

similar to previous values determined on intestines from fish acclimatized to 8°C, taken 21 h after injection of the same quantity of puromycin (Figure 2).

Another way of observing the effect of temperature acclimatization on electrical parameters of the goldfish intestine is to measure the incubation temperature at which glucose first causes a permanent increase in the steady transmural potential. This temperature has been found to be approximately equal to that at which glucose-evoked potentials change from a phase 1 to a phase 2 type of behaviour<sup>6</sup>. This temperature is not changed by injection of puromycin when the environmental temperature is kept constant at 8°C (12.5°C for control intestines; 13.1°C when injected 21 h previously with puromycin). However puromycin does retard the change in this temperature which occurs when fish acclimatized to 8°C are subjected to 25°C for 20 h (26.6°C for goldfish at 25°C for 20 h; 18.4°C for similar fish injected with puromycin 1 h before changing the environmental temperature). These values are each the mean of five determinations and the difference between the two populations is significant ( $t = 3.62$ ;  $P < 0.01$ ).

It is therefore suggested that a carrier for sodium ions, activated by glucose and thought to be situated in the

luminal membrane of the goldfish mucosa, is replaced by a different carrier under the stimulus of a changed body temperature and that, since puromycin stops this substitution, the carrier is either a protein or else dependent on the presence of protein molecules for its normal operation.

**Résumé.** Le potentiel transmural de l'intestin du poisson rouge, incubé *in vitro*, dépend partiellement de la présence de la glucose. Ce potentiel, dû à la glucose, est déterminé par la température d'acclimatation, et se réduit dans la mesure que cette température augmente. Cet aspect de l'acclimatation est complet 20 h après qu'on a élevé la température du milieu du poisson, et le puromycin entrave ces variations. Ces résultats suggèrent l'idée que les variations dans les propriétés des membranes de l'épithélium intestinal impliquent la synthèse des molécules de protéine.

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### Effect of Thymectomy on Immune Response in Adult Rats

MILLER<sup>1</sup> observed in 1962 that total body irradiation considerably decreased the immune response and increased the tolerance of skin grafts in thymectomized young adult mice. He concluded that the thymus was immunologically important, even in adult age. Many authors supported these results, but there were a lot of contradictory data<sup>2-5</sup>. We observed in earlier experiments<sup>6</sup> a significant rise of the immune response in rats a long time after they had been thymectomized. It was in view of such contradictory results that the present experiments were designed with the object of ascertaining the effect of age and that of time elapsed since thymectomy.

62 rats of the Wistar strain were used. They belonged to three age classes: 4, 9 and 13 months. The 4-month group contained only animals that had been thymectomized a week before the first immunization, while a part of the animals in the other two groups had also been thymectomized a week before, another part 6 months and still another part 10 months before the beginning of immunization. Intact animals of corresponding ages served as controls. The plan of the experiments is tabulated in Table I. (See the experimental and mathematical methods in <sup>6</sup>.)

Mathematical-statistical results (Table II): As regards primary response, the difference between controls and long thymectomized animals was somewhat above the level of significance in the 9-month group, and somewhat below significance in the 13-month group. Since the deviation of about one tube in favour of thymectomized animals, observed in both groups, is in good harmony with earlier observations<sup>6</sup>, immune response promoting tendency can be regarded as proved for both groups.

As regards secondary response, no significant deviation was registered in any group. This situation was found un-

changed 2 weeks after the irradiation without renewed antigenic stimulation.

As regards tertiary immune response, a significant decrease was registered in comparison to the controls in the freshly thymectomized members of the 4- and 13-month groups, a result in good agreement with those

Table I. Plan of the experiments

Time in weeks	Experiments
0	Immunization with sheep red cells (SRC) intravenously 100 · 10 <sup>6</sup> /100 g body weight
1	Hemagglutination <sup>a</sup>
2	—
3	Immunization with SRC 150 · 10 <sup>6</sup> /100 g
4	Hemagglutination <sup>b</sup>
5	350 r total body X-ray irradiation
6	—
7	Hemagglutination <sup>c</sup>
8	Immunization with SRC 100 · 10 <sup>6</sup> /100 g
9	Hemagglutination <sup>d</sup>
10	Hemagglutination <sup>e</sup>

The superior letters indicate the identical groups in Table II.

<sup>1</sup> J. F. A. P. MILLER, *Nature* 195, 1318 (1965).

<sup>2</sup> A. C. AISENBERG and B. WILKES, *J. Immunol.* 93, 75 (1964).

<sup>3</sup> K. E. FICHTELIUS, G. LAURELL, and L. PHILIPPSON, *Acta path. microbiol. scand.* 57, 81 (1965).

<sup>4</sup> R. B. TAYLOR, *Immunology* 7, 595 (1964).

<sup>5</sup> H. O. ZUNKER and H. A. AZAR, *Proc. Soc. Exp. Biol. Med.* 178, 423 (1965).

