Similar standard curves recorded at 30°C could be obtained for a number of oximes and for hydroxylamine. The ratio

$$\frac{\Delta \text{ absorbance}}{\text{oxime conc.}} \times 10^{-2}$$

indicating the slope of the standard curve, is stated below for the various compounds.

Pyridine-2-aldoxime-N-methiodide (pralidoxime iodide, P-2-AM), 3·3.
Pyridine-2-aldoxime-N-methyl methansulphonate (P-2-S), 3·0.
Pyridine-2-aldoxime-N-methylchloride, 3·7.
Pyridine-2-aldoxime, 0·4.
Pyridine-4-aldoxime, 1·2.
Diacetylmonoxime (DAM), 1·8.
Trimethylen-bis-(pyridine-4-aldoxime) dibromide (TMB-4), 9·6.
Hydroxylamine, 1·0.

The reproducibility of the standard curves indicates a method for quantitative determination of oximes in aqueous solution by the acetyl-thiocholine method.

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The induction of aminoazo dye N-demethylase in nonhepatic tissues by 3-methylcholanthrene

(*Received* 16 *January* 1963; *accepted* 23 *January* 1963)

THE intraperitoneal injection of polycyclic hydrocarbons such as 3-methylcholanthrene (3-MC) and 3,4-benzpyrene (BP) markedly and rapidly increases the activity of enzyme systems in rat liver microsomes which N-demethylate 3-methyl-4-monomethylaminoazobenzene (3-methyl-MAB) and which metabolize other foreign compounds and drugs.[1–3] Similar treatment of rats with drugs such as phenobarbital, barbital, aminopyrine, phenylbutazone, or orphenadrine also results in such increases in activity.[4]

Our laboratory has utilized 3-methyl-MAB as a model substrate that appears to be metabolized by the same microsomal enzymes in liver that metabolize a variety of drugs. The oxidative N-demethylation of this substrate to 3-methyl-4-aminoazobenzene (3-methyl-AB) requires molecular oxygen, NADPH* and NADH for maximum activity,[5] and changes in the activity of the azo dye, N-demethylase are paralleled by changes in the activity of several other drug-metabolizing enzymes in liver microsomes. The present investigation was undertaken to determine whether the administration of 3-methylcholanthrene or phenobarbital could stimulate drug-metabolizing enzymes in nonhepatic tissues.

METHODS

Male Sprague–Dawley rats, weighing 50 to 70 g, were maintained on a 22% casein diet containing high levels of vitamins.[6] The rats were injected intraperitoneally with 1 mg of 3-MC in 0·25 ml of corn oil for 2 days. Control rats received injections of corn oil. The animals were killed by decapitation and

* NAD^+, $NADP^+$, NADH, and NADPH, refer to the oxidized and reduced forms of nicotinamide andenine dinucleotide and nicotinamide adenine dinucleotide phospate respectively.

33% homogenates of the various tissues were made in ice-cold 0·25 M sucrose solution. The homogenates were centrifuged at 9000 × *g* for 15 min at 2°, and the resulting supernatant fraction was centrifuged at 105,000 × *g* for 1 hr to obtain microsomes. The microsomes were resuspended in 0·1 M KH_2PO_4 buffer (pH 7·4), and incubations were carried out aerobically with shaking at 37° in the presence of a cofactor mixture containing 0·20 ml glucose-6-phosphate (42 mg/ml),* 0·25 ml NADP (8 mg/ml), 0·10 ml NADH (20 mg/ml), 0·06 ml $MgCl_2$ (0·1 M), 0·11 ml Tris buffer (0·05 M, pH 7·4), 0·18 ml glucose-6-phosphate dehydrogenase (20 Kornberg units/ml), 0·10 ml of 3-methyl-MAB (1·5 mg/ml), and 2·0 ml of microsomal suspension. Microsomes from liver were incubated for 12 min; microsomes from the other tissues were incubated for 60 min. Formation of 3-methyl-AB was measured as previously described.[5] In each experiment, control flasks containing substrate, liver microsomes, and cofactors were inactivated at zero time. The amount of 3-methyl-AB present in these flasks averaged 0·4 ± 0·1 μg.† Incubation of substrate and cofactors for 60 min with lung microsomes heated at 100° for 10 min did not increase this blank value. The blank value obtained in each experiment was subtracted from all experimental values reported here.

RESULTS AND DISCUSSION

Microsomes of brain, testis, lung, or kidney obtained from control rats had little ability to N-demethylate 3-methyl-MAB (Table 1). However, treatment of rats with 3-MC for 2 days markedly elevated azo dye N-demethylase activity in lung and kidney but not in brain or testis (Table 1). The intraperitoneal injection of 37·5 mg of phenobarbital/kg twice daily for 4 days did not cause significant

TABLE 1. EFFECT OF 3-METHYLCHOLANTHRENE PRETREATMENT ON AMINOAZO DYE N-DEMETHYLASE ACTIVITY IN VARIOUS RAT TISSUES*

Tissue	Control (μg 3-methyl-AB formed)	3-MC (μg 3-methyl-AB formed)	Stimulation (%)
Brain	1·1 ± 0·3	0·8 ± 0·2	—
Testis	0·4 ± 0·2	0·5 ± 0·1	—
Lung	1·2 ± 0·1	9·9 ± 1·3	729
Kidney	0·4 ± 0·1	2·1 ± 0·3	425
Liver	14·8 ± 0·6	49·6 ± 3·0	234

* Rats were injected with 3-MC as described under Methods. Microsomes equivalent to 13·3 g brain, 4·0 g testis, 4·0 g lung, and 6·0 g kidney were incubated for 60 min; microsomes equivalent to 133 mg of liver were incubated for 12 min with 3-methyl-MAB in the presence of cofactors as described under Methods. N-demethylase activity is expressed as micrograms of 3-methyl-AB formed. Each value represents the average and standard error obtained from 5 experiments using pooled tissue from 10 rats in each experiment.

changes in the demethylation of 3-methyl-MAB by any of the nonhepatic tissues described in Table 1. The very low or complete lack of demethylase activity in brain microsomes obtained from normal, or from 3-MC- or phenobarbital-treated rats was of particular interest. Although these results agree with previous failures to find drug-metabolizing enzymes in brain, further attempts to detect low brain activity could be made by using more discrete areas of tissue.

* Glucose-6-phosphate, NADP, and NADH were dissolved in 0·1 M KH_2PO_4 (pH 7·4) buffer, magnesium chloride was dissolved in water, glucose-6-phosphate dehydrogenase was dissolved in 0·05 M Tris buffer (pH 7·4), and 3-methyl-MAB was dissolved in methanol.

† Standard error.

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*

(*Received* 24 *December* 1962; *accepted* 17 *January* 1963)

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

* Aided by Grant H-7249 from the U.S. Public Health Service.