TABLE 2. HEXOBARBITAL OXIDASE ACTIVITY IN LIVERS OF MALE RATS AFTER ONE ORAL DOSE OF ORGANOCHLORINE PESTICIDES

Pesticide	Dose in mg/kg (half LD_{50})	1 ml liver supernatant	3 ml liver supernatant
DDT	150	22* (2)†	76 (3)
dieldrin	30	40 (2)	61 (2)
lindane	75	46 (2)	82 (2)
dicofol	500	26 (2)	97 (2)
tetradifon	2000	44 (4)	70 (4)
chlorfenson	1000	57 (4)	100 (2)
dichlobenil	2000	35 (4)	45 (3)
Control	—	15·2 (19)	41 (13)

* Expressed as percentages of the standard value of hexobarbital.
† Number of animals; for measurements with 1 or 3 ml supernatant different animals were used.

For dicofol, tetradifon, chlorfenson and dichlobenil stimulation of hexobarbital oxidase has not been described hitherto. The stimulation found varies for the different pesticides. Perhaps it also varies with the dose level, but by giving a half LD_{50} we have tried to correct for the percentage uptake of the substance and to use a "physiological active" dose. Although a comparison of the stimulating potency is very difficult, it is probable that dichlobenil has the weakest potency.

From these results it was concluded that this method can be useful for measurement of the induction of hexobarbital oxidase in toxicological studies. The method has the advantage that by using a gaschromatographic determination of hexobarbital, an internal standard could be used so that only one extraction is necessary. When a gaschromatograph is present in the laboratory a simple and accurate determination of hexobarbital oxidase and possibly of other "drug metabolizing" enzymes can be carried out.

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Effect of phenoxybenzamine on the turnover rate of heart norepinephrine

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CHANGES in the turnover rate of norepinephrine (NE) are a much better index of sympathetic tone than are changes in the tissue concentration of the amine, which might remain constant or even decline despite an increased rate of synthesis. Recent reviews from this laboratory have emphasized

the importance of the turnover rate in studying the effects of drugs or of environmental changes on sympathetic nerve endings.[1,2] The turnover rate (synthesis rate) of NE may be measured from the steady state relationship in which the rates of formation and efflux of NE are equal:

$$\text{rate of synthesis} = kC_0$$

where k is the rate constant of efflux (fractional turnover rate constant) and C_0 is the steady state level of neurohormone.

The present paper examines the effect of phenoxybenzamine (Dibenzyline) on the turnover rate of NE in the rat heart. Administration of the drug for 4 days markedly accelerated the efflux of tritiated NE (NE-H^3) and increased the turnover rate of the catecholamine.

Phenoxybenzamine hydrochloride, dissolved in 0·5 ml of 50% ethanol–isotonic saline, was injected i.p. into male Sprague–Dawley rats (200 g) over a period of 4 days. *d,l*-NE-7-H^3 (0·156 μg/kg; specific activity 32 μc/μg) was injected i.v. and the fractional turnover rate constant (k) of heart NE was determined, from the rate of decline in specific activity Recent studies have shown that this dose of the labeled amine is a tracer dose in that the specific activity declines as a single exponential.[3,4] The NE-H^3 was injected 4 hr after the last dose of phenoxybenzamine to avoid the transient blockade of amine uptake produced by the drug.[5] The animals were killed at various times and the hearts were rinsed free from blood, blotted and assayed for NE and NE-H^3 as previously described.[3] The specific activity of each sample was determined by dividing the radioactivity (cpm/g) by the endogenous NE concentration (μg/g). The values for specific activity were logarithmically transformed for calculation of linearity of regression and S.E. of the regression coefficients.[6] The turnover rate (synthesis rate) of heart NE was obtained from the product of the steady state level and of k, the rate constant of decline in specific activity.[3,4]

After the administration of phenoxybenzamine (5 mg/kg every 12 hr) for 4 days, the concentration of NE in the heart declined to a new steady state level, about 65 per cent of the control value (Table 1).

TABLE 1. EFFECT OF PHENOXYBENZAMINE ON TURNOVER RATE OF HEART NE

Treatment	No. of experiments	Heart levels of NE (μg/g ± S.E.)	Rate constant of specific activity decline k(hr^{-1} ± S.E.)	Turnover rate of NE* (μg/g/hr)	Increase in turnover rate (%)
Control	3	1·03 ± 0·04	0·053 ± 0·005	0·055	
Phenoxybenzamine (5 mg/kg every 12 hr)	3	0·68 ± 0·03†	0·25 ± 0·03	0·17	+ 209
Control	2	1·11 ± 0·05	0·039 ± 0·01	0·043	
Phenoxybenzamine (6 mg/kg every 24 hr)	2	0·98 ± 0·05‡	0·085 ± 0·01	0·083	+ 93

* Product of NE level and rate constant of sp. act. decline.
† $P < 0·001$.
‡ $P > 0·05$.

After the injection of NE-H^3 in tracer doses (0·156 μg/kg), the average initial level of labeled amine in the heart of phenoxybenzamine-treated animals did not differ significantly from that in the controls. As shown in Fig. 1, after pretreatment with phenoxybenzamine, the specific actixity of heart NE-H^3 declined, with a half-life of about 3 hr compared to a control value of about 13 hr. The turnover rate, calculated from the product of the NE level and the rate constant of decline in specific activity, was 3 times greater in the phenoxybenzamine-treated animals than in the controls (Table 1).

