

activity in the liver of adults is much less than in young rats, only adult toads were used in the present investigation. But LEBLOND and CARRIERE³ and DISTEFANO and DIERMEIER⁴ have observed a strikingly reduced mitotic activity in the epithelium of crypts of Lieberkühn and in liver cells, respectively, as a result of hypophysectomy. So it may be concluded that the normal mitotic activity of these tissues may to some extent be associated with the functional activity of the hypophysis in the body. But it may also be mentioned here that after attaining the static mitotic activity in the adult, it is questionable whether the hypophysis has any role in adult cellular mitotic activity. LISON and VALERI⁵ have shown the diminution of hepatic nuclear volume in hypophysectomized male rats. Our observations of decrease of both cellular and nuclear volumes in liver as a result of hypophysectomy are in good agreement with their findings⁵. The hypophysectomized toads were kept for 8 days in the presence of water but without any food. Considering the effect of starvation, another group of normal toads (with hypophysis) was also kept in this condition for 8 days and was experimented upon. No significant change in either cellular or nuclear volume has been observed in comparison with normal non-hibernating season's toads. We cannot defi-

nately say at present if the changes as observed in the present investigation were due to changes in metabolic pattern as a result of hypophysectomy, or to any direct imbalance in hormonal action on the cell⁸.

Résumé. On a noté une décroissance significative dans les volumes des cellules hépatiques (et aussi dans leurs volumes nucléaires) du crapaud hypophysectomé par rapport au non-hibernant. Ceci est probablement dû à un changement du modèle-type métabolique ou, peut-être, à l'action directe hormonale déséquilibrée, exercée sur les cellules hépatiques à la suite de l'hypophysectomie.

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Serum-Protidopoyesis in Neonatally Thymectomized Rats Develops Normally

The importance of thymus in the development of immunological powers is proved by the following data: neonatally thymectomized animals accept heterologous skin homografts¹⁻³, and normal or neoplastic cell-grafts^{4,5}; they are unable to form antibodies owing to antigen introduction^{2,6-8}.

The absence of antibody formation is likely to be a phenomenon dissociated from the ability to synthesize γ -globulins: in new-born thymectomized animals treated with heterologous serum at the age of 2 months, AZAR et al.⁹ noticed a plasmocytic reaction and an increase of γ -globulins in the serum quantitatively comparable with those of a normal animal; on the contrary, there is no formation of antibodies. This fact confirms the remark of JANKOVIC et al.³ on the normality of the serum proteic picture in a neonatally thymectomized animal. On the contrary, MOWBRAY and JEEJEEBHAY¹⁰ appear to have noticed the disappearance of α -globulins in the serum of dogs thymectomized when grown up. We must say that this remark is in contrast with the data of other authors^{9,11,12}. The reported data suggest the interest of a complete study on the serum-protidopoyesis in new-born thymectomized animals.

36 Sprague-Dawley strain rats, thymectomized within the first 24 h after birth, were divided into 4 groups of 9 units. The animals of each group (those of the 1st group on the 5th day after thymectomy; those of the 2nd on the 15th day; those of the 3rd on the 30th day; and those of the 4th on the 60th day) received intraperitoneally an aqueous solution of glycine-¹⁴C (4 μ C/g of weight). Within each group, 3 rats were killed 1 h after the injection; 3 rats 2 h after it; 3 rats 24 h after it. Death was caused under ether anaesthesia by loss of blood from neck veins; necroscopy showed the absence of thymic remains. Just after

death a fragment of liver and one of spleen were taken and fixed in 10% formalin. Then strips of bone marrow were prepared.

On blood serum total proteins were determined by a biuret method, and paper electrophoresis was executed in double sample in the same separation chamber (barbital buffer, pH 8.7; 0.1 μ ; 2.5 V/cm; 12 h). One of the strips was used for the quantitative study of proteic fractions (staining with amido-black). The second strip, sown with 0.1 cm³ of serum, was employed to evaluate the radioactivity in each fraction (Geiger-Müller counter); the data obtained were expressed as a percentage of the total radioactivity present. The histological pieces, after paraffin inclusion, served for the preparation of autoradiograms (Ilford G5 emulsion; time of exposure 8–10 days).

