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Effects of Reserpine and Propranolol on Urinary Excretion of Histamine and 5-Hydroxytryptamine in Severe Cold Exposure in Normal and Cold-acclimated Guinea-Pigs

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Summary. The effects of cold-acclimation, reserpine and propranolol were investigated on the survival time, rectal temperature and urinary excretion of histamine and 5-HT in guinea-pigs at −20°C. Both reserpine and propranolol shortened survival time by 3 hours and 1.5 hours respectively, the shortest time being in the cold-acclimated reserpine-treated animals.

There was a trend in severe cold exposure to increased excretion of *histamine* both in the non-acclimated and in cold-acclimated animals.

Reserpine did not change the excretion but increased the concentration of histamine from 0.08 to 0.25 μg/ml. Propranolol proved to be a histamine liberator by increasing the excretion in non-acclimated from 0.10 to 1.40 μg/h and concentration from 0.10 to 4.52 μg/ml and in cold-acclimated animals the excretion from 0.20 to 2.85 μg/h and the concentration from 0.08 to 3.23 μg/ml.

Severe cold increased the excretion of *5-HT* in the non-acclimated animals from 0.08 to 0.21 μg/h and cold acclimation increased this to 0.17 μg/h.

Reserpine disminished the excretion from 0.08 to 0.03 μg/h in the non-acclimated animals, but propranolol had no effect.

The results showed that the excretion of histamine and 5-HT into urine are changed in cold and can be modified with drugs. The application of the findings in proving a cold stress deserves further study.

Key words: Cold exposure, excretion of histamine and 5-hydroxytryptamine – Survival time – Histamine excretion, cold exposure – 5-Hydroxytryptamine excretion cold exposure.

Zusammenfassung. Die Wirkungen der Kälteadaptation, des Reserpin und Propranolol wurden auf die Rektaltemperatur und auf die Exkretion des Histamin und 5-Hydroxytryptamin im Urin bei Meerschweinchen in −20°C gemessen. Reserpin

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und Propranolol verkürzten die Überlebenszeit respektiv mit 3 bzw. 1 1/2 Stunden. Die kürzeste Lebenszeit hatten die kälteadaptierten, mit Reserpin behandelten Tiere.

Die Kälte-Exposition in −20°C hatte etwas in Exkretion des *Histamin* sowohl bei den nichtadaptierten als bei den kälteadaptierten Tieren vermehrt. Reserpin hatte keinen Effekt auf die Exkretion, *erhöhte* aber die Histaminkonzentration im Urin um das Dreifache. Ein interessanter Befund war die Zunahme der Histaminexkretion bei den nichtadaptierten Meerschweinchen von 0.10 bis 1.40 μg/h und der Konzentration von 0.10 bis 4.52 μg/ml nach der Propranolol-Behandlung. Bei den kälteadaptierten Tieren betrugen die Zunahme der Histaminexkretion von 0.20 bis 2.85 μg/h und die Steigerung der Konzentration von 0.08 bis 3.23 μg/ml.

Die Kälte-Exposition vermehrte auch die Exkretion des *5-Hydroxytryptamin* bei den nichtadaptierten Meerschweinchen von 0.08 bis 0.21 μg/h und bei den kälteadaptierten Tieren von 0.08 bis 0.17 μg/h. Reserpin verminderte die Exkretion bei den nichtadaptierten Tieren. Propranolol hatte keinen Effekt.

Unsere Ergebnisse zeigen, daß die Exkretion des Histamin und 5-Hydroxytryptamin im Urin von der Kälte-Exposition und den Medikamenten beeinflußt werden. Für die Erklärung der Anwendbarkeit dieser Resultate für die Beurteilung des Kältestresses bei Menschen sind weitere Versuche erforderlich.

Schlüsselwörter: Unterkühlung, Histamin- und 5-Hydroxytryptaminausscheidung – Überlebenszeit – Histaminausscheidung, Unterkühlung – 5-Hydroxytryptaminausscheidung, Unterkühlung.

