decreased at 17 h after the blood was collected, although it increased to 7.15 afterward. On the other hand, the intracellular pH in hereditary spherocytes was slightly but distinctly lower than that in normal erythrocytes over a period from 20 to 60 h after the blood sample was drawn. These findings suggest that the lowering in intracellular pH may influence the metabolic fate of ADP, since Rapoport[3] reported that the metabolism of ATP and 2,3-diphosphoglycerate in normal erythrocytes was affected by changing the intracellular pH. Or it is also possible that disturbance in the utilization of ADP may be accompanied by lowering of intracellular pH. Thus, although it is not possible at the present time to derive any causal correlation between these findings: lower intracellular pH and high ADP level, this report may represent the first description of intracellular events in hereditary spherocytes using ^{31}p-NMR spectroscopy.

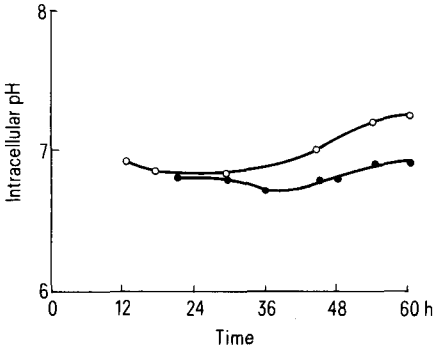

Fig. 2. Intracellular pH of normal erythrocytes (open circle) and hereditary spherocytes (closed circle) as obtained from chemical shift of Pi in ^{31}p-NMR-spectra.

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Studies of autoimmune induction in the rat lacrimal gland

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Summary. Induction of autoimmunity in the rat lacrimal gland was presently assessed. Antibodies to lacrimal gland extract were detectable in 58% of the immunized rats. Skin tests were positive at 4 week postimmunization in 70% of the animals. Histological observations revealed the presence of mononuclear cell infiltrates surrounding the ductal and acinar epithelium at 2-6 weeks.

The lacrimal gland, termed the exorbital gland in the rat, is a compound tubuloalvealar secretory gland containing fat droplets and serous granules, presumably lysozyme. Little is known concerning the induction of autoimmunity in the lacrimal and associated exocrine glands of the head and neck. Animal models of exocrine autoimmune disease, especially in salivary and lacrimal glands, could aid in understanding the pathogenesis of Sjögren's syndrome in the human[2]. Sjögren's syndrome is characterized by sialoadenitis and dacryoadenitis, in association with keratoconjunctivitis sicca, xerostomia, and fibrosis[3]. The present study described an attempt at inducing autoimmunity in the inferior lacrimal glands of the laboratory rat. Autoimmune induction was assessed by the following criteria: 1. the humoral immune response; 2. the delayed hypersensitive response; and 3. histopathological lesions.

Material and methods. The present study employed a total of 114 male and female young adult rats of the Wistar strain. The lacrimal glands from 50 nontreated Wistar rats of both sexes were removed at sacrifice and prepared as a 1:5 tissue-saline extract following the method of Witebsky and Rose[4]. The tissue extract was prepared in phosphate buffered saline (pH 7.2) employing a motor driven ground glass tissue homogenizer. The tissue suspensions were shaken throughout a 3-day period (phenol as preservative) at 4 °C in 50 ml Erlenmeyer flasks. The suspensions were spun in a refrigerated centrifuge at 10,000 rpm for 1 h. Lipid which flocculated to the surface of the supernant was removed by skimming and the pellet was discarded. Protein determination of the supernant extract was determined by the Christian-Warburg method[5] following readings on a Cary recording spectrophotometer. The saline extract, determined as 30 mg protein/ml, displayed a protein: nucleoprotein ratio of 15:1. Rat thyroid saline extracts, prepared as above, were used to test for organ specificity.

