Depletion and Repletion of Noradrenaline in Adrenergic Nerves of the Rat after Decaborane Treatment

Decaborane ($B_{10}H_{14}$) has been reported to deplete the rat brain[1] and peripheral organs of the rabbit[2,3] of noradrenaline (NA). The NA content is readily restored to about normal levels after an injection of NA[2-4] with an apparently normal subcellular distribution.

As to the mode of action of decaborane, MERRITT and SCHULTZ[5] have produced evidence to show that it inhibits the decarboxylation of DOPA, apparently by inactivation of the co-factor pyridoxal-5'-phosphate[6].

The depletion is enhanced by cold exposure[7] which increases sympathetic nerve activity[8]. Moreover, BYGDEMAN and EULER[9] have shown that electrical stimulation of sympathetic nerves in decaborane-treated animals rapidly becomes inefficient, indicating transmission failure owing to lack of available transmitter.

The presence of noradrenaline and other amines in the adrenergic nerves can be studied with the aid of the histochemical fluorescence method of FALCK and HILLARP, see[10-12]. Recent experiments suggest that this method can be used, even in a semiquantitative way, with good accuracy[13].

The aim of the present work was to study the effect of decaborane on the NA content in the adrenergic nerves of rat iris with the fluorescence histochemical method and the effect of exogenous NA after depletion with a particular view to its cellular localization.

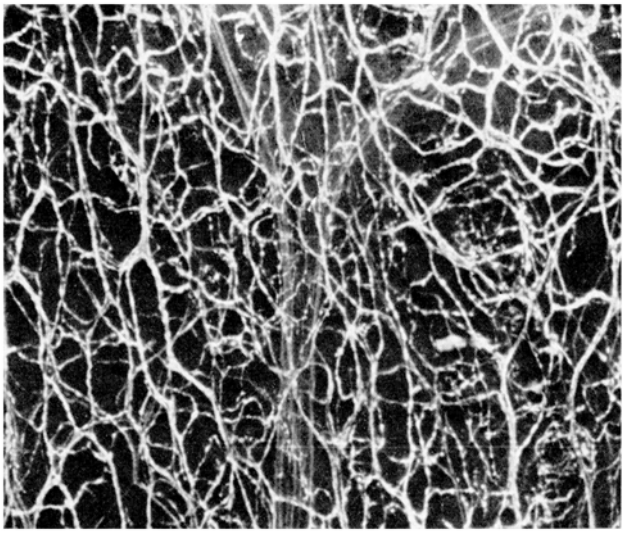
Material and methods. Sprague-Dawley rats weighing about 200 g were used. In some animals the left side iris and submaxillary gland were decentralized by cutting the sympathetic trunk at various time intervals before death. In some animals the right cervical sympathetic trunk was electrically stimulated for 80 min with 10 biphasic impulses per sec (for details cf. MALMFORS[10]).

After killing the animals by decapitation, the eyes were removed, and the irises prepared, stretched and mounted on a slide, dried and treated with formaldehyde gas of optimal humidity and examined in a fluorescence microscope (for details cf. MALMFORS[10]). From some animals the submaxillary glands were removed and analyzed fluorimetrically for NA.

The following drugs were used: Decaborane, kindly given to us by Dr. A. A. WYKES, was dissolved in a small amount of ethyl alcohol, diluted with saline and used immediately. It was given in doses of 15–30 mg/kg i.p. Reserpine (Serpasil®, 10 mg/kg i.p.). (−)-Noradrenaline bitartrate, 0.1 mg/kg calculated as base i.v., 15 min before death. Desipramine (DMI 25 mg/kg i.p.), 30 min before the NA. Nialamide (100 mg/kg i.p.), 1–6 h before death.

Results. The fluorescence of the adrenergic nerves due to their content of endogenous NA in normal rat iris (Figure 1) has been described earlier[10].

