RESEARCH PAPERS

A STEREOTYPED RESPONSE INDUCED BY MESCALINE IN MICE AS A MEANS OF INVESTIGATING THE PROPERTIES OF DRUGS ACTING ON THE CNS

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The effects of mescaline on spontaneous activity of mice placed in special cages have been studied. The typical response (stereotyped response) has been recorded and assessed in terms of percentage of animals showing the effect. Among drugs affecting the response chloropromazine shows the highest specificity. Promazine, hexobarbitone and pentobarbitone also show a definite but minor effect in doses that do not impair motor function. The response is little affected by phenobarbitone. Meprobamate, mephenesin and a thiadiazole derivative (L 1458) at non-paralyzing doses, and also azacyclonol, do not produce specific antagonistic effects. Nor is the effect significantly influenced by atropine, but it is enhanced by morphine.

MESCALINE has been intensively studied because of its impressive effect on the central nervous system of man and the laboratory animal. Mescaline induced hallucinations have been described in man[1] and experimental catatonia in animals[2]. Thus it may be possible to make use of the behavioural changes induced by mescaline to test the activity of drugs with reported beneficial effects in mentally-disturbed patients. The interest in studying the effect of mescaline and its antagonists has been intensified by the discovery and development of the so called "tranquillizing drugs". In the mouse, mescaline causes certain characteristic changes in behaviour, such as enhanced excitability and paroxysm of ear scratching[3]. Fellows and Cook[4] tested drugs for their ability to prevent or reduce the number of scratching episodes. Tripod[5] has described the antagonism of many sedative agents to mescaline-induced psychomotor stimulation in mice. But the "actographic" method of Hanschild[6] employed by Tripod[7] does not record movements[5].

It has been observed by us and others[8] that the scratching response may be induced by drugs other than mescaline, and it is known that psychomotor stimulation is a feature of the action of many CNS stimulants that produce hypermotility and hyperexcitability as, for instance, amphetamine does.

The present study has been undertaken to determine whether a quantitative characteristic behaviour response could be obtained in mice after mescaline.

Our observations were directed towards a quantitative evaluation of the integrated response of mice to mescaline treatment, using a simple apparatus,

and to assessing the ability of certain CNS depressants to prevent the effects of the drug.

Materials and Methods

Mice of both sexes of CFI strain and of our own breeding were used. Spontaneous activity was kymographically recorded by means of an apparatus first constructed some years ago by Mr. G. Ciuffi*. It consists of a cylindrical metal cage with a mobile floor fixed to the top of a spiral spring. A small rigid bar from the top of the spring is connected to a writing lever. This apparatus proved suitable for graphical recording of typical changes of the motility induced by mescaline in mice.

Aqueous solutions of the drugs used were injected intraperitoneally or intramuscularly. The intraperitoneal route was employed only for insoluble products, and these were suspended in an aqueous 10 per cent acacia gum solution.

Results

Effects of Mescaline

After administration of mescaline, 10 mg./kg. i.p. mice showed an increased sensitivity to touching and handling. Motor activity was slightly reduced. Movements were small and rapid and periods of intense scratching were observed. When animals were transferred from the cage to a new environment their usual curiosity and exploring activity was decreased.

After 30 mg./kg., mice showed more intense paroxysms of scratching of muzzle, ears and neck, with a further increase in sensitivity to touch. Spontaneous activity was abnormal. The movements of cleaning and periods of slight tremors and of tremors at rest were interrupted by short unidirectional movements. When stimulated to walk, some animals showed a slight motor incoordination. Righting reflex and muscular tone were normal. When higher doses were administered the symptoms appeared more marked and signs of autonomic involvement were noted (changes in rate and depth of respiration, lacrimation). A slight motor incoordination was present as well as a slight impairment of righting reflex. The maximum dose at which behavioural changes were still distinguishable from general toxic effects was commonly 100 mg./kg. The effects seen after i.p. or sub-cutaneous injection are similar.

When individual animals were placed in cages, observations and recordings of oscillations gave us a more detailed view of the phenomena.

