glycemia was: 135 ± 8, 98 ± 2 and 85 ± 4 mg/100ml. Hyperthyroid animals lost 24% of their body-weight during the treatment, whereas those injected with ^{131}I put on 34%.

T_3 caused an increase of the contents and concentration of NE in the heart and a decrease of E concentration in the 2 organs studied. In ^{131}I-treated animals, there was an increase of NE concentration and of E contents and concentration in the submaxillary glands, whereas E concentration increased in myocardium (Table I).

^{131}I did not alter the glycogen of the organs studied. Administration of isoproterenol to control rats significantly decreased glycogen both in the submaxillary gland and the heart 30 min after being injected, which effect was not observed in hypothyriod animals subjected to the same treatment (Table II).

Table II. Effects of isoproterenol and ^{131}I on the concentration of glycogen of the submaxillary gland and the heart

Experiment Glycogen		Submaxillary gland (μg/100 g ± SEM)	Heart (μg/100 g ± SEM)
Control	(8)	46.1 ± 1.8	88.3 ± 3.2
Isoproterenol	(8)	14.5 ± 1.1 [a]	28.5 ± 0.7 [a]
^{131}I	(8)	41.9 ± 3.6	104.6 ± 3.9
^{131}I + Isoproterenol	(8)	30.5 ± 4.2	98.4 ± 3.7

(), number of cases; SEM, standard error of the mean. [a], $P < 0.001$.

Discussion. T_3 caused in the rat's submaxillary glands a hyperactivity characterized by a maximum increase of secretory responses[16] and hypertrophy noticeable not only on account of the increase in weight but also owing to the histological modifications observed[17]. In the heart, there was an increase in weight. The fact that the amount and concentration of the heart NE is increased would indicate a decrease of its turn-over and release. The decrease of E concentration in both organs is partly to be attributed to the hypertrophy observed and to a higher release coinciding with the metabolic and hemodynamic effects of hyperthyroidism.

In ^{131}I-treted animals there was a decrease in the activity of both submaxillary gland and heart, with a loss of weight. The increase of NE concentration in the submaxillary gland may be attributed to the modifications in the weight of the gland because the amount keeps constant. On the other hand, there was a marked increase (concentration and content) of E which might indicate that its release or catabolism have decreased. The increase of E concentration in myocardium – probably owing to a lower release or catabolism – are in agreement with the metabolic and cardiocirculatory events of hypothyroidism.

Glycogen in the submaxillary gland and heart, which was not modified in ^{131}I-treated animals inspite of the variations in the concentration of endogenous catecholamines, was altered by an exogenous one, isoproterenol, in the organs of control animals, but this effect was not noticed in ^{131}I-treated animals. This suggests that thyroid hormones could modify the sensitivity of adrenergic receptors.

In short, it might be said that the heterogeneity of opinions regarding the thyroid and sympathetic function might derive from the different behavior of the several tissues having their own metabolism, so that NE and E turn-over would be affected according to the predominance of their metabolic or hemodynamic functions. It is possible that the action of thyroid hormones on catecholamines operates at different levels: modification of storages, increase in the sensitivity of receptors and inhibition of their catabolism.

Resumen. Se estudió en ratas Wistar macho, el efecto de la triiodotironina y el ^{131}I sobre el contenido y concentración noradrenalina y adrenalina, en glándula submaxilar y corazón. Se estudió también, el efecto del isoproterenol sobre el glucógeno de ambos órganos en animales controles e hipotiroideos experimentales. Se observó un comportamiento diferente en los depósitos de noradrenalina y adrenalina en los órganos estudiados. La triiodotironina produjo una disminución en la liberación o catabolismo de las catecolaminas y el ^{131}I parece causar el efecto opuesto y una disminución de la sensibilidad de los recptores adrenérgicos.

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Triiodothyronine and Thyroxine: Induction of Mitosis in Adult Frogs[1]

Triiodothyronine (T_3) and thyroxine (T_4) are able to induce metamorphosis in larval amphibia[2]. In so doing, these hormones, as part of their overall effect, instigate mitotic activity in a number of tissues[3–6]. KALTENBACH and HOBBS[6] indicated that the action of thyroxine upon tadpole ocular tissue, such as retina, cornea and lens, might be direct. When cholesterol pellets containing thyroxine were introduced into the orbit, mitosis was stimulated in the tissues mentioned. While there are also reports showing the iodinated phenols can trigger proliferation in adult mammalian tissues[7–9], it has not been considered likely that these materials effect the same phenomenon

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in adult amphibian tissue[10]. This opinion is incorrect. As our evidence will show, T_3 and T_4 elicit hyperplastic responses in a variety of adult amphibian organs.

