trapped in the outlet of the ovariole (Figure E) being unable to pass out. In other instances, the eggs had apparently been dispatched but the vitellarium failed to shrink and remained sack-like (Figure D). On the other hand, the shrinkage of the vitellarium sometimes began before the terminal oocyte was mature enough to be deposited (Figure C).

The effects of juvenile hormone on the differentiation processes in the ovaries of *Thermobia* resemble the morphogenetic action exerted by the hormone during metamorphosis. The hormone influences only the tissues that have not yet passed a certain point of developmental determination[6]. The differentiation processes in the gonads of pterygote insects seem to be determined and often also accomplished prior to the imaginal ecdysis and are therefore affected only if the hormone or its analogues are administered to larvae[7–9] or pupae[10]. In the firebrat, the differentiation of oogonia and prefollicular cells continues in the adult stage[11] and, as shown in the present study, may therefore be fully impeded by the juvenile hormone. The defects in egg deposition are difficult to interpret. Their possible cause may be either malformation of the follicular epithelium (incomplete number of follicular cells etc.) or disturbances in the hormonal interplay that controls the individual phases of reproduction.

Zusammenfassung. Applikation von 1–100 µg irgendeines der *2 Cecropia*-Juvenilhormone auf die erwachsenen Weibchen von *Thermobia domestica* verursacht Störungen des Differenzierungs- und Eiablageprozesses in den Ovarien und erniedrigt demzufolge die Vermehrungsfähigkeit der Tiere.

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RNA Metabolism in Brain of Suckling Normal and Hypothyroid Rats[1]

It is well established that in the rat, neonatal thyroid deficiency markedly affects cerebral protein synthesis[2,3]. Although this alteration has been considered as the underlying factor of the biochemical, histological and behavioural alterations accompanying neonatal hypothyroidism[4], the mechanisms by which thyroid hormone would stimulate protein synthesis in developing brain have not yet been clearly elucidated.

It is known that RNA metabolism plays a key role in the control of processes of growth and development, and thyroid hormone regulates such processes by stimulating RNA synthesis in its target tissues[5]; it has also been demonstrated that RNA synthesis is very high in the immature brain, decreasing rapidly during early postnatal maturation[6,7]. Considering that in the rat brain, the maturational effects of thyroid hormone take place during the first 10–12 postnatal days[8], it seemed interesting to study the effects of neonatal thyroidectomy in the rat upon brain RNA metabolism, at 10 days of age, when brain morphogenesis is very active.

Materials and methods. Rats from a highly inbred Wistar strain were used throughout, normal controls and neonataly thyroidectomized animals being prepared and kept as previously reported[4]. 5-(^{3}H)-orotic acid (sp. act. 15 Ci/mmole) was obtained from the Commissariat à l'Energie Atomique (France), and all other chemicals were reagent grade.

At 10 days of age, each animal was injected s.c. with a labelled orotic acid solution (1 µC/g body weight), and killed by decapitation 1, 3 or 24 h after the injection. The cerebral hemispheres from 3 brains were pooled in each experiment and slightly homogenized in 10 volumes of cold 0.32 *M* sucrose, containing 1 m*M* $MgCl_2$ and 0.4 m*M* potassium phosphate buffer (pH 6.7). The homogenate (HT) was used to isolated crude nuclear (CN), crude mitochondrial (MIT), microsomal (MIC), and supernatant (S) fractions according to SEMINARIO et al.[9], except that pellets were washed with homogenizing solution and crude nuclei were washed 3 times. Purified nuclei (PN) were obtained from CN following the procedure of BALAZS and COCKS[10]. The final pellets were resuspended in homogenizing solution; aliquots of these suspensions, as well as one of S and HT, were used to determine RNA according to MUNRO and FLECK[11]. Radioactivity incorporated in RNA was measured in each fraction as follows: cold trichloroacetic (TCA) solution was added to give a final TCA concentration of 10%; after centrifugation in the cold, the precipitate was washed once with cold 10% TCA and