¹ J. F. A. P. MILLER, *Lancet* 1961 ii, 748.

² J. F. A. P. MILLER, *Ann. N.Y. Acad. Sci.* 99, 340 (1962).

³ B. D. JANKOVIC, B. H. WAKSMAN, and B. G. ARNASON, *J. exp. Med.* 116, 159 (1962).

⁴ D. M. V. PARROTT, *Transplant. Bull.* 29, 102 (1962).

⁵ C. MARTINEZ, J. KERSEY, B. W. PAPERMASTER, and R. A. GOOD, *Proc. Soc. exp. Biol. Med.* 109, 193 (1962).

⁶ R. A. GOOD, A. P. DALMASSO, C. MARTINEZ, O. K. ARCHER, S. C. PIERCE, and B. W. PAPERMASTER, *J. exp. Med.* 116, 773 (1962).

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⁹ H. A. AZAR, R. W. SNYDER, and J. WILLIAMS, *Fed. Proc. Fed. Am. Soc. exp. Biol.* 22, 600 (1963).

¹⁰ J. F. MOWBRAY and H. JEEJEEBHAY, reported by R. M. ZOLLINGER, M. C. LINDEM, R. M. FILLER, J. M. CORSON, and R. E. WILSON, *New Engl. J. Med.* 270, 704 (1964).

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The strips of marrow were stained with May-Grunwald-Giemsa. All these experiments were then repeated in 36 neonatally sham-thymectomized rats of the same strain. Furthermore, on the serum of 4 animals (2 thymectomized; 2 sham-operated) an ultracentrifugal analysis was performed on the 15th and on the 30th day.

In thymectomized rats the γ -globulin synthesis proceeds in a manner qualitatively analogous to that of sham-operated animals (Figure 1); if we consider that the total proteic concentration and the electrophoretic distribution of the serum-proteic fractions are superimposable (Table), we may conclude that the phenomenon has quantitative dimensions which are also practically identical. The values of the γ -globulins increase progressively till the 30th day, by which time they reach levels like those of an adult animal; the incorporation of glycine- ^{14}C in this fraction is greater after the 15th day. The study of the ultracentrifugal picture (of a merely indicative importance) shows that the macromolecular component is identically formed in thymectomized rats and in the controls, and that the G-component (to use a terminology analogous to that employed for human serum) becomes appreciable on the 30th day (Figure 2). Finally our experiments show that the hepatic and splenic distribution of a marked

amino acid appears to be qualitatively analogous in two groups of animals (Figure 3) and that the increase of the number of plasmocytes in bone marrow is of the same order (Table).

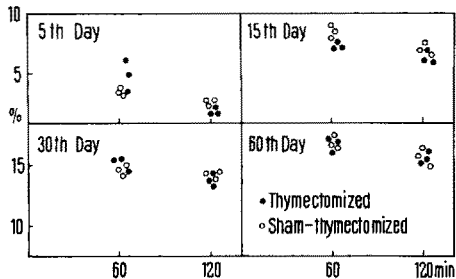
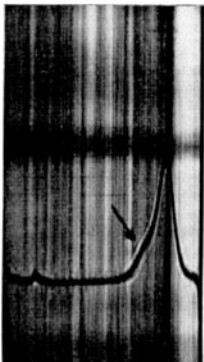
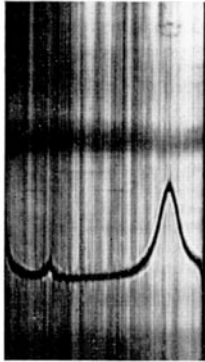


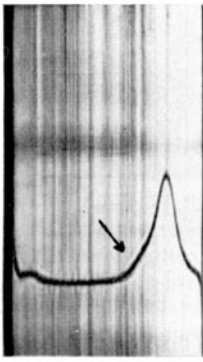
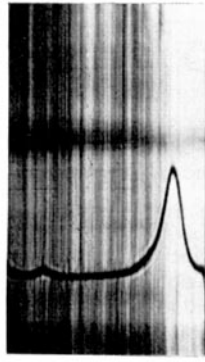
Fig. 1. Incorporation of the glycine- ^{14}C in the serum γ -globulins, 1 h and 2 h after introduction of amino acid.