The effect of 15 mg./kg. i.p. of mescaline depended upon the initial condition of the mouse. Mice with a high initial spontaneous activity usually quietened and gave the typical scratching response. Animals at rest before administration, showed an increased motor activity. In some mice, after 15 mg./kg. of mescaline we observed a rhythm in motor behaviour when periods of fine movements were followed by small and rapid displacements inside the cage. After 20 mg./kg. this rhythm took

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on a more definite character, and became distinguishable on the kymographic tracings (Fig. 1).

The observed changes consist of slow rhythmic waves, caused by unidirectional and regular movements of the animal, around the circumference of the cage by short stages. These movements were alternated with intense characteristic scratching or scratching-like movements. The frequency of these waves depends upon the duration of the scratching episodes and the amplitude of each stage of circular movement. We assigned to this behaviour the term *stereotyped response*. The behaviour of a single animal was found to be constant during the effect of mescaline.

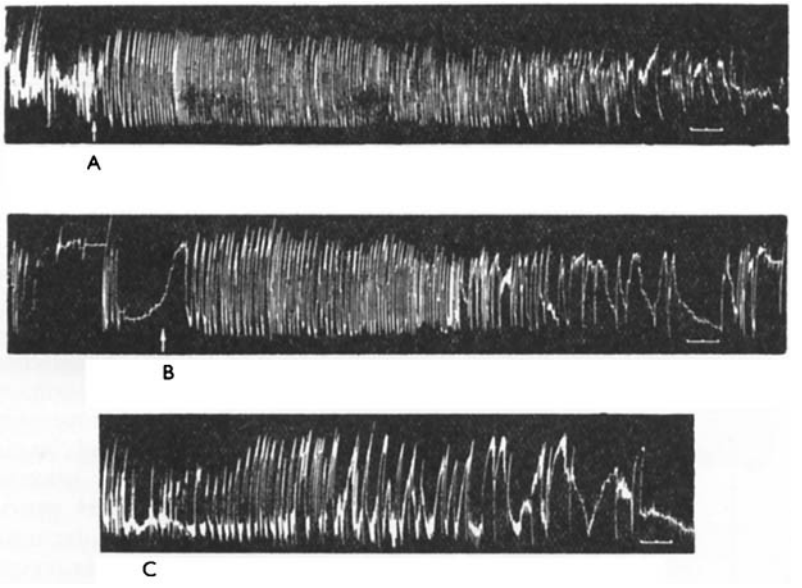

FIG. 1. Stereotyped response in mice following mescaline: A, B (at arrow), mescaline sulphate 50 mg./kg. i.p.; C mescaline sulphate 30 mg./kg. i.p.
Time = 5 minutes.

We have systematically investigated this phenomenon and concluded that it is one of the most prominent features of the action of mescaline that could be instrumentally recorded. By considering it as a quantal effect, experiments with a large number of animals have shown a linear relation between the log of the dose and probits of mice showing a "stereotyped response".

Figure 2 shows the straight line that reveals this dose effect function (according to Litchfield and Wilcoxon[9]). The resultant ED50 was 24·7 mg./kg. (19/20 confidence limits: 19·4–31·4).

An attempt to establish the average duration of the response for each dose used, failed because of individual variability and the difficulty of ascertaining the end of the response which always disappeared gradually.

However we observed that the duration of effect increased with increasing doses. At the level of the LD50 it lasted 90–150 minutes.

In Table I original data and the more important steps of our calculation are given.

TABLE I

CHANGES IN MOTOR BEHAVIOUR OF MICE FOLLOWING MESCALINE: EXPERIMENTAL DATA OF DOSE-ACTIVITY CURVE ACCORDING TO LITCHFIELD AND WILCOXON[9]

Doses of mescaline mg./kg.	Animals/ dose	Effective doses (ED) and confidence limits (C.L.)			Slope and C.L.	Heterogenity test χ^2 for P = 0·05	
		ED16	ED50	ED84		χ^2 found	χ^2 tabular
15–20 25–30 40–50 60	57	15·9 (21·9–11·5)	24·7 (31·4–19·4)	38·3 (52·8–27·7)	1·55 (1·95–1·23)	10·7	11·1

Effects of Other Drugs acting on CNS

We have compared under similar experimental conditions the effect of mescaline with that of drugs well known for their exciting effect on CNS and motor activity in mice.

