similar concentration of crystalline insulin. These differences may represent the presence in bile of small amounts of ILA not detectable immunologically. Bile ILA could not be suppressed completely upon incubation with an excess of AIS. The most likely explanation for these results seems to be that insulin may undergo some modification while passing through the liver.

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Atrophy of the Lymphoid Tissues of Mice Induced by Extracts of the Submaxillary Gland. (32404)

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Extracts of the submaxillary gland of the mouse induce several unique biological effects, including the stimulation and differentiation of sympathetic nerve cells, an action on the growth and keratinization of the epidermis, the induction of granulocytosis, a renin-like action, etc.(1). Because of the presence of the last-named substance(2), we studied the effect of infarction of the submaxillary gland on the blood pressure and noted that no change in blood pressure was induced by this operation but that the thymus underwent striking atrophy. The experiments have been extended to include the effect of intraperitoneal injections of saline extracts of the gland on the lymphoid tissues and blood counts of A/Jax and C3H strain mice.

Materials and methods. The submaxillary gland of 60- to 70-day-old A/Jax mice was infarcted by ligating the artery and salivary duct of the gland with a silk thread. The animals were killed 7 days later by dislocation of the cervical vertebra, and their tissues weighed and examined histologically. Blood obtained from the tail vein was stained with May-Grünwald and Giemsa solution and 200 cells or more were counted. The data were analyzed by Student's "t" test. Extracts of the submaxillary gland were prepared by

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TABLE I. Effect of Ligation of Artery and Duct of Salivary Gland on Weight of the Lymphoid Tissues and on Peripheral Leucocyte Counts in A/Jax Mice. Results given as means ± standard error.

	No. and sex of animals	Body wt in g		Organ weights in mg/g of final body wt			Blood counts per mm³			
							Lymphocytes		Neutrophils	
		Initial	Final	Thymus	Spleen	Mesenteric lymph node	Initial	Final	Initial	Final
Sham operated	6 ♂	23.6 ±.59	22.9 ±.37	1.19 ±.108	6.12 ±.586	2.43 ±.116	4250 ± 916	5730 ± 831	1780 ± 246	840 ± 121
Ligation	7 ♂ *	22.1 ±.71	20.9 ±.86	.54 ±.079†	5.93 ±.643	1.92 ±.193	4600 ± 725	3350 ± 356‡	2090 ± 556	1610 ± 225
Sham operated	5 ♀	21.9 ±.34	21.8 ±.34	1.23 ±.105	5.37 ±.288	2.53 ±.159	4100 ± 563	5120 ± 518	2150 ± 182	1570 ± 173
Ligation	5 ♀	20.8 ±.49	21.5 ±.47	1.22 ±.140	6.04 ±.213	2.93 ±.176	3720 ± 367	5830 ± 749	1680 ± 218	1990 ± 260

* Blood cell counts were made on only 6 animals of this group.
† Statistical significance of difference from sham operated group $p < .01$.
‡ Statistical significance of difference from sham operated group $p < .05$.

TABLE II. Effect of Intraperitoneal Administration of Submaxillary Gland and Other Tissue Extracts on Weight of Lymphoid Organs and Peripheral Leucocyte Counts in Male A/Jax and C3H Mice. Results given as mean ± standard error.

Strain	Extract injected	No. of animals	Total dose of extract in mg of wet tissue per mouse	Body wt in g		Organ wt in mg/g of final body wt			Counts per mm³			
									Lymphocytes		Neutrophils	
				Initial	Final	Thymus	Spleen	Mesenteric lymph node	Initial	Final	Initial	Final
A/Jax	Saline†	5		17.5 ±.79	18.9 ±.59	1.32 ±.105	4.81 ±.157	2.82 ±.474	3870 ±702	4670 ±490	1330 ±240	2420 ±627
	Liver	6	137.9	17.0 ±.95	18.4 ±.65	1.56 ±.145	5.38 ±.205	3.01 ±.168	4540 ±390	3750 ±819	1650 ±267	2020 ±304
	Kidney	5	159.6	16.4 ±.68	17.9 ±.64	1.46 ±.099	5.20 ±.453	2.53 ±.037	3650 ±156	3150 ±354	1690 ±263	1970 ±38
	Submaxillary gland	6*	138.6	16.9 ±.70	13.7 ±.44	.26 ±.041	2.78 ±.180	2.51 ±.150	3820 ±684	1510 ±257	2210 ±321	3890 ±780
C3H	Saline†	5		16.3 ±.58	18.4 ±.84	2.09 ±.210	8.06 ±.537	2.86 ±.231				
	Liver	7	259.0	17.2 ±.31	18.1 ±.53	1.72 ±.337	9.04 ±1.217	3.40 ±.151				
	Kidney	4	245.7	16.9 ±.65	17.5 ±.85	2.21 ±.173	7.94 ±.998	3.84 ±.321				
	Submaxillary gland	6	284.9	16.2 ±.60	17.5 ±.74	.33 ±.033	5.27 ±.502	2.57 ±.107				

* Blood counts were made on only 4 members of this group. † Control.

homogenizing the glands of 2- to 6-month-old A/Jax and C3H male mice in 5 ml of cold normal saline per gram of wet tissue and centrifuging at 10,000 r.p.m. for 10 minutes. The supernatant fluid was injected intraperitoneally into 36-day-old A/Jax and C3H male mice for 7 days. Female mice respond to the extract as do males but their salivary glands contain less of the active principle than do those of male mice.