The radioactivity values of the γ -globulinic fraction, electrophoretically separated, are expressed as $\%$ of the total radioactivity present on the strip (radioactivity of the 5 electrophoretic fractions).

Sham-thymectomized



Thymectomized



15th Day 30th Day

Fig. 2. Ultracentrifugal separation of serum-proteins shows that the macromolecular component is equally present in thymectomized and sham-thymectomized rats. On the 30th day a third component analogous to the G-component of human serum is appreciable (arrows).

Electrophoretic separation of serum-proteins and number of plasmocytes in bone marrow in thymectomized and sham-thymectomized rats; the obtained values are similar in the two groups of animals. Average values and standard deviation have been calculated

Days after operation	No. of animals	Total protein (g%)	Albumin (g%)	Globulins (g%)				No. of plasmocytes in bone marrow (% ₀₀)
				α ₁	α ₂	β	γ	
Thymectomy								
5	3	4.51 ± 0.23	2.36 ± 0.07	0.60 ± 0.02	0.35 ± 0.02	0.80 ± 0.03	0.40 ± 0.03	3.3 ± 0.2
15	3	4.76 ± 0.21	2.31 ± 0.05	0.68 ± 0.03	0.43 ± 0.02	0.87 ± 0.04	0.47 ± 0.02	3.7 ± 0.6
30	3	5.31 ± 0.25	2.26 ± 0.05	0.75 ± 0.04	0.50 ± 0.02	0.94 ± 0.02	0.86 ± 0.06	5.3 ± 0.4
60	3	5.41 ± 0.18	2.19 ± 0.03	0.79 ± 0.04	0.51 ± 0.01	0.96 ± 0.04	0.96 ± 0.04	9.0 ± 1.2
Sham-thymectomy								
5	3	4.40 ± 0.20	2.31 ± 0.04	0.56 ± 0.01	0.39 ± 0.03	0.71 ± 0.05	0.43 ± 0.02	2.7 ± 0.3
15	3	4.81 ± 0.23	2.26 ± 0.03	0.71 ± 0.04	0.46 ± 0.04	0.85 ± 0.03	0.53 ± 0.03	4.0 ± 0.5
30	3	5.33 ± 0.20	2.21 ± 0.04	0.80 ± 0.03	0.51 ± 0.02	1.00 ± 0.05	0.81 ± 0.04	5.0 ± 0.5
60	3	5.54 ± 0.17	2.23 ± 0.04	0.81 ± 0.02	0.50 ± 0.02	0.99 ± 0.04	1.01 ± 0.07	8.3 ± 1.1