Amphetamine sulphate, 3–10 mg./kg. s.c. showed the well known constant exciting effect with sudden enhancement of motor activity characterised by fast and continuous multidirectional movements. Swift scratching and cleaning movements were observed but only for short periods and were soon interrupted by the nearly paroxysmal hypermotility. The tracings (Fig. 3) show no similarity with those obtained for mescaline-treated mice.

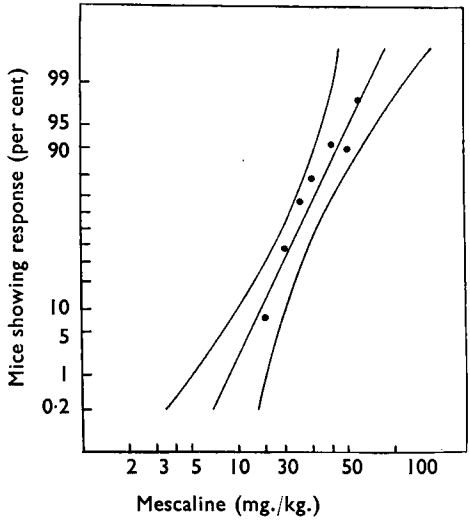

FIG. 2. Effects of increasing doses of mescaline on the frequency of stereotyped response in groups of mice (19/20 C.L. according to Litchfield and Wilcoxon).

After *caffeine* (10–20 mg./kg. i.p.) all mice showed an increased motor activity irrespective of the conditions before injection. Paroxysmal scratching movements were present in all mice but a regular succession of displacements and scratching—as seen with mescaline was never observed (Fig. 3).

Morphine induces a moderate hyperactivity at low doses (1 mg./kg.) and initially also with higher ones (3–5 mg./kg.) as well as the typical and well known rigidity and extension of the tail. However, it appears that the

reactions to handling and manipulating are decreased. Some morphine-treated mice developed a stereotyped motor behaviour, with circling movements. However, when the animals were placed in the cages and their activity recorded the tracings were unlike those obtained with mescaline because of the high frequency of the circling movements, the absence of rhythm and the great variability of the response (Fig. 3).

Nicotine tartrate-induced changes in motor activity also differed from those given by mescaline. After nicotine tartrate 5 mg./kg., spontaneous activity of mice was commonly reduced. The animals occasionally showed tremors and muscular twitches; with higher doses (7·5 mg./kg.) clonic convulsions appeared. Such observations are fully in accord with the effects of nicotine in the rat at the same dose level as reported by Blum[10]. But we have never seen patterns of motility similar to those seen with mescaline.

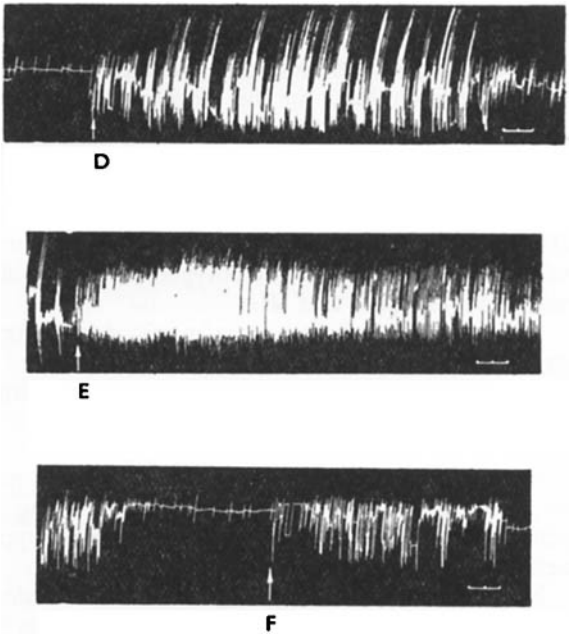

FIG. 3. Changes of motor activity in mice following: D (at arrow), caffeine 20 mg./kg.; E (at arrow), amphetamine sulphate 10 mg./kg.; F (at arrow), morphine hydrochloride 3 mg./kg. Time = 5 minutes.