Results. Infarction of the submaxillary gland induced marked atrophy of the lymphoid tissues and a significant decrease in the blood lymphocyte counts of A/Jax mice (Table I). The neutrophil and red cell counts showed no constant change. No effects were noted in the female animals.

The submaxillary gland extract (Table II) induced changes in A/Jax and C3H mice comparable to those observed following infarction of the gland as well as a decrease in the size of the submaxillary and parotid glands and an increase in the size of the adrenals. There was a marked difference in the reaction of the two strains, the C3H mice requiring twice as much extract to induce a comparable effect as compared to the A/Jax strain. There was a statistically significant difference ($p<0.01$) between the effects of the submaxillary gland extract on the weights of the thymus and spleen compared to extracts of kidney and liver and saline injections in the A/Jax mice. Similar results were obtained with the C3H mice except for the submaxillary gland extract as compared to the kidney extract ($P<0.05$). There was also a significant decrease in the peripheral lymphocytic count and an increase in the neutrophil count of the A/Jax mice injected with submaxillary gland extract as compared to those receiving saline or kidney extract ($p<0.01$).

Microscopic examination of the atrophic thymus (Fig. 1) revealed scanty thymocytes with a dominance of reticuloepithelial elements but no necrosis. Examination by electron microscopy revealed no inclusion bodies or virus-like particles as might be expected if the observed atrophy were a consequence of viral infection(3).

To exclude the possibility that the observed

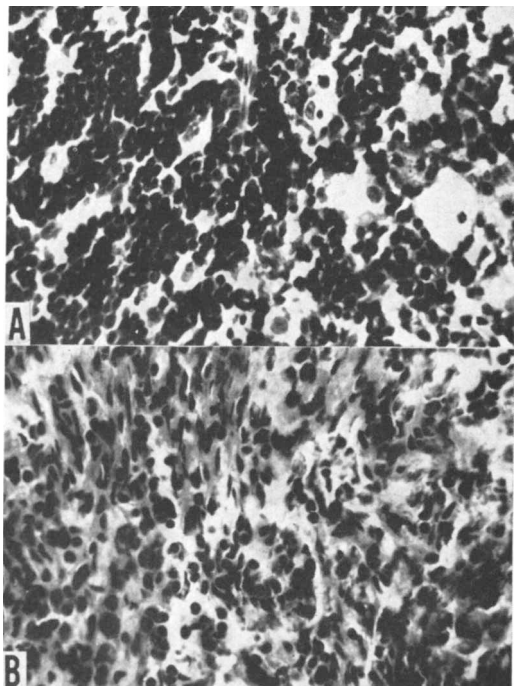

FIG. 1. Microscopic appearance of the thymus of a C3H mouse receiving saline as a control, A, compared to B, receiving an extract of the submaxillary gland for 7 days. Note scanty thymocytes and dominance of reticuloepithelial elements in the treated animal. (× 240 of 6 μ section).

effects were due to a stress reaction, the experiments were repeated on adrenalectomized mice (Table III), maintained on saline with and without the implantation of a pellet of desoxycorticosterone. The results indicate that the observed atrophy of the lymphoid tissues is not mediated through the adrenals. To demonstrate that the observed effects were not induced by a virus, such as the Molomut-Padnos lymphocytopenic virus(4) which is known to cause atrophy of the lymphoid organs in mice, the experiments were repeated with a filtrate of submaxillary gland extract through a 100 mμ millipore filter membrane. The thymus glands of animals treated with the filtrate of the submaxillary gland extract weighed 1.48 ± 0.11 mg as compared to 2.26 ± 0.13 mg per g final body wt for the controls ($p<0.001$) and histological and electron microscopic studies revealed the presence of no necrosis, inclusion bodies, or virus-like particles. These results indicate that

TABLE III. Effect of Intraperitoneal Administration of Submaxillary Gland Extract on Weight of Lymphoid Organs in Male Adrenalectomized A/Jax Mice With or Without Substitution Therapy with Desoxycorticosterone (DOCA). All animals received 1% NaCl and 5% glucose as drinking water. Results given as means ± standard error.