The experimental design consisted of 4 groups, each comprising 16 animals. The rats were immunized with either 1.0 mg/ml (group I), 5.0 mg/ml (group II), or 10.0 mg/ml (group III); group IV consisted of age and sex-matched nontreated animals. Following immunization with the 3

The dose response levels of rats in the various treatment groups immunized with lacrimal gland extract is demonstrated

Interval tested	Antigen* dilution	Treatment groups (dose levels) Group I 1.0 mg/ml		Group II 5.0 mg/ml		Group III 10.0 mg/ml		Totals	(%)
A Serological response									
2 weeks	none	3/12	(25%)	4/12	(33%)	0/12	(0%)	7/12	(58%)
4 weeks	none	0/10	(0%)	2/10	(20%)	0/12	(0%)	2/10**	(20%)
B Skin test response									
4 weeks	1:10	3/10	(30%)	2/10	(20%)	2/10	(20%)	7/10**	(70%)
	1:100	1/10	(10%)	0/10	(0%)	2/10	(20%)	3/10**	(30%)

Percentages indicate numbers of animals responding by either (A) antibody production or (B) skin reaction. *Antigen = lacrimal gland saline extract. **2 animals of this group died.

previously determined antigen dose levels[2], 4 animals from each group were sacrificed at 2, 4, 6, and 8 weeks intervals. The rats were injected with 0.1 ml of tissue extract previously emulsified in equal proportions with complete Freund's adjuvant. Injections were administered on 2 consecutive days utilizing 1 fore- and 1 hind-footpad on day 1 and the alternate footpads on day 2. On the 2nd day, an additional dose of 0.1 ml of Bordetella pertussis (Difco) antigen was administered s.c. into the left flank region. Each rat received a total of either 1.0, 0.5, or 0.1 mg of the immunogen.

Histopathological sections and sera were obtained at biweekly intervals throughout an 8-week period. Detection of autoantibodies to tissue extracts was performed by Ouchterlony double diffusion in agar. Skin tests, utilizing 0.01 and 0.001 mg/ml antigen per injection site, were performed on the 30th day postimmunization as previously described by Flax et al.[5] and Mizejewski et al.[6]. Criteria for the skin tests included erythema and induration observed after a 24-h period. H and E sections of the lacrimal gland, cut at 5 µm, were examined for histopathology. In addition, several lacrimal glands were studied in thin section by light (0.1 µm) and electron microscopy (EM).

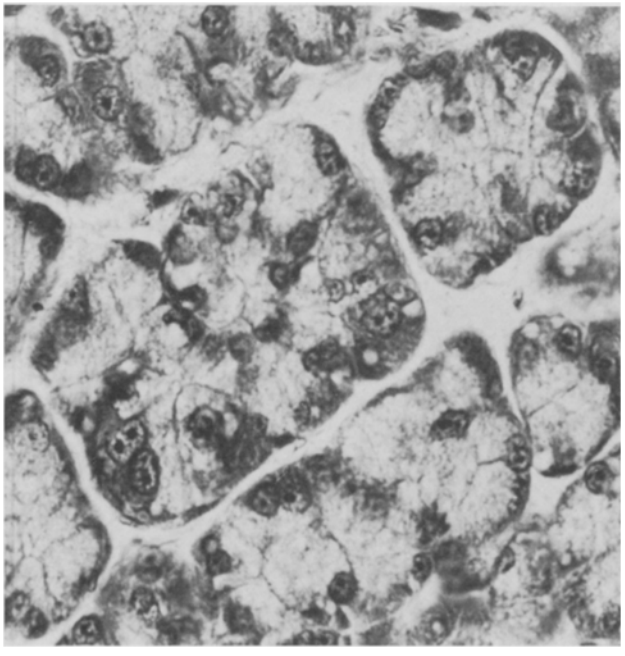
Results and Discussion. Antibodies to the lacrimal gland extract, detectable as a single band in agar immunodiffusion, were present in animals of groups I and II at the 2 and 4 week intervals concomittant with the appearance of tissue lesions (see below). At the 2 week interval, antibodies were detected in 7 of 12 (58%) animals in groups I and II and in 2 of 10 (20%) animals at 4 weeks in group II only (table, A). Antibodies were not detected in group III at all time intervals studied; after 4 weeks postimmunization, antibodies were absent in all treatment groups. Absorption of the antiserum with nonimmune rat serum (8 parts antiserum, 2 parts serum) did not alter the results; however, similar absorption with the lacrimal gland extract eliminated the reaction in agar. The antibodies appeared to be organ specific in that no reaction was detected against rat thyroid gland extract.

The skin tests (4 week postimmunization) were predominantly positive in animals of groups I and II, less in group III, and absent in group IV (table, B). Skin tests were most intense at the lower dose levels (group I) with some measuring 8×6 mm in crossed dimensions. Positive tests of delayed hypersensitivity were detected in 7 of 10 (70%) animals at 1:10 antigen dilution and in 3 of 10 (30%) at 1:100 dilution.