Depletion with decaborane. The effects of decaborane, 15 and 30 mg/kg, on the fluorescence intensity of the adrenergic nerves in the rat iris are shown in Table I: 24 h after the highest dose of decaborane the nerve fluorescence was very markedly reduced and only faint streaks of fluorescence could be seen, mainly in the varicosities and in the non-terminal axons (Figure 2). A few single terminals[14] showed a somewhat stronger fluorescence (cf. MALMFORS and SACHS[14]). In the decentralized iris from the decaborane-treated animal the fluorescence was almost normal (Table I, Figure 3). The fluorescence of the adrenergic nerves in the animals given decaborane 24 h and nialamide 6 h before death was clearly stronger than that in the control animals not given nialamide (Table I).



Stretch-preparations of rat irises mounted whole. The ground-plexus of the adrenergic terminals above the dilator muscle is seen with varying fluorescence intensity due to the pretreatment of the animals. Besides the ground-plexus strands of smooth-looking ground-plexus can be seen. All figures ×200.

Fig. 1. Normal animal. Strongly fluorescent terminals with pronounced varicosities and weakly fluorescent non-terminal axons.

Table I. The fluorescence intensity of the adrenergic nerves in the rat iris after treatment with decaborane

Dose mg/kg	Time after the injection (h) 24	48	72
15	++[a] (3)	++ (3)	
30	(+) (10)	(+) (4)	+ (3)
30 and nialamide 100 mg/kg 6 h before death	++ (4)		
30 and decentralization	+++ (6)		

Number of animals in parenthesis. [a] Key to the symbols: +++, strong fluorescence; ++, medium fluorescence; +, weak fluorescence; (+), very weak fluorescence.

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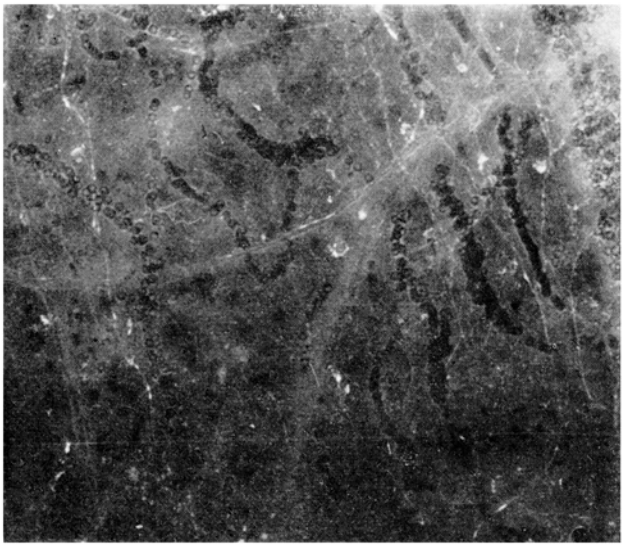

Fig. 2. Decaborane 30 mg/kg for 24 h, intact iris. Very weakly fluorescent terminals, mainly varicosities, and non-terminal axons.

Fig. 3. Same animal as Figure 2 but iris decentralized shortly before the administration of decaborane. The fluorescence is the same as in the normal animal.

Table II. Restitution of the specific fluorescence of the adrenergic nerves of rat iris with NA (0.1 mg/kg i.v. given 15 min before death) with or without preceding nialamide (100 mg/kg 1–2 h before). All animals were given decaborane (30 mg/kg 24 h before) and in some cases reserpine (10 mg/kg i.p. 30 min before the NA) or DMI (24 mg/kg i.p. 30 min before the NA)

Pretreatment	NA	Nialamide + NA
Decaborane	+++[a] (7)	+++ (7)
Decaborane + reserpine	(+) (3)	+++ (3)
Decaborane DMI	(+) (3)	+

Number of animals in parenthesis. [a] Key to the symbols: see Table I.

Table III. NA content, μg/g, in the normally innervated and the 7–10 days previously decentralized rat submaxillary gland after 15–20 mg/kg decaborane i.p. 24 h prior to removal of glands

Normal gland	NA (μg/g)
	1.4 ± 0.12
After decaborane	
Control	0.55 ± 0.11
Decentralized	1.0 ± 0.06

M ± S.E.M.