In a separate report published elsewhere[11], we showed there is a seasonal variation in mitotic activity in the lenses of frogs. As part of an effort to determine the cause(s) of the phenomenon, the effects of hormones on mitotic activity have been investigated. The possibility was considered that seasonal variation might be due to a triggering of activity in the hypothalamus and pituitary; the products of the latter might then cause secretion of materials which would affect proliferative activity in the lens. Among the hormones which we tested were T_3 and T_4. Both of these were found to raise the mitotic index of the lens in adult *Rana pipiens* and *Rana catesbeiana*.

Materials and methods. Frogs received daily i.p. injections of 0.5 µg/g triiodothyronine suspended in 1% Tween 80. ^{3}H-Thymidine was administered via the same route at a dose of 1.5 µc/g (spec. activity 6.0 Ci/m*M*). For exposure to ^{3}H-actinomycin D, the eyes were enucleated; the lenses where then removed and incubated for 30 min in Medium A-199 containing the isotope at a concentration of 3.0 µc/ml (spec. activity 8.4 Ci/m*M*). To determine mitotic and isotope labelling activity, lens epithelial whole mounts were prepared and autoradiographed as has been previously described[12]. All other tissues were fixed in Bouins solution, embedded in paraffin and sectioned at 10 µm. Labeled DNA was extracted from fresh tissue by a modification of the SCHNEIDER nucleic acid isolation technique[13] and quantified by the diphenylamine reaction[14]. The ^{3}H-Thymidine incorporation was determined by scintillation counting in a Packard Tri-carb liquid scintillation spectrometer.

Results and discussion. There is a population of *Rana pipiens*, obtainable from certain regions of South Dakota which manifest virtually no mitotic activity in the germinative zone of the lens epithelium. In these exceptional animals, injection of triiodothyronine for 2 weeks raised the mitotic count to approximately 300 figures. The pertinent data are shown in Figure 1. Labelling experiments with tritiated thymidine show DNA synthesis begins about 5 days after hormone treatment has been initiated. In populations of frogs which already show mitotic activity prior to hormone administration, the prereplicative period usually amounts to 2 days. In continuous labelling experiments (^{3}H-Thymidine twice a day for 6 days), better than 50% of the lens epithelial cells were found to contain isotope.

It did not seem likely that the lens would be the only organ which would be affected by the presence of T_3. A study of a variety of organs from winter (January-February) South Dakota animals, including liver, kidney, skin intestine, cornea, oviduct, nictitating membrane and spleen, showed that the T_3 was also able to evoke DNA synthesis and mitosis in them. Autoradiographic and biochemical analyses showed that in all the tissues investigated, save for muscle and brain, there is a strong increase in incorporation. The Table reveals the amount of DNA

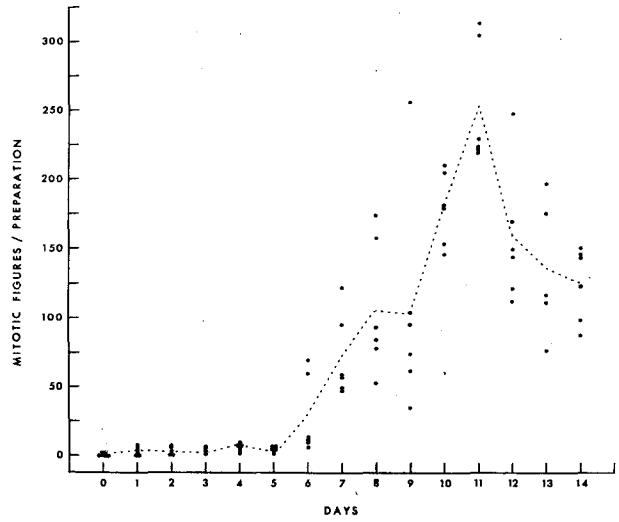

Fig. 1. The effect of daily i.p. injections of T_3 (0.5 µg/g) on lenticular mitotic activity in South Dakota *Rana pipiens*. Each point represents the total number of mitotic figures for a single epithelial whole-mount preparation. The dashed line intercepts the mean values.

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The incorporation of ^{3}H-Thymidine, expressed as counts/min/µg DNA, into various tissues of T_3-treated (experimental) animals and untreated controls

Tissue	No. of experiments	Experimental (counts/min/µg DNA)	Control (counts/min/µg DNA)	E/C
Cornea	2	13.72[a]	0.15[a]	91.47
Intestine	2	60.45 ± 20.84	1.38 ± 1.25	43.68
Kidney	2	79.45 ± 49.55	1.21 ± 0.35	65.66
Liver	2	22.04 ± 11.45	0.29 ± 0.24	76.00
Nictitans	2	26.85[a]	0.86[a]	31.11
Oviduct	2	9.70 ± 0.01	0.40 ± 0.06	24.48
Skin	2	49.19 ± 2.06	0.67 ± 0.08	73.41
Spleen	2	71.93[a]	0.53[a]	136.23

The treated frogs received daily i.p. injections of T_3 (0.5 µg/g) for 6 days. 2 h prior to sacrifice both treated and control frogs were injected with ^{3}H-Thymidine (1.5 µCi/g; spec. activity 6.0 Ci/m*M*). Each value represents the means of 2 animals ± standard error of the mean. [a] Indicates that the sample was pooled.

