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Effects of drugs acting alone and in combination on the motor activity of intact mice

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Summary

1. When administered to intact white mice, the central depressants—diphenhydramine, promethazine, chlorpromazine, gammahydroxybutyrate, gammabutyrolactone, hyoscine, and pethidine—produced sedation in small doses, but excitement and convulsions in higher doses. When given to mice pretreated with subanaesthetic doses of phenobarbitone these drugs abolished the righting reflex both in convulsant doses (hyoscine excepted) and in non-convulsant doses. These effects are similar to the effects previously observed with local anaesthetics.

2. Meprobamate, diazepam and chlorpromazine produced a loss of righting reflex both when given alone and following phenobarbitone. When given alone in higher doses, chlorpromazine induced convulsions.

3. The central stimulants bemegride and picrotoxin antagonized the loss of righting reflex produced by phenobarbitone, but nikethamide, caffeine and strychnine did not alter the depressant effects of phenobarbitone.

4. On the basis of these and previous studies with intact white mice a tentative classification of drugs having generalized depressant and stimulant effects on the central nervous system was proposed and discussed.

Introduction

It has been suggested that all anaesthetic drugs whether local or general act by blocking the production of action potentials in excitable tissue (Inoue & Frank. 1962). Frank & Sanders (1963) found that local anaesthetics acted like general anaesthetics both in intact animals and on isolated slabs of cerebral cortex *in situ*. They also found that local anaesthetics such as procaine which have prominent excitatory action on the central nervous system differed markedly in their mechanism of action from a classical central stimulant such as leptazol, and suggested that the excitatory effect of local anaesthetics was an exaggerated form of the stage II of general anaesthesia.

In addition to the local and general anaesthetics, a large number of centrally acting drugs currently classified according to their therapeutic use as analgesics, tranquillizers, sedatives and central muscle relaxants also are capable of producing a generalized depression of the nervous system. Although this property has been widely recognized in their clinical use, particularly as adjuvants in general anaes-

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thesia, the mechanisms underlying their depressant actions are not known. The theories of anaesthesia proposed so far rarely consider drugs which ordinarily cannot be used clinically to produce anaesthesia. It is possible that failure to produce anaesthesia may not be due to a lack of intrinsic anaesthetic property but rather to a lack of potency coupled with over-riding toxic effects. If these centrally acting drugs do possess anaesthetic properties, then it would be possible to explain part of their action in the central nervous system on the basis of this property. In the present investigation tests were carried out on intact mice to study the effects of these drugs and of various central nervous system stimulants, in order to compare their effects with those of anaesthetics observed in a previous study (Frank & Sanders, 1963).

Methods

All experiments were carried out on white Swiss Albino female mice weighing between 18–30 g. All drugs (except meprobamate and diazepam) were prepared in normal saline and administered intraperitoneally in volumes of 0·1–0·5 ml. Meprobamate was given in a 50% solution of polyethylene glycol and diazepam was administered in the form of Diazepam injection (Hoffmann-La Roche & Co.). Control tests were carried out with the solvents used for each of these drugs. These solvents had no effect on motor activity in intact mice. The drugs used were:

Depressants: Diphenhydramine hydrochloride, promethazine hydrochloride, chlorpromazine hydrochloride, gammahydroxybutyrate (GHB), gammabutyrolactone (GBL), hyoscine hydrobromide, pethidine hydrochloride, meprobamate, diazepam, and gamma aminobutyric acid (GABA).

Stimulants: Bemegride, picrotoxin, nikethamide, caffeine, strychnine sulphate, and glutamic acid.

The anaesthetic response in these tests was characterized by a reversible loss of the righting reflex without the loss of a spinal reflex (the withdrawal of the foot upon pinching). The doses of the various drugs used in interaction tests with phenobarbitone were obtained from the dose-response curves derived for each drug given alone in separate tests conducted before interaction experiments. The end point for these experiments was either the loss of the righting reflex, or convulsions or both, depending upon the drug in question. In the interaction tests the time interval between the administration of phenobarbitone and the test drug was adjusted so that their maximum individual effects coincided (see Frank & Jhamandas, 1969). Dose-response curves were analysed by the methods of Litchfield & Wilcoxon (1949).

