

High Noradrenaline Content in the vas deferens of the Cock and the Tortoise

Recently it has been shown that the vas deferens of different mammals contains high amounts of noradrenaline^{1,2}. This is probably due to the very dense distribution of adrenergic nerves found in this organ³⁻⁵. It seemed therefore of interest to examine the catecholamine content of the tortuous vas deferens of birds and reptiles. The cock (*Gallus domesticus*) and the Greek tortoise (*Testudo graeca*) were chosen for the examination.

Methods. Adult animals were used. The cocks were of the White Leghorn strain. The animals were killed by decapitation and the vasa deferentia were removed. Their

spermatozoa content was squeezed out as much as possible. After weighing the organs, the catecholamines were extracted and estimated according to EULER and LISHAJKO⁶.

Results. The results are shown in the Table.

Conclusions. As shown in the Table the vas deferens of the cock and the tortoise contain high amounts of noradrenaline. The tissue content of noradrenaline is of the same magnitude as that seen in most mammals. Thus the cock vas deferens has about the same content as that of the hedgehog and the guinea-pig, while the vas deferens of the tortoise has a content similar to that of the marmoset.

A more detailed discussion of the significance of the high noradrenaline content of the vas deferens is given elsewhere^{2,7}.

Zusammenfassung. Der Noradrenaliningehalt des Samenleitergewebes von Hahn und Schildkröte ist gleich hoch wie bei Säugetieren.

N. O. SJÖSTRAND

Department of Physiology, Karolinska Institutet, Stockholm (Sweden), October 29, 1964.

Catecholamines in the vas deferens of the cock and the tortoise					
Animal	Number	Body weight	Weight of pair of vasa deferentia	Nor-adrenaline	Adrenaline
		kg	g	µg/g tissue	µg/g tissue
Cock	1	1.7	0.14	8.6	2.3
	2	1.9	0.15	9.3	1.2
	3	2.1	0.20	9.5	0.85
Mean		1.9	0.16	9.1	1.4(5)
Tortoise	1	0.7	0.30	15.2	—
	2	0.7	0.36	16.3	—
	3	0.9	0.42	17.8	—
Mean		0.77	0.36	16.4	—

- ¹ N. O. SJÖSTRAND, Acta physiol. scand. 56, 376 (1962).
- ² N. O. SJÖSTRAND, Acta physiol. scand., in press (1965).
- ³ B. FALCK, Acta physiol. scand., Suppl. 197 (1962).
- ⁴ B. FALCK, CH. OWMAN, and N. O. SJÖSTRAND, Exper. 21, 98 (1965).
- ⁵ CH. OWMAN and N. O. SJÖSTRAND, Z. Zellforsch., in press (1965).
- ⁶ U. S. v. EULER and F. LISHAJKO, Acta physiol. scand. 51, 348 (1961).
- ⁷ This work has been supported by a grant from Stiftelsen Magnus Bergwalls minne.

Study of the Mechanism of Inhibition Produced by Hexoses on Histamine Release Activity of Dextran

In a previous paper, BERALDO et al.¹ showed that dextran induces mast cell damage and histamine release from rat tissues and that both processes were inhibited by glucose, mannose and fructose. Since dextrans are polysaccharides composed solely of glucose, a competitive mechanism between those hexoses and dextran at level of the mast cell was suggested.

To test this hypothesis, peritoneal mast cell suspension was incubated in the presence of different concentrations of dextran with and without hexoses, and the results were submitted to the double-reciprocal plot, as described by LINEWEAVER and BURK.

Peritoneal mast cell suspensions were obtained by injecting 15 ml of glucose-free Tyrode solution, pH 7.5, containing 5 µg/ml heparin², into the peritoneal cavity of Wistar rats of either sex, weighing 150–200 g. 5 min later, the animals were anaesthetized with ether, decapitated, the abdomen opened and the fluid withdrawn. For each experiment, a pool of mast cell suspensions obtained from 3 rats was used, in which the histamine content and mast cell number were determined.

For histamine determination 0.2 ml of 0.1N HCl were added to 1.0 ml mast cell suspensions, the mixture was boiled for 5 min and neutralized with 0.1N NaOH. The histamine was assayed on the guinea-pig ileum, according to FELDBERG and TALESNIK³. The histamine values were used as base. The mast cells were counted using a Neubauer chamber, according to the principle used for leucocytes. The mast cell suspension was diluted and stained in saline containing 3% acetic acid and 0.003% toluidine blue.

The results obtained, using samples from twenty different pools, are shown in the Table.

Using suspension samples containing known amounts of histamine and mast cells, the experiments made to study the inhibition produced by hexoses on the histamine release activity of dextran were carried out as follows: to a series of 15 ml centrifuge tubes, containing 2.6 ml mast cell suspension, was added either 0.2 ml glucose-free Ty-

- ¹ W. T. BERALDO, W. DIAS DA SILVA, and A. D. LEMOS FERNANDES, Brit. J. Pharmacol. 19, 405 (1962).
- ² Nutritional Biochemical Co.
- ³ W. FELDBERG and J. TALESNIK, J. Physiol. (Lond.) 120, 550 (1953).

rode solution (pH 7.5) or 0.2 ml Tyrode solution containing $1.1 \cdot 10^{-5} M$ glucose²; in another series of tubes, the glucose was replaced by galactose² in the same concentration. The mixtures were incubated at 37°C. 10 min later, 0.2 ml saline, containing the desired amount of dextran⁴, were added and the incubation continued for a further 15 min. Similar samples, to which dextran was not added, were used as controls. All the tubes were then centrifuged at 2000 rpm at 2°C for 10 min and the histamine was assayed in the supernatants as above. The results were expressed as percentages of the total histamine originally present in each sample.