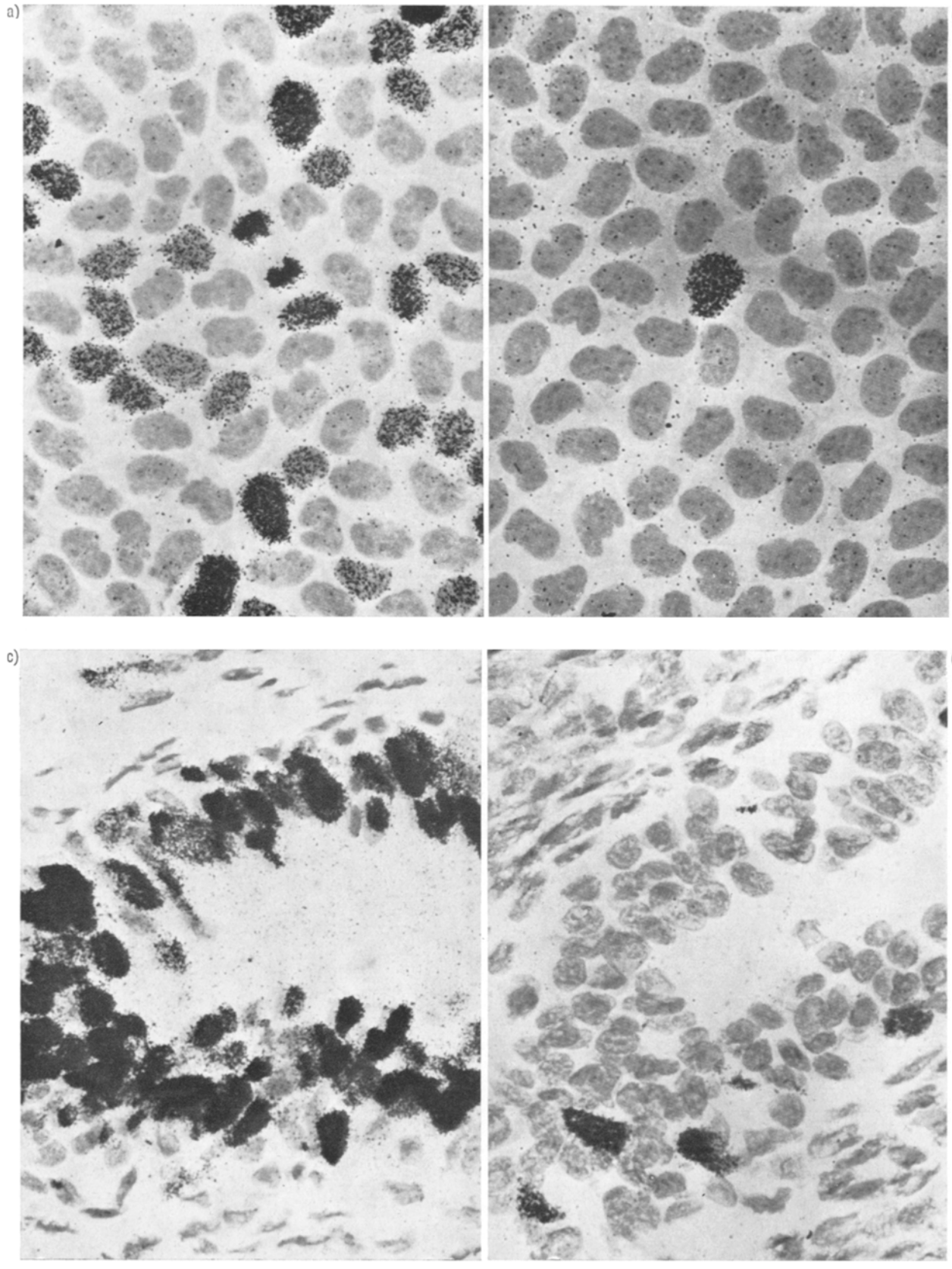

Fig. 2. Autoradiograms of tissues after they had been exposed to ^{3}H-Thymidine for 48 h (1.5 μCi/g; spec. activity, 6.0 Ci/mM). The treated animals received daily i.p. injections of T_3 (0.5 μg/g) for 6 days prior to sacrifice. The control animals received only the vehicle (1% Tween 80). a) Whole-mount preparation of the lens epithelium of a treated frog. Note the labeled mitotic figures. b) Epithelial whole-mount of a control. Harris Hematoxylin. ×500. c) Cryostat section (10 μm) of the small intestine of an experimental animal. d) Again, the number of incorporating nuclei is greater in the experimental than in the control. Feulgen. ×500.

synthesis as determined by ^{3}H-Thymidine incorporation. (The isotope was administered 2 h prior to sacrifice.) Removal of RNA did not decrease the ratio of experimental to control radioactivity.