The urinary excretion of histamine increases within two days up to seven fold when rats are exposed to cold (+6°C) (LeBlanc, 1963). The increase levels off during the following months (LeBlanc, 1963). At the same time, excretion of 5-hydroxytryptamine (5-HT) increases by about 50% and remains elevated (LeBlanc, 1963). A trend towards increased urinary excretion of histamine has also been reported to occur during one hour's exposure in cold-adapted men but not in non-adapted ones (LeBlanc et al., 1964). The source of the urinary histamine and 5-HT is not known, but it could be the gastric mucosa, where acute severe cold stress causes erosions and depletion of the cellular stores of these amines in the rat (Hirvonen and Elfving, 1974). Other possible sources for histamine are mast cells in the lungs and skin.

The measurements of histamine and 5-HT in urine and blood can be used at necropsy as tests for reaction to antemortem stress. Postmortem preservation of the amines is quite good in urine, which increases their practical importance (Laves and Berg, 1965).

The present work is part of a series of experiments designed to study the reactions of mammalian organism to severe cold exposure ending in death. The aim was to investigate whether the excretion of histamine and 5-HT would show any regular patterns which could also be demonstrated in necropsy material. Drugs affecting the storage of the biogenic amines or their effects at the receptor level have been useful tools in studies of thermoregulation (for references, see Schönbaum and Lomax, 1973) and were thus applied to affect survival in cold.

Material and Methods

Test Animals. The animals were non-acclimated or cold-acclimated guinea-pigs of Dunkin-Hartley strain of both sexes weighing from 600 to 1000 g. The non-acclimated animals, were kept at 22°C in normal laboratory colony. These 28 animals served as controls for the cold-acclimated groups. The cold-acclimated animals consisted of 28 guinea-pigs which were kept at +4°C for two months in individual plastic cages bedded with cutter chips. All animals received pellets, vegetable and water ad libitum.

Non-acclimated Guinea-Pigs. The reserpine group consisted of 10 animals. The injection of 1 mg/kg reserpine (Ciba-Geigy A.G., Basel, Switzerland) was given s.c. about 19 h before the exposure to cold.
The propranolol group of 10 animals was given 25 mg/kg of propranolol (Medipolar Ltd., Oulu, Finland) i.p. 30 min before the exposure.
The control group, 8 animals, received 0.5 ml of NaCl 30 min before the exposure to cold.

Cold-acclimated Guinea-Pigs. The reserpine group and *propranolol* group consisted of 10 animals. Eight animals served as *controls.*
The drug and saline injections were given as stated above.

Assays of Histamine and 5-HT

Histamine in the urine was determined principally with the method of Anton and Sayre (1969). Histamine was extracted from the urine directly into isoamylalcohol without trichloracetic acid. The chloroform step was also omitted. These modifications had been tested beforehand so as not to affect the results obtained with the original method (unpublished data). The orthophtal-dialdehyde (Fluka AG, Buchs Switzerland) histamine fluorochrome was measured in an Aminco-Bowman spectrofluorometer (excitation at 360 nm, emission at 450 nm).

5-HT was extracted from the urine into butanol with borate buffer. The fluorochrome was developed with orthophtal-dialdehyde (Fluka) according to Curzon and Green (1970) and assayed in an Aminco-Bowman spectrofluorometer (excitation at 260 nm and emission at 420 nm).

Experimental Procedure

To determine the basal urinary excretion of histamine and 5-HT the urine was collected for 24 h in the control groups, in non-acclimated (at +22°C) and acclimated control animals (at +4°C). The effect of the drugs, reserpine and propranolol on the urinary excretion of the amines was first tested without exposure to severe cold both in the non-acclimated and acclimated animals. It was noted in preliminary experiments that the survival time of the propranolol-treated animals at −20°C was around four hours. The urine of the animals was accordingly collected for four hours after the propranolol injection. Similarly, the urine of the reserpine treated animals was collected for 19 hours after the injection, whereafter the animals were exposed to the severe frost. The collection of urine was always carried out at the same hours of the day. The hypothermia experiments were made with the same guinea-pigs in which the basal excretion of the amines had previously been measured to obtain more reliable information about the effect of severe cold and the drugs.

