fluorescence in the absence of formaldehyde treatment. The brain stems from rats pretreated with reserpine (5 mg/kg i.p. 12 hr) were homogenized and fractionated concurrently with those from normal animals. These smears were free of fluorescent spots.

DISCUSSION

Because of the small dimensions of the isolated particles, it was not possible to determine whether the fluorescent spots were all green to yellow-green (NA or dopamine) or whether a few had a yellow fluorescence (5-HT). Nevertheless, the size of the fluorescent spots and the specificity of the fluorescence reaction leave little doubt that they contain monoamines and indicate that they are identical with the varicosities of the nerve terminals. It is thus clear that amine-containing fluorescent structures, presumably the varicosities, follow the separation of the NEPS fraction by density gradient centrifugation, since the P_2–A fraction consists mainly of myelin fragments, P_2–B of NEPS, and P_2–C of mitochondria.[8] 5-HT and NE determinations of the subfractions of P_2[6, 7] are in general accordance with the present results.

Electron microscopic observations have shown that the synaptosomes[9] and the synaptic structures of autonomic nerves seen in tissue sections[10, 11] have the same structural characteristics. There is strong evidence that these latter structures are identical with the varicosities of the nerve terminals observed in the fluorescence microscope.[3, 4] The fluorescent spots seen in the present study are thus in all probability identical with the varicosities of the nerve terminals seen in the fluorescence microscope, with amine-containing synaptosomes obtained by density gradient centrifugation, and with the presynaptic widenings of the nerves seen in electron microscopic sections. All these structures thus in all probability represent the synaptic structures of the monoaminergic nerve terminals.

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Release of cardiac noradrenaline by decaborane in the heart-lung preparation of guinea pig

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MERRIT *et al.*[1] reported the ability of decaborane ($B_{10}H_{14}$) to deplete noradrenaline from the rat brain. Recently Euler and Lishajko[2] have found that decaborane (10^{-5} M) releases catecholamines from isolated adrenergic nerve granules. A striking decrease of the noradrenaline content in the rabbit organs 24–48 hr after the administration of 4 mg/kg of this drug has been shown by the same authors. The depletion of the heart catecholamines was found to be 80–90 per cent. The action of decaborane was previously studied *in vivo* by i.p. or s.c. administration. With this procedure it is difficult to follow the first phase of the depletion process. In an attempt to gain further information

on this problem the effects of acute administration of decaborane on the release of ^{3}H noradrenaline from the heart were studied using the heart–lung preparation of guinea pig.

EXPERIMENTAL PROCEDURE

Male guinea pigs, weighing 200–300 g were used. The heart–lung preparation was prepared as previously described.[3, 4] The venous reservoir was filled in each experiment with 35 ml of heparinized, freshly collected blood; 5 μc of ^{3}H noradrenaline (New England Nuclear Corp., specific activity 7 mM, chromatographically purified prior to use) were infused at the bottom of the venous reservoir during a 10 min period. After waiting for 20 min the amount of ^{3}H noradrenaline present in the blood was determined by extracting 0·5 ml. of blood with 2 ml. 10% trichloroacetic acid (TCA); 0·5 ml. of the TCA soluble fraction were used for the radioactivity assay in a Packard Liquid Scintillation Spectrometer. Decaborane was dissolved in a small volume of ethanol which was then diluted with phosphate buffer, pH 6·5, 1:1. 0·01 ml. of this solution, containing 3·5 μg of the drug (0·10 μg/ml. blood) were added to the reservoir. Tyramine HCl was used at the concentration of 0·14 μg/ml. blood. After the addition of the drugs to the reservoir, blood samples (0·5 ml.) were taken every 5 min and the changes of the blood radioactivity were measured as described.

At the end of the experiment (110 min after the infusion of ^{3}H noradrenaline) the heart was immediately homogenized in 15 ml. 10 per cent TCA. Heart catecholamines were adsorbed on to an aluminium oxide column, eluted with 0·25 N acetic acid and fluorimetrically determined according to the method of Euler and Lishajko;[5] the ^{3}H noradrenaline uptake into the heart was calculated from the count/min present in 0·5 ml of the eluate from the aluminium oxide column. Another group of experiments was made using preparations obtained from animals treated with 4 mg/kg i.p. of decaborane 24 hr previously: this dose was found to cause, in 24 hr, a 95 per cent depletion of the heart noradrenaline content. The cardiac uptake of ^{3}H noradrenaline was estimated, in this group, 110 min after the tracer was infused.