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twice with ethanol: ethyl ether (3:1 v/v). The residue, which contained RNA, was dissolved in a small volume of 2 *N* NaOH, at 37 °C and transferred to a counting vial containing 12 ml of BRAY's solution[12]. 700 mg of Cab-o-Sil (Cabot, Argentina) were added with the purpose of obtaining a homogeneous suspension. Further purification of RNA was considered unnecessary in view of the observation of BALAZS and COCKS[10], showing that ^{14}C of DNA was less than 1% of that of RNA 23 h after the injection of ^{14}C orotic acid. Furthermore, in some parallel experiments, the results were compared with those obtained by phenol-dodecyl sulphate extraction and ethanol precipitation of RNA according to MURTHY[13], showing a very close agreement. In each subcellular fraction, the specific radioactivity (SR) was expressed as dpm/mg RNA.

Radioactivity of the acid-soluble pool was measured in the TCA supernatant of an aliquot of HT after 5 washings with ethyl ether and drying the aqueous phase under vacuum at low temperature; the residue dissolved in 12 ml of BRAY's solution and transferred to a counting vial. The results, expressed in dpm/mg wet weight of tissue, was considered as an indication of the entry into the brain of labelled orotic acid and its metabolites. Although isotopic exchange cannot be discarded, this consideration is valid for the purpose of comparison of both experimental groups. The total radioactivity of HT, also expressed in dpm/mg wet weight of tissue, was estimated by the sum of that present in the acid-soluble and acid-insoluble fractions. All samples were counted in a Packard Spectrometer (model 3003), with an efficiency of 18–25%. Counts were corrected to 100% efficiency by the channel's ratio method.

Results. At 10 days of age, both the brain and body weights were unaffected by neonatal thyroid deficiency; thus, the brain weight calculated as a percent of the body weight was similar in both groups (4.25 ± 0.31 for normal, and 4.60 ± 0.26 for hypothyroid rats).

The total brain RNA content per gram was slightly lower in hypothyroid than in normal animals, the difference not being significant. However, in the microsomal fraction, a significant decrease (14%) was observed in hypothyroids as compared to normals (Table I).

In both groups, the total ^{3}H content increased at similar rates following the injection of labelled orotic acid (Figure 1A). The percent of total ^{3}H incorporated into RNA was similar in both groups throughout 24 hs. It increased slowly and at the longest time interval represented only 45% of the total radioactivity (Figure 1B). Although the rates of increase in the labelling of the acid-soluble pool were similar in both groups, the ^{3}H content of this pool was higher in hypothyroid than in normal brains 1 h (11%) and 3 h (16%) after the injection, the difference disappearing at 24 h (Table II).

No significant difference was observed in the percentage distribution of the label among subcellular fractions with time (Figure 2). In both groups, at the earliest time interval, more than 85% of the RNA in subcellular fractions was recovered in the nuclear fraction (CN), and subsequent losses of the label from the nuclei in the first 24 h can be accounted for by the increased labelling of cytoplasmic RNA, which at 24 h represents 55–65% of the ^{3}H incorporated into RNA.

The values of SR also showed that in both groups the labelling of RNA in PN was much more rapid than that of the other fractions (Table II). As a result of thyroid deficiency it was found that 1 h after injection the value of the SR of RNA in PN was significantly lower (20%) than that found in normals. In MIC, the increase of SR of RNA was also slower in hypothyroid animals. Thus at 1 and 3 h lower values were obtained as compared to normals (44% and 13%, respectively). All these differences, however, disappeared at 24 h. The SR of mitochondrial RNA was lowered by hormonal deprivation, but only after a longer time interval: 3 h (27%) and 24 h (32%). The values of soluble RNA were similar for both animal groups.

