 32.3	1495.5±222.7
	♂ 5	758.3± 58.5	603.9± 90.9	935.6± 84.2

Table 7. Number ($\times 10^{-6}$) of nucleated cells per 100 g body weight in lymphoid organs of C_{57}BL strain mice gonadectomized at 7 and 21 days of age and autopsied at 45 days of age. Mean ± standard error.

Age at gonadectomy	Sex and no. of animals	Thymus	Mesenteric lymph nodes	Spleen
7 days	♀ 7	1937.9± 60.6	722.2± 62.7	1750.7±107.6
	♂ 7	1810.0±152.5	756.4± 47.8	1915.4±246.2
21 days	♀ 8	1315.8±115.3	1207.5±212.5	1528.9± 99.1
	♂ 6	1478.8±138.8	565.2± 71.6	1400.7± 67.3
Controls (45 days of age)	♀ 7	1079.4±134.8	872.6±126.3	1306.8±106.8
	♂ 7	1004.9± 98.6	759.6±161.9	1339.1±228.6

Table 8. Number ($\times 10^{-6}$) of nucleated cells per 100 g body weight in lymphoid organs of C_{57}BL strain mice 14 and 30 days following gonadectomy at 18 months of age. Mean ± standard error.

Days after gonadectomy	Sex and no. of animals	Thymus	Mesenteric lymph nodes	Spleen
14 days	♀ 3	162.5± 42.6	275.2±108.3	1207.5±168.6
	♂ 5	215.8± 66.9	200.2± 19.4	804.9± 91.2
30 days	♀ 5	195.6± 41.2	227.8± 65.6	755.5±186.0
	♂ 4	226.2± 43.8	549.0±201.1	616.8±138.6
Controls (18 months of age)	♀ 6	34.7± 31.9	368.2± 74.9	500.6±199.9
	♂ 6	23.9± 11.1	319.9± 74.2	584.3± 80.6

Table 9. Weight (mg) of lymphoid organs and of the liver per 100 g body weight of CF #1 strain mice treated with cortisone acetate.
Mean ± standard error.

Group*	No. of animals	Thymus	Mesenteric lymph nodes	Spleen	Liver
♀ Normal	5	335.9±34.9	249.9±60.9	517.5±24.0	7374.1±307.2
♀ Cortisone-treated	5	113.7±10.2	103.3± 6.5	321.1±31.7	9144.6±299.1
♂ Normal	5	276.8±47.6	244.7±24.1	719.2±44.6	7636.0±101.9
♂ Cortisone-treated	4	85.9±14.5	155.6±34.4	248.7±72.9	11915.9±233.2

*Normal: Non-operated female and male mice 35 days of age.
Cortisone-treated: Female and male mice which were injected intramuscularly with a single dose of 10 mg per 100 g body weight of cortisone acetate at 34 days of age and examined 24 hours later.

Table 10. Number ($\times 10^{-6}$) of nucleated cells per 100 g body weight in lymphoid organs of CF #1 strain mice treated with cortisone acetate.
Mean ± standard error.

Group*	No. of animals	Thymus	Mesenteric lymph nodes	Spleen
♀ Normal	5	1194.3±180.2	534.5±152.7	1193.0±108.2
♀ Cortisone-treated	5	211.6± 26.2	299.2± 32.5	630.1± 74.6
♂ Normal	5	1081.9±212.2	444.0± 54.7	1561.9±136.2
♂ Cortisone-treated	4	161.8± 34.1	343.7± 62.6	757.3±119.0

*See table 9.

Table 11. Weight (mg) of lymphoid organs and of the liver per 100g body weight of CF #1 strain mice subjected to adrenalectomy and cortisone treatment.
Mean ± standard error.

Group*	No. of animals	Thymus	Mesenteric lymph nodes	Spleen	Liver
Normal	5	276.8±47.6	244.7±24.1	719.2± 44.6	7636.0±101.9
Adrenalectomized	4	321.3±36.9	346.5±38.4	1435.4±580.2	6968.6±202.5
Adrenalectomized +Cortisone-treated	4	105.9± 8.4	182.9±32.3	580.6±134.8	9861.2±427.4

*Normal: Non-operated male mice 35 days of age.
Adrenalectomized: Male mice 7 days afrer the adrenalectomy at 28 days of age.
Adrenalectomized+Cortisone-treated: Male mice which were adrenalectomized at 28 days of age and injected intramuscularly with a single dose of 10 mg per 100 g body weight of cortisone acetate 6 days after the adrenalectomy. Twenty four hours later these mice were examined.

Table 12. Number ($\times 10^{-6}$) of nucleated cells per 100 g body weight in lymphoid organs of CF #1 strain mice subjected to adrenalectomy and cortisone treatment. Mean ± standard error.

Group*	No. of animals	Thymus	Mesenteric lymph nodes	Spleen
Normal	5	1081.9±212.2	444.0± 54.7	1561.9± 136.5
Adrenalectomized	4	1540.4±304.7	911.9±155.3	3664.8±1245.9
Adrenalectomized +Cortisone-treated	4	232.4± 29.2	513.3± 61.1	1475.3± 344.1

*See table 11.

Table 13. Weight (mg) of lymphoid organs and of the liver per 100 g body weight of CF #1 strain mice subjected to gonadectomy and cortisone treatment. Mean ± standard error.

