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Résumé

A l'aide de techniques chromatographiques on a pu mettre en évidence dans la peau de *Bufo* adulte, outre plus de pigments fluorescents déjà connus, le bufo-chrome donnant de l'acide 2-amino-4-hydroxy-ptéridine-6-carboxylique par oxydation, le bufo-chrome 2 et deux substances à fluorescence jaune, le bufo-yellow 1 spécifique de l'espèce *Bufo* et le bufo-yellow 2 qu'on retrouve chez d'autres amphibiens.

High Fat Diet and Mast Cell Count in Rat Mesenterium

The relationship between mast cells (heparinocytes), fat metabolism and atherosclerosis has attracted attention in recent years¹. However, the question as to whether mast cells have a fundamental role in lipid metabolism still remains unanswered.

It was therefore of interest to ascertain whether a high fat diet would show any effect on the mast cell system.

Mast cell count in the mesenterium of rats maintained on diets with a varied fat content

Group	Composition of diet	No. of animals	Average number of mast cells/animal
I. Low fat	46 Cal. % protein 12 Cal. % fat 42 Cal. % carbohydrate	20	1727.3* S.D. = 319.08
II. High fat	13 Cal. % protein 80 Cal. % fat 7 Cal. % carbohydrate	18	1572.4 S.D. = 315.05
III. High fat + cholesterol	as in group II., however, 3% fat replaced by cholesterol	19	1470.1* S.D. = 234.6

* The difference between groups I and III significant for $P < 0.01$.

57 male Wistar rats, average weight 150 g, raised under identical conditions, were divided into 3 groups, each maintained on an isocaloric diet differing in lipid content (Table). All the rats had a normal growth curve. After 8 weeks, the rats were sacrificed and their mesenterium was fixed in Schaffer's solution (2 parts 80% alcohol to one part 40% formaldehyde), and stained by the standard method with toluidine blue².

From the mesenterium of each animal three specimens were taken, always from corresponding *loci*. By means of

an adapted ocular, 50 square fields (each of 0.01225 sq. mm area) were examined at a magnification of $200\times$. The mast cell counts given in the table are averages from one animal (i.e. the number of mast cells from 150 fields, 50 fields from each specimen).

It can be seen that the high fat diet containing cholesterol produced a significant decrease in mast cell count after 8 weeks. A decrease, although not statistically significant, can be observed in the group kept on the high fat diet without the addition of cholesterol.

After completion of these experiments, GRUNBAUM *et al.*³ published a report in which is apparent a small, statistically insignificant fall of mast cell count in the tissue of external ear of rats, fed a high fat diet.

A publication, cited above⁴, has shown that there is a much lower mast cell count in the myocardium of atherosclerotics than in controls of the same age.

It is suggested that the effects of a high fat diet on the mast cell count is conditioned by loading and exhaustion of its secretory activity.

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Zusammenfassung

57 Ratten mit isokalorischer Diät und verschiedenem Fettgehalt wurden auf 3 Gruppen verteilt. Die Ratten der Gruppe mit Diät von hohem Fett- und Cholesterolgehalt zeigten eine statistisch signifikante Verminderung der Heparinocytenzahl in den Mesenterien.

³ B. W. GRUNBAUM *et al.*, Proc. Soc. exp. Biol. Med. 94, 613 (1957).

⁴ A. CAIRNS and P. CONSTANTINIDES, Science 120, 105 (1954).

On the Catechol Amine Levels in Blood Plasma after Stimulation of the Sympathoadrenal System

Improved techniques have made possible the chemical determination of adrenaline and noradrenaline in blood plasma. In the experiments described below such determinations were performed after stimulation of the sympathoadrenal system.

Cats of both sexes, weighing about 3.5 kg, were anesthetized with nembutal (25 to 50 mg/kg body weight intraperitoneally and later small intravenous doses when required). Carotid pressure was recorded using heparine as an anticoagulant. Injections were made in the jugular vein. Blood samples (18 ml) were taken through a polyethylene tubing in the femoral artery and collected in a 50 ml polyethylene bottle containing 2 ml of 1% disodium versenate in physiological saline. The bottle was kept in ice water during the collection of blood. The blood was immediately centrifuged at about $15000\times g$ for 10 min in a refrigerated International centrifuge. (Under these conditions no uptake of catechol amines in platelets was detectable.) The plasma was sucked off, and the blood corpuscles suspended in saline and reinjected. Perchloric acid extracts of plasma were neutralized by K_2CO_3 . The precipitate was removed by centrifugation. The extracts

¹ A. CAIRNS and P. CONSTANTINIDES, Science 120, 105 (1954). – P. CONSTANTINIDES, Science 117, 505 (1953). – J. FODOR and Z. LOJDA, Physiologia Bohemoslov. 5, 275 (1956).

² A. E. G. PEARSE, *Histochemistry Theoretical and Applied*, J. & A. Churchill Ltd., London (1954).

