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PHARMACOLOGICAL METHODS OF ESTIMATING INHIBITION OF DRUG OXIDATION IN THE MOUSE

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Three pharmacological techniques for measuring inhibition of " non-specific " oxidase activity in the mouse are described: (1) potentiation of pentobarbitone hypnosis, (2) potentiation of chlorpromazine hypothermia; and (3) reduction in the toxicity of octamethylpyrophosphorodiamide (schradan). Iproniazid, isoniazid, and β-diethylaminoethyl 3,3-diphenylpropylacetate (SKF 525A) gave comparable results in all three tests.

Experiments *in vitro* have shown that mammalian liver slices carry out the oxidative transformation of a wide range of drugs of unrelated chemical structure, such as pentobarbitone, pethidine, chlorpromazine, amidopyrine, ephedrine, codeine and phenacetin. The addition of β-diethylaminoethyl 3,3-diphenylpropylacetate (SKF 525A) inhibits many of these transformations. The literature concerning this " non-specific " enzyme system has recently been reviewed (Brodie, 1956; Brodie, Gillette and La Du, 1958).

β-Diethylaminoethyl 3,3-diphenylpropylacetate potentiates the hypnotic effects of pentobarbitone in mice and rats (Cook, Toner and Fellows, 1954) by reducing the rate of bio-transformation of the pentobarbitone (Axelrod, Reichenthal and Brodie, 1954). It also potentiates the hypothermic action of chlorpromazine in mice (Lessin and Parkes, 1957a). Kamm, Gillette, and Brodie (1958) have shown that chlorpromazine is metabolized by the " non-specific " oxidase into the relatively inactive chlorpromazine sulphoxide.

The same mammalian liver enzyme system can, *in vitro* and *in vivo*, convert octamethylpyrophosphorodiamide (schradan) into a highly toxic cholinesterase inhibitor (Gardiner and Kilby, 1950; Dubois, Doull and Coon, 1950; Cheng, 1951; Aldridge and Barnes, 1952). Rats pretreated with a suitable dose of β-diethylaminoethyl 3,3-diphenylpropylacetate survived doses of schradan that were fatal to normal animals (Davison, 1955).

Other drugs will inhibit the " non-specific " oxidase. For example, Fouts and Brodie (1956) and O'Brien and Davison (1958) showed that the potentiation of barbiturates by iproniazid reported by Allmark (1953), by Goldin, Dennis, Venditti, and Humphreys (1955), and by Sturtevant (1956), was due to the reduction in the liver oxidase activity. Isoniazid has also been reported by some of these workers to potentiate the effects of barbiturates. In view of the structural resemblance between isoniazid and iproniazid, it is possible that both inhibit the liver enzyme in the same way.

The activity of agents inhibiting this enzyme system has been investigated pharmacologically. The methods were based on measurement of the effects of drugs known to be substrates for this enzyme system, namely the hypnotic effects of pentobarbitone, the hypothermic action of chlorpromazine, and the toxicity of schradan. Potentiation of pentobarbitone hypnosis and chlorpromazine hypothermia results from inhibition of enzymic inactivation, whereas reduction of schradan toxicity results from inhibition of enzymatic activation.

METHODS

Male mice (LAC greys) weighing 16 to 25 g. were used. Drugs were injected intraperitoneally, except pentobarbitone which was given intravenously in a volume of 0.1 ml./10 g. of body weight. Room temperature was maintained at 22°. The doses of iproniazid, isoniazid, and β-diethylaminoethyl 3,3-diphenylpropylacetate used have no effect on the body temperature of the mice.

Pentobarbitone Sleeping Time.—This was the interval, in minutes, between giving 25 mg./kg. of pentobarbitone and the time at which the animals

were able to right themselves. The mean sleeping time of groups of 10 mice was determined.

Chlorpromazine Hypothermia.—Groups of mice were treated with 2.5 mg./kg. of chlorpromazine. The body temperature was measured 1 hr. later by a method previously described (Lessin and Parkes, 1957b) and the mean for the group determined. The test drugs were given at various intervals before the chlorpromazine.