In 3 control preparations the uptake of ^{3}H noradrenaline by the heart was determined with the same procedure.

RESULTS AND DISCUSSION

The addition of 0·10 μg/ml. of decaborane to the venous reservoir resulted in a release of ^{3}H noradrenaline from the heart, represented by an 18·3 $\pm$ 1·73 per cent increment in the blood radioactivity (Fig. 1). This effect lasted for about 30 min. After this period the radioactivity slowly decreased and 50 min later the increase in the count/min in the blood was only 2·5 $\pm$ 0·28 per cent. At this time the addition of a second dose of decaborane to the reservoir induced a 12·5 $\pm$ 1·24 per cent increment

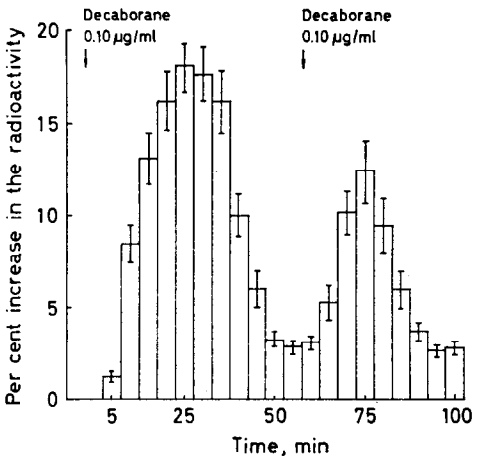

FIG. 1. Effects of two additions of decaborane (0·10 μg/ml. blood) on the release of ^{3}H noradrenaline from the heart. The time of the addition of the drug to the blood reservoir of the heart–lung preparation is marked by the arrows. Ordinates show the per cent increase of the radioactivity in 0·5 ml. blood samples taken each 5 min from the reservoir. Each point represents the mean $\pm$ S.E. of 3 Expts.

in the radioactivity: this rise was significantly smaller ($P < 0.05$) as compared with the effect of the first dose. The total increase in the blood radioactivity induced by decaborane can also be expressed as per cent of ^3H noradrenaline released from the heart: the first and the second dose of decaborane released 29·4 ± 1·06 per cent and 20·1 ± 0·76 per cent repectively of the amount of ^3H noradrenaline that was present in the heart as determined at the end of the experiment.

In a second group of preparations the effect of tyramine on the release of noradrenaline by decaborane was tested: tyramine HCl 0·14 μg/ml.) induced a 9·10 ± 0·96 per cent increase in the blood radioactivity. The effect of decaborane, administered after tyramine, was not altered (16·3 ± 0·42 per cent increase in the blood radioactivity).

In the experiments in which two consecutive doses of decaborane were administered (Table 1, Group 2), the ^3H noradrenaline uptake by the heart and the endogenous noradrenaline content were not significantly different as compared with the control group. Again, in the experiments in which tyramine and decaborane were administered (Table 1, Group 3) these patterns did not significantly vary from control values.

TABLE 1. EFFECT OF THE ACUTE ADMINISTRATION OF DECABORANE AND TYRAMINE AND OF THE PRETREATMENT WITH DECABORANE ON THE HEART NORADRENALINE CONTENT AND ^3H NORADRENALINE UPTAKE IN THE HEART–LUNG PREPARATION OF GUINEA PIG

Group	Type of treatment	Heart noradrenaline (μg/g)	P	Per cent ^3H noradrenaline uptake	P
1	none	1·10 ± 0·06		32·6 ± 0·12	
2	decaborane + decaborane	1·16 ± 0·07	>0·05	31·7 ± 0·08	>0·05
3	tyramine + decaborane	1·08 ± 0·11	>0·05	32·3 ± 0·13	>0·05
4	pretreatment with decaborane (4 mg/kg 24 hr previously)	0·06 ± 0·002	<0·001	31·8 ± 0·15	>0·05

All values are mean of 3 Expts. ± S.E. The P values relate to Student's test of significance. In the Group 2 two consecutive doses of decaborane (0·10 μg/ml.) were added to the blood reservoir 20 min after the infusion of ^3H noradrenaline (see Experimental Procedure). In the group 3 decaborane (0·10 μg/ml.) was administered after a dose of 0·14 μg/ml. of tyramine HCL (free base) following the described procedure. In all the groups the endogenous content of noradrenaline and the ^3H noradrenaline uptake by the heart were estimated 110 min after the infusion of the tracer in the blood reservoir.