Plasma Levels of Adrenaline and Noradrenaline in Cats after Stimulation of Sympathoadrenal System by Carbachol. Levels are given in $\mu\text{g/l}$ plasma

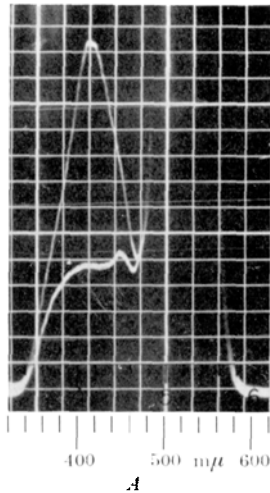
Cat no.	Adrenal-ectomy	Carba-chol ¹ mg/kg	Total catech-amines	Adren-aline ²	Nor-adren-aline ²
1.	Before	—	0.7	—	—
	Before	0.2	19.2	14.8	4.4
	After	—	0.8	—	—
2.	After	1.0	8.5	2.4	6.1
	Before	—	0.2	—	—
	Before	0.2	12.9	7.6	5.3
3.	After	—	0.9	—	—
	After	1.0	15.8	2.1	13.7
	Before	—	0.6	—	—
4. (Reserpine 7 mg/kg)	Before	0.2	46.5	33.3	13.2
	After	—	1.0	—	—
	After	0.2	1.2	—	—
5. (Reserpine 7 mg/kg)	After	1.0	10.2	2.0	8.2
	Before	—	0.7	—	—
	Before	0.2	5.1	2.3	2.8
6. (Reserpine 5 mg/kg)	After	—	0.0	—	—
	After	1.0	0.0	—	—
	Before	—	1.1	—	—
7. (Reserpine 5 mg/kg)	Before	0.2	1.9	—	—
	After	—	0.5	—	—
	After	1.0	0.5	—	—
8.	Before	—	2.6	—	—
	Before	0.2	14.0	5.4	8.6
	After	—	0.2	—	—
9.	After	0.2	0.2	—	—
	After	1.0	0.5	—	—

were passed through Dowex 50 columns. The eluates (obtained with *N* HCl) were assayed for adrenaline and noradrenaline (as trihydroxyindoles) using an Aminco-Bowman spectrophotofluorimeter. Details of the method will be published elsewhere.

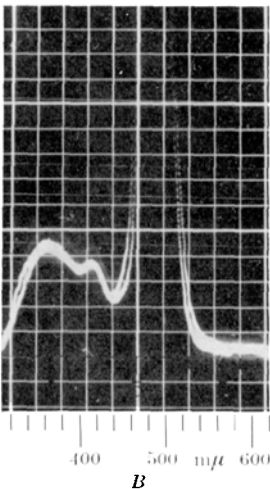
Stimulation of the sympathoadrenal system was performed by injecting carbachol (0.2 or 1 mg/kg) 10 to 15 min after atropine sulfate (2 mg/kg). At stimulation, significant amounts of adrenaline and noradrenaline appeared in the blood plasma (Table). In animals with intact adrenals more adrenaline than noradrenaline was found. After removal of these glands noradrenaline predominated, supporting the view that noradrenaline is the chief adrenergic transmitter³.

Reserpine has been shown to cause the disappearance of tissue catechol amines. Owing to lack of transmitter, the adrenergic nerves thus cease to function⁴. In cats given reserpine (5 to 7 mg/kg subcutaneously 18 h before the experiments) and deprived of their adrenals, stimulation by carbachol caused no detectable (in cat no. 6 perhaps a slight) release of catechol amines (Table, Figure). In animals with intact adrenals, some release took place even after treatment with reserpine. (Noradrenaline seemed to predominate.) These glands are fairly resistant to reser-

Activation Spectra of Plasma Samples of Adrenalectomized Cats after Stimulation by Carbachol (1 mg/kg).



(A) Non-reserpinized cat (no. 3, Table). Upper record: sample. Note peak at 410 $m\mu$, which is identical with that obtained from noradrenaline. Lower record: 'tissue blank' (sample in which adrenolutin and noradrenolutin have 'faded' by oxidation in alkali; after 'fading' ascorbic acid was added in the same amount as in the sample, in which oxidation was prevented by adding the ascorbic acid at an earlier stage).



(B) Reserpinized cat (7 mg/kg 18 h before experiment; cat no. 4, Table). Records of sample and 'tissue blank' are almost indistinguishable.

Fluorescent wave length 535 $m\mu$. Wavelengths are uncorrected instrumental values.

pine, particularly in the cat, and thus still contained some catechol amines (about 10% of the normal value).

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Zusammenfassung

Stimulation des sympathicoadrenalen Systems der atropinisierten Katze mit Carbacholin ergab signifikante Mengen von Adrenalin und Noradrenalin im Blutplasma. Bei intakter Nebenniere dominierte Adrenalin, nach Adrenalectomie Noradrenalin. Die Freisetzung von Adrenalin oder Noradrenalin unterblieb bei mit Reserpin vorbehandelten adrenalectomierten Tieren.

¹ Blood samples taken between 30 and 90 s after rapid carbachol injection.
² Values not calculated at total catechol amine levels below 3 $\mu\text{g/l}$ plasma.
³ U. S. V. EULER, *Noradrenaline. Chemistry, Physiology, Pharmacology and Clinical Aspects* (Springfield, Illinois, 1956), p. 248.
⁴ Å. BERTLER, A. CARLSSON, and E. ROSENGREN, *Naturwissenschaften* 43, 521 (1956). – A. CARLSSON, E. ROSENGREN, Å. BERTLER, and J. NILSSON, in S. GARATTINI and V. GHETTI, *Psychotropic Drugs* (Amsterdam 1957), p. 363. – E. MUSCHOLL and M. VOGT, *J. Physiol. (London)* 136, 7 P (1957).