Figure 1 shows that the concentrations of dextran ranging between $0.5 \cdot 10^{-5} M$ and $3.5 \cdot 10^{-5} M$ were the most effective for releasing histamine and they were used to study the mechanism of inhibition produced by hexoses. The influence of temperature was studied incubating suspension samples of mast cells with $1.25 \cdot 10^{-5} M$ dextran at 5°, 10°, 20°, and 37°C. It was verified that at 5° and

10°C there was no histamine release; at 20° and 37°C the amounts of histamine released were 7 and 37%, respectively.

GOTH and KNOOHUIZEN⁵ claimed that dextran was unable to release histamine from rat peritoneal cell suspensions; however, all their experiments were performed at 20°-24°C.

The kinetics of the inhibition produced by hexoses on histamine release are shown in Figure 2. As the relationship between the percentage of histamine released (V) and the dextran concentrations (S) fitted the double-reciprocal plot described by LINEWEAVER and BURK, it is suggested that dextran releases histamine by associating with receptors in mast cells. The apparent KM for this process was found to be $1.47 \cdot 10^{-5} M$. The dextran-receptor complex formation was competitively inhibited by glucose, and the dissociation constant (K_I) was found to be $7.45 \cdot 10^{-4} M$. Galactose, on the other hand, was found to be ineffective as an inhibitor^{6,7}.

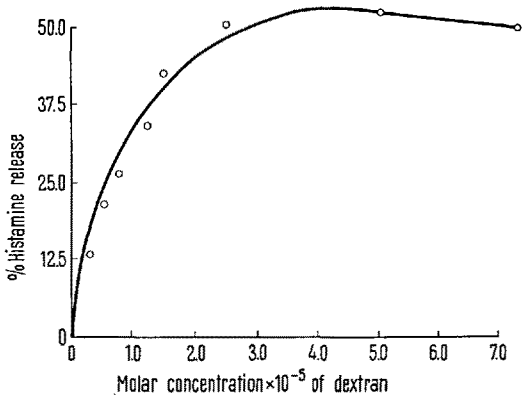


Fig. 1. Release of histamine from rat peritoneal mast cell suspensions incubated with varying concentrations of dextran.

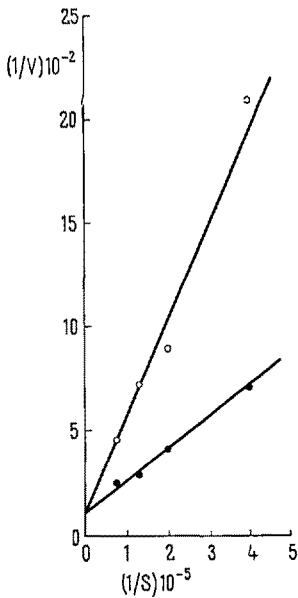


Fig. 2. Relationship between the reciprocals of the percentage of histamine released (1/V) and the dextran concentrations (1/S). Upper line (o—o—o) with $1.1 \cdot 10^{-5} M$ glucose and lower line (●—●—●) without glucose.

Histamine and mast cell content of peritoneal cell suspensions

Pool No.	Histamine (µg/ml)	Mast cell number · 10 ³ (per ml)
1	1.80	175
2	0.55	37
3	1.14	100
4	0.68	77
5	1.70	175
6	0.55	37
7	1.13	100
8	1.10	90
9	0.69	79
10	0.44	46
11	0.68	150
12	0.44	47
13	1.10	90
14	0.68	150
15	2.20	182
16	2.10	179
17	2.30	145
18	2.30	143
19	0.50	125
20	0.80	110
Mean	1.14 µg	111.9

Zusammenfassung. Die Hemmung der Histaminfreisetzung durch Hexosen wurde untersucht. Es gelang, nachzuweisen bzw. graphisch darzustellen, dass ein Zusammenhang zwischen dem Prozentgehalt der Histaminfreisetzung und der Dextrankonzentration besteht, wenn nach LINEWEAVER und BURK analysiert wurde.

W. DIAS DA SILVA and A. D. LEMOS FERNANDES

Faculdade de Medicina, Universidade de Minas Gerais, Belo Horizonte (Brazil), August 5, 1964.

⁴ Pharmacia, Uppsala (Sweden), having an average molecular weight of 40.000.

⁵ A. GOTH and M. KNOOHUIZEN, Life Sciences 9, 459 (1962).

⁶ This work was supported in part by funds received from the Rockefeller Foundation and the Conselho Nacional de Pesquisas do Brasil.

⁷ For comments and criticism during the preparation of the manuscript we are indebted to Prof. Dr. W. T. BERALDO.