The hypothermia was induced in a cold chamber at −20°C. The animals were kept there, each in its own cage, until dead. The cage was funneled for collection of the excreted urine. The urine found in the bladder at necropsy was pooled with the collected urine and the whole amount was frozen until analyzed. In spite of pooling the amount of urine was sometimes too small for the assays, which explains the small size of the reserpine groups. In addition a number of the cold acclimated reserpine treated animals died before the exposure.

Rectal temperature was measured every half hour and the moment of death was determined by monitoring the cessation of heart beats.

Statistics. A modified t-test using F-comparison was used in the statistical analysis.

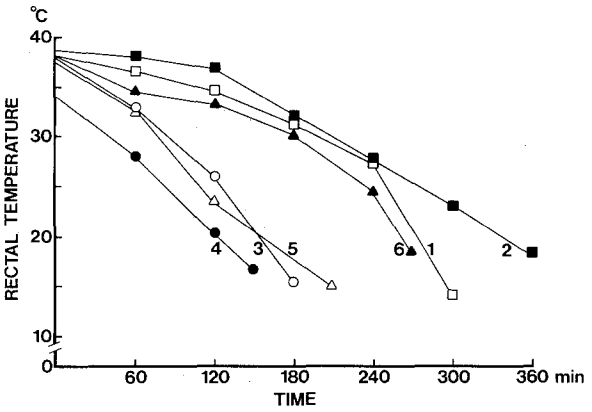


Fig. 1. The average survival times and the fall of rectal temperature in the six groups of guinea-pigs. Symbols for the groups: 1 = non-acclimated controls; 2 = cold-acclimated controls; 3 = non-acclimated reserpine-treated; 4 = cold-acclimated reserpine-treated; 5 = non-acclimated propranolol-treated; 6 = cold-acclimated propranolol-treated

Results

Survival Time and Rectal Temperature

The average survival time and the rate of temperature fall are given in Figure 1. The survival time was longest (on average 6 h) in the cold-acclimated control group and shortest in the cold-acclimated reserpine-treated group (on average 2.5 h). Propranolol shortened survival by about 1.5 h both in the non-acclimated and acclimated animals. With the exception of the reserpine-treated group the survival time of the cold-acclimated animals was longer than that of the non-acclimated animals and the rectal temperature at death was 2–4°C higher. In the reserpine-treated group the cold-acclimated animals died about 0.5 h earlier than the non-acclimated ones.

The temperature fell at the same rate, c. 2°C/h, in the non-acclimated control group and cold-acclimated propranolol-treated group and by c. 5°C/h in the non-acclimated propranolol- and reserpine-treated groups. The rate was fastest, c. 7°C/h, in the cold-acclimated reserpine-treated group. The reserpine animals were already slightly hypothermic before the cold exposure.

Urinary Excretion and Concentration of Histamine (Table 1)

The basal urinary excretion of histamine increased twofold when guinea-pigs were acclimated to cold at +4°C for two months, but the concentration did not change. In the *severe cold* (−20°C) the excretion increased, both in non-acclimated and cold-acclimated animals, but the change was non-significant. The concentration did not change.

Reserpine alone did not increase the excretion in either group but the concentration was increased significantly in both groups.

