Animals thymectomized at birth failed to show the lymphocytic infiltration of the meninges after challenge with LCM virus (*6*), even though their numerical complement of lymphocytes was normal at the time of virus challenge. Mice which had been implanted intraperitoneally with Millipore diffusion chambers containing thymus and which died after virus inoculation showed the pathognomic lesions of the disease. Therefore, it would appear that the contained thymic tissue produced a noncellular or humoral factor which restored ability to react to infection with a lymphocytic infiltration.

Gowans (*12*) has recently presented evidence that there may be two classes of small lymphocytes: those which are "immunologically committed," in that they already have reacted to antigen, and those which are "uncommitted," in that they are free to establish a new line of dividing antibody-producing cells in response to a new antigenic stimulus.

In accordance with this view, then, the neonatally thymectomized mouse, while not lacking peripheral blood lymphocytes and not showing lymphoid organ atrophy, is unable to produce a "clone" of small lymphocytes to react to the new exposure with LCM virus.

A preliminary report (*3*) showed that a Millipore diffusion chamber containing isologous thymic tissue implanted into neonatally thymectomized mice could prevent the lymphoid atrophy and wasting syndrome which otherwise characterize such animals. This restorative action was attributed to a noncellular or humoral factor that diffused from the thymic tissue in the chamber, the pore size of which did not allow the passage of intact cells.

The data presented here provide further support for a humoral theory of thymic action. The most probable source of this humoral factor is the epithelial-reticular cells of the thymus, which have been shown to survive in the chamber up to 60 days (*3*). Evidence has been reported for activity of the epithelial component of the thymus in lymphocyte production (*13*).

The present data do not permit judgment on whether the diffusible factor is produced by thymus alone or by other tissues as well.

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## Possible Cytoplasmic Change in an Immunologically Competent Tissue of the Chicken

Abstract. *The weight of the bursa of Fabricius of chicks hatched from eggs dipped in a 0.67 percent solution of testosterone propionate in ethyl alcohol was significantly reduced. Also, bursa weights of offspring one and two generations removed from the chickens hatched from the dipped eggs were significantly reduced. The change appears to be cytoplasmic.*

Immunological competence appears to be a function of the mammalian thymus (*1*) and avian bursa of Fabricius (*2*), a lymphoepithelial gland. The weight of the bursa and antibody response of chickens has been eliminated or markedly reduced by dipping eggs in alcohol solutions of testosterone propionate (*3*). Chickens hatched from eggs dipped in testosterone propionate (TP) produced offspring with a reduced bursa size (*4*). The reduced bursa size of the offspring could be attributed to a gene (chromosome) or cytoplasmic change. Data supporting the idea that TP acted by way of the cytoplasm is presented in this paper.

In both of the trials, the parental generation ($P_1$) refers to chickens hatched from eggs dipped in either ethyl alcohol (EA) or in a 0.67-percent solution of testosterone propionate in ethyl alcohol. In all other generations ($F_1$ and $F_2$), the eggs were not dipped. The $P_1$ generation was produced by removing fertile eggs from the incubator on the 3rd day of incubation and immersing, for 5 seconds, 3.2 cm of the pointed end of the egg in cold solutions (5°C ± 4°C) of TP or EA. The data were analyzed by the analysis of variance. The analysis of covariance was used to analyze bursa weights where body weights differed significantly. Duncan's (*5*) new multiple range test was used to determine significant differences between any two means. Significant difference refers to a statistical difference at the 5 percent level of probability. All means are given with standard deviations.

In the first trial, the $P_1$ chickens were raised to maturity and mated within their own group to produce the $F_1$ generation, that is, EA males were crossed with EA females and TP males with TP females. Individuals of the $F_1$ generation were raised to maturity and mated within their own group to produce the $F_2$ generation. The bursae were significantly reduced in size in chicks hatched from TP-dipped eggs ($P_1$) (Table 1). The TP chicks one generation ($F_1$) and two generations ($F_2$) removed from the dipping also possessed significantly reduced bursae at hatching. The body weight of the EA and TP females that produced the $F_1$ and $F_2$ generations did not differ significantly.

A second trial was conducted to investigate the possibility that the reduction in bursa size in the $F_1$ and $F_2$ generations was induced by a change in the cytoplasm. When the $P_1$ generation was produced, the expected significant reduction in bursa size at hatching was observed in the $P_1$ chicks (Table 2). An $F_1$ generation was produced by mating EA males with EA females, EA males with TP females, and TP males

Table 1. The weights of the bursa and body at hatching of $P_1$, $F_1$, and $F_2$ chicks. Significant differences are apparent for the bursa in all three cases.

| Ethyl alcohol | | Testosterone propionate | |
|---|---|---|---|
| Bursa (mg) | Body (g) | Bursa (mg) | Body (g) |
| | *$P_1$ (12 birds)* | | |
| 46±12* | 44±3 | 13±8 | 44±4 |
| | *$F_1$ (20 birds)* | | |
| 43.0±7.7* | 35.0±3.0 | 31.9±7.7 | 35.0±3.0 |
| | *$F_2$ (55 birds)* | | |
| 49.0±13.0* | 42.0±3.0 | 38.0±9.0 | 43.0±3.0 |