<sup>6</sup> G. CSABA, M. BODOKY, J. FISCHER, and T. ÁCS, *Experientia*, in print.

obtained in MILLER's<sup>1</sup> mouse experiments. The value of immune response seems to have dropped in the 13-month group below that of the controls, owing also to the action of the old thymectomy, and this tendency remained perceptible even at the second measurement of the tertiary response (although the effect of the fresh thymectomy had disappeared by that time).

To sum up: (1) Results of the present experiments on rats support those of MILLER's mouse experiments. Total

body irradiation of thymectomized adult rats reduced the immune response below that of the unimpaired controls, provided the antigenic stimulation was repeated. Such reduction was, however, neither durable nor did it occur in all age groups. (2) In general, the length of time elapsed since the removal of the thymus affected the immune response more than the age of the thymectomized animal. (3) Present results have substantiated the earlier observation that the primary immune response, instead of diminishing, becomes decidedly stronger if the time between thymectomy and immunization is long, a phenomenon presumably due to a liberation of another – immune substance producing – part of the lymphatic system from the inhibitory influence of the thymus. (4) The present results should be taken as a warning not to indulge in generalizations regarding the immunological role of the adult thymus. Although thymectomy performed on adult animals was followed by a diminution of immune response, the role of the adult thymus is still questionable and is not always effective, its effect was inhibitory rather than stimulatory and the role of the adult thymus by no means compares with the significance of this organ at the embryonic or neonatal stage.

*Zusammenfassung.* Röntgenbestrahlung thymectomierter Ratten ergab eine abgeschwächte Immunreaktion. Für eine Immunreaktion adulter Tiere scheint jedoch der Thymus nicht notwendig zu sein. In Abhängigkeit von Zeit und Alter der Tiere zeigten sich Unterschiede in der Stärke der Immunreaktion.

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Table II. Number of determinations (*n*) and the average critical tubes ( $\bar{x}$ )

Age of animals		Primary <sup>a</sup>	Secondary <sup>b</sup>	Secondary <sup>c</sup>	Tertiary <sup>d</sup>	Tertiary <sup>e</sup>
4 months						
	<i>n</i>	9	9	9	8	8
Controls	$\bar{x}$	5.89	5.56	4.44	6.00	4.25
Fresh thymectomy	<i>n</i>	8	7	6	6	5
	$\bar{x}$	6.25	5.57	5.00	4.50	4.40
9 months						
	<i>n</i>	8	6	4	4	4
Controls	$\bar{x}$	4.88	4.17	4.75	4.25	3.50
Fresh thymectomy	<i>n</i>	9	7	7	7	7
	$\bar{x}$	5.44	4.71	4.86	4.57	4.10
Thymectomy 6 months before	<i>n</i>	7	7	7	5	4
	$\bar{x}$	5.86	5.00	4.71	5.00	4.25
13 months						
	<i>n</i>	7	7	6	5	5
Controls	$\bar{x}$	5.29	5.43	5.17	6.00	5.20
Fresh thymectomy	<i>n</i>	9	7	6	5	4
	$\bar{x}$	4.44	4.86	5.00	4.20	5.25
Thymectomy 10 months before	<i>n</i>	5	5	5	5	5
	$\bar{x}$	6.20	5.40	4.60	5.00	4.00

## The Effect of Hyperthermia Applied in the Given Stages of Pregnancy on the Number and Form of Vertebrae in the Offspring of White Mice

In his former communication<sup>1</sup> the author has described the experiment which showed that in female white mice, hypothermia applied in the given stages of pregnancy caused inborn anomalies of the vertebral column, i.e. changes in the number of vertebrae in the given region, and occurrence of fused asymmetric or wedge-shaped vertebrae.

In 1964 the author started the second part of his experiment, which involved the investigation of the effect of hyperthermia applied in analogous stages of pregnancy; the material and methods of determining pregnancy, preservation, staining of skeletons and clearing being in both experiments identical<sup>1</sup>. The body temperature of the females was increased by 3–4 °C, ranging within 40–41 °C. For this purpose the females were put into a specially adapted thermostat for 20 h<sup>2</sup>. The experimental females were assigned to one of six groups (denoted by the letters A–F). In group A the body temperature was in-

creased on day 7½ of pregnancy, in group B on day 8½ etc., until in group F hyperthermia was applied on day 12½ of pregnancy. The total number of pregnant females amounted to 150, of which 53 died during the heat shock, and 75 gave birth to 372 youngsters (of which 22 were born dead).

So far 186 specimens have been examined, of which 48 showed inborn anomalies of the vertebral column. As in the former experiment, two kinds of malformation have been found: (1) Changes in the number of vertebrae in the given region; e.g. 6 instead of 7 cervical vertebrae, 12 (Figure 1) or sometimes 11 thoracic vertebrae instead of 13, 5 lumbar vertebrae (Figure 1) instead of 6. (2) Teratological changes consisting in fusion of the centra and arches of two or more neighbouring vertebrae, presence of split-out centra, and occurrence of wedge-shaped half-vertebrae (Figure 2) that caused scoliosis. Sometimes the

<sup>1</sup> M. LECYK, *Experientia* 21, 452 (1965).

<sup>2</sup> L. FERNANDEZ-CANO, *Fert. Steril.* 9, 455 (1958).