In Figure 2 radioactive nuclei can be seen in the experimental (2a) but not in the control; radioactive mitotic figures were also seen more frequently in the former than in the latter.

Experiments pursued during the springtime (March-April) on populations of northern Canadian origin gave different results. These animals had almost no mitotic figures in their lenses when they first arrived at the laboratory but the mitotic index began to increase during the ensuing weeks. When the organisms were given T_3 only the lens, among the organs studied, displayed more hyperplastic activity. This result may be due to a temperature change witnessed by the animals in their native locale or in transit to the laboratory.

In a previous report, we suggested that the increase in temperature occuring during the spring might cause anterior pituitary outflow mediated by hypothalamic releasing factors[15]. The 'non-responsive' organisms may have already produced enough TSH to arouse considerable mitotic activity in most tissues so that additional T_3 could produce no further increase in the magnitude of the effect. This possibility will be tested in experiments upon hypophysectomized and thyroidectomized specimens but is, in any event, a prediction of the thermoendocrine hypothesis we presented elsewhere[15].

The question whether the amount of T_3 and T_4 administered is in the physiological as opposed to the pharmacological range has yet to be satisfactorily resolved. The frogs do, however, have enough endogenous T_3 and/or T_4 to produce the effects observed. Thus, when TSH (0.002 U/g) was injected, mitotic activity in the lens increased by a factor of three. So far we have not studied this response in other organs.

Despite much study the mechanism of action of T_3 and T_4 is unclear[16]. Initial data we have secured suggests that genomic activation may account for our findings. Within an hour after T_3 administration, the nuclei of lens epithelial cells exposed to ^{3}H-actinomycin D showed an increased level of binding of the antibiotic. It has been suggested that actinomycin D can be used as a probe for gene activation[17]. In earlier studies of injured and cultured frog lenses, we have shown that increased ^{3}H-actinomycin D binding takes place in advance of heightened syntheses of RNA[18–20]. Whether this will also prove to be the case in the T_3 stimulated system is at present unknown but the matter will be investigated shortly.

On the basis of the initial data it seems, the increase in the number of division figures takes place because more cells have entered upon active traverse of the generative cycle (i.e., the growth fraction has been amplified by the hormone). In the South Dakota frogs which do not possess any measurable mitotic index in the lens, this must be the case for almost all the cells (as determined by microspectrophotometry and autoradiography) exist in G_1. As we will show explicitly, in a later publication, introduction of T_3 shifts many cells through the S, G_2 and M states.

Zusammenfassung. Nachweis, dass bei adulten, reifen Fröschen Thyroxin und Triiodothyronin die mitotische Aktivität und die DNS-Synthese in verschiedenen Organen (Augenlinse, mit Epithel, Niere Darm, Leber, Milz, Haut, Horn- und Nickhaut) zu stimulieren vermag.

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A Comparative Study of the Calorigenic Action of Noradrenaline in the Rat and Ground Squirrel Adapted to Different Temperatures

The effect of noradrenaline was examined in rat, guinea-pig and mouse[1–4, 5], but in hibernators this effect was not systematically studied. Some data on the seasonal changes concern the sensitivity of a hibernator to injected noradrenaline[6].

Materials and methods. Observations were made on 4 groups of adult albino male rats of Wistar strain and on 4 groups of ground squirrels weighing 200–260 g, each consisting of 8 to 10 animals. Rats were adapted to 4 °C, 30 °C or 36 °C and the ground squirrels to 30 °C or 36 °C for about 4 weeks. One group of ground squirrels was examined after being artificially aroused from hibernation in October. Noradrenaline (Galenika) was injected i.p. in doses of 1.6 mg/kg of body wt. Control groups were injected only with physiological solution. The oxygen consumption was measured individually in a gas analyser[7], 30 min before the injection of noradrenaline and after the injection over the period of the duration of the effect. All measurements were realised at 30 °C.

Results and discussion. Results are expressed in calories per m^2/24 h and summarized in Figures 1 and 2 together with the initial values obtained prior to the injection. In the rats adapted to 4 °C or to 30 °C, a significant increase in the heat production was observed 10 min after the injection of noradrenaline, being 46% and 30% respectively. Compared with the data of some other authors

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