Changes in Mescaline-induced Stereotyped Response by different Drugs

The results convinced us that the method of recording this specific stereotyped activity induced by mescaline was suitable for studying interactions between mescaline and other drugs.

We therefore investigated the influence of drugs, given to mice half an hour after mescaline, upon the stereotyped response. To express the activity of the various substances, we inferred the expected effect of known doses of mescaline alone, from the fitted dose/activity curve.

The effect of a single dose of each of the drugs was then compared with the expected response after mescaline and the inhibition calculated. A per cent inhibition was plotted on log-dose probability paper and the ED50 calculated as well as its 19/20 confidence limits. Throughout our experiments, we constantly checked the effect of mescaline alone.

There were three types of response of the mice under the new conditions.

First a normal response to mescaline with a proportion of the mice presenting the stereotyped response for all doses within the limits of variability of expected effects of mescaline alone as seen with inactive drugs.

Secondly, non appearance of the stereotyped response in a significant proportion of mice treated with mescaline together with the drug being tested. This is evidence for specific block.

Thirdly, absence or disappearance of the stereotyped response due to failure of motor function either directly observed or demonstrated by a period of immobility in the tracings of the activity of mice. This is evidence of unspecific block.

We also observed, as a result of the effect of a drug, a potentiating action of the mescaline-induced responses. This potentiation was demonstrated by the increase either in the number of mice responding to the ED25 and ED50 of mescaline or in the duration of its effect. The effects of drugs on the stereotyped response induced by mescaline are shown in Tables II and III.

The response is little or not modified by atropine sulphate 2·5 and 5 mg./kg.

Morphine hydrochloride produces a potentiating effect that is easily demonstrable at 3 mg./kg. The response is increased by about 40 per cent, and its duration is much increased. The response to 20 mg. of mescaline lasting normally about 1–2 hours, is prolonged with morphine to 3 and even 4 hours (Fig. 4). With 5 mg./kg. of morphine some inhibition was seen as well as a significant suppression of spontaneous activity.

Meprobamate, 20 to 200 mg./kg., caused a block of the response only in doses that markedly affect motor function and caused immobility (Fig. 5).

Mephenesin, and also those other centrally acting muscle relaxants chemically related to the thiadiazoles[11,12] such as L 1458 (5-thienyl-2-amino-1 : 3 : 4-thiadiazole), causes a similar unspecific block.

An unexpected confirmation of the lack of specific inhibition by meprobamate and mephenesin was given by the reappearance of the stereotyped response, after a duration of immobility of about 10 to 15 minutes with higher doses of mephenesin and after half an hour with meprobamate (Fig. 5).

No specific action on the stereotyped response was observed with azacyclonol in doses one third of the reported LD50[13].

Phenobarbitone sodium only partially blocked the response. With doses that do not produce marked improvement of motor mechanism resulting in immobility, block was never complete (Fig. 5). Plotting the per cent inhibition and per cent immobility on log-dose/probability paper, the two straight lines converge at the top near the 60 per cent effects (Fig. 6).

Pentobarbitone sodium produces some specific block. At dose of 10 mg./kg. no mouse showed immobility, but the response was inhibited in a high proportion of all animals.

TABLE II

EFFECTS OF DIFFERENT DRUGS ON MESCALINE INDUCED RESPONSE AND MOTILITY

Compounds and doses mg./kg.		No. of animals	Per cent inhibition of response	Per cent immobility	LD50 mg./kg. mouse
Meprobamate	20	14	3	0	i.p. 719 [745–694][12]
	40	3	0	0	
	50	7	0	0	
	80	15	0	0	
	100	23	10	15	
	200	13	100	96	
Mephenesin	25	6	42	35	i.p. 518 [559–480][12]
	50	5	44	50	
	100	3	83	83	
L 1458	12·5	3	0	0	i.p. 374 [408–343][12]
	25	3	0	0	
	35	18	0	11	
	60	18	32	28	
	80	12	100	100	
Pentobarbitone	5	8	0	0	i.p. 128·76 ± 2·75[20]
	10	25	79	0	
	20	12	100	62	
Azacyclonol	40	9	0	0	i.p. 220 ± 31[13]
	80	15	11	7	
Atropine sulphate	2·5	6	23	0	i.p. 250 [190–330][21]
	5	12	19	0	
Morphine hydrochloride	1	3	−2*	0	s.c. 531[22]
	3	6	−42*	0	
	5	21	7	14	

* Potentiation of response.