When the cervical sympathetic trunk was electrically stimulated for 80 min, 60 min after 30 mg/kg decaborane, the fluorescence in the stimulated iris disappeared to a greater extent than in the unstimulated one which still had nearly normal fluorescence.

Restitution of the NA content after decaborane by NA-injection. When decaborane-pretreated animals (24 h before) were given NA 0.1 mg/kg i.v. 15 min before sacrifice, there was a marked restitution of the fluorescence (Table II, Figure 4). The intensity of the fluorescence was about the same as in an untreated animal. Furthermore the varicose appearance of the adrenergic terminals was about the same in both cases and the same as in untreated animals, whereas the non-terminal axons were rather weakly fluorescent both in untreated and in animals treated with NA. After DMI (25 mg/kg), NA caused no restitution of the fluorescence (Table II, Figure 5). After reserpine there was a clear restitution of the fluorescence in the iris depleted by decaborane only if the NA was preceded by nialamide (Table II, Figure 6), although the appearance was different from the normal one. The terminals were smooth and the non-terminal axons were pronounced[10].

Depletion of NA in the normal and decentralized rat submaxillary gland after decaborane. As shown in Table III, sympathetic decentralization of the gland (7–10 days before) greatly reduces the depleting effect of decaborane, which is in agreement with the results on iris fluorescence. A maximal depletion of NA was not aimed at in these animals.

After decentralization the submaxillary glands showed an increase in weight of about 10%[15].

Discussion. The depletion of the adrenergic transmitter in the sympathetic nerves of the rat iris by decaborane as demonstrated by the fluorescence histochemical method, is in good agreement with earlier reported findings from other organs in which the NA content was directly determined[2]. The weak fluorescence of the iris nerves after high doses of decaborane might even indicate a reduction below 10% of the normal value[16].

The time course and especially the morphological picture of the remaining fluorescence with a few strongly fluorescent axons and terminals is similar to that seen after the tyrosinehydroxylase inhibitor H44/68, the

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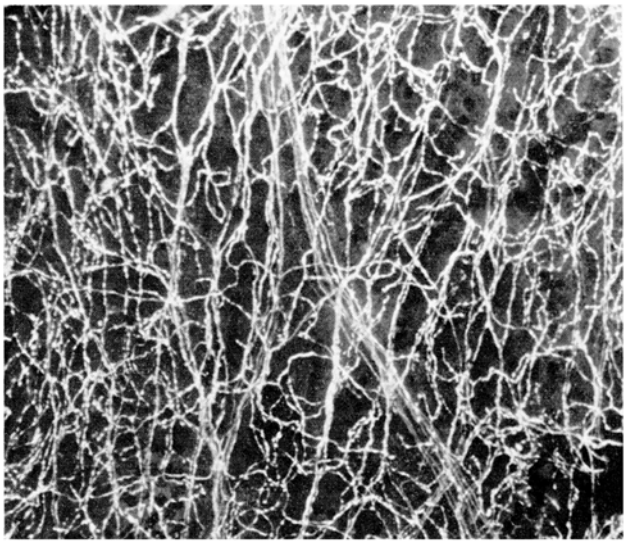

Fig. 4. 24 h after decaborane 30 mg/kg, NA (0.1 mg/kg i.v.) has been given 15 min before death. The restituted fluorescence is the same as in a normal animal.