Group*	No. of animals	Thymus	Mesenteric lymph nodes	Spleen	Liver
Normal	5	355.9±34.9	249.7±60.9	517.5±24.0	7374.1±307.2
♀ Gonadectomized	4	408.5±30.0	237.6± 6.8	743.9±65.9	6282.7±191.3
Gonadectomized +Cortisone-treated	5	102.4± 2.2	101.4± 6.1	152.7± 8.1	5036.8±386.5
Normal	5	276.8±47.6	244.7±24.1	719.2±44.6	7636.0±101.9
♂ Gonadectomized	5	374.4±15.4	288.1±30.6	750.0±83.2	6903.5±477.2
Gonadectomized +Cortisone-treated	4	113.5± 3.4	231.4± 9.7	322.9±45.8	9440.6±340.4

*Normal: Non-operated female and male mice 35 days of age.
Gonadectomized: Female and male mice 14 days after gonadectomy at 21 days of age.
Gonadectomized + Cortisone-treated: Female and male mice which were gonadectomized at 21 days of age and injected intramuscularly with a single dose of 10 mg per 100 g body weight of cortisone acetate 13 days after the gonadectomy. Twenty four hours later these mice were examined.

Table 14. Number ($\times 10^{-6}$) of nucleated cells per 100 g body weight in lymphoid organs of CF #1 strain mice subjected to gonadectomy and cortisone treatment. Mean ± standard error.

Group*	No. of animals	Thymus	Mesenteric lymph nodes	Spleen
Normal	5	1194.3±180.1	534.5±152.7	1193.0±108.2
♀ Gonadectomized	4	1855.8± 77.9	773.3± 38.7	2193.7±495.4
Gonadectomized +Cortisone-treated	5	219.3± 18.2	280.5± 21.1	343.2± 30.0
Normal	5	1081.9±212.2	444.0± 54.7	1561.9±136.5
♂ Gonadectomized	5	1428.1± 99.7	735.2± 42.2	1764.0±214.2
Gonadectomized +Cortisone-treated	4	198.7± 33.8	680.5± 27.9	849.4±120.5

*See table 13.

Table 15. Weight (mg) of lymphoid organs and of the liver per 100 g body weight of CF #1 strain mice subjected to gonadectomy and sex hormone treatment. Mean ± standard error.

Group*	No. of animals	Thymus	Mesenteric lymph nodes	Spleen	Liver
Normal	5	355.9±34.9	249.7±60.9	517.5±24.0	7374.1±307.2
♀ Gonadectomized	4	408.8±30.0	237.6± 6.8	743.9±65.9	6282.7±191.3
Gonadectomized +Estradiol-treated	5	161.4±20.8	307.4±45.0	565.1±70.0	8133.5±193.4
Normal	5	276.8±47.6	244.7±24.1	719.2±44.6	7636.0±101.9
♂ Gonadectomized	5	374.4±15.4	288.1±30.6	750.0±83.2	6903.5±477.2
Gonadectomized +Testosterone-treated	6	203.4±18.1	218.1±10.8	700.2±37.3	6127.2±498.1

*Normal: Non-operated female and male mice 35 days of age.
Gonadectomized: Female and male mice 14 days after gonadectomy at 21 days of age.
Gonadectomized + Estradiol-treated: Female mice which were ovariectomized at 21 days of age and injected intraperitoneally with 2.5 mg per 100 g body weight of estradiol benzoate per day for 3 days, beginning on the 11th day after the ovariectomy. Twenty four hours after the last injection these mice were examined.
Gonadectomized + Testosterone-treated: Male mice which were orchiectomized at 21 days of age and injected intramuscularly with 5 mg per 100 g body weight of testosterone propionate per day for 5 days, beginning on the 9th day after the orchiectomy. Twenty four hours after the last injection these mice were examined.

Table 16. Number ($\times 10^{-6}$) of nucleated cells per 100 g body weight in lymphoid organs of CF #1 strain mice subjected to gonadectomy and sex hormone treatment. Mean ± standard error.

Group*	No. of animals	Thymus	Mesenteric lymph nodes	Spleen
Normal	5	1194.3±180.1	534.5±152.7	1193.0±108.2
♀ Gonadectomized	4	1855.8± 77.9	773.3± 38.7	2193.7±495.4
Gonadectomized +Estradiol-treated	5	594.2±133.4	653.8±140.2	1399.3±155.5
Normal	5	1081.9±212.2	444.0± 54.7	1561.9±136.5
♂ Gonadectomized	5	1428.1± 99.7	735.2± 42.2	1764.0±214.2
Gonadectomized +Testosterone-treated	6	795.6± 57.1	562.3± 31.5	1682.4± 42.3

*See table 15.

Table 17. Number ($\times 10^{-6}$) of nucleated cells per 100 g body weight in lymphoid organs of CF #1 strain mice fed a protein free diet from 21 to 35 days of age. Mean ± standard error.

	Group	No. of animals	Thymus	Mesenteric lymph nodes	Spleen
♀	Protein free diet	7	115.6± 16.2	304.5±36.3	330.0±54.3
	Regular diet	6	1860.1±141.6	778.4±73.1	1349.2±56.9
♂	Protein free diet	7	38.8± 8.6	195.1±27.9	260.7±33.7
	Regular diet	6	1089.6±106.5	587.5±83.5	981.9±38.8