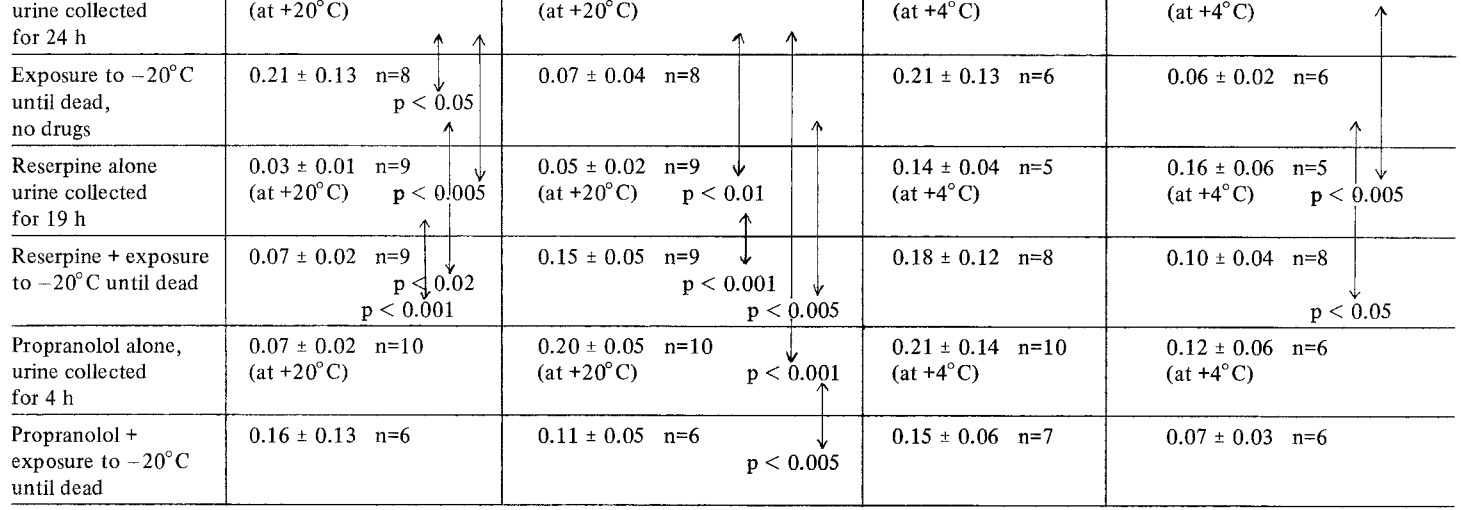
After reserpine and severe cold both the excretion and the concentration were increased by about twofold in the non-acclimated group compared to the reserpine

Table 1. Urinary excretion (μg/h) and concentration (μg/ml) of *histamine*. Mean ± SD. Histamine is expressed as free amine. For the survival times and the fall of rectal temperature in the groups see Figure 1. The arrows with the p-values indicate the statistical significance level between the two groups with the modified t-test

Experimental groups	Non-acclimated animals		Cold-acclimated animals	
	excretion (μg/h)	concentration (μg/ml)	excretion (μg/h)	concentration (μg/ml)
Basal value urine collected for 24 h	0.10 ± 0.04 n=8 (at +20°C)	0.10 ± 0.02 n=8 (at +20°C)	0.20 ± 0.09 n=7 (at +4°C)	0.08 ± 0.03 n=8 (at +4°C)
Exposure to −20°C until dead, no drugs	0.31 ±0.20 n=6	0.10 ± 0.06 n=6	0.40 ± 0.62 n=6	0.10 ± 0.11 n=6
Reserpine alone urine collected for 19 h	0.10 ± 0.02 n=9 (at +20°C)	0.14 ± 0.03 n=9 (at +20°C) $p < 0.01$	0.22 ± 0.05 n=4 (at +4°C)	0.25 ± 0.05 n=4 (at +4°C) $p < 0.001$
Reserpine + exposure to −20°C until dead	0.20 ± 0.03 n=6 $p < 0.001$	0.24 ± 0.05 n=8 $p < 0.001$ $p < 0.001$	0.22 ± 0.14 n=10	0.12 ± 0.06 n=10 $p < 0.005$
Propranolol alone, urine collected for 4 h	1.40 ± 0.43 n=9 (at +20°C) $p < 0.001$	4.52 ± 1.12 n=9 (at +20°C) $p < 0.001$	2.85 ± 0.88 n=10 (at +4°C) $p < 0.001$	3.23 ± 0.28 n=10 (at +4°C) $p < 0.001$
Propranolol + exposure to −20°C until dead	1.47 ± 1.13 n=6	3.32 ± 1.46 n=6 $p < 0.005$	1.67 ± 0.72 n=4 $p < 0.05$ $p < 0.02$	1.26 ± 0.47 n=4 $p < 0.001$ $p < 0.02$

Table 2. Urinary excretion (μg/h) and concentration (μg/ml) of *5-HT*. Mean ± SD. 5-HT is expressed as free amine. For the survival times and the fall of rectal temperature in the groups see Figure 1. The arrows with the p-values indicate the statistical significance level between the two groups with the modified t-test

