

essentially identical to those shown for the immunized-only mice (group A). Toxicity in terms of deaths was the same for both cytoxan and the cytoxan plus HPO treated mice, namely, 1 death out of 8 experimental animals.

Our previous studies⁵⁻⁷ have suggested that mycobacterial adjuvant functions to enhance the transformation of small lymphocytes to immunopotential stem cells, thereby providing larger numbers of progenitor cells capable of responding to a primary immune stimulus. This adjuvant effect has been observed to continue over a period of 30 days or more in the mouse following a single injection of adjuvant⁵. It might be speculated that immunization with heterologous erythrocytes³, on the other hand, influences the differentiation and proliferation of the available stem cell pool; but any small lymphocyte transformation which may occur does so after the primary inductive role of this antigen is terminated, and is thus largely a passive result of the need to replenish the stem cell pool size. It would appear then, that the potentiating action of HPO on cytoxan induced immunosuppression in adjuvant-BSA immunized mice, and absent in SRBC immunized animals, is directed towards interference with the small lymphocyte to stem cell transformation. Since stimulation of this type cell transformation may also be fundamental to leukemic processes⁷, an analogy between

the high pressure oxygen effect in depressing an adjuvant-immunization system and that in retarding leukemogenesis¹ would appear consistent with this interpretation⁸.

Zusammenfassung. Die intermittierende Anwendung hyperbarischen Sauerstoffs erhöht die die Antikörper unterdrückende Wirkung von Cytoxan bei Mäusen, die vorher mit Ochsen Serum-Albumin und Freund's Adjuvans immunisiert worden waren. Der Wirkungsmechanismus des Sauerstoffs wird als Verhinderung der Transformation von kleinen Lymphocyten in immunbiologisch aktive Stammzellen gedeutet.

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(Oregon 97201, USA), 20th March 1967.

⁵ J. I. MORTON and B. V. SIEGEL, *Vox Sang.* 11, 570 (1966).

⁶ B. V. SIEGEL and J. I. MORTON, *Immunology* 10, 559 (1966).

⁷ B. V. SIEGEL and J. I. MORTON, *Blood* 29, 585 (1967).

⁸ Supported by U.S. Atomic Energy Commission Contract No. RLO-1927-13.

Effect of Hypothalamic Lesions on MSH Content of the Intermediate Lobe of the Pituitary Gland in the Rat

It has been reported that, as in amphibians, interruption of the hypothalamo-hypophysial connections in mammals results in a hypertrophy of the intermediate lobe of the pituitary gland¹. This suggests that the central nervous system may exert a mainly inhibitory influence on the secretion of melanophore-stimulating hormone (MSH).

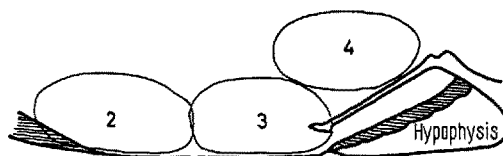
We have produced lesions in the hypothalamus of the rat in order to investigate whether the amount of MSH in the intermediate lobe would be affected by this procedure.

Experimental. Female albino rats, weighing between 120 and 140 g, were placed in a stereotaxic instrument and a radio-frequency coagulation was performed in several parts of the hypothalamus. Control animals were sham-operated, but no electrode was introduced into the brain. Each experiment consisted of 4 groups of animals, receiving: (1) a sham-operation; (2) a midline lesion in the region between optic chiasm and median eminence; (3) a midline lesion destroying the posterior part of the median eminence and the hypophysial stalk area; (4) a bilateral lesion in the mammillary bodies. The position of these lesions is illustrated in the Figure.

After 1 week the rats were decapitated. The posterior lobe (including pars intermedia and pars nervosa) was carefully separated from the anterior lobe, and homogenized in 0.5 ml of 0.1 N HCl. The acid extract was then diluted with Ringer solution, and the MSH concentration determined in vitro on colour change in pieces of skin of the lizard *Anolis carolinensis*². This sensitive assay can detect a concentration of about 0.1 ng/ml of synthetic α -MSH. As a reference standard synthetic α -MSH has

been used, so that the contents could be expressed as μ g α -MSH.

Each experiment was repeated 5 times, and the results were pooled according to the site of the lesion afterwards



Sagittal section through the hypothalamus, indicating the areas common to the lesions within a given experimental group (2, 3, 4).