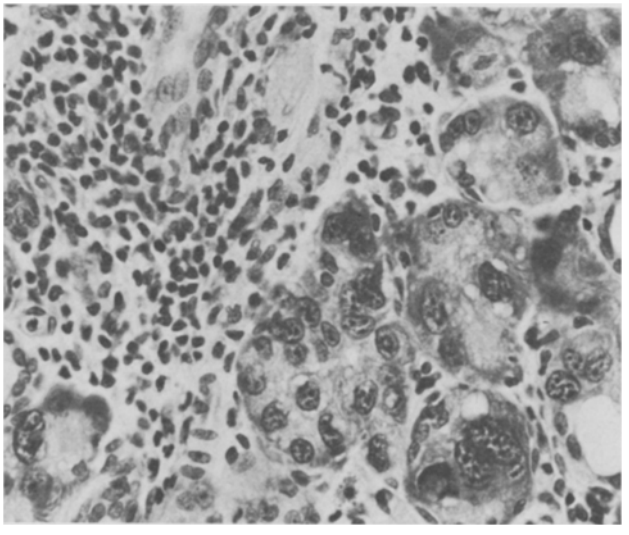
The histopathological gland lesions (dacryoadenitis) consisted of mononuclear cell infiltrates which appeared mainly at the 2–4-week interval (figure). The infiltrates were first apparent in the area of the ductal epithelium and associated vasculature. The ductal epithelium appeared disorganized and hyperplastic during this period. From this area, the infiltrates appeared to spread in and among the acinar gland architecture during the 4–6-week period. The acinar cells did not appear disrupted; however, these cells appeared to contain only the serous, dense granules rather than both serous and nonserous secretory components. The most severe histopathology was confined to rats receiving the higher antigen doses, notably, animals in group III.

The EM observations which will be described in more detail in a later publication[8], confirmed the presence of lymphoid cells surrounding the ductal epithelium. Lymphoblast and plasma-like cells, characterized by dilated endoplasmic reticulum cisternae containing dense material, appeared to be penetrating between the acinar cells in the later time periods (4–6 week). Finally, the cells appearing to contain foamy looking cytoplasm in the H and E sections had a large number of holes in epon embedded 1.0 µm sections.

The 3 basic requirements for induction of an autoimmune disease have been satisfied by the present animal model. First, a serological response was observed which demonstrated the existence of circulating antibodies against a homologous lacrimal gland extract. Secondly, a delayed hypersensitive response exemplified by positive skin test reactions signified the presence of a circulating population of sensitized lymphoid cells in the body of the host.



a



b

Fig. a. The normal histology of the rat lacrimal gland is displayed. Note the abundance of fat vacuoles and compact cytoplasm of the acinar cells. $\times 25$. Fig. b. The lacrimal gland from a rat in treatment group II (5 mg/ml) is demonstrated at 4 weeks postimmunization. Note heavy mononuclear cell infiltrates at left of photo surrounding ductal epithelium and associated vasculature. The presence of empty vacuoles representing depletion of fat droplets in acinar cells is also represented.

Thirdly, histopathological lesions observed by both light and EM indicated the presence of mononuclear cell infiltrates in and among the epithelial components (acinar and ductal) of the lacrimal gland. Confirming evidence that an autoimmune state had indeed been induced in the rat lacrimal gland must await further experimentation employing adoptive immunity. This could be accomplished by the passive transfer of immune cells and/or serum into nonimmunized syngeneic rats.

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Plasma triiodothyronine, thyroxine and thyrotrophin levels in germfree rats[1]

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Summary. Plasma T_3, T_4 and TSH levels in developing germfree rats were high, low and normal as compared with those in conventional counterparts. The high T_3/T_4 ratio in germfree rat plasma was lowered by cholestyramine feeding.

Thyroid function in germfree animals has been studied by determining the metabolic rate, the iodine uptake and the circulating thyroxine (T_4) etc., leading to the hypotheses of a hypo-thyroidal function in the young germfree rat and a hypo- or euthyroidal function in the adult germfree rat[2-4]. However, little information is available on triiodothyronine (T_3) in germfree rats except our preliminary report[5]. The significance of T_3 in maintenance of euthyroid status has recently been recognized[6]. In addition, circulating T_3 and T_4 levels are known to be affected by their enterohepatic metabolism at different rates[7].