Schradan Toxicity.—A graded response method was used. The mean survival time of groups of mice was determined after doses of from 25 to 50 mg./kg. of this compound. The interval between injection and death was called the survival time.

Results

Pentobarbitone Potentiation.—The potentiation of pentobarbitone sleeping time in mice given iproniazid (30 mg./kg.), isoniazid (30 mg./kg.) or β-diethylaminoethyl 3,3-diphenylpropylacetate (5 mg./kg.) 5 min., 1 hr., 2 hr. and 4 hr. before the pentobarbitone, is shown in Table I. Iproniazid and isoniazid exert their greatest potentiating action in 5 min., whereas the effect of β-diethylaminoethyl 3,3-diphenylpropylacetate appeared more slowly.

TABLE I

POTENTIATION BY IPRONIAZID, ISONIAZID, AND SKF 525A OF (*a*) PENTOBARBITONE SLEEPING TIME AND (*b*) CHLORPROMAZINE HYPOTHERMIA

Means ±S.E. of groups of 10 mice. (A) Sleeping time in min. after 25 mg./kg., i.v.; (B) body temp. 1 hr. after 2·5 mg./kg., i.p.

Potentiating Agent Given Before	Iproniazid (30 mg./kg.)	Isoniazid (30 mg./kg.)	SKF 525A (5 mg./kg.)
A. Pentobarbitone			
5 min. ..	17·8±1·1	16·3±1·4	12·0±1·3
1 hr.	18·7±1·1	17·0±1·1	22·4±1·6
2 ,, ...	15·7±1·3	13·6±1·4	22·6±1·6
4 ,, ...	9·7±0·6	12·0±1·3	21·2±1·3
No potentiator	7·5±0·5		7·1±1·0
B. Chlorpromazine			
5 min. ..	33·9±0·8	34·0±0·8	35·0±0·8
1 hr.	33·5±0·4	34·1±0·4	32·4±0·6
2 ,, ...	32·9±0·3	34·4±0·7	32·7±0·3
4 ,, ...	34·2±0·7	34·8±0·6	34·9±0·2
No potentiator	36·2±0·8	36·2±0·5	
,, treatment	37·4±0·06	37·7±0·2 (5 mice)	

TABLE II

EFFECT ON SURVIVAL TIME OF IPRONIAZID, ISONIAZID, AND SKF 525A GIVEN BEFORE SCHRADAN (50 MG./KG.)

Mean survival time±S.E. in min. (No. of mice used in parentheses)

Schradan Given After	Iproniazid (30 mg./kg.)	Isoniazid (30 mg./kg.)	SKF 525A (5 mg./kg.)
5 min. ..	21·1±1·0 (8)	23·6±1·8 (8)	16·2±0·4 (5)
30 ,, ..	20·2±0·9 (8)	22·9±0·9 (8)	19·9±0·8 (7)
1 hr. ..	19·0±0·9 (8)	21·7±0·7 (8)	22·2±0·9 (8)
4 ,, ..	18·7±1·3 (8)	21·7±1·3 (8)	20·1±1·3 (5)
24 ,, ..	14·9±0·6 (8)	14·1±0·7 (8)	
No inhibitor	14·8±0·4 (7)	14·9±0·7 (8)	13·9±0·06 (9)