TABLE III

EFFECTS OF DIFFERENT DRUGS ON MESCALINE INDUCED RESPONSE AND MOTILITY. EXPERIMENTAL DATA OF DOSE-ACTIVITY CURVES ACCORDING TO LITCHFIELD AND WILCOXON[9]

Compound	Number of doses	Animals/ dose	ED50 and C.L.	Slope and C.L.	Heterogenity test χ^2 for P=0·05		LD50 mg./kg. mouse
					χ^2 found	χ^2 tabular	
Chlorpromazine							
Block of response	4	12	0·16 (0·10–0·25)	2·33 (1·12–4·84)	4·70	5·99	i.p. 225–250[23]
Immobility	4	16	1·07 (0·82–1·38)	1·97 (1·31–2·95)	4·35	5·99	
Promazine							
Block of response	8	15	1·70 (1·36–2·12)	1·93 (1·62–2·30)	10·75	12·60	i.p. 200[23]
Immobility	5	14	4·00 (3·20–5·00)	1·50 (1·17–1·93)	2·78	7·82	
Hexobarbitone							
Block of response	4	14	11·40 (8·90–14·59)	2·21 (1·64–2·99)	1·25	5·99	i.p. 280 ± 20·4[24]
Immobility	3	16	53·00 (39·25–71·55)	2·02 (1·48–2·74)	0·72	3·84	
Phenobarbitone							
Block of response	5	15	26·3	3·06	8·14	7·82	i.p. 235 ± 12[25]
Immobility	5	15	53·2 (43·25–65·43)	1·30 (1·06–1·58)	0·30	3·84	

The activity of hexobarbitone was still more marked. As shown by Figure 6, the distance, on the dosage scale, between immobility and blocking is great and highly significant.

Chlorpromazine has shown high specificity in counteracting the response. As shown in Table III and in Figure 6, the abolition of the response has been observed within a range of doses almost completely independent of those that produce immobility. The scratching response and the alternating rhythmic activity were seen to be greatly reduced.

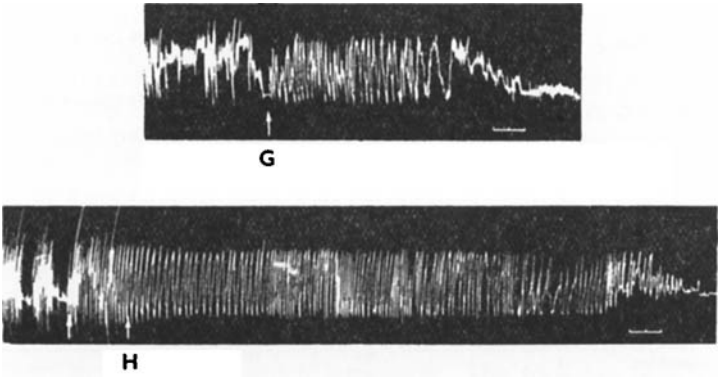

FIG. 4. Stereotype response in mice following mescaline: G (at arrow), mescaline 20 mg./kg.; H (at first arrow), mescaline 20 mg./kg., (at second arrow), morphine hydrochloride 3 mg./kg.
Time = 5 minutes.

Promazine also causes a specific block but it is much less effective than chlorpromazine (ED50 is about ten times that of chlorpromazine) and the specificity is less marked.

The median effective doses (ED50) abolishing the response and motor activity of the most extensively investigated compounds are given in Table III.

By analysis of the dose: activity relation, it was found that slopes of the linear functions representing respectively the block and the immobility are not statistically different with chlorpromazine, promazine, hexobarbitone and phenobarbitone. However, it has been noted that the slope of dose stereotyped response block curves was always greater than the slope of dose: immobility curves.

DISCUSSION

In our opinion the stereotyped response represents an aspect of total motor patterns concerned with the activity of mescaline. Only mescaline produces in mice a typical motor behaviour characterised by small movements—always or nearly in the same direction and scratching periods —in definite succession. Under suitable conditions the alternation is well defined and gives a characteristic kymographic record. The doses of mescaline used by us (within a range from 15 to 60 mg./kg. s.c. or i.p.) to demonstrate the response were apparently free from toxic effect.

