The immediate effect of thyroxine on metabolic rate in the pig at 1–11 days of age

	O_2 consumption ml/kg/min		O_2 consumption in % of initial value		
	Saline	Thyroxine	Saline	Thyroxine	P
Before injection	19.6 ± 0.6 (13)	17.5 ± 0.6 (14)	100%	100%	
Time after injection (h)					
1–2	17.3 ± 0.8 (13)	18.1 ± 0.9 (14)	91.8 ± 2.0	97.5 ± 1.5	$0.01 < P < 0.05$
3	18.1 ± 0.6 (13)	19.1 ± 1.2 (14)	92.6 ± 2.4	108.7 ± 5.4	$0.01 < P < 0.05$
4	18.0 ± 0.7 (13)	20.1 ± 1.3 (14)	91.4 ± 1.4	114.3 ± 5.5	$P < 0.001$
5	17.4 ± 0.5 (13)	19.2 ± 1.2 (14)	89.1 ± 1.9	109.5 ± 5.6	$0.001 < P < 0.01$
7	17.2 ± 0.8 (13)	16.7 ± 0.7 (13)	88.0 ± 1.9	97.2 ± 3.4	$0.01 < P < 0.05$
10	16.2 ± 0.5 (13)	16.0 ± 0.6 (13)	82.4 ± 1.6	92.2 ± 3.0	$0.001 < P < 0.01$
13–15	16.1 ± 0.6 (9)	16.0 ± 0.6 (10)	80.0 ± 2.2	91.8 ± 1.9	$P < 0.001$

Means and standard errors are given, with numbers of animals in brackets. P, comparison between saline and thyroxine results.

following birth, and to 30 °C for animals of 7–11 days of age. An equilibration period of 30 min was allowed before the initial level of oxygen consumption was measured. Following the s.c. injection of either thyroxine solution or saline (for the controls) a further period of measurement was begun. In some experiments rectal temperature instead of oxygen consumption was measured at 30 min intervals.

A solution of L-thyroxine (L. Light and Co. Ltd.) in normal saline was prepared immediately prior to injection. Both the thyroxine solution and the saline for the control animals were adjusted between pH 7.5 and 8.0 by addition of 0.1 N sodium hydroxide.

In unsuckled pigs during the first post-natal day, thyroxine did not produce elevation of either rectal temperature or oxygen consumption rate. In suckled pigs of 1–3 days of age, however, increases occurred in both quantities, relative to the saline controls, with the peak effects at 3–4 h after injection ($P < 0.01$); the approximate increase in rectal temperature was 0.5 °C. The effect on metabolic rate was also present at 8 days of age, with the peak at 5–7 h after injection ($P < 0.01$), although a rise in rectal temperature did not take place either in this age group or in 19-day-old pigs.

Mean values for the rate of oxygen consumption at intervals up to 15 h following injection have been calculated from the collected results obtained from pigs up to 11 days of age, but excluding the unsuckled new-born, which showed no response. These figures are given in the Table, both as ml oxygen/kg min and as percentages of the initial values, in order to facilitate comparison between animals showing variation in absolute levels. A significant effect is sustained throughout the period of observation.

The immediate effect of thyroxine in the intact animal, as opposed to the commonly observed latent effect, does not appear to have been described previously, although such an effect has been observed in hypophysectomized or thyroidectomized rats[5]. The effect is evident during the first week after birth, indicating the highest sensitivity of the animal to thyroxine at this time. In the older pig the absence of the rise in rectal temperature and the rather later peak in metabolic rate could be related to the increased binding of injected free thyroxine by α-globulin, with consequent delay in the hormone's action. The absence of the effect in the first part of the post-natal period may be related to lack of development of target organs at that time. This hypothesis is consistent with the observation of the maximum effect at about 5 days of age. Another possibility is that the effect is due to thyroxine stimulating the release or augmentation of action of adrenaline, to which the new-born pig responds with a rise in metabolic rate[6].

Résumé. Deux augmentations, l'une immédiate et l'autre latente, s'observent dans la consommation d'oxygène après injection sous-cutanée de thyroxine au porcelet nouveau-né. L'augmentation immédiate présente un maximum environ 4 h après l'injection. Elle a été la plus forte chez les porcelets de l'âge 1 à 4 journées.

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The Corticotroph Cells of the Anterior Pituitary Gland of a Reptile: *Cnemidophorus l. lemniscatus* (Sauria, Teiidae)

Up to the present, corticotroph cells have not been identified in the reptile hypophysis, although the existence of corticotrophin therein is known[1]. The available data indicate 2 types, both acidophilic, of non-mucoproteinaceous secretory cells in the pars anterior of reptiles: one localized in the caudal zone and composed of numerous and generally small elements, which are usually considered as alpha cells, and the other situated rostrally and formed by elongated cells, named X on account of their uncertain function[2]. The same two types of cells were found in the above-mentioned species[3]. The