When phenoxybenzamine was given to the rats in a lower dosage (6 mg/kg every 24 hr for 4 days), there was no change in the concentration of NE in the heart, but the turnover rate was almost double the control value (Table 1).

At the steady state, the NE level remains constant because the rate of synthesis is balanced by efflux (intraneural metabolism + loss at receptors).[1,2] Any alteration of neural function which enhances the efflux of NE, such as an increase in sympathetic tone (increased NE release), will tend to lower the

intraneuronal concentration of neurohormone. According to the hypothesis that a negative feedback mechanism regulates the synthesis of NE,[7] a tendency for a lower NE level will lead to an increased rate of amine synthesis.

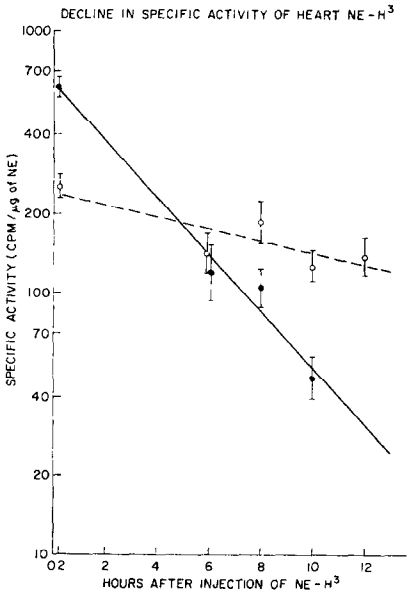

FIG. 1. The specific activity of NE-H^3 in heart at various times after administration of *d,l*-NE-H^3 (0·156 µg/kg). Open circles represent mean values of at least 5 control animals and bars represent ± S.E.; closed circles indicate mean values of animals pretreated with phenoxybenzamine (5 mg/kg every 12 hr) for 4 days. Slopes of the decline were calculated by the method of least squares.

In interpreting the present results, it is helpful to review some current concepts of the action of phenoxybenzamine on sympathetic neurons. Brown and Gillespie first observed that phenoxybenzamine and other adrenergic blocking agents increase the amount of NE appearing in the venous blood of the spleen when its sympathetic nerve is stimulated.[8] The increased efflux of NE was first explained as an overflow of NE that escapes fixation by the receptors, the assumption being that a substance which blocks access of NE to receptors also prevents its access to sites of inactivation. This view was abandoned when phenoxybenzamine was found to be one of a large group of drugs, including cocaine, imipramine and desipramine, that blocks the uptake of circulating NE by nerve endings.[9,10] The increased output of NE after phenoxybenzamine was then attributed to a cocaine-like effect of the drug on the presynaptic (neuronal) membrane.[11]

However, Boullin *et al.*,[5,12] using the isolated cat colon, showed that the cocaine-like action of phenoxybenzamine on the neuronal membrane is only transient and has little bearing on the overflow of the nerve-released amine. The present data confirm their finding that administration of phenoxybenzamine 4 hr before the injection of NE-H^3 does not interfere with the accumulation of the amine. The overflow of NE after nerve stimulation observed by Boullin *et al.* is associated with the persistent occupation of receptors by phenoxybenzamine. Thus, at a time when the uptake of perfused NE is no longer blocked, phenoxybenzamine causes a 4-fold increase in the overflow of NE from the nerve endings during electrical stimulation. Even in the absence of nerve stimulation, the drug elicits a 50 per cent increase in the spontaneous outflow of NE. It was concluded that phenoxybenzamine, by preventing the neurotransmitter from combining with receptor sites, blocks the re-uptake of NE and thereby increases the net efflux of released amine into the circulation.

It is possible that the accelerated turnover rate of heart NE observed in the present studies results from an increased overflow of NE from receptors. This idea is compatible with the observation that phenoxybenzamine administration increases the amount of NE in urine and plasma[13,14] and causes

a gradual depletion of NE from certain tissues[15,16] including the heart (Table 1). A valid objection to this view is that the heart has mainly beta receptors[17] which are not blocked by phenoxybenzamine.*

It seems more likely that the increased turnover rate of heart NE may be due to an increase in sympathetic activity caused by the hypotensive action of phenoxybenzamine.[19] In favor of this view are recent studies showing that sympathetic stimulation accelerates the synthesis of the neurohormone. For instance, exercise and cold exposure, which enhance sympathetic tone, cause an increase in the turnover rate of NE in heart[20,21] and brain.[22] Similarly, electrical stimulation of sympathetic nerves increases the synthesis of radioactive NE from labeled tyrosine.[23,24] From the present data, it is impossible to determine the relative contribution of increased sympathetic stimulation and of blockade of noradrenergic receptors to the effects of phenoxybenzamine on NE turnover in the heart. By either mechanism the drug would increase the net efflux of NE from nerve endings into the circulation, and the synthesis rate would have to accelerate in order to maintain the tissue level of neurohormone.

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* Recently, however, Govier has presented evidence that phenoxybenzamine acts on alpha receptors in the heart.[18]

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