The present study was undertaken to evaluate the economy of thyroid hormones in germfree rats, which have large pools of cholesterol[8] and bile acids[9] in contrast to those in conventional rats. We describe plasma T_3, T_4 and thyrotrophin (TSH) levels in germfree and conventional rats and lowering of the plasma T_3/T_4 ratio by cholestyramine feeding in germfree rats. *Materials and methods.* Young (30-day-old) and adult (75-day-old) germfree and conventional male rats[10] were used. In experiment 1, the young rats were fed for 5 days a cholesterol diet containing 0.35% cholesterol[8] or a cholestyramine diet containing 0.35% cholesterol and 5% cholestyramine[11]. Aortic blood was drawn from the fed rats under hexobarbital anesthesia. Plasma was separated and stored at −20 °C until use for the radioimmunoassay of T_3, T_4[12] and TSH[13]. In experiment 2, the adult rats on a normo-cholesterol diet (CL-2)[14] were decapitated after a 20-h fast. Other procedures were done in the same way as described above.

Results. Plasma T_3 levels in young germfree rats on a cholesterol diet were higher than those in conventional counterparts, while plasma T_4 levels in the former were lower than those in the latter (table 1). Cholestyramine feeding reduced the mean T_3 level from 125.5 to 75.3 ng/100 ml and elevated the mean T_4 level from 2.53 to 3.40 μg/100 ml in young germfree rats, but did not significantly change these levels in conventional counterparts.

Adult germfree rats on a normo-cholesterol diet had also higher T_3 and lower T_4 levels in plasma, in contrast to those in conventional counterparts (table 2). However, plasma TSH levels were not significantly different between germfree and conventional rats.

Discussion. The present data indicating a lower plasma T_4 level in the germfree rat are consistent with the results on serum T_4 in 30- and 60-day-old germfree rats obtained by Sewell et al.[4]. However, they observed slightly higher T_4 levels in 100-day-old germfree rats, and suggested a delayed maturation of thyroid function in the young germfree rat. Although a delayed maturation has been reported on the pituitary-adrenal axis[15] and the testicular function[16] in germfree rodents, this may not account for a higher T_3 level in germfree rat plasma.

The high T_3/T_4 ratio in plasma was always associated with normal plasma TSH level in the germfree rat (table 2),

Table 1. Plasma T_3 and T_4 levels in 30-day-old germfree (GF) and conventional (CV) rats fed a 0.35% cholesterol diet (CONT) or a 0.35% cholesterol-5% cholestyramine diet (CHOL)

Group of rats		T_3 (ng/100 ml)	T_4 (μg/100 ml)	T_3/T_4 (%)
CONT-GF	(4)	125.5 ± 14.0[a]	2.53 ± 0.06[b]	5.0 ± 0.6[a]
CONT-CV	(4)	63.8 ± 2.4	3.08 ± 0.08	2.1 ± 0.1
CHOL-GF	(3)	75.3 ± 5.4[c]	3.40 ± 0.17[d]	2.3 ± 0.3[d]
CHOL-CV	(4)	66.5 ± 5.3[e]	2.90 ± 0.01[e]	2.3 ± 0.2[e]

Mean ± SE for number of rats indicated in parentheses. [a,b]Different from the CONT-CV group at 1% and 0.1% levels, respectively. [c,d]Different from the CONT-GF group at 5% and 1% levels, respectively. [e]Not significantly different from the CONT-CV group and the CHOL-GF group ($p > 0.05$).

Table 2. Plasma T_3, T_4 and TSH levels in 75-day-old germfree (GF) and conventional (CV) rats fed a normo-cholesterol diet

Group of rats		T_3 (ng/ml)	T_4 (μg/100 ml)	T_3/T_4 (%)	TSH (ng/100 ml)
GF	(4)	90.0 ± 7.1[a]	2.97 ± 0.05[b]	3.1 ± 0.2[b]	292.0 ± 7.3[c]
CV	(5)	64.0 ± 4.0	4.00 ± 0.06	1.6 ± 0.1	294.0 ± 8.7

Mean ± SE for number of rats indicated in parentheses. [a,b] Different from the CV group at 2% and 0.1% levels, respectively. [c] Not significantly different from the CV group ($p > 0.05$).