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 ± 1.22 (6)	55.6 ± 0.71 (5)	56.9 ± 1.08 (6)	55.3 ± 1.54 (6)	55.9 ± 0.65 (5)	74.8 ± 1.74 (6)

Numbers are mean values ± 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 ± 0.80 (5)	37.2 ± 1.54 (6)	29.0 ± 1.14 (5)	70.3 ± 2.17 (6)

Numbers are mean values ± 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 dye[6]. 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 mesenchimal 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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^{3}H-Vitamin A and Rat Thyroid: Autoradiographic Observations

As part of a general study of the autoradiographic localization of ^{3}H-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 ^{3}H-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 µg of retinyl-11,12-$^3H_2$ acetate (^{3}H-vitamin A acetate), specific activity — 213 µCi/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 µm thick were cut. Autoradiographs were prepared using a liquid emulsion technique similar to that described by Messier and Leblond[1] 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 Appleton[2]. 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

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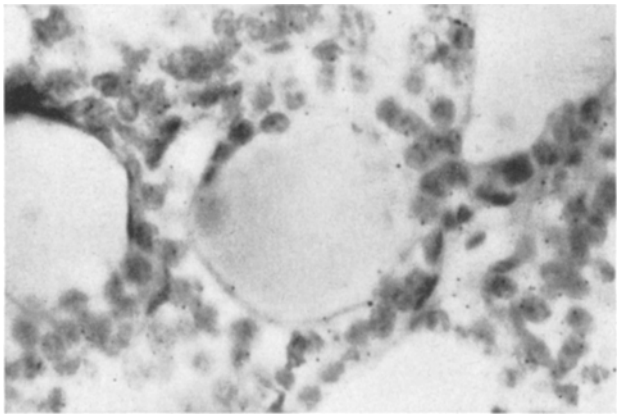

Fig. 1. Ordinary autoradiograph of rat thyroid. Scattered radioactivity derived from ^{3}H-retinyl acetate is seen associated with follicular cells and stroma. Stained with hematoxylin and eosin. ×1000.

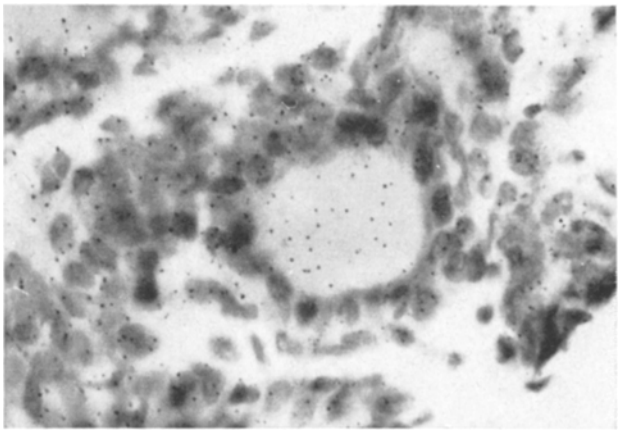

Fig. 2. Soluble-compound autoradiograph of rat thyroid. A higher concentration of radioactivity derived from ^{3}H-retinyl acetate is seen over follicular cells and stroma. Note particularly, concentrations of radioactivity over the colloid. Stained with hematoxylin and eosin. ×1000.

status of the animal can influence the size, weight and/or histological condition of the thyroid[3–10]. For example, JUNGHERR et al.[7] reported that thyroid hyperplasia occurred in bull calves as a result of vitamin A deficiency. In contrast, CARPENTER and SAMPSON[8] found that hypervitaminosis A produced thyroid follicles which were reduced in size, irregular in shape and contained only small amounts of colloid. Despite the apparent relationship, attempts to demonstrate the presence of vitamin A in the thyroid were not successfull. POPPER[11] and POPPER and GREENBERG[12] were unable to detect vitamin A fluorescence in rat thyroid using fluorescence microscopy techniques. The present study is the first to show that radioactivity derived from ^{3}H-retinyl acetate may be autoradiographically located in the follicular cells and colloid of rat thyroid. Furthermore, it appears that the colloid radioactivity is in a relatively soluble form, since it is seen only in freshly frozen tissue and is lost upon fixation and washing of the tissue, while at least a portion of the radioactivity associated with the follicular cells seems to be bound to the cells.

It is unfortunate that the results of the present study can do no more than locate radioactivity derived from injected vitamin A and not tell us more about the nature of the substance(s) it represents. It may be that the radioactivity does represent very small amounts of the vitamin A molecule itself, but it is equally as likely that the radioactivity represents a metabolic derivative of the vitamin or only a fragment of the original molecule or even a totally unrelated substance to which the ^{3}H isotope has been attached or has been exchanged for unlabeled hydrogen. In any event, the results of the present experiment warrant further investigation[13].

Résumé. Des études autoradiographiques démontrent la présence de radioactivité dans les cellules folliculaires et dans la colloïde de la thyroïde du rat à la suite de l'injection intrapéritonéale de l'acétate rétinyl-^{3}H (acétate de la vitamine A). La radioactivité de la colloïde se manifeste seulement dans le tissu congelé vivant; elle disparaît lors de la préparation histologique ordinaire. Par contre, une partie de cette radioactivité reste liée aux cellules folliculaires, même après la préparation histologique habituelle.

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Specific Stages of Cellular Response to Homeostatic Control During Diethylnitrosamine-Induced Liver Carcinogenesis

After application of a carcinogen, a latency period usually precedes the appearance of morphologically recognizable tumors. Experimental observations indicate that this preneoplastic period is a non-uniform interval which can be subdivided into specific stages by analyzing the stepwise cellular disorientation from organ-specific homeostatic control mechanisms.

Experiments were performed to obtain insight into the growth-behaviour of preneoplastic cells by the combined application of autoradiography and enzyme histo-