Results

Interaction between phenobarbitone and other central depressants

When given alone in small doses phenobarbitone produces a reversible loss of righting reflex in mice but does not cause a loss of spinal reflexes (Frank & Sanders, 1963). Most of the central depressants used in this study—namely diphenhydramine, promethazine, gammahydroxybutyrate, gammabutyrolactone, hyoscine and pethidine—produced only signs of central nervous system stimulation (for example, convulsions) when administered by themselves in high doses. When the drugs

were given to mice pretreated with subanaesthetic doses of phenobarbitone in either convulsant or non-convulsant doses, central depression rather than stimulation was observed. Dose-response curves were derived for phenobarbitone when given alone (control curve) and when given in combination with a fixed dose of another drug (test curve). A separate phenobarbitone control curve was obtained for each of the drugs used in interaction experiments. These curves were tested for parallelism and the ED50 for phenobarbitone was derived in each case. The results obtained with the various central depressants tested are presented below.

Diphenhydramine and promethazine. When given alone to mice in small doses these drugs produced no discernible effect on the gross motor activity. Higher doses of either drug produced excitement and convulsions in the mice. When given in convulsant (130 mg/kg, 170 mg/kg) and non-convulsant doses (50 mg/kg) after phenobarbitone, promethazine produced only a loss of righting reflex (Fig. 1), and reduced significantly the ED50 of phenobarbitone (Table 1). Diphenhydramine produced essentially similar effects when given after phenobarbitone (Table 1).

Chlorpromazine. Administered by itself, chlorpromazine in doses of 10–100 mg/kg produced a reversible loss of righting reflex, whereas doses of 120 mg/kg

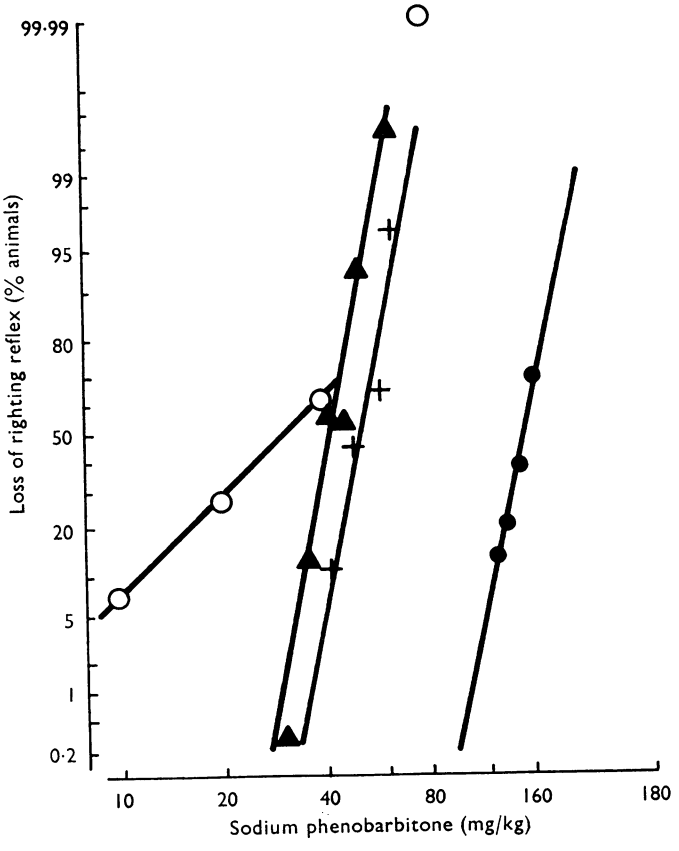

FIG. 1. Central depression produced by promethazine (●, control ; +, 50 mg/kg ; ▲, 130 mg/kg; ○, 170 mg/kg) in mice pretreated with subanaesthetic doses of phenobarbitone. Logarithmic probability plots. Each point was obtained from twelve to fifteen mice.

or more caused excitement and convulsions similar to those produced by diphenhydramine and promethazine. A subanaesthetic dose of chlorpromazine (5 mg/kg) when given to mice previously treated with small doses of phenobarbitone potentiated the effects of the latter as shown by a significant parallel shift of the dose-response curve to the left (Fig. 2 ; Table 1). A slightly higher dose of chlorpromazine (10 mg/kg), which when given alone caused only 8% of the animals to lose the righting reflex, produced a further but non-parallel shift in the dose-response curve to lower doses of phenobarbitone. Chlorpromazine also potentiated the effect of phenobarbitone when given in a convulsant dose (200 mg/kg) after the barbiturate (Fig. 2).