Speck[14] studying toxicity and physiological effects of mescaline reported that in fasting rats, i.p. doses of 58 mg./kg. did not consistently reduce the blood glucose content and only slightly affected the heart rate. Moreover we found the LD50 by i.p. administration in mice to be 230 mg./kg. (19/20 confidence limits: 213–248).

Chlorpromazine was the most effective drug specifically blocking the response and its activity has been demonstrated in a range of doses close to those commonly used in man.

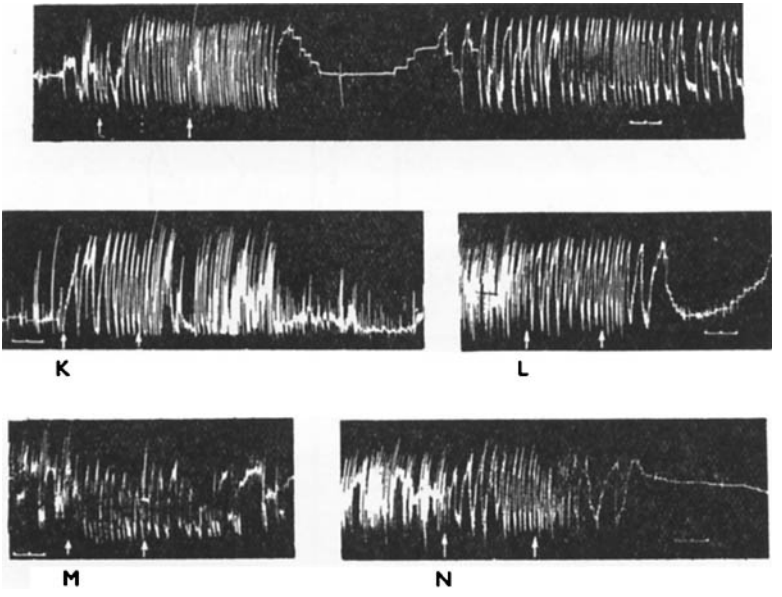

FIG. 5. Stereotyped response in mice following mescaline: J (at first arrow), mescaline 40 mg./kg., (at second arrow), meprobamate 100 mg./kg.; K (at first arrow), mescaline 40 mg./kg., (at second arrow), chlorpromazine 0·20 mg./kg.; L (at first arrow), mescaline 40 mg./kg., (at second arrow), chlorpromazine 1·20 mg./kg.; M (at first arrow), mescaline 40 mg./kg., (at second arrow), phenobarbitone 25 mg./kg.; N (at first arrow), mescaline 40 mg./kg., (at second arrow), phenobarbitone 50 mg./kg.
Time = 5 minutes.

On the other hand significant impairment of motor activity has been observed after doses as great as six times those that block the response.

According to Courvoisier[15] chlorpromazine in the rat also antagonizes all the symptoms that follow the administration of mescaline and Denber[16] reported that chlorpromazine was the most active agent in counteracting the psychic effects of mescaline in man.

Fellows and Cook[4] studying the scratching response in mice treated with mescaline reported it to be antagonized by chlorpromazine, reserpine, serotonin and morphine, but not by barbiturates or meprobamate.

According to Tripod[5] the motor stimulating activity of mescaline in

mice is antagonized by chlorpromazine, promazine, reserpine and serotonin, but also by meprobamate, 450 mg./kg. orally, and by azacyclonol, 200 mg./kg. orally. Also in the same paper the author reports that "les stimulations psychomotrices" induced by mescaline are enhanced by phenobarbitone and atropine.