Experimental groups	Non-acclimated animals		Cold-acclimated animals	
	excretion (μg/h)	concentration (μg/ml)	excretion (μg/h)	concentration (μg/ml)
Basal value urine collected for 24 h	0.08 ± 0.03 n=8 (at +20°C)	0.08 ± 0.02 n=8 (at +20°C)	0.17 ± 0.09 n=8 (at +4°C)	0.06 ± 0.03 n=8 (at +4°C)
Exposure to −20°C until dead, no drugs	0.21 ± 0.13 n=8	0.07 ± 0.04 n=8	0.21 ± 0.13 n=6	0.06 ± 0.02 n=6
Reserpine alone urine collected for 19 h	0.03 ± 0.01 n=9 (at +20°C)	0.05 ± 0.02 n=9 (at +20°C)	0.14 ± 0.04 n=5 (at +4°C)	0.16 ± 0.06 n=5 (at +4°C)
Reserpine + exposure to −20°C until dead	0.07 ± 0.02 n=9	0.15 ± 0.05 n=9	0.18 ± 0.12 n=8	0.10 ± 0.04 n=8
Propranolol alone, urine collected for 4 h	0.07 ± 0.02 n=10 (at +20°C)	0.20 ± 0.05 n=10 (at +20°C)	0.21 ± 0.14 n=10 (at +4°C)	0.12 ± 0.06 n=6 (at +4°C)
Propranolol + exposure to −20°C until dead	0.16 ± 0.13 n=6	0.11 ± 0.05 n=6	0.15 ± 0.06 n=7	0.07 ± 0.03 n=6



alone and the same was found in the concentration compared to the effect of cold alone. In the cold-acclimated group the concentration was only half of that after reserpine alone, but the excretion remained unchanged.

After the injection of propranolol an about fortyfold increase above normal was found in the concentration within four hours and a more than fourteenfold increase in the excretion both in non-acclimated and cold-acclimated animals.

Severe cold together with propranolol did not significantly change the excretion and concentration in the non-acclimated group, when compared to the propranolol alone effect, but in the cold-acclimated group both the excretion and concentration decreased from the propranolol alone value. In the groups both the excretion and concentration were more elevated than after exposure alone, except the excretion in the non-acclimated animals.

Urinary Excretion and Concentration of 5-HT (Table 2)

The basal excretion of 5-HT was in the cold-acclimated animals about twofold compared to the non-acclimated animals.

In severe cold (−20°C) the excretion was increased in the non-acclimated group by about threefold but not significantly in the cold-acclimated group. The concentration did not vary. *Reserpine* decreased significantly both the excretion and concentration in the non-acclimated group but increased by about threefold the concentration in the cold-acclimated group.

After reserpine and severe cold the excretion and concentration were elevated in the non-acclimated animals compared to the effect of reserpine alone. The concentration was about twofold but the excretion about one third of that after cold alone. In the cold-acclimated group the concentration was increased when compared to the effect of cold alone.

Propranolol alone increased the concentration by twofold but it was significant only in the non-acclimated animals. The excretion was not affected.

Severe cold and propranolol together decreased the concentration in both groups when compared to the effect of propranolol alone, but the change was significant only in the non-acclimated animals. Compared to the cold effect alone only the concentration was increased in the non-acclimated group.

Discussion

Cold stress activates the sympathetic nervous system, seen as increased catecholamine excretion into urine (Cottle 1960, Leduc, 1961; LeBlanc and Nadeau, 1961). The response is regarded as rather specific, since it causes both thermoconservation by cutaneous vasoconstriction and thermogenesis by hyperglycaemia and lipolysis.

Treatment with propranolol inhibits lipolysis, an important factor in nonshivering thermogenesis, by blocking the β-receptors in adipose tissue. Adrenergic activity and the ability to resist cold is also reduced by reserpine treatment. Thus it was not unexpected that both drugs shortened survival time at −20°C. In control and propranolol groups, cold acclimation increased survival time but shortened it in the reserpine animals, an observation already made by us previously (Hirvonen et al., 1976). One explanation for the reversed effect could be that during cold-acclimation the guinea-

pigs developed a high level of sympathetic tuning which normally improves the ability to withstand cold (Hsieh and Carlson, 1957; Depocas, 1960; Heroux, 1961). The new status seems somehow to be sensitive to reserpine, but the exact mechanism is still unknown. The inability of thermoregulation was evident in the rapid fall (7°C/h) of the body temperature in the cold-acclimated reserpine treated animals. It might be useful to take the invalidisation of thermoregulation by adrenergic inhibitors into consideration in man, too, because β-blocking agents and reserpine are widely used in cardiovascular diseases, especially hypertension.