GHB and GBL. When given alone in small doses (GHB 200 mg/kg or less and GBL 100 mg/kg or less) these drugs produced no clearly discernible effects except possibly a slight sedative effect. Higher doses produced excitement and myoclonic jerks at low frequencies. Both these drugs potentiated the effects of phenobarbitone when they were given in either convulsant or non-convulsant doses following the barbiturate. The ED50 of phenobarbitone was reduced significantly in all doses tested (Table 1).

Hyoscine. Given in doses 250 mg/kg or lower, hyoscine did not affect the motor activity of intact mice. Doses of 300 mg/kg or higher produced hypermotility, exopthalmos, hyperventilation and cyanosis followed by convulsions and death.

TABLE 1. *Effect of some central depressants on ED50 of sodium phenobarbitone*

	Phenobarbitone (control)		Phenobarbitone and drug (test)	
Drug	ED50 mg/kg	95% confidence limits	ED50 mg/kg	95% confidence limits
Diphenhydramine				
60 mg/kg*	148·0	139·6–156·9	114·0	104·1–124·8
100 mg/kg†	148·0	139·6–156·9	93·5	87·8– 99·6
Promethazine				
50 mg/kg	151·0	141·1–161·5	51·3	42·8– 61·6
130 mg/kg*	151·0	141·1–161·5	41·7	38·9– 44·7
Chlorpromazine				
5 mg/kg	126·0	118·9–133·6	79·4	73·4– 85·9
Gammahydroxybutyrate				
100 mg/kg	126·0	118·3–134·1	90·2	77·1–105·5
200 mg/kg	126·0	118·3–134·1	53·0	46·5– 60·4
1,000 mg/kg†	126·0	118·3–134·1	22·4	18·7– 24·9
Gammabutyrolactone				
50 mg/kg	135·0	125·9–144·7	85·1	76·0– 95·3
100 mg/kg	135·0	125·9–144·7	41·7	35·3– 49·2
370 mg/kg†	135·0	125·9–144·7	20·0	13·3– 30·0
Hyoscine				
250 mg/kg	127·6	119·2–136·5	96·6	89·4–104·3
Pethidine				
50 mg/kg	138·0	128·3–148·3	92·3	88·3– 96·5
75 mg/kg*	138·0	128·3–148·3	99·5	95·6–103·5
Meprobamate				
100 mg/kg	164·0	149·0–180·0	61·0	54·2– 68·6
Diazepam				
8 mg/kg	164·0	149·0–180·0	64·6	58·7– 71·1

In every case the difference between control and test ED50 of phenobarbitone at 95% probability level was significant.
* Dose producing convulsions in 50% of animals receiving the drug.
† Dose producing convulsions in 99·5% of animals receiving the drug.

A non-convulsant dose of hyoscine (250 mg/kg) potentiated the depression induced by phenobarbitone (Table 1). In contrast, convulsant doses of hyoscine (500 mg/kg and 750 mg/kg) did not demonstrate an interaction effect with phenobarbitone (Table 2). Moreover, pretreatment with phenobarbitone did not modify the lethality of hyoscine (Table 2).

