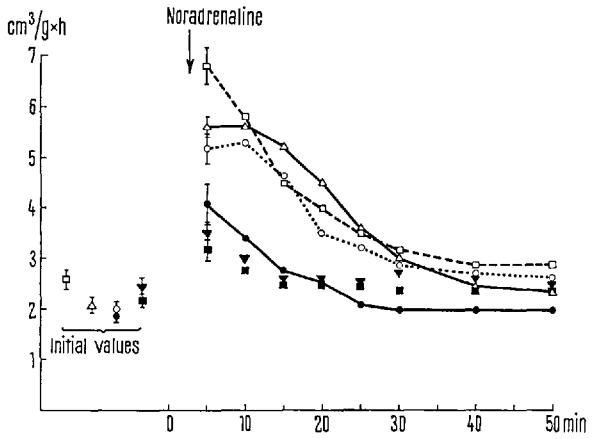
reactions to handling of the animals outside the chamber. This circumstance means that the oxygen consumption after doses lower than approximately 0.8 mg/kg body weight can no longer manifest itself, because it does not exceed the increase caused by the adaptation reaction to the intervention. The limit of maximum oxygen consumption after the injection is distinctly marked only for higher doses in the groups of normal mice, while in the hairless animals the plateau is indistinct. After this higher dose, about 40% mortality of animals in all 3 groups occurs on the second to the third day after the injection. The difference in hairiness manifests itself in the reaction to noradrenaline primarily in the achieved maximum increase in oxygen consumption over the normal animals kept at 24 °C and also over the animals adapted to 17 °C ($p < 0.05$), where the slight excess over the non-adapted group is only insignificantly indicated. The more marked reaction of the hairless animals corresponds to the findings[4-6] that adaptation to cold, accompanied by a simultaneous elevation of the rest values of respiratory metabolism, causes a multifold higher calorigenic effect of noradrenaline. Owing to the rapid fading of the reaction, the question remains how much higher is the dose necessary for producing the maximum effect in mice than in rats or guinea-pigs, where it is 0.2–0.4 mg/kg.



Oxygen consumption before and after i.p. injection of L-noradrenaline in mice. □—□ Hairless (1.6 mg/kg); △—△ normal adapted to 17 °C (1.6 mg/kg); ○····○ normal kept at 24 °C (1.6 mg/kg); ●—● normal kept at 24 °C (0.8 mg/kg); ■ controls (distilled water); ▼ controls (needle prick only).

Unlike rats or guinea-pigs it has been found that in mice the calorigenic effect of noradrenaline is very intensive also in adult individuals. In rats kept at 25 °C, JÁNSKÝ et al.[4] report an elevation of 25%, while in adult guinea-pigs kept at 30 °C the increase lies about 20% over the value in the thermoneutral zone[7]. In the adult mice measured by us the % excess over the value in the thermoneutral zone after a dose of 1.6 mg/kg for mice of the H strain kept at 24 °C makes 155%, for H-strain mice adapted to 17 °C 170%, and for hairless animals 195%. The question arises how the method of administering noradrenaline, which had been applied i.m. in the cases cited, influences the overall reaction. It appears that owing to the association between the response to noradrenaline and the extent of non-shivering heat production, demonstrated distinctly in guinea-pigs[5], the intensity of non-shivering heat production in adult mice reaches markedly higher values compared with other mammals.

Zusammenfassung. Bei ausgewachsenen Mäusen wurde nach i.p. Noradrenalininjektion die Steigerung der O_2-Aufnahme über den Grundumsatz in der Thermoneutralzone verfolgt. Nach einer Dosis von 1,6 mg/kg wurde ein bedeutender Unterschied ($p < 0,05$) der O_2-Aufnahmesteigerung zwischen der Gruppe normaler (155%) und normaler, auf 17 °C adaptierter Mäuse (165%) und der Gruppe haarloser Mäuse (195%) festgestellt.

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Effect of Cardiac Catecholamine Depletion Through Sympathectomy on Spontaneous Ventricular Fibrillation During Induced Hypothermia in Cats

Much evidence has accumulated to show that the sympathetic nerves are highly involved in the spontaneous development of ventricular fibrillation, a common complication during induced deep hypothermia in homeothermic mammals. Thus, blockade of the adrenergic neurons with bretylium[1], depletion of the neuronal noradrenaline stores with prenylamine[2,3] or reserpine[4], and blockade of the adrenergic β-receptors with INPEA[5] effectively prevents the spontaneous ventricular fibrillation constantly developing in untreated cats subjected to hypothermia to about 21 °C body temperature.

In the present series of experiments, an attempt was made to obtain a more limited interference with the adrenergic mechanisms by way of cardiac sympathectomy. In detailed investigations on the sympathetically innervated nictitating membrane of the cat, it has been demonstrated that the denervation supersensitivity starts to build up during the first 2 days following operation, accompanying the progressive degenerative changes and

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loss of transmitter in the adrenergic nerve terminals[6]. Thus, in order to obtain a reasonable reduction of the noradrenaline stores of the heart with as little supersensitivity of the effector cells as possible, the animals were cooled shortly (2–3 days) after the operation.