Discussion. In the present work, brain RNA synthesis has been studied after the subcutaneous administration of labelled orotic acid. In spite of the poor cerebral uptake, our results are in good agreement with those obtained in autoradiographic studies following the subcutaneous injection of ^{3}H-uridine into 10-days-old mice[14]. In normal and hypothyroid brains, they show the nuclear origin of newly synthetized RNA and subsequent transfer to the cytoplasm, which is in accordance with previous observations of other workers[10,15], who used the intracerebral injection of the labelled precursor. Our finding showing that the SR of nuclear RNA is by far the highest, even after 24 h of the injection, also coincides with results reported by BALAZS and COCKS[10], who found that the SR of nuclear RNA does not fall below that of cytoplasmic fractions after 48 h of the injection. This differenciates the brain cells from those of other tissues, like the liver[16], in which the SR of nuclear RNA increases and decreases rapidly.

The most significant alteration observed in hypothyroid brain was the decrease in the SR of both nuclear and mi-

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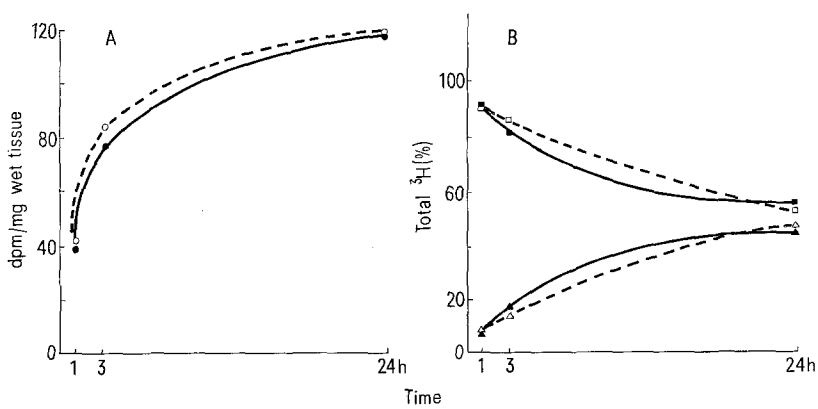

Fig. 1. Total radioactivity in whole brain and its distribution between acid-soluble and acid-insoluble fractions. A) Total ^{3}H content: (●—●) normal controls; (○--○) hypothyroids. B) Percentage distribution of total ^{3}H. Acid-soluble pool: (■—■) normal controls; (□--□) hypothyroids. Acid-insoluble residue (RNA): (▲—▲) normal controls; (△--△) hypothyroids. Each point represents the mean of 4–5 experiments.

Table I. RNA content in brain and its subcellular fractions from normal and hypothyroid rats

Fraction	Normal	Hypothyroid	*P* value
HT	2.78 ± 0.13	2.57 ± 0.07	
CN	0.51 ± 0.08	0.48 ± 0.01	
(PN)	(0.22 ± 0.01)	(0.23 ± 0.01)	
MIT	0.47 ± 0.02	0.45 ± 0.01	
MIC	1.05 ± 0.06	0.91 ± 0.07	<0.001
S	0.29 ± 0.01	0.27 ± 0.01	
Recovery	73.0%	75.5%	

The results are expressed in mg/g wet weight of tissue, and are the means ± S.E.M. of 9 individual experiments for each group. They were compared by means of the Student's *t*-test, and when the difference is significant, *P* value is indicated in the Table. Abreviations as in the text.

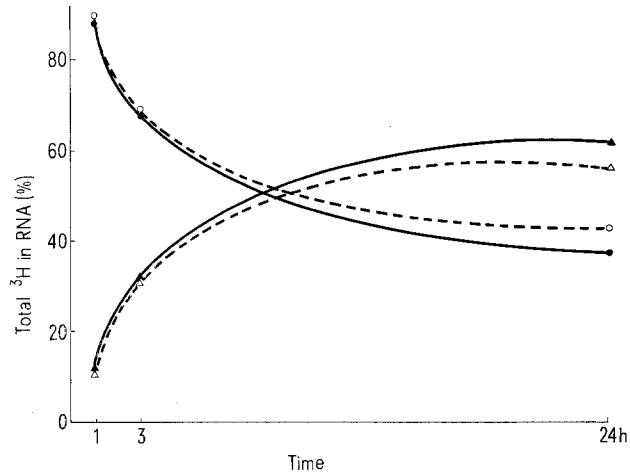

Fig. 2. Percentage distribution of ^{3}H in the RNA of subcellular fractions. The results are expressed in percentage of ^{3}H recovered in the main fractions (CN + MIT + MIC + S). Nuclear RNA (CN): (●—●) normal controls; (○---○) hypothyroids. Cytoplasmic RNA (MIT + MIC + S): (▲—▲) normal controls; (△---△) hypothyroids. Each point represents the mean of 4–5 experiments.

