Thus the only action found was a parasympatholytic effect, ten times less than that of atropine. No reactivating properties were detected with this substance.

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N-hydroxylation of carcinogenic amines *in vivo* and *in vitro* with liver microsomes

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THE N-oxidation of aromatic amino compounds was first demonstrated *in vivo* by Kiese.[1] Cramer *et al.*[2] isolated N-hydroxy-2-acetylaminofluorene from the urine of rats fed with 2-acetylaminofluorene, and Wyatt *et al.*[3] found N-hydroxy-4-acetylaminodiphenyl as an urinary metabolite of 4-acetylaminodiphenyl. Troll and Nelson[4] detected a derivative of 2-naphthylhydroxylamine in the urine of dogs and patients exposed to 2-naphthylamine.

In the organism, the reactive N-oxidation products lead to the formation of methaemoglobin and other toxic symptoms. Formation of methaemoglobin following application of amino compounds gives strong evidence that the formation of amines to hydroxylamino and nitrosocompounds has occurred in the body.[5]

We have shown that N-hydroxylation of aromatic amines and N-alkylamines is catalysed *in vitro*, in the presence of TPNH and oxygen, by isolated liver microsomes.[6] N-Hydroxylation of 2-aminofluorene[7] and 2-naphthylamine[8] by such a system has been reported. The combination of hydroxylamino and nitroso compounds with proteins may produce antigenic substances.[9] It seems, that the reactive N-oxidation products play important roles in the carcinogenicity of aromatic amines and their derivatives. Therefore, we have investigated N-hydroxylation of several other carcinogenic amines *in vivo* and *in vitro*.

METHODS

Cats were injected intraperitoneally with the amines (0·5 m-mole/kg), dissolved in a mixture of 10% gum arabic, 5% 1:2-propylene glycol and 85% NaCl (0·9%). Blood samples were withdrawn from the carotid artery. Methaemoglobin was estimated, after haemolysis and addition of cyanide, at 550 mμ. Microsomes were prepared from homogenates of rat livers in a phosphate buffer (0·1 M; pH 7·4) by centrifugation for 1 hr at 78,000 × *g* and washed twice with the phosphate buffer. Incubation mixtures were prepared as indicated in Fig. 2. 4-Nitrosodiphenyl was synthesized from 4-aminodiphenyl by oxidation.[10] Hydroxylamino and nitroso compounds were estimated together, after extraction with CCl_4 in the presence of Fe^{3+} and removing amines, by comparing their adsorption spectra with that of synthetic products or, alternatively, by spectrophotometric analysis of the compounds resulting from diazotization and coupling.[11]

RESULTS AND DISCUSSION

Following injections of carcinogenic amines, methaemoglobin was found in the cat, a fact which can be explained by N-oxidation (Fig. 1). It should be mentioned here that 4-aminostilbene and 2-aminofluorene are effective only when dissolved in 1:2-propylene glycol, but haemoglobin is not oxidized by injections of 1:2-propylene glycol alone. The rates of haemoglobin oxidation, however, cannot accurately be compared without taking into consideration the significant differences in blood amine concentrations.

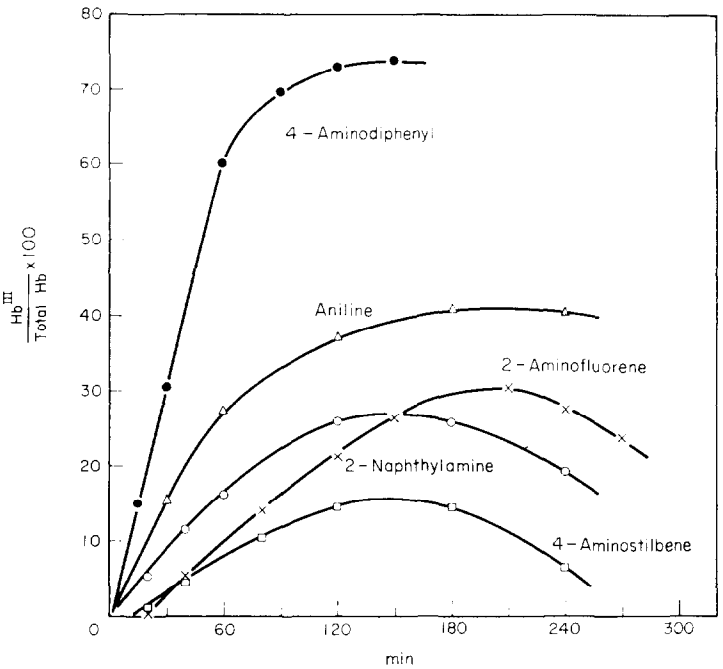

FIG. 1. Methaemoglobin formation in the cat following injection of 0·5 m-mole/kg amine intraperitoneally. Aniline △, 2-naphthylamine ○, 2-aminofluorene + 4-aminostilbene □, 4-aminodiphenyl ●.

