Drug retention by the isolated diaphragm preparation

SIR,—Experiments have been made using the isolated rat phrenic nerve diaphragm preparation (Bulbring, 1946) to investigate protection by a reversible carbamate anticholinesterase from the effects of the irreversible organophosphate compound, paraoxon. The effects of paraoxon alone on the rat diaphragm preparation have been described by Barnes & Duff (1953).

Hemi-diaphragms from male albino rats of 300–400 g were prepared and the phrenic nerve supported by a Perspex assembly designed for use with the nerve electrodes immersed in the bath fluid. This support was constructed of sections of Perspex cemented together with wires to the electrodes lying in the cavities between the sections. Krebs perfusion fluid at 37° was used in all experiments. The organ bath volume was 100 ml. A twitch response to 50/sec stimulation followed by failure to hold tetanus was used as an indication of a severe degree of cholinesterase inhibition.

In these experiments it was found that 2×10^{-6}M of paraoxon alone produced a complete failure to hold a 50/sec tetanus within 30 min. After three washings at 5 min intervals no recovery was observed during the ensuing 2–3 hr. But if the diaphragm was exposed to 2×10^{-6}M of 3-isopropyl-*N*-methyl carbamate for 30 min, then washed several times, the anticholinesterase effects of 2×10^{-6}M paraoxon given subsequently could be reversed by washing.

However, this recovery from paraoxon poisoning was followed by a gradual secondary failure over 2 hr. The secondary failure was thought to be due to retention of paraoxon by the apparatus or diaphragm despite washing.

Experiments were made to identify where retention occurred.

Experiment (*i*). A hemi-diaphragm was set up in the bath containing 2×10^{-6}M paraoxon, while the other hemi-diaphragm from the same rat was kept in oxygenated Krebs at 37°. After 30 min the bath was washed three times. The hemi-diaphragm showed complete failure to hold a 50/sec tetanus 2½ hr after washing. It was replaced by the other hemi-diaphragm which, in the same bath fluid, showed complete failure to hold a 50/sec tetanus 1½ hr later.

Experiment (*ii*). The bath fluid contained 2×10^{-6}M paraoxon and the diaphragm-nerve holder was immersed in it but no diaphragm was set up. After 30 min the bath was washed three times and a hemi-diaphragm set up in it. This preparation showed a partial failure to hold a 50/sec tetanus after 2 hr. The results of this experiment showed that paraoxon was being retained by the apparatus despite washing.

Experiment (*iii*). In this the diaphragm-nerve holder was not immersed in the bath fluid containing the paraoxon. After three washes of the bath a hemi-diaphragm was set up in the bath fluid and this did not show any signs of failure after 2 hr. Thus the diaphragm-nerve support was shown to be the most likely place retaining the paraoxon.

Experiments (ii) and (iii) were repeated and the bath fluid assayed for paraoxon by a sensitive colorimetric method (Aldridge, 1964) 2½ and 6 hr after setting up the hemi-diaphragm. In addition, another experiment (iv) was done in the same way as (ii) except that a dummy diaphragm-nerve holder, without electrodes, made of solid Perspex was used. Experiment (v) was done as experiment (iii) except that no hemi-diaphragm was set up after washing.

From a comparison of the results (Table 1) of experiments (v) and (iii) it appears that the diaphragm itself retains some paraoxon during washing but this amount is small compared with that retained by the original Perspex holder. The lower concentrations of paraoxon at 6 hr were thought to be due to degradation of paraoxon by the nerve.

TABLE 1.

Experiment	Concentration of paraoxon in the bath fluid	
	2½ hr after setting up the diaphragm	6 hr after setting up the diaphragm
(ii) Diaphragm-nerve holder immersed in the bath fluid containing 2×10^{-6}M paraoxon	$1{\cdot}75 \times 10^{-8}$M	not measured
(iii) As above but holder not immersed in fluid	4×10^{-9}M	2×10^{-9}M
(iv) Dummy holder made of Perspex in one piece immersed in bath fluid containing 2×10^{-6}M paraoxon	$2{\cdot}5 \times 10^{-9}$M	1×10^{-9}M
(v) As expt (iii) but no diaphragm set up after washing	5×10^{-9}M	$4{\cdot}3 \times 10^{-9}$M

It is therefore suggested that the diaphragm-nerve holder be constructed of solid Perspex or glass, so that drugs cannot diffuse into the cavities containing the electrode connections.

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Disulfiram and the effect of catecholamines on neuroleptic-induced catalepsy in mice and rats

SIR,—We have found that chlorpromazine- or haloperidol-induced catalepsy in mice and rats could be reversed by dopa or monoamine oxidase- and catechol-*O*-methyltransferase inhibitors or both (Maj & Zebrowska, 1966a,b). We therefore wished to know whether noradrenaline or dopamine was involved in this anticataleptic action. For this purpose we used disulfiram which inhibits the β-hydroxylation of dopamine to noradrenaline in various tissues (Goldstein, Anagnoste, Lauber & McKereghan, 1964; Musacchio, Goldstein, Anagnoste, Poch & Kopin, 1966).

Catalepsy was examined in white mice according to Zetler & Moog (1958) and in Wistar rats according to Courvoisier, Ducrot & Julou (1957). In mice, reserpine was given intraperitoneally 3·5 hr, chlorpromazine and haloperidol subcutaneously 1·5 hr, disulfiram intraperitoneally 2 hr and DL-dopa, intraperitoneally 0·5 hr before the experiment. Rats were given reserpine intraperitoneally 3·5 hr, chlorpromazine and haloperidol subcutaneously 1 hr and disulfiram intraperitoneally 2 hr and DL-dopa intraperitoneally immediately before the test. Nialamide was injected in both species 18 hr before the experiment. Observations were made at 5 min intervals for 1 hr (13 observations) in mice and at 10 min intervals (7 observations) in rats. The number of cataleptic animals and the number of cataleptic responses were recorded. The animal was considered to be cataleptic after 7 or more positive responses in mice, or 4 or more in rats.

Dopa and nialamide were not antagonistic towards reserpine-chlorpromazine or haloperidol-induced catalepsy in mice pretreated with disulfiram (Table 1). In experiments with chlorpromazine catalepsy was not seen after disulfiram, since in 5 mice the righting reflex had been abolished.

In rats (Table 2) the administration of dopa and nialamide counteracted the cataleptic action of reserpine and haloperidol. Only in animals receiving