In agreement with earlier results (LeBlanc, 1963) we found increased urinary excretion of histamine and 5-HT during cold-acclimation. Acute severe cold exposure increased the histamine excretion both in non-acclimated and cold-acclimated animals, but the change was not statistically significant although the trend looked quite clear. During the cold stress increased excretion of these amines is perhaps unspecific stress reaction. Histamine possibly originates in gastric mast cells which are degranulated in cold stress (Hirvonen and Elfving, 1974). Other sources would be the skin mast cells (LeBlanc, 1963) and platelets (Tuomisto, 1968). Many kinds of stress reactions induce release of histamine (for review, see Beaven, 1976a). The excretion of histamine was increased by cold stress but the concentration was not elevated in the present experiments. A large dose of reserpine given 19 h earlier did not modify histamine excretion in the non-acclimated animals at basal temperature. Because the concentration was increased, it seems possible that reserpine had a marked antidiuretic effect (Nechay and Sanner, 1961). Similarly, during cold exposure with reserpine, the concentration was increased both in non-acclimated and cold-acclimated animals. In the former group the total excretion had also doubled, possibly because the antidiuretic effect of reserpine was counteracted by cold-induced diuresis.

Propranolol treatment at basal temperature and during cold exposure caused a marked increase both in histamine excretion and in its urinary concentration. The change in the excretion did not become significant in the non-acclimated animals because of the great variation. This is a new observation because propranolol has not been decribed as a histamine liberator.

One possible explanation for the mechanism is that adrenaline and other adrenergic stimulators are known to inhibit histamine release from mast cells (for review, see Beaven, 1976b). When this inhibition is abolished by a β-adrenergic blocker, propranolol, an outflux of histamine can occur. The increased excretion was more marked in cold-acclimated than in non-acclimated animals. This could be due to increased stores of histamine in cold-acclimated animals, for instance in the skin mast cell pool (LeBlanc, 1963) and stomach. Whether histamine has any function in thermoregulation is still unknown. It causes hypothermia when injected into the rostral hypothalamus or when its precursor histidine is injected into the lateral ventricle (Cox et al., 1976). Histamine release might even be unbeneficial because it causes peripheral vasodilatation and thus loss of heat.

The excretion of 5-HT, 90% of which originates in the enterochromaffin cells was increased in cold-acclimation and in non-acclimated animals during cold exposure. No change in the concentration of 5-HT in the urine was observed, which indicates that the increased excretion was perhaps keeping pace with the increased diuresis. Previously it has been reported that 5-HT was increased in the blood when rats were exposed to +3 – +4°C (Gordon, 1961). Whether the changes of 5-HT are significant to

survival is not known, but large doses of 5-HT as such diminshed the rat's ability to withstand cold stress (Zilberstein, 1960). An interesting and unexpected finding was the reduced excretion of 5-HT in reserpine treated non-acclimated animals. Reserpine is known to induce 5-HT release from thrombocytes (Brodie 1958, Paasonen 1965) and therefore increased excretion would have been more logical. The reduced excretion may be due to the decreased amount of urine because of the antidiuretic effect of reserpine. In cold-acclimated animals an elevated concentration was found. During cold exposure with reserpine the 5-HT excretion and concentration were increased, perhaps by cold diuresis antagonizing the antidiuretic effect of reserpine. The 5-HT excretion in non-acclimated animals in cold environment was doubled, obviously due to the increased diuresis, not seen in cold-acclimated animals. Propranolol treatment at basal temperature did not change the excretion of 5-HT, but increased concentrations were found in the non-acclimated group. The concentrations were levelled off when the propranolol treated animals were exposed to severe cold.