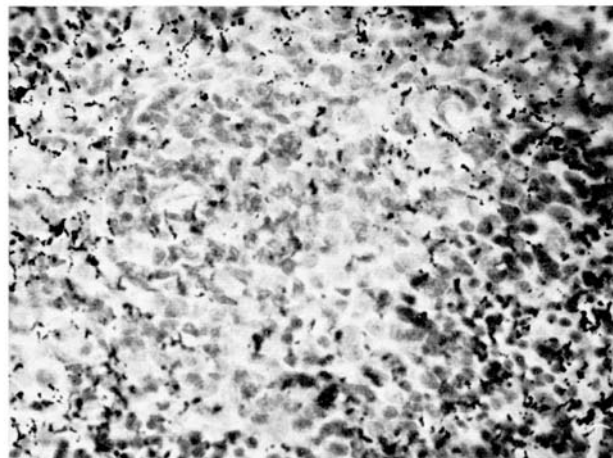
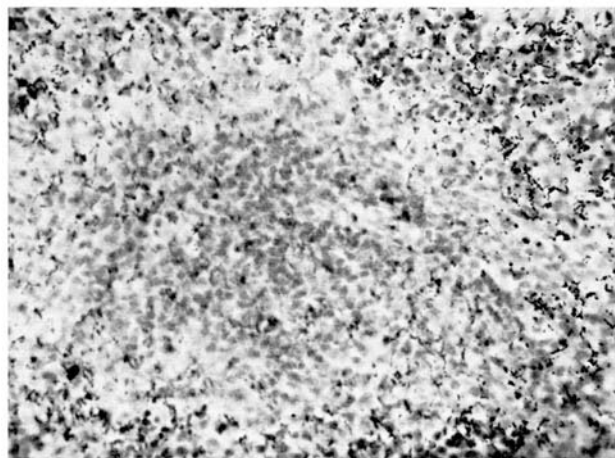


Fig. 3. 1 h after the injection of glycine- ^{14}C the splenic tissue becomes visibly black. The amino acid distribution is analogous in sham-thymectomized (above) and in thymectomized rats (below). It is possible to notice that the lymphatic follicles acquire glycine in a very low quantity. 30th day of experiment.

The data obtained confirm that the serum-proteic picture of a neonatally-thymectomized animal is unchanged; they also confirm that the process of serum-protidopoiesis remains within the limits of normality; particularly the synthesis of γ -globulins proceeds normally. Thus it is not in this way that the thymus influence on immunogenesis is mediated. The data on the 'humoral' intervention of thymus on immunogenesis¹³⁻¹⁵ and the consideration that the γ -globulinopoietic device is probably safe in the thymectomized animal, would suggest attributing to thymus a 'starter' role: in any case, the mode and the exact course of this start to the power of immunological response is still unknown.

Riassunto. In ratti timentomizzati alla nascita e seguiti per 2 mesi non si osservano alterazioni a carico del quadro sieroproteico: in particolare i valori delle γ -globuline e l'incorporazione di glicina- ^{14}C in tale frazione sono analoghi a quelli trovati in animali sottoposti a falsa timentomia.

Pure qualitativamente simile appare la distribuzione dell'aminoacido marcato nel parenchima splenico e in quello epatico dei ratti di entrambi i gruppi.

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Peristaltic Movement of the Tortoise Intestine

While 5-hydroxytryptamine (5-HT) has an excitatory effect on various intestinal musculatures^{1,2}, it was shown that on isolated tortoise intestine³ 5-HT had a weakly excitatory as well as an inhibitory effect. The different responsiveness to 5-HT in the tortoise intestine prompted us to study the nature of the peristaltic movement and its modification by 5-HT in this animal. In the present study intestinal strips of the tortoise, *Amyda japonica*, which is found in the waters in Korea, were used. To study peristaltic activity, the upper part of the intestine (ca. 5 cm in length) was dissected out and suspended in oxygenated Ringer solution at 25°C, according to the method of TRENDLENBURG⁴, or BELESIN and VARAGIC⁵. To investigate differences in responsiveness of longitudinal and circular muscle of the tortoise intestine, three preparations (circular, longitudinal, and the usual Magnus preparation) were suspended in the same 100 ml organ bath and their responses were examined simultaneously. The circular

preparation was made as follows: after cutting the intestine so as to make a ring of an intestinal segment 5 mm in width, 3 such rings were connected through cotton thread to make a chain, and the chain was suspended in the bath. The longitudinal preparation was made by cutting the intestine in the direction of the longitudinal fibres 5 cm in length and 5 mm in width.

Unlike mammalian intestinal strips, the peristaltic movement could not be induced either by increasing intraluminal pressure (up to 80 mm H₂O) or by the simultaneous application of pressure and 5-HT (up to 1 mg/ml) on the intraluminal surface (9 experiments). Intraluminal

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