about 5 weeks, using, say, thirty males (ten controls and ten at each of two dose-levels) and 360 females (four females per male per week).

Rats may also be used as test animals but, being larger, more accommodation is required to obtain the same information. The vaginal smear has to be used to detect inseminations instead of the convenient mating plug. Moreover, we have found rats unable to mate regularly with more than two females per week; so that more males are needed to produce the same number of pregnancies.

This technique has now been used to study the mutagenic action of X-rays[2], neutrons[5], radioisotopes[6], triethylenemelamine[4] and alkane sulphonic esters[7,8]. Following reports that the radio-opaque medium diodone might be mutagenic we have tested this compound with negative results[9]. Negative results have also been obtained for caffeine[10] by other workers using a similar test.

It is obviously preferable to use a mammal rather than an insect as the test animal for drug assays. The technical simplicity and the few weeks required for the dominant lethal test in mice make it a very suitable system for testing the mutagenicity of any new drug.

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Reactivation of Paraoxon-inactivated Cholinesterase in the Rat Cerebral Cortex by Pralidoxime Chloride

IT has been demonstrated by Firemark, Barlow and Roth[1] that the concentration of pralidoxime (PAM) in the rat brain cortex after a single intravenous injection of PAM-^{14}C is significantly higher than in other brain regions. The present communication reports an attempt to see whether such a relatively small dose of PAM as used by these authors (20 mg/kg) is able to reactivate satisfactorily the phosphorylated cholinesterase in the brain cortex of rats poisoned with lethal doses of paraoxon.

Female albino rats weighing 200–250 g were given 10 mg/kg of atropine sulphate followed 20 min later by a subcutaneous injection of paraoxon. Ten minutes after injecting paraoxon, 20 mg/kg of PAM (chloride) was given intravenously. One hour later the animals were killed by decapitation. Brains were quickly removed and immediately chilled in ice. The cerebral cortex (parietal region) was placed on a frosted glass slide and sliced with a razor blade. Cholinesterase activity was measured manometrically at 37° C with the following reaction mixture: 0·15 M sodium bicarbonate, 0·164 M sodium chloride and 0·01 M acetylcholine chloride in a total volume of 2·0 ml.; pH 7·4, gas phase nitrogen + carbon dioxide (95 : 5). Enzyme activity was expressed in µl. carbon dioxide liberated per mg of tissue (dry weight) during the first 30 min (Q value). All results were corrected for non-enzymatic hydrolysis.

The results presented in Table 1 show that 1 h after the injection of PAM the mean enzymatic activity in the cerebral cortex of rats poisoned with paraoxon was considerably higher than the activity in the cortex of rats injected with paraoxon only. The differences at each dosage group are statistically significant ($P < 0·05$). With a dose of paraoxon of 0·25 mg/kg the reactivation was on average 54 per cent. With higher doses of paraoxon (0·5 and 1·0 mg/kg) the mean reactivation was 45 per cent and 25 per cent, respectively. In the last group all animals injected only with paraoxon died 10 min after poisoning, and had a cholinesterase activity which was significantly lower than that in PAM-treated animals which survived and were killed 1 h after injection of oxime.

Table 1. CHOLINESTERASE ACTIVITY IN THE BRAIN CORTEX OF RATS INJECTED WITH PARAOXON FOLLOWED BY PAM*

Paraoxon (mg/kg)	Cholinesterase activity: Without PAM Q	Per cent	With PAM Q	Per cent	Reactivation per cent
0	12·25 ± 0·17	100	12·54 ± 0·70	100	—
0·25	5·26 ± 1·04	42	9·13† ± 1·32	73	54
0·5	2·51 ± 0·71	20	6·60† ± 1·20	53	45
1·0	3·76‡ ± 0·45	30	5·95† ± 0·30	48	25

* Each value represents the mean and standard error of the activity measured at least in five brains.
† Mean values differ significantly ($P < 0·05$) as determined by the t-test.
‡ Dead 10 min after poisoning.

These data are in contrast to the general belief that PAM, owing to its quaternary structure, does not penetrate the blood–brain barrier in a concentration which is sufficient to reactivate phosphorylated cholinesterase in the brain (reviewed in ref. 2). However, this conclusion is based mainly on results obtained with homogenates of whole brain and makes no allowance for the uneven distribution of PAM in the brain. The use of cortical slices instead of homogenates of whole brain has the advantage that the tissue slices contain most cells in an intact form. This may result in a greater effectiveness of PAM in reactivating the so-called functional cholinesterase on the cell surface than the reserve cholinesterase within the cells. The latter accounts for more than 95 per cent of the enzymatic activity in the isolated frog rectus abdominis muscle[3]. The situation in the brain might be similar and the ability of PAM to reactivate paraoxon-inhibited functional cholinesterase in the rat cerebral cortex would provide an explanation for some of its central actions which have been observed both in animals and human beings poisoned with various organophosphorus anticholinesterase agents[4–6].

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PATHOLOGY

Effect of Environmental Temperature on the Metabolic Response to Injury

THE pattern of the metabolic response by man and the rat to injury (for example, fracture of a long bone) has been under investigation for some time. The reaction is characterized by a marked rise in the urinary excretions of nitrogen, sulphur, phosphorus, potassium and creatine, which reach a peak in the rat at the third day after the injury and then slowly decline[1–3]. It was early noted that in man once shock had subsided there was also a rise in

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