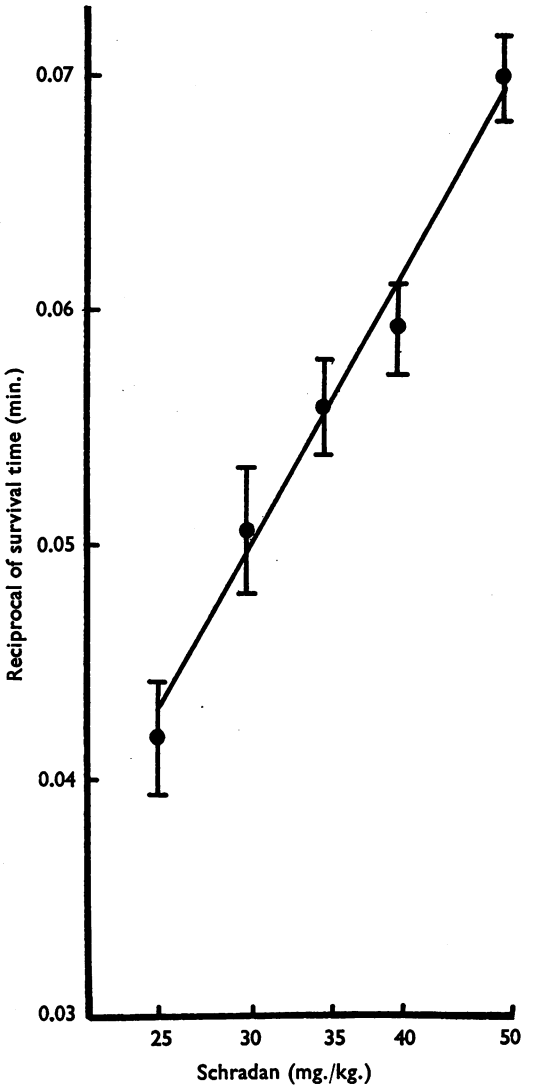

FIG. 1.—Relationship of schradan dose to survival time. n=55, r=0.80, b=0.091±0.019 (95% limits).

Chlorpromazine Hypothermia. — Chlorpromazine (2.5 mg./kg.) produced a fall in body temperature of 1.2 to 1.5° whereas in the pretreated animals the fall was 4.5 to 5.0°. As with pentobarbitone sleeping time, the first two compounds acted almost immediately, whereas the third took longer to exert its full effect (Table I).

Schradan Survival Time.—The doses ranged from 25 to 50 mg./kg.: below 25 mg./kg. animals did not die and above 50 mg./kg. there was little further shortening of the survival time (Fig. 1).

When β-diethylaminoethyl 3,3-diphenylpropylacetate (5 mg./kg.) was given to groups of mice at various intervals before 50 mg./kg. of schradan, the inhibitor exerted maximum protection when this interval was 1 to 2 hr. (Fig. 2). Iproniazid (30 mg./kg.) and isoniazid (30 mg./kg.) protected the animals from the toxic effects of schradan, although, as in the previous tests, the two hydrazides exert their full effect within a few minutes (Table II).

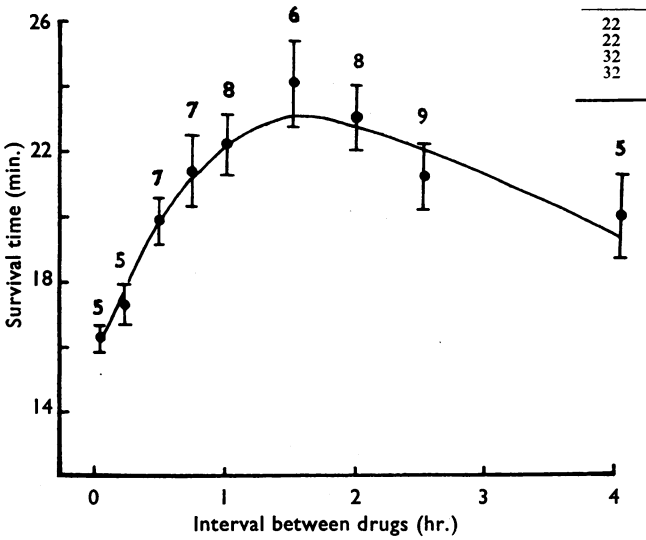

FIG. 2.—Effect on survival time of varying the interval between SKF 525A (5 mg./kg.) and schradan (50 mg./kg.). Range marks indicate S.E. and numerals the number of mice. 9 untreated mice survived 13.5 to 14 min.

TABLE III

EFFECT OF CHLORPROMAZINE AND RESERPINE UPON THE SURVIVAL TIME OF MICE TREATED WITH SCHRADAN (50 MG./KG.)

5 mice in each group. Effect of both drugs at 22° significant ($p < 0.01$).