Pethidine. When injected in doses of 50 mg/kg or lower, pethidine had no discernible effect on the gross motor activity of intact mice, but in higher doses it

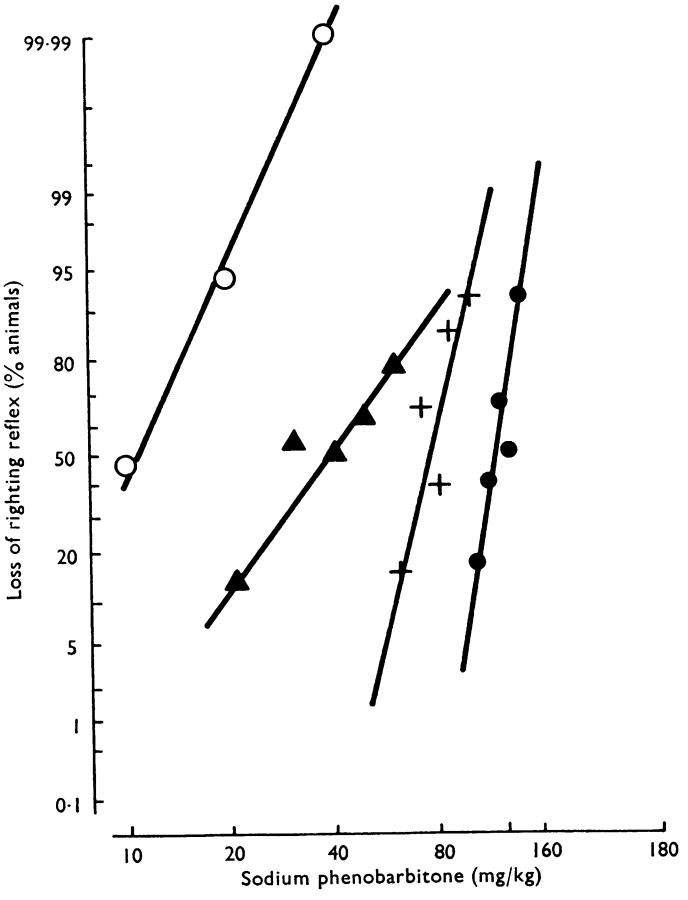

FIG. 2. Central depression produced by chlorpromazine (●, control ; +, 5 mg/kg ; ▲, 10 mg/kg ; ○, 200 mg/kg) in mice pretreated with subanaesthetic doses of phenobarbitone. Logarithmic probability plots. Each point obtained from twelve to fifteen mice.

TABLE 2. *Interaction between phenobarbitone and convulsant doses of hyoscine*

Phenobarbitone mg/kg	Hyoscine mg/kg	L.R.R.†	Deaths
0	500	0/9	11/20
135*	500	2/11	9/20
150*	500	0/8	12/20
0	750	0/0	11/12
135*	750	1/1	11/12
150*	750	0/0	12/12

* 135 mg/kg= ED25; 150 mg/kg=ED50.
† L.R.R. Number of animals showing a *reversible* loss of righting reflex. Only animals which did not subsequently die are included.

produced overt excitement and convulsions. In the interaction experiments pethidine potentiated the anaesthetic effect of barbiturate both when it was given in a convulsant dose (75 mg/kg) and in a non-convulsant dose (50 mg/kg) as shown in Fig. 3. Although both test curves showed a parallel shift to the left of the control curve (Fig. 3), the smaller dose of pethidine produced a greater potentiation than did the larger convulsant dose (Table 1).

Meprobamate and Diazepam. Meprobamate 150–300 mg/kg and diazepam 10–40 mg/kg produced a loss of righting reflex when given alone. In both cases the pinch-withdrawal reflex (the spinal reflex) was lost a short time after the loss of the righting reflex. When given in subanaesthetic doses, meprobamate (100 mg/kg) and diazepam (8 mg/kg) produced a loss of the righting reflex in mice previously treated with subanaesthetic doses of phenobarbitone. In both cases the drugs caused a parallel shift to the left of the phenobarbitone dose-response curve and the ED50 of phenobarbitone was reduced significantly (Table 1).

GABA. When given intraperitoneally in doses as high as 2,000–3,000 mg/kg GABA did not affect the gross activity. Such doses given after phenobarbitone also had no effect on the responses of the mice.