crosomal RNA, occurring only at the earliest time after the injection of the labelled precursor. This difference cannot be explained in relation to the availability of ^{3}H, since the radioactivity of the acid-soluble pool was higher in hypothyroid animals than in normals (Table II). Since the entry of isotopic precursors into the brain is relatively slow, it is therefore probable that the observed changes at the earliest time might be reflecting an alteration in the synthesis of the so-called 'rapidly labelled RNA'. TATA and WIDNELL[16] have previously reported a similar finding in growing-rat liver after thyroidectomy. It is well documented[17] that in mammalian brain cell nuclei, rapidly labelled RNA comprises mostly rRNA and mRNA precursors and tRNA; our results do not allow us, at present, to determine if one or several kinds of RNAs are altered. However, it is clear that the synthesis of a minor fraction of total RNA (which may be necessary to furnish normal protein synthesis) is diminished. In long periods, that difference could be masked by the bulk of RNAs.

Considering that, 1 h after the injection, the difference between the SR in microsomal RNA of normals and hypothyroids is higher than that of SR in nuclear RNA (44% and 20%, respectively) and, moreover in hypothyroids, the SR of microsomal RNA was still lower than in normals at 3 h, it is possible to assume that the passage of nuclear RNA to the cytoplasm is also altered. The lowest concentration of RNA in the microsomal fraction of hypothyroids (Table I) support this view. Both conclusions, lower nuclear synthesis and transport of RNA which arose from the results presented here are in good agreement with those obtained by us, utilizing the intracerebral injection of ^{3}H-orotic acid[18].

The decreased SR of mitochondrial RNA occurring after longer labelling times cannot be clearly interpreted with the present data; it must be noted that our MIT fraction is a crude preparation which contains also nerve endings and myelin fragments[9]. The possibility that the synthesis of autonomous RNA in brain mitochondria might be affected by neonatal thyroid deficiency cannot be ruled out.

Table II. ^{3}H content of acid-soluble pool in brain and specific radioactivity (SR) of RNA in subcellular fractions

		Time after injection (h)		
		1	3	24
a) Acid-soluble pool				
	N	35.71 ± 0.78	62.93 ± 3.79	65.50 ± 4.10
	H	39.40 ± 0.99[b]	73.51 ± 2.41[a]	63.83 ± 1.92
b) SR				
PN	N	8213 ± 230	20748 ± 408	44999 ± 2533
	H	6596 ± 86[b]	21097 ± 190	49053 ± 3605
MIT	N	448 ± 50	3071 ± 87	21484 ± 1031
	H	390 ± 43	2242 ± 109[a]	14675 ± 616[a]
MIC	N	457 ± 37	1265 ± 44	12478 ± 824
	H	256 ± 23[c]	1110 ± 41[a]	14496 ± 1097
S	N	1026 ± 110	2744 ± 225	19296 ± 1897
	H	961 ± 86	2757 ± 295	21868 ± 765

The results are the means ± S.E.M. of 4–5 individual experiments and are expressed: a) in dpm/mg wet wight of tissue and b) in dpm/mg RNA. Statistical analysis as in Table I. *P* values are: [a] 0.05; [b] 0.02; [c] 0.005; [d] 0.001. N, normal controls; H, hypothyroid rats. Abbreviations of subcellular fractions as in text.

Resumen. Se estudió la incorporación de radioactividad en RNAs de fracciones subcelulares de cerebro de ratas normales y neonatalmente tiroprivas de 10 días de edad, luego de la administración subcutánea de ácido orótico tritiado. El hipotiroidismo afecta la síntesis de RNA nuclear «ràpidamente marcado» y probablemente su transporte al citoplasma.

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