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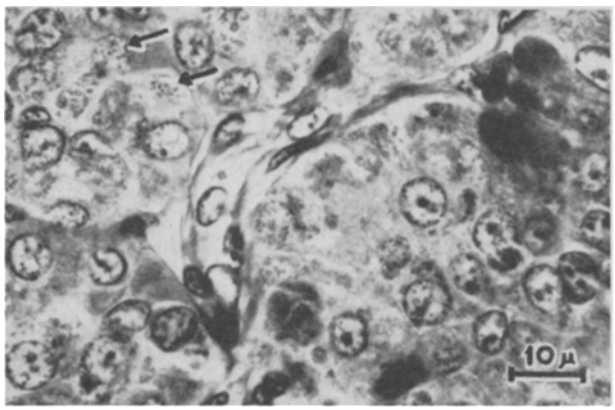

Fig. 1. A group of corticotroph cells in the anterior pituitary of an animal given 14 daily doses of metopirone. The arrows indicate their granules. At right, appearing with dark cytoplasms, some X cells.

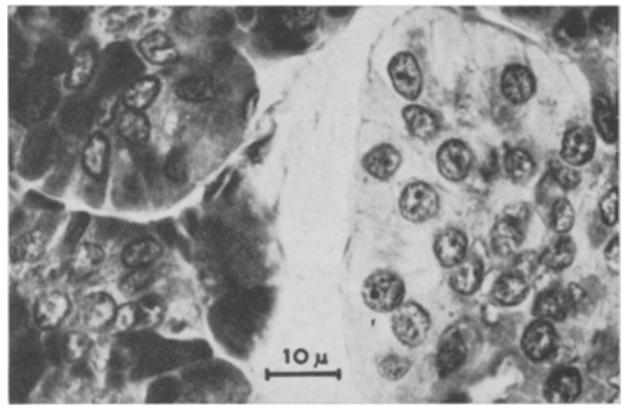

Fig. 2. At right, a group of corticotroph cells of a control animal. At left, different chromophilic cells.

aim of the present work was to inquire what cells are responsible for secreting corticotrophin in this animal; for that, metopirone was used, an inhibitor of the 11β-hydroxilation which blocks the synthesis of cortisol and aldosterone in the interrenal tissue and consequently brings about an increase of the corticotrophin secretion at the pituitary level. By means of this substance, corticotroph cells could be identified in mammals[4], birds[5], amphibians[6] and fishes[7].

Ten adult male individuals of the lizard, *Cnemidophorus l. lemniscatus* were used; their weights varied between 10.5 and 15.5 g. The animals were divided into 2 equal groups; those in the first group received daily i.p. injections of 2.5 mg of metopirone ditartrate CIBA dissolved in 0.25 ml of distilled water; those in the second group served as controls and were given only the solvent. 24 h after the last injection, animals which had received 2, 8, 11 or 14 doses, were anaesthetized by ether and then decapitated. The pituitary glands were fixed in a fluid composed of 2% chromium trioxide, 100 ml; 1.8% sodium chloride, 100 ml; formalin, 100 ml, and acetic acid, 12 ml. They were embedded in paraffin and their sections, 4 µ thick, stained with Alcian blue–periodic acid–leucofuchsin–orange G after permanganic oxidation[8], and with progressive iron haematoxilin–erythrosine–orange G–aniline blue[9].

The animals treated with metopirone, especially those which had received a greater number of injections, showed, together with a considerable hypertrophia of the interrenal tissue, a great hypertrophia and hyperplasia of certain cells situated in the rostral region of the anterior pituitary gland; these cells evidently are the corticotrophs (Figures 1 and 3). In their cytoplasm they exhibit relatively large and scarce granules, which take orange G as those of X cells do with the employed technique, but differ from these by presenting a moderate affinity to iron haematoxilin. They have large and vesicular nuclei with considerably developed nucleoli. Their cytoplasm frequently appears vacuolated. In control animals, these elements correspond to chromophobic cells which have a similar localization but are somewhat smaller, less numerous and on the whole occupy a smaller volume; they are negative to periodic acid–leucofuchsin and do not take the acid stains which were utilized (Figure 2); some zones of their cytoplasm are lightly coloured by haematoxilin. Their nuclei are generally ovoid and smaller than those of activated cells. In treated animals alterations are not observed in both previously described types of non-mucoproteinaceous secretory cells, which probably are somatotroph and lactotroph; on the other hand, the presence of prolactin in the reptile hypophysis has been demonstrated[10].