Interaction between phenobarbitone and central stimulants

The stimulants tested in this study were bemegride, picrotoxin, strychnine, caffeine, nikethamide, and glutamic acid. With the exception of glutamic acid, when given alone to mice in appropriate doses these drugs produced major convulsions resulting in death. Bemegride, picrotoxin and nikethamide produced clonic convulsions, strychnine caused tonic convulsions and caffeine produced both clonic and tonic convulsions. In the interaction experiments with phenobarbitone, the stimulants were given in a convulsant dose following pretreatment with

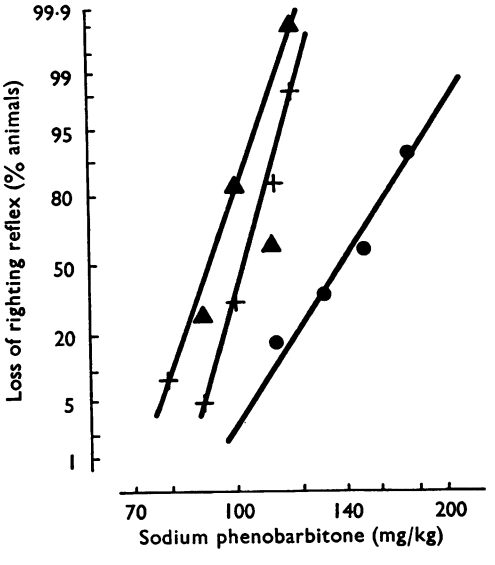

FIG. 3. Central depression produced by pethidine (●, control ; ▲, 50 mg/kg ; +, 75 mg/kg) in mice pretreated with subanaesthetic doses of phenobarbitone. Logarithmic probability plots. Each point was obtained from twelve to fifteen mice.

anaesthetic doses of phenobarbitone. The results obtained with the various drugs are presented below.

Bemegride and picrotoxin. Both these drugs antagonized the depressant effects of phenobarbitone. This antagonism is reflected in the shift of the dose-response curve to phenobarbitone to the right (Fig. 4). The control and test curves were parallel and the ED50 for phenobarbitone was significantly increased (Table 3). None of the animals receiving either stimulant drug convulsed or died during the interaction experiments.

Strychnine, caffeine and nikethamide. These drugs neither antagonized nor potentiated the depressant effect of phenobarbitone (Table 3). The dose-response curves obtained by giving them after phenobarbitone pretreatment overlapped with

TABLE 3. *Effect of central stimulants on ED50 of sodium phenobarbitone*

	Phenobarbitone (control)		Phenobarbitone and drug (test)		
Drug	ED50 mg/kg	95% confidence limits	ED50 mg/kg	95% confidence limits	Significance at 95% probability
Bemegride 45 mg/kg*	127·6	116·0–140·4	195·0	175·6–216·4	+
Picrotoxin 15 mg/kg*	127·6	116·0–140·4	165·2	154·3–176·7	+
Caffeine 250 mg/kg*	127·6	116·0–140·4	129·1	110·3–151·0	–
Strychnine 1·6 mg/kg*	127·6	116·0–140·4	121·9	103·3–143·8	–
Nikethamide 300 mg/kg*	127·6	116·0–140·4	117·5	98·0–131·0	–

+ Indicates presence of a significant difference; and – indicates absence of a significant difference between control and test ED50 of phenobarbitone at 95% probability level.
* Dose producing convulsions in 50% of the animals receiving the drug.