Treatment	No. of animals	Duration of treatment in days	Total doses of extract in mg of wet tissue/mouse	Body wt in g		Organ wt in mg/g of final body wt		
				Initial	Final	Thymus	Spleen	Mesenteric lymph node
Saline controls	5	3	—	19.2 ± 1.17	14.9 ± .74	2.35 ± .156	8.93 ± 1.169	2.66 ± .319
Extract	3	3	30.0	20.5 ± 1.29	16.8 ± .60	.96 ± .010	4.64 ± .558	1.51 ± .143
p value of difference						<.01	<.05	<.05
DOCA + saline	4	7	—	17.8 ± 1.93	19.3 ± 2.04	1.33 ± .100	8.15 ± .608	2.54 ± .205
DOCA + extract	4	7	64.0	19.3 ± .93	17.8 ± .36	.78 ± .118	7.28 ± .603	2.37 ± .083
Saline only	3	7	—	18.2 ± 1.56	19.4 ± 1.46	1.39 ± .216	6.61 ± .620	2.46 ± .032
Extract only	3	7	80.0	18.5 ± 1.28	17.6 ± 1.24	.65 ± .078	4.94 ± .207	2.16 ± .189

the presence of a virus was not responsible for the observed changes.

Discussion. Our results suggest that the submaxillary gland of the male mouse contains a substance which induces generalized atrophy of lymphoid tissues, particularly of the thymus, and a moderate degree of lymphopenia. Bueker and Schenkein(5) have also noted that the stimulating protein of nerve growth prepared from the submaxillary glands of mice induces moderate atrophy of the lymphoid organs after 49 days of treatment. Although this atrophy, as suggested by Bueker and Schenkein, may have been due to the stress evoked by the long administration of the protein, it also, in part, may have been due to the presence of an inhibitor of lymphoid tissues. The nature of the substance responsible for this action is unknown but appears to be a protein-like material. The active principle loses its biological activity on boiling for 10 minutes, is non-dialyzable, and is precipitated at 40 to 60% saturation with ammonium sulfate. Most of the activity remains in the supernatant fluid after one hour centrifugation at 105,000 *g*. Its relationship to the immune process and to the lymphocytosis-stimulating factor of Metcalf(6) as well as its role in the homeostasis of the lymphoid tissues remain to be determined. The observed increases in neutrophil count confirm the recently reported results of Angeletti *et al*(7).

Summary. Infarction of the submaxillary glands of male A/Jax mice or the administration intraperitoneally of an extract of these glands induced atrophy of the thymus, spleen and mesenteric lymph node and a moderate degree of lymphopenia of A/Jax and C3H mice. The observed response was also noted following adrenalectomy and following ultrafiltration or ultracentrifugation of the extract and is not attributable, therefore, to a stress reaction or viral infection, but rather to the presence of a factor which inhibits lymphoid tissues.

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Buphthalmia in the Rabbit: Effect of Age and Repeated Testing on the Cornified Cell Count Diagnostic Technique.* (32405)

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Buphthalmia in the rabbit is believed to be an autosomal recessive mutation (*bu*)(1) with incomplete penetrance(2). It is of great interest because of its resemblance to congenital glaucoma in humans. Fox and Babino (3) reported a technique for the early identification of buphthalmic rabbits (bu/bu) based on the degree of corneal cornification. Their procedure involved the enumeration of cells removed from the intact anesthetized rabbit eye. From their data they concluded that in the normal (+/+) eye an increase in the number of cornified cells was observed at puberty. The number then declined and stabilized. They also reported that testing the same eye at one-week intervals resulted in an increasing number of cells counted with each successive test. Both of these observations, however, were based on limited data. The present study was made to determine more accurately the effect during the first year of age and the effect of repeated testing on the cornified cell counts, using the normal (+/+) rabbit eye.

Materials and methods. One hundred forty-two normal (+/+) rabbits from The Jackson Laboratory rabbit colony were used. These were all from Strain III, a partially inbred population of New Zealand White origin. Cell counts were made by the procedure of Fox and Babino(3). In brief, this procedure consists of swabbing the anesthetized eye to remove the cornified cells, placing these cells on a clean glass slide, staining the cells with Giemsa, and counting the number and type of cells present in a given microscopic field. The individual eye was used as the unit of observation. Only healthy appearing eyes were used. Two studies were made. In the first study on the effect of age on the cornified cell count, observations were made on 262 eyes at 24 different ages ranging from 22 to 360 days of age. Each eye was assayed only once. In the second study on the effect of repeated testing on the cornified cell count the 22 eyes used were assayed 4 times with one week between each assay. The repeated measurements were made during a stable prepuberal period, from 50-80 days of age [see Fig. 1 and (3)].

Results and discussion. The mean number of cornified cells per eye at ages up to one year is shown in Fig. 1. The overall mean was 22.4. This compares well with the mean of 20.6 previously reported(3). The picture in general is one of stability from 35 to 100 days of age, an unstable period of higher counts at puberty, followed by subsequent stability with the exception of one eye with a count of 100 at 245 days. The cause of this high count is unknown. Beginning infection of the lacrimal duct or other factors might be involved.

The effect of repeated measurements on the cornified cell count is shown in Table I. From the data and the statistical analysis it may be seen clearly that repeated testing at one-week intervals has no observable effect on the cornified cell count when taken during this stable prepuberal period.

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