In the preparations obtained from guinea pigs pretreated with decaborane (Table 1, Group 4) the cardiac uptake of ^3H noradrenaline was not significantly different from that in the controls while the pretreatment with this drug resulted in a significant decrease of the endogenous noradrenaline content ($P < 0.01$).

The present results demonstrate that decaborane acutely releases noradrenaline from the heart stores. It has been previously shown that tissues can be refilled with catecholamines after noradrenaline stores have been depleted by decaborane.[2] This study clearly demonstrates that pretreatment by this drug does not prevent the heart noradrenaline uptake. This fact is also supported by the finding that the ^3H noradrenaline acutely released from the heart by decaborane can be again taken up.

Previous studies have suggested that the release of catecholamines by decaborane could be explained by the formation of borane–catecholamine complexes:[1, 2] since boranes are readily hydrolyzed,[6] it is possible to explain the re-uptake of the released noradrenaline by an inactivation of these complexes in the blood. In this regard the importance of the recirculation of the blood and drugs through the heart, using the heart–lung preparation, must be underlined.

After the administration of a first dose of decaborane a second dose of the drug induced a smaller release of ^3H noradrenaline. This finding can be explained by: (1) the conversion of the ^3H noradrenaline taken up by the heart from an "available" to a more firmly bound storage form; or (2) the existence of a tachyphylaxis mechanism. The inability of tyramine to reduce the response to decaborane

seems to exclude the second process. In this regard further research is being conducted in this laboratory using the isolated perfused dog spleen.

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Metabolism of organophophorus insecticides—VI. Mechanism of detoxification of Dipterex* in the rat

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DIPTEREX, being a potent anticholinesterase agent, is widely used as an insecticide. Since mammals—including man—are subjected to possible hazards, it seemed highly desirable to investigate the mechanism of detoxification of the insecticide in the rat. The metabolism of Dipterex in the rat has been recently investigated *in vivo* by Hassan and Zayed;[1] using ^{14}C-labelled insecticide in which the two methyl groups are ^{14}C-labelled. The fate of Dipterex was followed during 24 hr, at the end of which 28 per cent of the administered dose could be recovered as $^{14}CO_2$ in the expired air and 32 per cent was eliminated into the urine. ^{14}C-formate and ^{14}C-dimethylphosphate in the urine constituted 2 per cent and 22 per cent of the injected dose respectively.

For further investigation of the metabolic pattern of Dipterex in the rat, ^{32}P-labelled insecticide has been used. A sublethal radiodose in saline (100 mg/kg body weight) was injected (i.p.) and the urine was collected for 48 hr. ^{32}P-activity recovered from the urine amounted to 75–85 per cent of the administered dose. The total activity proved to be products of acidic character; since it could not be recovered from Dowex (1-x8, Cl^-) by hydrochloric acid of pH 3·0 and was readily and completely eluted at pH 1·5. The acidic eluate was paper chromatographed in three different solvent systems (cf. Table 1). Radiometric assay of the chromatograms revealed the presence of three ^{32}P-labelled metabolites. Two substances were identified as mono- and dimethylphosphates, as they possess *Rf* values similar to those of authentic samples run alongside as reference substances. The third metabolite, however, remained unidentified. It cannot possibly be orthophosphate or monodemethylated Dipterex (cf. Table 1).

From several chromatograms, it has been estimated that mono-methylphosphate accounts for 20–30 per cent, and dimethylphosphate for 60–70 per cent of the total metabolites output. The unidentified metabolite contributes to about 10 per cent. After acid hydrolysis of the total ^{32}P-metabolites, only mono- and dimethylphosphates could be detected by paper chromatography. Whether the

* 0,0-dimethyl-2,2,2-trichloro-1-hydroxyethyl phosphonate.