On the basis of our results it can be concluded that the release of histamine and 5-HT are increased in non-acclimated animals and excreted into the urine during severe cold exposure and that this can be modified by drugs. The increase is perhaps an unspecific stress reaction, but too little is known about the pathophysiology of these amines to permit more accurate conclusions. The measurement of these amines, however, can be used at necropsy, when antemortem cold stress needs to be shown. Preliminary analyses in victims of accidental hypothermia have shown increased histamine values in some cases.

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References

Anton, A. H., Sayre, D. F.: A modified fluorometric procedure for tissue histamine and its distribution in various animals. J. Pharmacol. Exp. Ther. **166**, 285–292 (1969)

Beaven, M. A.: Histamine (First of two parts). New Engl. J. Med. **294**, 30–36 (1976a)

Beaven, M. A.: Histamine (Second of two parts). New Engl. J. Med. **294**, 320–325 (1976b)

Brodie, B. B.: Storage and release of 5-hydroxytryptamine (5-HT). In: 5-hydroxytryptamine Lewis, G. P. (Ed.), pp. 64–83. London: Pergamon Press 1958

Cox, B., Green, M. D., Lomax, P.: Thermoregulatory effects of histamine. Experientia **32**, 498–499 (1976)

Cottle, W.: Urinary noradrenaline in cold acclimated rats. Proc. Can. Fed. Biol. Soc. **3**, 20 (1960)

Curzon, G., Green, A. R.: Rapid method for the determination of 5-hydroxytryptamine and 5-hydroxy indoleacetic acid in small region of rats brain. Brit. J. Pharmacol. **39**, 653–655 (1970)

Depocas, F.: The calorigenic response of cold-acclimated white rats to infused noradrenaline. Canad. J. Biochem. Physiol. **38**, 107–114 (1960)

Gordon, P.: Changes in blood serotonin during exposure to cold. Nature **191**, 183–184 (1961)

Heroux, O.: Comparison between seasonal and thermal acclimation in white rats. Canad. J. Biochem. Physiol., 1829–1836 (1961)

Hirvonen, J., Elfving, R.: Histamine and serotonin in the gastric erosions of rats dead from exposure to cold. A histochemical and quantitative study. Z. Rechtsmed. **74**,273–281 (1974)

Hirvonen, J., Huttunen, P., Vapaatalo, H.: Serum glucose, serum fatty acids and adipose tissue lipids after fatal hypothermia of cold acclimatized, reserpine or propranolol treated guinea-pigs. Z. Rechtsmed. **77**, 177–189 (1976)

Hsieh, A. C. L., Carlson, L. D.: Role of adrenaline and noradrenaline in chemical regulation of heat production. Am. J. Physiol. **190**, 243–246 (1957)

Laves, W., Berg, S.: Agonie. Lübeck: Schmidt-Römhild 1965

LeBlanc, J.: Secretion and activity of histamine and serotonin during cold adaptation. Am. J. Physiol. **204**, 520–522 (1963)

LeBlanc, J., Nadeau, G.: Urinary excretion of adrenaline and noradrenaline in normal and cold adapted animals. Can. J. Biochem. Physiol. **39**, 215 (1961)

LeBlanc, J., Pouliot, M., Rheume, S.: Thermal balance and biogenic amine excretion in Gaspi fishermen exposed to cold. J. Appl. Physiol. **19**, 9–12 (1964)

Leduc, J.: Catecholamine production and release in exposure and acclimation to cold. Acta Physiol. Scand. **53**, Suppl. 183 (1961)

Nechay, B. R., Sanner, E.: Interference of reserpine with the diuretic action of theophylline and hydrochlorothiazide on the chicken. Acta Pharmacol. (Kobenhavn) **18**, 339 (1961)

Paasonen, M. K.: Release of 5-hydroxytryptamine from blood platelets. J. Pharm. Pharmacol. **17**, 681–697 (1965)

Schönbaum, E., Lomax, P. Ed.: The pharmacology of thermoregulation. Basel: S. Karger 1973

Tuomisto, J.: Release of histamine by reserpine from rabbit blood platelets. Ann. Med. Exp. Fenn. **46**, 441–446 (1968)

Zilberstein, R. M.: Effects of reserpine, serotonin and vasopressin on the survival of cold-stressed rats. Nature **185**, 249 (1960)

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