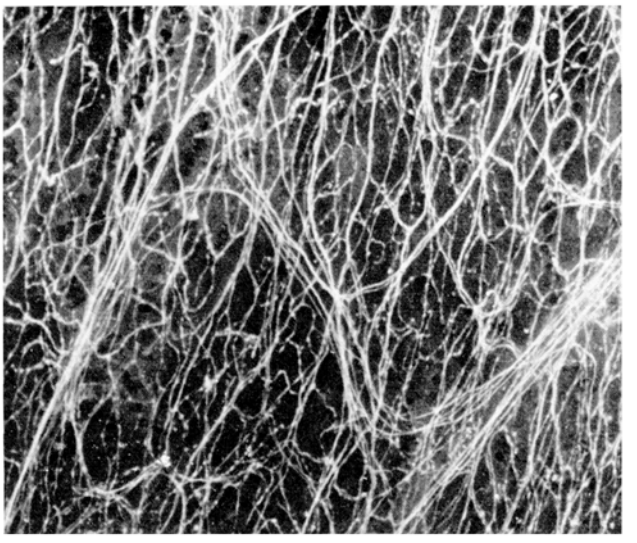

Fig. 6. The same treatment as in Figure 4 but in addition nialamide (100 mg/kg) and reserpine (10 mg/kg) was given 2 h and 30 min before the NA respectively. There is a marked restitution of the fluorescence but the terminals are not as varicose as in Figure 4 and the non-terminal axons are more pronounced.

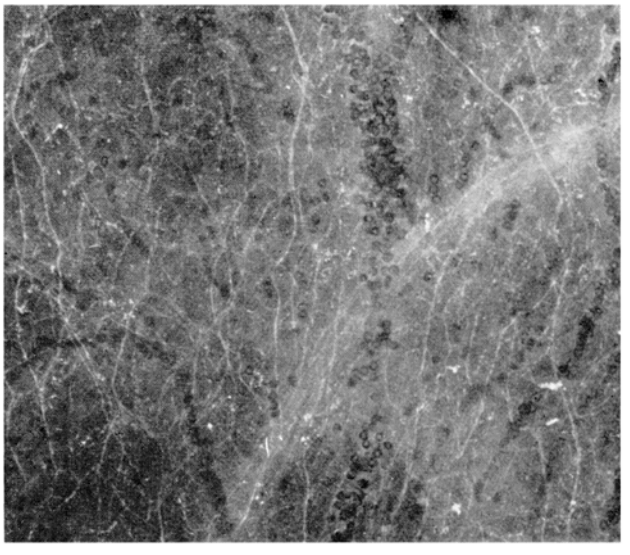

Fig. 5. The same treatment as in Figure 4 but DMI (25 mg/kg i.p.) has been given 30 min before the administration of NA. The fluorescence is about the same as in the animal treated with decaborane alone for 24 h (cf. Figure 2).

methyl-ester of α-methyl-*p*-tyrosine[17]. Furthermore, the effect of decentralization and stimulation was the same after both drugs. Obviously the normal nerve impulses cause a more rapid depletion after inhibition of NA synthesis. This effect is further enhanced by electrical stimulation[9,18].

From the present and previous observations, it appears that decaborane does not impair the uptake and storage mechanisms[2,3] in the adrenergic nerves, since the transmitter content can be easily restored in an apparently normal fashion by exogenous NA. Furthermore, the cellular and subcellular distribution of the exogenous NA seems to be the same as in a normal animal[3] as also indicated by the present experiments. When the uptake of NA into the amine storage granules is impaired, as after reserpine, NA is taken up and stored in all likelihood extragranularly. This accumulation is markedly potentiated by inhibition of MAO. After decaborane, however, MAO has very little effect on the reappearance of fluorescence after injection of NA, suggesting that in this case uptake of NA is essentially in the granules. Even when decaborane is given shortly before the administration of NA, it does not seem to affect the uptake mechanisms of the axon.

The evidence obtained by the histochemical fluorescence technique thus supports the conclusion that, in agreement with previous findings, decaborane inhibits resynthesis of the adrenergic neurotransmitter but does not impair the uptake and storage mechanisms in the axon membrane or the intraaxonal granules[19,20].

Zusammenfassung. Mit Hilfe der Fluoreszenz-Technik werden die Veränderungen in sympathischen Fasern der Ratteniris nach Noradrenalin-Depletion durch Boron-Hydrid Decarboran (Dopa-Dekarboxylase-Hemmer) analysiert.

T. Malmfors and U. S. von Euler

Departments of Histology and Physiology, Karolinska Institutet, S-10401 Stockholm 60 (Sweden), 23 November 1970.

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