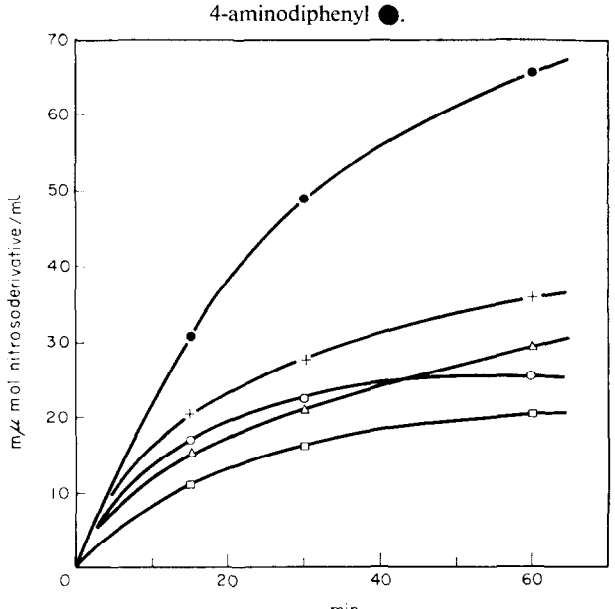

FIG. 2. N-Oxidation of carcinogenic amines with isolated liver microsomes and TPNH. In 10 ml incubation mixture: 80 mg microsomal proteins, 120 μmole $MgCl_2$, 240 μmole nicotinamide, 60 μmole glucose-6-phosphate, 10 μmole TPN, 2 Kornberg units glucose-6-phosphate dehydrogenase, 10 μmole substrate dissolved in 0·2 ml 1:2-propylene glycol and methanol 1:1 (v/v); volume completed with 0·1 M phosphate buffer (7·4); 37 °C, shaken in air.
Aniline △, 2-naphthylamine ○, 2-aminofluorene + 4-aminostilbene □, 4-aminodiphenyl ●.

After injections of 4-aminodiphenyl, we were able to detect the presence of 4-nitrosodiphenyl in the blood by comparing the adsorption spectra of CCl_4 extracts with that of the synthetic product.

Fig. 2 illustrates N-hydroxylation of carcinogenic amines with isolated rat liver microsomes and TPNH. We were unable to effect a complete dissolution of 4-aminostilbene and 2-aminofluorene in the incubation mixtures; therefore, rates of N-oxidation may be assumed to be even higher.

Hultin[12] has proved that ^{14}C-2-aminofluorene is bound to the proteins of isolated liver microsomes in the presence of TPNH. The kinetics of N-hydroxylation of aromatic amines in such a system shows the same characteristics.[5]

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BOOK REVIEW

Advances in Pharmacology: edited by S. GARATTINI and P. A. SHORE, Vol. 1. Academic Press, New York and London, 1962, 475 pp.

IN the preface to this first volume of *Advances in Pharmacology* the editors state that they had charged the contributors to present papers that would not be merely highly detailed review articles written by experts for the benefit of other experts, but also would be of such a nature as to allow "the initiate to ground himself readily in new research areas." Furthermore, the various authors were encouraged by the editors to formulate and consider hypotheses and concepts. From these two points of view, *i.e.* acceptability to the student and formulation of hypotheses, this book in general succeeds remarkably well. However, since the book consists of contributions from many authors, the extent to which the two criteria are met varies from paper to paper.

The selection of topics by the editors for this first volume is timely and one finds a subtle thread of continuity tying together the various papers. J. H. Burn and M. J. Rand, in an extremely lucid discussion, present their concept of the functioning of the adrenergic nerve fiber. This paper, which readily attains the two goals mentioned above, might well be offered as a selected reading to medical students who frequently exhibit great interest and no small amount of confusion in autonomic pharmacology. Continuing the interest in autonomic pharmacology, B. J. Haverback and S. K. Wirtschafter present a paper on the role of biogenic amines in gastrointestinal motility and gastric secretion; included is a section dealing with the metabolism of the pharmacologically active amines in patients with malignant carcinoid syndrome or various malabsorption diseases. Another aspect of biogenic amines is considered in a scholarly paper by J. P. Green on the binding and release of these compounds in tissues. This paper is probably the least easy for the neophyte in pharmacology to digest,