Studies of the cofactor requirements of the N-demethylase in lung microsomes have shown its similarity to the analogous enzyme in liver. Thus, the omission of NADP and NADH or glucose-6-phosphate and glucose-6-phosphate dehydrogenase from the incubation mixture markedly inhibited the microsomal N-demethylation of 3-methyl-MAB by lung. Heating lung microsomes at 100° for 10 min also resulted in loss of enzyme activity.

Recent studies by Wattenberg and co-workers, utilizing an extremely sensitive analytical assay procedure, have demonstrated low levels of NADPH-dependent benzpyrene hydroxylase in the adrenal, kidney and small intestine of normal rats.[7, 8] They found that the administration of various polycyclic hydrocarbons to rats caused large increases in benzpyrene hydroxylase activity in the latter two tissues and caused the appearance of activity which was previously too low to be detected in the thyroid, lung, testis, and skin. Other studies by Dutton and Stevenson showed increased glucuronide synthesis in the skin of mice painted with 3,4-benzpyrene.[9]

The presence of inducible N-demethylase, hydroxylase, and glucuronyl transferase in nonhepatic tissues suggests that these enzymes as well as their hepatic counterparts may play a role in detoxifying drugs and other foreign compounds and that changes in the low activity of these enzymes at or near a receptor site may alter the pharmacological action of drugs that have escaped metabolic conversion by the liver.

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Increased retention of exogenous norepinephrine by cat atria after electrical stimulation of the cardioaccelerator nerves*

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THE retention of unchanged ^{3}H-norepinephrine (^{3}H-NE) by the sympathetically innervated structures of the eye is greatly decreased by chronic, superior cervical ganglionectomy.[1] On the other hand electrical stimulation of the splenic nerves of cats, previously given intravenous injections of ^{3}H-NE increased the output of the amine and its major metabolite, normetanephrine, from the organ.[2] These observations, together with recent radioautographic localization of ^{3}H-NE almost exclusively over sympathetic nerve endings,[3] indicate that the retention of tissue catecholamine is dependent upon the integrity of sympathetic nervous structures. This being so, it was of interest to determine

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whether *increased* sympathetic activity immediately before intravenous injections of ^{3}H-NE led to the increased tissue incorporation of the amine. For this purpose the cat heart was used.

Cats (1·2 to 2·3 kg), anesthetized with pentobarbital (30 to 40 mg/kg, i.p.), were prepared for stimulation of the right cardioaccelerator nerves by means of a right thoracotomy, followed by resection of the second rib.[4] Test animals and operated control animals were maintained on artificial respiration. Stimulation of the postganglionic fibers from the stellate ganglion was carried out, via shielded, bipolar platinum electrodes, using a 30 V stimulus (1 msec duration and 20 stimuli/sec) for 30 sec.* The heart rate was monitored with an electrocardiograph (lead II), recorded on a Grass polygraph. Chromatographically pure ^{3}H-NE,† as a solution of the hydrochloride (New England Nuclear Corp.), with a specific activity of 29·76 μc/μg base, was given via a jugular cannula in a dose of 2 μg base/kg body weight, either immediately after or 1 min before the 30-sec period of stimulation. The heart was removed 15 min after the administration of the ^{3}H-catecholamine, and the atria and ventricles were homogenized separately in chilled perchloric acid (0·4 N), prior to the estimation of ^{3}H-NE by a method similar to that described by Crout *et al.*[5] Aliquots of both the original extract after centrifugation and the acid-eluate from the alumina (which contained the ^{3}H-NE) were used for the determination of total radioactivity (^{3}H-total) and that accounted for by unchanged amine, respectively. Radioactivity was measured in a liquid scintillation counter, with a dioxane-phosphor;[6] corrections for quenching were made by adding internal standards of tritiated water to each sample.

The results of these experiments are shown in Table 1. Despite a rather wide range of individual values, especially in the ventricles, it can be seen that supramaximal sympathetic stimulation, immediately before administration of the amine, significantly increased the total amounts of tritium and ^{3}H-NE specifically in the atria. This increase was unrelated to altered heart rate since, in the unstimulated control animals, the administration of ^{3}H-NE always produced tachycardia, whereas the combined effect of sympathetic stimulation followed by the amine ranged from varying degrees of tachycardia (as great as that seen in control animals) to bradycardia. If altered functional activity

TABLE 1. THE INFLUENCE OF SYMPATHETIC NERVE STIMULATION ON THE TOTAL AMOUNT OF TRITIUM AND THE AMOUNT OF ^{3}H-NOREPINEPHRINE IN CAT ATRIA AND VENTRICLES

Results are expressed as disintegrations per min ± SEM. (× 10^5) per gram.