Methods. 18 adult cats of either sex, weighing 2.2–4.5 kg, were sympathectomized by bilateral excision of the sympathetic chains between and including the superior cervical and stellate ganglia[7,8] under nembutal anaesthesia (30 mg/kg i.p.). 2 or 3 days later the animals were anaesthetized as above, and cooled by submersion in an ice-water bath[3]. Arterial blood pressure, ECG, respiration and rectal temperature were continuously recorded. The animals were given artificial respiration by a pump respirator when indicated. Methoxamine (Vasoxine, Burroughs Wellcome & Co.; 0.4 mg/ml of 0.9% saline) was administered intermittently into the arterial catheter to maintain the blood pressure above 60 mm Hg at reduced body temperatures[3,5]. Methoxamine was particularly chosen because it selectively acts upon the adrenergic α-receptors and has little cardiac stimulant properties. Those animals not developing ventricular fibrillation were re-warmed to normothermia in warm water. 10 unoperated control animals were subjected to hypothermia in the same way.

When the experiments were finished, the animals were bled and the heart was removed for fluorimetric determination of noradrenaline[9,10].

In order to determine any effect of methoxamine on the cardiac noradrenaline stores, 5 animals were anaesthetized with nembutal (30 mg/kg i.p.) and slowly injected intra-arterially with a total of 1.2 mg methoxamine (cf. Table) after which they were maintained at normothermia for $2^1/_2$ h (corresponding to the cooling time in the hypothermia experiments) and then killed by bleeding. 6 animals killed immediately after induction of the nembutal anaesthesia served as controls. The heart tissue was prepared for fluorimetric analysis[9,10].

Results and discussion. All unoperated control animals developed ventricular fibrillation at a mean rectal temperature of 20.5 ± 0.5 °C (S.E.M.) in agreement with previous series of experiments on animals receiving no pretreatment[1,3–5,8]. A rhythmic cardiac activity could be rapidly restored by the intra-arterial administration of the adrenergic β-blocking agent INPEA[5,11] (20 mg of the D(−)-form to each animal). 7 of the animals had to receive methoxamine in amounts varying between 0.4–2.0 mg to maintain an adequate blood pressure at reduced body temperature (Table).

Five of the 8 animals cooled 2 days after sympathectomy, and 8 of the 10 animals cooled 3 days postoperatively, developed ventricular fibrillation. The fibrillation temperature of the 2-day group (21.7 ± 0.2 °C) was significantly higher ($0.02 < P < 0.05$) than that in the control group; the temperature of the 3-day animals (20.9 ± 0.6 °C) did not differ statistically from the controls ($P > 0.05$). As with the unoperated control animals, treatment with INPEA reversed the fibrillation into rhythmic heart activity. The animals that did not fibrillate were re-warmed to normothermia without complications. The total amount of methoxamine given to each animal is stated in the Table.

The fluorimetric determinations of cardiac noradrenaline (Table) revealed that administration of methoxamine alone to anaesthetized, normothermic animals did not significantly affect the noradrenaline concentration ($P > 0.05$). However, cooling of the animals produced a significant reduction ($0.02 < P < 0.05$) in cardiac noradrenaline by 39%, which agrees with previous observations that cold exposure enhances the catecholamine release from the heart[3,12].

There is much evidence that a considerable sympathetic activation takes place during induction of hypothermia. Thus, animals with an intact sympatho-adrenal system show a marked increase in blood pressure upon submersion into the ice-water bath[1,3–5,8], and cooling produces an augmented output of catecholamines from the adrenal medulla[13,14] and an increase in circulating catechola-

Synopsis of the lowest rectal temperature reached, rate of ventricular fibrillation, amount of methoxamine administered, and level of cardiac noradrenaline in animals from the various groups

Lowest temperature reached	Ventricular fibrillation	Methoxamine (mg)	Cardiac noradrenaline (μg/g)	% decrease[a]
Normothermic, untreated				
–	–	–	1.37 ± 0.10	–
Normothermic, methoxamine				
–	–	1.2	1.46 ± 0.16	–
Cooled control animals				
20.5 ± 0.5	Yes	0–2.0	0.90 ± 0.17	39
Sympathectomized 2 days, cooled				
17.6	No	1.2	0.40	73
17.6	No	1.6	0.46	68
17.6	No	0.8	0.65	55
21.9	Yes	–	0.84	42
21.6	Yes	0.8	1.02	30
23.4	Yes	1.2	1.21	17
22.2	Yes	–	1.57	0
19.2	Yes	0.8	–	0[b]
Sympathectomized 3 days, cooled				
16.9	No	1.2	0.06	96
17.7	No	1.2	0.06	96
20.8	Yes	0.8	0.10	93
22.2	Yes	–	0.13	91
23.0	Yes	1.2	–	90[b]
21.0	Yes	–	0.45	69
21.0	Yes	1.2	0.62	58
18.0	Yes	1.0	–	50[b]
22.0	Yes	1.8	–	50[b]
19.2	Yes	1.2	0.76	48

[a] Calculated from the mean value of cardiac noradrenaline in the group of normothermic, methoxamine-treated animals (see text). [b] No fluorimetric determinations available. No. of nerves remaining estimated by fluorescence microscopic analysis[17].