Room Temp.	Drug	Dose (mg./kg.)	Interval between Drug and Schradan	Mean Survival Time ± S.E. (min.)
22	Chlorpromazine	10	1 hr.	All >30
22	—	—	—	14·5±0·8
32	Chlorpromazine	10	1 hr.	18·7±0·7
32	—	—	—	17·2±1·2
22	Reserpine	2	4 hr.	21·9±1·4
22	—	—	—	15·3±0·7
32	Reserpine	2	4 hr.	14·0±0·3
32	—	—	—	14·6±0·6

To determine the effect of chlorpromazine on schradan toxicity, the animals were given 10 mg./kg. of chlorpromazine 1 hr. before schradan. When the experiment was carried out at an environmental temperature of 22°, chlorpromazine exerted a marked protecting action (Table III). On the other hand, when the environmental temperature was raised to 32° chlorpromazine no longer had any effect. Reserpine (2 mg./kg.) gave similar results, and afforded protection when the experiment was carried out at 22° but not at 32°.

In the last series of experiments various doses of iproniazid, isoniazid and β - diethylaminoethyl 3,3 - diphenyl - propylacetate were given at the appropriate interval before a fixed dose of schradan (50 mg./kg.), The dose/response curves for the three inhibitors are contained in Figs. 3, 4, and 5 respectively. The curves for iproniazid and isoniazid are similar and do not differ significantly from each other, but β-diethylaminoethyl 3,3-diphenylpropylacetate was clearly much more active than the other two.

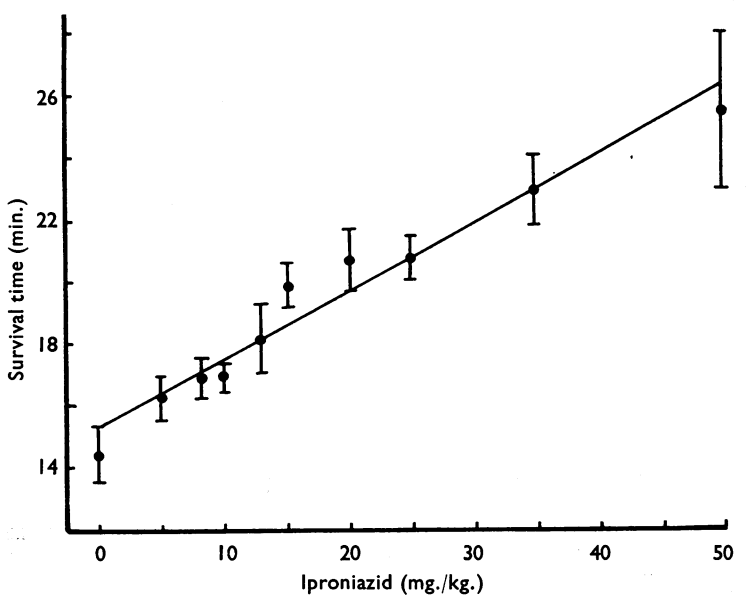

FIG. 3.—Effect of varying the dose of iproniazid upon survival time of mice treated with schradan (50 mg./kg.). Iproniazid 10 min. before schradan. n=83, r=0.71, b=0.22±0.015 (95% limits).

DISCUSSION

The results show that in mice iproniazid, isoniazid and β-di-

ethylaminoethyl 3,3-diphenylpropylacetate potentiated the pentobarbitone sleeping times and the hypothermic effects of chlorpromazine, and antagonized the toxic effects of schradan. In all three tests, iproniazid and isoniazid exerted their maximum potentiating effects very rapidly, whilst the peak effect with β-diethylaminoethyl 3,3-diphenylpropylacetate occurred about 1 to 2 hr. after its administration.

In all three tests β-diethylaminoethyl 3,3-diphenylpropylacetate was about 10 times more active than iproniazid or isoniazid.

The comparable results obtained for iproniazid and β-diethylaminoethyl 3,3-diphenylpropylacetate supported the view that potentiation of the pentobarbitone sleeping time and of the chlorpromazine hypothermia and the antagonism of schradan toxicity by these drugs was due to their known ability to inhibit the "non-specific" oxidase system. The results obtained with isoniazid resemble those with iproniazid, and it is reasonable to conclude that isoniazid is also an inhibitor of this enzyme system.