* $P \leq .05$

Table 2. The weights of the bursa and body at hatching of EA and TP chicks ($P_1$), offspring from EA chicks ($F_1$), and offspring from reciprocal crosses of EA and TP chicks ($F_1$). The numbers in parentheses refer to the number of birds.

| $P_1$ | | $F_1$ | | | | | |
|---|---|---|---|---|---|---|---|
| | | Hatch 1 | | Hatch 2 | | Hatch 3 | |
| Bursa (mg) | Body (g) | Bursa (mg) | Body (g) | Bursa (mg) | Body (g) | Bursa (mg) | Body (g) |
| | | | *Testosterone propionate* | | | | |
| 19.3±10 (20) | 44.2±4.8 | | | | | | |
| | | | *Ethyl alcohol* | | | | |
| 59.9±14.0 (20) | 45.6±3.8 | 43.0±8.8 (96) | 38.4±3.9 | 42.3±11.7 (54) | 41.7±3.7 | 44.3±10.0 (79) | 41.1±3.0 |
| | | | *TP males × EA females* | | | | |
| | | | | 42.2±14.4 (21) | 38.6±2.2 | 41.9±10.0 (68) | 41.0±2.5 |
| | | | *EA males × TP females** | | | | |
| | | 39±10.6 (39) | 38.3±2.9 | 36.8±8.5 (39) | 39.2±3.8 | 34.2±9.7 (39) | 39.1±3.6 |

* Within hatches the bursa means of this group are significantly different from all other bursa means.

with EA females. No significant differences in $F_1$ bursa weights were found in chicks whose dams hatched from EA dipped eggs (Table 2). However, chicks produced by dams that hatched from TP dipped eggs (EA × TP) showed a significant reduction in bursa weight in all three hatchings.

The reduced size of the bursa in the $F_1$ and $F_2$ generations of both trials may have resulted from a gene or chromosomal change, or a change transmitted through the cytoplasm occurring in the $P_1$ chicks. The results of the reciprocal matings (Table 2) suggest that a cytoplasmic change had occurred. This change could be inherited through the cytoplasm, in which case the reduction in the size of the bursa would be expected to remain as a stable effect, or as a dauermodification—a temporarily inherited alteration produced by environmental means. Many generations will need to be studied to determine the stability of the bursa change. The influence that TP exerts on the embryo's bursa is reflected in a reduced capacity of the hatched chicken to produce circulating antibody (*2; 6*).

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## Thermoperiodism in Sea-run Cutthroat Trout (Salmo clarki clarki)

Abstract. *Thermal tolerances from square-wave cycled temperature acclimation were compared with those from constant acclimation temperatures. A response to the maximum temperature of the square-wave cycle occurred. The acclimation thermoperiod was varied from one-fourth to twice the 24-hour cycle, and a relative maximum of tolerance resulted for the 24-hour thermoperiod. This suggests physiological adaption to natural 24-hour thermoperiods.*

Thermal acclimation and tolerance studies have been conducted more commonly with constant acclimation temperatures (*1*). Fewer workers (*2–7*) have considered cycled temperature acclimation more like that occurring in natural environments.

Of these, Brett (*2*) compared field with laboratory temperature tolerances and acclimation in *Ameiurus* and found that tolerances in the field would have had corresponding laboratory acclimation temperatures more like the maxima than the average of the environmental temperatures. Fry *et al.* (*3*) gave evidence that fish were better able to withstand upper extremes when subjected to fluctuating rather than constant acclimation. Lowe and Heath (*4*) were able to obtain higher tolerances with fish subjected to cyclic variation of temperature in the field over a 3-month season than with any form of constant temperature acclimation. They also found that fish cycle themselves behaviorally so that near-lethal temperatures are preferred for limited periods of the day, a characteristic well known in reptiles (*8*). Hillman (*5*) showed that in tomatoes damage caused by constant light could be obviated by cycling the temperature. Hubbs (*6*) had better development for fish from habitats with greater thermal fluctuation when using controlled fluctuating temperatures in the laboratory.

Studies were made with sea-run *Salmo clarki clarki* to compare laboratory-cycled temperature with constant-temperature acclimation and to determine the effect of cycle length (thermoperiod) on temperature tolerance (*9*). Yearling hatchery fish of spawn taken from adults ascending the Nooksack River, Whatcom County, Washington, February 1962, were used (*10*). Control for the square-wave acclimation patterns (Fig. 1*A*) was within ± 0.05° C of the designated temperatures (*11*). The square-wave pattern was used since in its case the major difference between cycled and constant acclimation is the alternating nature of the pattern; the shape of the particular temperature-time curves of natural environments would be an added variable. Three aquaria at a time could be cycled independently with various cycle periods as shown (Fig. 1*A*) while two or more control aquaria were held at constant temperatures. There were 15 fish per 4 gallons of filtered hatchery water (samples were occasionally reduced by escape or accident). Hatchery food was fed three to four times daily, and the photoperiod was 24 (12:12) hours, coinciding with the 24-hour thermoperiod. Fish were held at 15°C for one week before experiments were begun (except for acclimation samples at 10°C). The *p*H varied from 7.1 to 8.2, and oxygen was kept near saturation throughout the experiments.

The measurement of temperature tolerance was the CTM (critical thermal maximum or maxima) as defined by Lowe and Vance (*12*). The methods for obtaining them were similar to those formerly used (*13*). The heating rate for the CTM was 0.4 ± 0.1°C per minute in a glass chamber the same