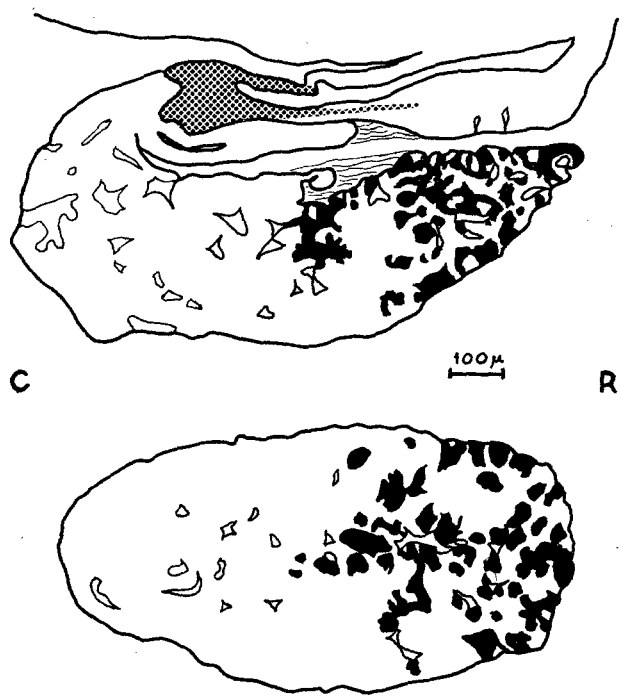

Fig. 3. In black, localization of the corticotroph cells in the hypophyses of animals which have received 14 daily doses of metopirone. Above, sagittal section. Below, frontal section through the pars anterior. C, caudal; R, rostral; shaded, the neural lobe.

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Until now the corticotroph cells of reptiles have evidently been interpreted as undifferentiated cells; such a mistake can also easily occur in other vertebrates because of the fineness and scarcity of the specific granules[11-13]. On the contrary, they are clearly distinguishable from the X cells, which are also situated in the rostral zone of the pars anterior, not only by their different affinity to stains, as has already been mentioned, but by their morphological features. Normally the X cells are cylindric and the supranuclear zone of their cytoplasm appears full of closely packed granules; these granules are small but easily discernible with the light microscope, and are definitely orangeophilic with the staining methods used. The rostral localization of the corticotroph cells in the animal studied agrees with that which has been observed in birds[5] and fishes[7]. In the latter, cytoplasmic vacuolation as well as nuclear and nucleolar hypertrophy have also been noticed as an effect of metopirone administration[14]. The affinity of cytoplasmic zones for iron haematoxilin can be explained by the usual abundance of ergastoplasm in this cellular type[12].

In conclusion, a third type of non-mucoproteinaceous secretory cells, responsible for corticotrophin production and not previously described in reptiles, must be admitted in the anterior pituitary gland of *Cnemidophorus l. lemniscatus*.

Resumen. Mediante la administración de metopirona pudo demostrarse en la hipófisis anterior de *Cnemidophorus l. lemniscatus* la existencia de un tercer tipo de células secretoras no mucoprotídicas, responsables de la producción de corticotrofina y no identificadas hasta ahora en reptiles. Estas células se encuentran en la porción rostral del lóbulo y, en los animales testigos, resultan cromófobas con las coloraciones efectuadas. Por acción de la metopirona sufren considerable hipertrofia e hiperplasia y aparecen en su citoplasma gránulos gruesos y relativamente escasos que presentan moderada afinidad hacia la hematoxilina férrica.

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The Course of Lymphocytic Choriomeningitis Virus Infection in Mice Treated by Phytohaemagglutinin

The effect of phytohaemagglutinin on the antibody response and the homograft rejection has been investigated by several authors[1]. The experimental data and the conclusions of the authors concerning the effect of phytohaemagglutin treatment on the immunological responses seem to be contradictory. Considering that the consequences of lymphocytic choriomeningitis (LCM) virus infection depend on the immune status of the mice and that the fatal choriomeningitis fails to develop in animals depressed immunologically[2-8], intracerebral infection with LCM virus was chosen as the experimental method for studying the effect of phytohaemagglutinin on immunological reactivity.

Materials and methods. 5-week-old, inbred mice of strain 'A', weighing on the average 15 g, were used in the experiments. The total quantity of phytohaemagglutinin applied in the treatment, as well as the time of the virus infection varied per experiment. In each experiment 30 mice were injected i.p. with 0.4 ml (0.4 mg) of Phytoclin (Wellcome Research Laboratories), while an equal number of controls received 0.4 ml of physiological NaCl solution. Each time half of the mice in every experiment were injected intracerebrally with the pre-titrated 100 LD_{50} dose of LCM virus. Each experiment thus covered 4 groups, as specified in the Table. Absolute lymphocyte counts were taken at intervals and also the weight was checked several times of the mice of PHA and control groups. In the mice of PHA + LCM and C + LCM groups, the typical neurological symptoms of the infection with subsequent death of the animals were observed. The brain of the dead animals was studied histologically using hematoxylin and eosin staining.

Results and discussion. The experimental data are summarized in the Figure. In experiments 1 and 2 the animals were injected with 3 times 0.4 mg and 6 times 0.4 mg of phytohaemagglutinin respectively. The LCM virus was inoculated on the last day of the phytohaemagglutinin treatment. In experiments 3 and 4 the treatment with phytohaemagglutinin was started on the 3rd and 4th day after the LCM virus infection, injecting 3 times 0.4 mg and 4 times 0.4 mg of phytohaemagglutinin, respectively.

As regards the effect of the phytohaemagglutinin treatment on the course of the LCM virus infection, the result was the same in each of the experiments. The

Groups	PHA	PHA + LCM	Control	C + LCM
Treatment	PHA i.p.	PHA i.p.	Phys. NaCl i.p.	Phys. NaCl i.p.
Infection with LCM virus	–	100 LD_{50} i.cer.	–	100 LD_{50} i.cer.

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