MSH content of pituitaries from rats bearing lesions at several locations in the hypothalamus

Group	No. of rats	Lesion site	MSH content μ g/posterior lobe	Difference from controls
1	27	No lesion	1.1 ± 0.1	—
2	36	Anterior-basal	0.7 ± 0.1	$P < 0.05$
3	23	Stalk region	0.9 ± 0.2	Non significant
4	15	Posterior-dorsal	1.9 ± 0.4	$P = 0.08$

¹ R. L. HOLMES, *J. Endocr.* 24, 53 (1962); J. H. ADAMS, P. M. DANIEL and M. M. L. PRICHARD, *J. Path. Bact.* 87, 1 (1964); F. A. LÁSZLÓ, M. A. DÁVID and K. KOVÁCS, *Medna exp.* 10, 307 (1964).

² A. C. J. BURGERS, *Endocrinology* 68, 698 (1961).

observed. Since lesions affecting the median eminence caused a decrease in weight of the posterior lobe (mainly, if not exclusively, due to atrophy of the pars nervosa), MSH content is not expressed per mg tissue, but per posterior lobe.

Results are shown in the Table. It appears that lesions interrupting the connections between hypothalamus and hypophysis did not alter significantly the MSH content in the posterior lobe, which suggests that the integrity of these connections is not obligatory for the hormonal function of the pars intermedia. Damage to the median eminence region, while the hypophysial stalk remained intact, resulted in a decreased MSH concentration. On the other hand, several lesions dorsal to the mammillary bodies induced a conspicuous rise in MSH content, but due to great variations within this group of lesions the main difference with control animals was of borderline statistical significance ($p = 0.08$). The results would suggest, however, that the hypothalamus may exert

stimulatory as well as inhibitory influences on the function of the intermediate lobe.

Since assays of hormonal contents of a gland cannot differentiate between a change in release or in synthesis, further elucidation of the hypothalamic control of MSH production has to await the development of MSH determinations in blood.

Zusammenfassung. Es wird gezeigt, dass Elektrokoagulation in verschiedenen Teilen des basalen Hypothalamus einen Einfluss auf den Melanophorenhormongehalt im Hypophysenzwischenlappen der Ratte hat.

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The Nucleus Lateralis Tuberis in the Fresh Water Teleost, *Clarias batrachus* Linn.

The occurrence of the nucleus lateralis tuberis as a neurosecretory centre in addition to the preoptic-nucleus is peculiar to fishes¹⁻³.

I have examined the brains of catfish *Clarias batrachus* with a view to studying the histochemical reactions of the nucleus preopticus and also to ascertaining if the lateralis nucleus contributes neurosecretory material.

In the catfish examined the 2 neurosecretory nuclei, viz. the preoptic and the lateralis tuberis, are present and the latter is posterior to the optic decussation. The neurons of the preoptic nuclei are flask-shaped cells, each with a prominent axon. Sections clearly show the fibres from this region passing dorsally to the optic tract into the neurohypophysis.

Posterior to the optic decussation a large group of small lateralis neurosecretory cells are found, some of them in close proximity to the ventricle and the others near the capillaries. Fibres originating from this nucleus also proceed into the neurohypophysis. During their passage, large neurosecretory cells are found in them; in these, the neurosecretory granules stained by chrome-alum hematoxylin (CAH)⁴, and only feebly by aldehyde-fuchsin (AF)⁵ are paranuclear, which may indicate their nuclear origin.

In the ovariectomized (gravid) female catfish both the preoptic and the lateralis nuclei show heavily granulated neurosecretory cells, possibly because the ultimate target organ has been ablated. In the ovariectomized catfish receiving 5 mg of testosterone-propionate, there was degranulation in both the nuclei, and the AF positive granules could be very clearly made out in the fibrous-tract and also in the neurohypophysis. The Figure shows the neurohypophysis wherein AF positive material is found in large concentration, probably representing the neurohypophysial hormone.

In the intact gravid catfish, injections of the same quantity of androgen brought about degranulation in the neurons of both the sets of nuclei. Degranulation was also brought about when injected with 5 mg (total dose) of oestradiol dipropionate in spayed and normal catfish.

In inactive female catfish, the cells of the lateralis nuclei appeared to be not active, whereas a high secretory activity was observed in the cells of the nucleus preopticus.

2 types of secretory cells in the nucleus preopticus were noticed: 1 which is AF positive, and the other which is azocarmine positive and very feebly AF positive. In the nucleus lateralis, the feebly AF positive cells are always



Neurohypophysis of the oestrogen treated *Clarias batrachus* showing AF-material in large concentration, sagittal section, AF. $\times 320$.

¹ J. M. DODD and T. KERR, Symp. Zool. Soc. Lond. 9, 5 (1963).

² A. STAHL, C. r. hebd. Séanc. Acad. Sci., Paris 239, 1855 (1954).

³ N. ÖZTAN, Int. Congr. Zool. 2, 150 (1963).

⁴ G. GOMORI, Am. J. Path. 17, 395 (1941).

⁵ B. H. LANDING and H. E. HALL, Lab. Invest. 5, 256 (1956).