

Le retard de l'induction neurogène dans l'aire opaque est au moins en grande partie imputable à la différence de structure existant entre l'ectoblaste de l'aire opaque et celui de l'aire pellucide. Une étude ultrastructurale mise en œuvre présentement dans notre laboratoire laisse entrevoir des différences de structure nettement perceptibles au moins dès le stade de la jeune ligne primitive. Cette différence réside évidemment dans le fait que ces deux sortes d'ectoblaste ont une destination totalement différente, l'ectoblaste périphérique participant par la suite à la formation des annexes fœtales. Cet ectoblaste, du fait de ses tendances intrinsèques, présente donc une certaine inertie au stimulus inducteur. D'après nos données plus anciennes (GALLERA²), la transformation de cet ectoblaste en ectoblaste qui aura le caractère de celui de l'aire pellucide ne s'observe qu'après 4 h environ de contact avec l'inducteur. Après 6 h, il a pris l'aspect d'un jeune neurectoblaste qui n'est pourtant pas capable de ce différencier de façon autonome. En effet, si l'action inductrice est interrompue à ce

moment, sa différenciation préneurale ou bien avorte complètement ou bien se traduit par la formation exclusive de la crête neurale.

Summary. Two grafts of Hensen's node were implanted on the same host blastoderm the first graft in the area pellucida, the second in the area opaca. At various intervals, the grafts were detached from the ectoblast and host blastoderms were allowed to grow in order to assess the type of inductive response produced by the ectoblast. These experiments show that 2–6 h were necessary to elicit neural induction in the ectoblast of the area pellucida, while 7–9 h were required to obtain neural induction in the area opaca.

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Effect of Thymectomy on the Differentiation and Phagocytic Activity of the Liver Sinusoidal Cells

Different effects of thymectomy on the reticulo-endothelial system have been reported in recent years. According to OSOBA and MILLER¹ and MILLER and HOWARD², thymectomized mice often show an increase in size and number of the Kupffer cells, accounting for the marked increase in phagocytic activity as measured by the clearance from the blood of colloidal carbon. However, a significant reduction in radiogold clearance was found in the thymectomized mice by FRIDRICH and SCHÄFER³. A similar reduction, although not statistically significant, was shown by FUMAROLA et al.⁴ in rats after thymectomy. Little difference in the clearance of colloidal carbon was observed by MORROW and DI LUZIO⁵; the rate of removal from the blood after an injection of 8 mg/100 g body weight was about the same in thymectomized and in sham-operated rats. Experiments with rats by CORSI et al.^{6,7} showed that after a single injection of 16 mg carbon/100 g body weight the rate of removal is significantly reduced after thymectomy: after repeated injections the granulopoietic index was appreciably increased. Under the latter conditions a much larger storage in the spleen was observed in comparison with normal animals^{7,8}. The increased phagocytic activity of the spleen could not account for the increased granulopoietic index, because the largest amount of the injected carbon was stored in the liver, both in normal and thymectomized animals⁸. On the other hand, no change in the hepatic endothelial cells was observed in non-injected thymectomized rats by WAKSMAN et al.⁹ and by CORSI et al.⁷.

A morphological study of the endothelial cells in the rat liver after i.v. injections of trypan blue is reported here.

Wistar albino rats were thymectomized 2 days after birth. At 5–6 weeks of age the animals were injected i.v. with 0.9% trypan blue in saline, 2 ml/100 g body weight, and were killed 1 h later. In a number of cases the same dose of dye was repeated with a 1 h interval: the animals were killed 1 h after the second injection. Normal rats of the same age and body weight were used as controls. The liver was fixed in the Susa solution, embedded in paraffin and stained with haematoxylin and eosin. The cells of the hepatic sinusoids were studied with a view to evaluate the percentage of the cells showing a swollen

cytoplasm and a large and pale nucleus, i.e. the peculiarities of the Kupffer cells. When the characters were not typical, density of the nucleus, rather than the size and shape of the cell, was used as a criterion for classification: cells with a pale nucleus were classified as Kupffer cells. An evaluation was made also of the percentage of sinusoidal cells containing the injected dye. The counts were made in both principal lobes, on 400 cells in each animal. The results are summarized in Tables I and II.

No significant difference was observed between normal and thymectomized animals after 1 injection of the dye. The number of Kupffer cells was about the same as in normal and thymectomized rats which received no injection. A marked increase in the number both of Kupffer cells and of vitally stained cells was observed in the thymectomized rats after 2 injections of trypan blue. It is worth noting that transitional appearances between the typical endothelial cells and the typical Kupffer cells were frequent in all livers. The dye could be detected in most Kupffer cells and occasionally also in typical endothelial cells.

The present results seem to favour the view that the endothelium-like cell of the liver sinusoids may transform under circumstances into a Kupffer cell^{10,11}. Differentiation into Kupffer cells is not increased by thymectomy

¹ D. OSOBA and J. F. A. P. MILLER, *J. exp. Med.* 119, 177 (1964).

² J. F. A. P. MILLER and J. G. HOWARD, *J. Reticuloendothelial Soc.* 1, 369 (1964).

³ R. FRIDRICH and M. SCHÄFER, *Experientia* 22, 552 (1966).

⁴ D. FUMAROLA, L. MARCUCCIO and S. SALAMANNA, *Haematologica* 53, 481 (1968).

⁵ S. H. MORROW and N. R. DI LUZIO, *Nature, Lond.* 205, 193 (1965).

⁶ A. CORSI and G. V. GIUSTI, *Nature, Lond.* 213, 618 (1967).