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mines[15,16]; the signs of sympathetic activation in the heart[3,12] have already been mentioned. It has recently been shown[8] that the cardiac catecholamine stores are not reduced during hypothermia if the animals have undergone sympathectomy according to the present procedure immediately before cooling. It can therefore be assumed that the reduction of cardiac catecholamines during hypothermia requires an intact sympathetic neuron link to the heart. Hence, it seems that the decrease in cardiac noradrenaline registered in the present sympathectomized animals (Table) reflects a decrease caused mainly by the denervation rather than by the additional hypothermia. For this reason, the percental decrease in noradrenaline of the hearts in the Table has been calculated from the values obtained in the normothermic, methoxamine-treated group of animals.

Since the reduction in cardiac noradrenaline was generally more pronounced in the 3-day group (Table), it appears that cardiac noradrenaline continues to decrease also beyond the second day after sympathectomy. In view of these considerations it is obviously impossible to correlate the catecholamine level remaining in the heart 2–3 days after sympathectomy to the frequency of ventricular fibrillation during deep hypothermia. For example, it is probable that denervation in the 3 surviving animals from the 2-day group has been more complete than indicated by the noradrenaline figures (55, 68 and 73% reduction), and that the amount of cardiac noradrenaline representing *intact* sympathetic nerves was in fact lower.

The frequency of ventricular fibrillation during hypothermia is considerably lower in animals subjected to sympathectomy by the same operation procedure immediately before cooling, even though the level of cardiac noradrenaline remains unchanged[8]. Hence, there is strong reason to believe that the high rate of ventricular fibrillation in the present series – in spite of a pronounced reduction of the cardiac noradrenaline concentration – is caused by the progressive development of supersensitivity of the myocardial receptors after denervation[6]. The fact that the surviving 5 animals (Table) had the lowest noradrenaline concentration in the 2-day and 3-day groups, respectively, indicates that the denervation has been most complete in these animals. It is therefore conceivable that the progressive supersensitivity of the myocardial receptors to catecholamines released from the intact cardiac adrenergic nerves has been the most important factor in the development of ventricular fibrillation in animals subjected to hypothermia 2–3 days following cardiac sympathectomy. If circulating catecholamines from other parts of the sympatho-adrenal system had been of any importance in the supersensitivity mechanism it could have been expected that all animals should have developed ventricular fibrillation under the present conditions.

In conclusion, the present series of experiments show that hypothermia per se results in a reduction in cardiac noradrenaline, probably by a central activation mechanism. Attempts to reduce the cardiac noradrenaline stores through sympathectomy 2 or 3 days before cooling does not afford any significant protection against spontaneous ventricular fibrillation. This is probably due to the rapid development of supersensitivity of the myocardium to catecholamines, notably the noradrenaline released from the cardiac adrenergic nerves if the denervation has not been sufficiently complete[18].

Zusammenfassung. Versuche mit Katzen haben gezeigt, dass Hypothermie in einer Reduzierung des Noradrenalingehaltes im Herzen resultiert (zentrale Aktivierung). Der Versuch, den Noradrenalingehalt im Herzen durch Sympathektomie 2 oder 3 Tage vor Unterkühlung zu reduzieren, ergibt aber keinen signifikanten Schutz gegen spontanes Ventrikelflimmern. Dieses Verhalten beruht wahrscheinlich auf der schnellen Entwicklung der Empfindlichkeitssteigerung des Myokards gegenüber Katecholaminen und besonders Noradrenalin, das von adrenergen Herznerven freigesetzt wird (bei unvollständiger Denervierung).

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Detection of Antibody and Antigen in the Milkweed Bug, *Oncopeltus fasciatus*

Most of the attempts to demonstrate antibody production in insects have been with microorganisms or particulate antigens and the tests have been the standard serological ones[1–3]. Kamon and Shulov[4] immunized locusts with scorpion venom but they could not demonstrate any antibody production.

We have used 2 complex soluble antigen systems, rabbit serum or goat globulin, and 2 very sensitive detection tools, the fluorescent antibody technique[5] and the Ouchterlony technique[6], in an attempt to detect antibody production and to trace the fate of the antigen in the large milkweed bug, *Oncopeltus fasciatus* (Dallas). The antigen injection was always given with a microinjector and a 30 gauge needle at the base of a metathoracic leg of an insect which was 24 h past the molt into the last nymphal stadium. The control insects were injected with

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