The toxicity of schradan is due almost entirely, as far as is known, to its metabolite, the phosphordiamide-*N*-oxide (Casida, Allen, and Stahmann, 1954). The production of this metabolite depends upon the integrity of the converting system. Chlorpromazine and reserpine have been shown to influence this system, when administered in circumstances allowing a fall in body temperature. On the other hand these two agents were ineffective when the temperature of the animals was not allowed to fall. Fuhrman (1947) showed that a lowered body temperature enhanced the hypnotic effects of pentobarbitone and he attributed this to the effect of lowered temperature upon the detoxicating enzyme.

Like that of many other enzyme systems, the activity of the liver oxidase system is therefore sensitive to changes in temperature, and caution is therefore indicated when evaluating inhibitors of this system by the pharmacological techniques described. The method based on schradan survival time is probably the most satisfactory to use once the effect of a fall in body temperature has been excluded.

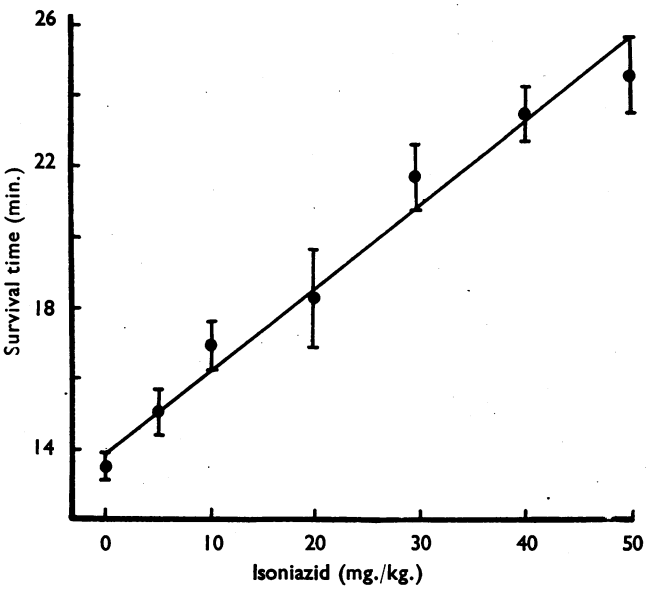

FIG. 4.—Effect of varying the dose of isoniazid upon survival time of mice treated with schradan (50 mg./kg.). Isoniazid 10 min. before schradan. n=57, r=0.88, b=0.23±0.01 (95% limits).

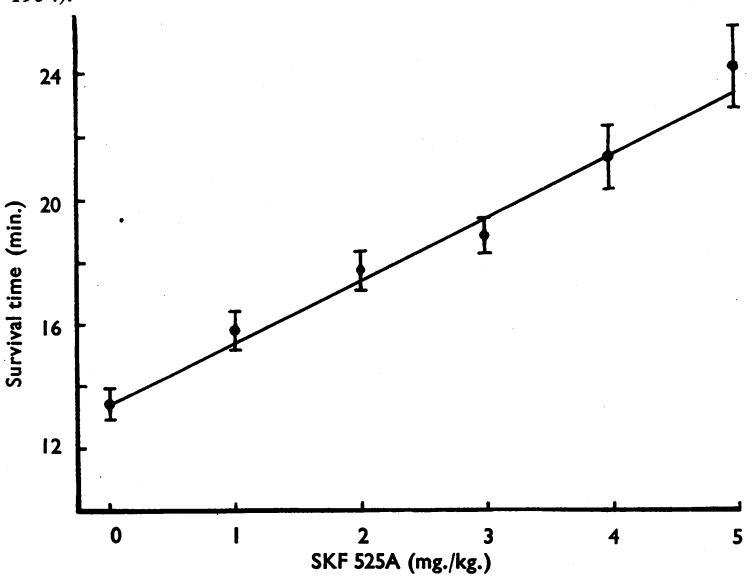

FIG. 5.—Effect of varying the dose of SKF 525A upon survival time of mice treated with schradan (50 mg./kg.). SKF 525A 1 hr. before schradan. n=61, r=0.76, b=1.98±0.14 (95% limits).

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