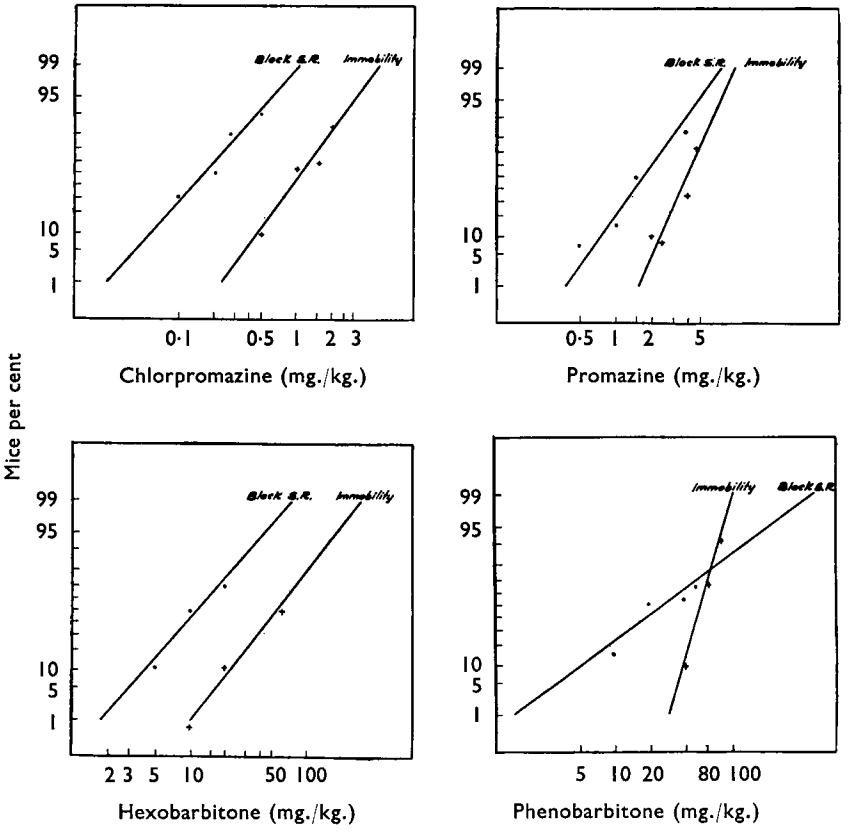

FIG. 6. Dose-activity curves of chlorpromazine, promazine, hexobarbitone and phenobarbitone. ●, block of stereotyped response; +, immobility.

Our results are not in full agreement with those of the authors quoted.

The most effective drug is chlorpromazine followed by promazine. The failure of meprobamate in specifically abolishing the stereotyped response, as we observed, and the scratching response, as found by Fellows and Cook[4]—contrasts with the antagonism of mescaline-induced "stimulations psychomotrices" reported by Tripod[5]. In our opinion the ED50 of meprobamate reported by this author is such to affect motor function, and can hardly be assumed to block specifically the effect of mescaline.

The three barbiturates studied, always inhibited the response and no

enhancement has been seen. Speck[14] also observed that phenobarbitone-induced anesthesia in rats may be easily and promptly counteracted by mescaline.

Among the drugs tested only morphine clearly enhanced the mescaline stereotyped response.

Fellows and Cook[4] found morphine to antagonise the scratching response and we also observed this effect in some animals. However the complex patterns of mescaline-induced motor behaviour as shown by the stereotyped response are, according to our observations, clearly potentiated by morphine. Also morphine alone is capable of inducing a stereotyped behaviour in mice, usually characterised by circling movements around the cage.

It may be interesting to remember that Cook and Weidley[17], studying the effect of drugs on conditioned avoidance response of rats, found that mescaline failed to produce any significant block of the conditioned response, but "in most instances" slightly enhanced the blocking activity on the conditioned response of promazine, reserpine, serotonin and morphine. On the other hand according to Wikler[18] mescaline-induced anxiety in man is relieved by barbiturates and not by morphine.

A poor effect on the response is given by azacyclonol, which is reported by Sturtevant and Drill[19] to change the psychomotor response of cats injected with mescaline from a catatonic to an excitatory type.

The many different technical approaches to testing such antagonism in mice has caused other authors to make use of the scratching response or "les stimulations psychomotrices". The study of the ability of drugs to abolish the effects of mescaline shows that different techniques may give different results. Further work is necessary to assess the usefulness of the systemic study of the stereotyped response as a means of differentiating the mode of action of certain CNS depressants.

From the practical point of view it must be said that although the method used by us is simple, the present investigation has required a relatively large number of animals to get statistically significant data.

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