	No. of experiments	Ventricle		Atria	
		^{3}H-total	^{3}H-norepinephrine*	^{3}H-total	^{3}H-norepinephrine*
Control† (unstimulated)	5	17·26 ± 1·49	12·28 ± 1·92	8·49 ± 0·63	5·02 ± 0·32
Stimulated for 30 sec immediately *before* injection of ^{3}H-NE	5	16·27 ± 1·03	11·09 ± 0·96	12·84 ± 0·85‡	8·81 ± 0·53‡
Stimulated for 30 sec 1 min *after* injection of ^{3}H-NE	4	13·72 ± 1·69	9·71 ± 1·07	10·74 ± 0·61	5·78 ± 0·12

* ^{3}H-norepinephrine values are *uncorrected* for recovery.

† No surgery was carried out on two of the control animals. After the time usually taken to prepare for sympathetic stimulation (45 min), ^{3}H-NE was administered intravenously. The values for the total amount of tritium (^{3}H-total) and the amount of ^{3}H-NE in both atria and ventricles fell within the range of values obtained with the other control animals and, therefore, have been used in the calculation of mean control values.

‡ Significantly different ($P < 0·01$) from corresponding control values.

* American Electronics stimulator (model 751-B) was used.

† DL-Norepinephrine-7-^{3}H.

of the heart had been responsible for the increased retention of amine, it could be expected that this would be reflected particularly in the ventricles in view of their greater tissue mass. In fact, however, the total amounts of tritium and of ^{3}H-NE in the ventricles were not measurably influenced by stimulation immediately before the amine injection. Since both atria and ventricles receive postganglionic sympathetic innervation,[7] the absence of effect in the ventricles was unexpected. One explanation for this finding may be the greater ratio of tissue mass to nerve endings in the ventricles, which might act to obscure any change in the retention of ^{3}H-NE after sympathetic nerve stimulation.

Whitby *et al.*[8] have shown that *o*-methylation is the major metabolic pathway for norepinephrine in the cat heart. For the following reasons it appears that supramaximal sympathetic stimulation before the administration of ^{3}H-NE results in a decreased *o*-methylation of the amine retained by the tissue. The aluminum oxide extraction method is specific for those chemical structures containing a catechol nucleus[9] and, therefore, the difference between the total amount of tritium and the ^{3}H-NE may be assumed to reflect the extent of conversion of administered norepinephrine to normetanephrine. If the individual values for ^{3}H-NE were corrected for the 70% average recovery of the amine actually obtained in this laboratory, it was found that 85·0 $\pm$ 3·6% of the total amount of tritium in control atria was present as unchanged ^{3}H-NE; the corresponding figures for atria stimulated before the administration of the amine were 98·3 $\pm$ 2·6%, and the difference between these values is significant ($P < 0{\cdot}05$).

Stimulation of the cardioaccelerator fibers 1 min after terminating the administration of ^{3}H-NE caused no significant alteration in either the total amount of tritium or the amount of ^{3}H-NE in either atria or ventricles. If ^{3}H-NE was liberated by nerve stimulation, the amount involved may have been too small to cause a measurable decrease in tissue ^{3}H-NE. Alternatively, the 1-min interval between administration of ^{3}H-NE and stimulation of the cardioaccelerator nerve may have been insufficient to allow penetration of the amine to sites from which it could be liberated by nerve stimulation. In this regard it should be considered that, in the work of Hertting and Axelrod[2] referred to above, 3 hr elapsed between the administration of ^{3}H-NE and stimulation of the splenic nerve, with subsequent liberation of labeled amine from the organ. Even though the *total* ^{3}H-NE of heart is as high at 2 min as at 2 hr after administration of the amine[8], there may be within that time a slow movement of retained amine toward the sites intimately concerned with sympathetic nerve activity. The decreased extent of *o*-methylation of retained ^{3}H-NE may indicate that this normally slow movement of exogenous amine can be accelerated if the stores of NE associated with sympathetic transmission are first depleted by stimulation of the sympathetic nerves.

Siegal *et al.*[10] have shown recently that continued submaximal stimulation of canine cardiac sympathetic nerves causes at least partial depletion of endogenous myocardial catecholamine, which then may be repleted by circulating amines. The results presented here are compatible with these findings.

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