⁷ A. CORSI, G. V. GIUSTI, A. L. GRANATA and L. RODIGHIERO, *Sperimentale* 118, 19 (1968).

⁸ G. V. GIUSTI and A. CORSI, *Nature, Lond.* 214, 916 (1967).

⁹ B. H. WAKSMAN, B. G. ARNASON and B. D. JANKOVIC, *J. exp. Med.* 116, 187 (1962).

¹⁰ E. LETTERER, *Allgemeine Pathologie* (Thieme, Stuttgart 1959).

¹¹ W. BLOOM and D. W. FAWCETT, *A Textbook of Histology* (Saunders, London 1968).

Table I. Percentage of sinusoidal cells differentiated into Kupffer cells in the liver of normal and thymectomized rats

Normal			Thymectomized		
Not injected	After 1 dose	After 2 doses	Not injected	After 1 dose	After 2 doses
55.9 \pm 1.22 (6)	55.6 \pm 0.71 (5)	56.9 \pm 1.08 (6)	55.3 \pm 1.54 (6)	55.9 \pm 0.65 (5)	74.8 \pm 1.74 (6)

Numbers are mean values \pm S.E. The figures in brackets refer to the numbers of animals.

Table II. Percentage of vitally stained sinusoidal cells in the liver of normal and thymectomized rats injected with trypan blue

Normal		Thymectomized	
After 1 dose	After 2 doses	After 1 dose	After 2 doses
30.2 \pm 0.80 (5)	37.2 \pm 1.54 (6)	29.0 \pm 1.14 (5)	70.3 \pm 2.17 (6)

Numbers are mean values \pm S.E. The figures in brackets refer to the numbers of animals.

in not abnormally stimulated rats. However, one consequence of thymectomy is an increase in differentiation under the influence of repeated stimuli. This is coincidental with a more extensive phagocytic response of the sinusoidal cells and seems to account well for the increased rate of removal of colloidal carbon from the blood in rats after repeated injections of the dye⁶. The change observed in the liver sinusoidal cells may be related to the changes caused by thymectomy on haemopoiesis, both in the spleen and in the bone marrow^{2, 12, 13}. It appears that the thymus has a wide spread influence upon differentiation of cells with mesenchymal potentialities, also outside the lymphoid organs.

Riassunto. La timectomia non provoca iperplasia del sistema reticolo-endoteliale nel fegato del ratto. Tuttavia

nel ratto timectomizzato, sottoposto allo stimolo di ripetute iniezioni di blu tripan, la risposta fagocitaria da parte delle cellule dei seni epatici e la corrispondente differenziazione morfologica in cellule di Kupffer sono maggiori che di norma. Il fenomeno è una riprova dell'estesa azione del timo sulla differenziazione delle cellule mesenchimali.

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¹² J. F. A. P. MILLER, M. BLOCK, D. T. ROWLANDS and P. KING, *Proc. Soc. exp. Biol. Med.* 118, 916 (1965).

¹³ A. CORSI and G. V. GIUSTI, *Nature* 216, 493 (1967).

³H-Vitamin A and Rat Thyroid: Autoradiographic Observations

As part of a general study of the autoradiographic localization of ³H-vitamin A and/or its metabolic derivatives in animal tissues, the thyroid gland was examined. This report presents autoradiographic evidence for the presence of radioactivity derived from ³H-vitamin A in the follicular cells and colloid of rat thyroid.

Materials and methods. Weanling albino rats of both sexes were injected i.p. with 500 μ g of retinyl-11,12-³H₂ acetate (³H-vitamin A acetate), specific activity — 213 μ Ci/mg, prepared as an aqueous dispersion with 15% Tween 20. The animals were killed by cervical dislocation 4 h after injection. The thyroid glands were removed and processed for autoradiographs (ARGs) by 2 methods:

a) Ordinary ARGs. Half the thyroid was immediately fixed in Bouin's solution and processed by the standard histologic techniques. Paraffin sections at 4–6 μ m thick were cut. Autoradiographs were prepared using a liquid emulsion technique similar to that described by MESSIER and LEBLOND¹ and were exposed in total darkness for 4 months at 5°C. The solvents used in this procedure remove unbound lipid and water-soluble tissue components. Therefore, exposed grains of the ARGs represent only the insoluble labeled compounds bound to the tissue.

b) Soluble-compound ARGs. The other half of the fresh thyroid tissue was immediately frozen in isopentane immersed in liquid nitrogen and further processed by the cryostat-microtomy technique described by APPLETON².

The ARGs were exposed in total darkness for 4 months at — 25°C. Since this procedure does not require the use of solvents or fixatives until after the period of exposure is completed, the exposed photographic grains of these ARGs represent both soluble and insoluble labeled material preserved in situ in the thyroid.

Results. Figure 1 illustrates an ordinary ARG of the thyroid. Sparsely scattered radioactivity is seen associated with the follicular cells and an occasional grain is present at the extreme periphery of the colloid adjacent to the apical surface of the follicular cell. In addition, small numbers of grains are found in the stroma, especially in vascular channels.

In contrast, the soluble-compound ARG of the thyroid (Figure 2) shows a generally higher concentration of radioactivity associated with the follicular cells and in the stroma as compared to the ordinary ARG. The most striking feature of this soluble-compound ARG preparation is the presence of many grains directly over the follicular colloid.

Discussion. Studies of the relationship between vitamin A and the thyroid gland have indicated that the vitamin A

¹ B. MESSIER and C. P. LEBLOND, *Proc. Soc. exp. Biol. Med.* 96, 7 (1957).

² T. C. APPLETON, *Jl R. microsc. Soc.* 83, 277 (1964).