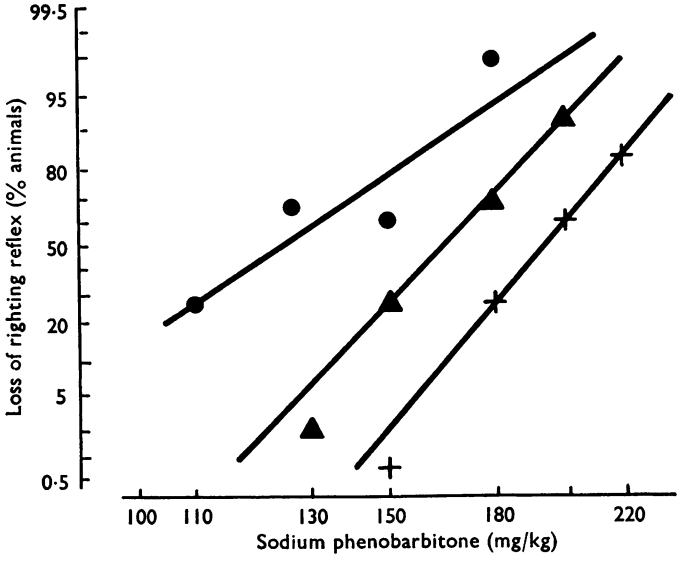

FIG. 4. Central depression antagonized by bemegride and picrotoxin in mice pretreated with anaesthetic doses of phenobarbitone. ●, phenobarbitone alone; ▲, phenobarbitone+picrotoxin 15 mg/kg; +, phenobarbitone+bemegride 45 mg/kg. Logarithmic probability plots. Each point was obtained from twelve to fifteen mice.

each other and the curves for phenobarbitone alone. None produced a significant change in the ED50 for phenobarbitone (Table 3). However, the anaesthetic doses of phenobarbitone prevented the convulsions produced by these drugs when given alone and none of the animals died.

Glutamic acid. Given by itself or following phenobarbitone treatment, glutamic acid in doses as high as 2,000–3,000 mg/kg had no effect on the motor activity of intact mice.

Discussion

In this study, a number of centrally acting drugs, both depressants and stimulants, have been examined for anaesthetic properties in tests on intact mice using the loss of righting reflex without the loss of the spinal reflexes as the index of anaesthetic activity. Although this criterion is not completely compatible with the state of clinical anaesthesia, it nevertheless did establish the absence or the presence of an experimental condition in animals which resembled general anaesthesia in man.

The main aim of this study was to attempt to classify drugs which act on the central nervous system on the basis of their ability or lack of ability to produce this anaesthetic-like effect. Obviously even without conducting studies of this type certain drugs, for example, clinical anaesthetics or leptazol, could be readily and properly classified according to this simple bimodal pattern. On the other hand many other drugs are not classified so readily even under this simple schema. Thus most of the drugs we tested and ultimately classified as general depressants (Table 4) did not produce " anaesthesia " when given alone regardless of the dose used but readily produced the anaesthetic-like effect when administered with small subanaesthetic doses of phenobarbitone.

In the present study it was obvious why these drugs failed to produce anaesthesia when given alone ; when given in higher doses they produced convulsions and caused death. Thus even though they did possess anaesthetic-like properties, these properties could not be demonstrated because of their severe toxic effects. In effect, then, the lethal dose was lower than the dose required to anaesthetize the animal. By first treating the animal with a small, subanaesthetic dose of phenobarbitone presumably the central nervous system is brought closer to the " anaesthetic state " even though no gross changes in motor activity can be observed, and then the small additional effect of the second drug would anaesthetize the animal.

It was suggested by Frank & Sanders (1963) that the strong central stimulant action of the local anaesthetics represented an exaggerated form of the excitement stages of anaesthesia (stage II). Presumably a similar suggestion would hold for the general depressants in this study which produce convulsions when given alone. However, it should be pointed out that the reduction or disappearance of these convulsions, and the ability of the animals to survive higher doses of these drugs in the interaction studies, cannot be taken as proof of the above suggestion. Once the animals have become " anaesthetized ", excitement and convulsions due to an action of the drug in the central nervous system will be depressed regardless of the mechanism of action of the drug in producing that effect. Thus anaesthetic doses of phenobarbitone depressed and eliminated the convulsions produced by the " general stimulants " included in this study (see also Sanders, 1967). Therefore, it is possible that some of the " general depressants " may have a central stimulant

effect by a mechanism of action other than an exaggeration of stage II of general anaesthesia.

We have based our classification of centrally acting drugs (Table 4) mainly on the ability (general depressants) or the lack of ability (general stimulants) to shift the phenobarbitone dose-response curve to smaller doses. In addition all the general stimulants produced central convulsions when given alone. However, the latter by itself is not a distinguishing feature because many of the general depressants had a similar effect. It was also interesting that only about half of the general stimulants were able to antagonize the anaesthetic effect of phenobarbitone ; that is, shift the dose-response curve to higher doses.

The results obtained with a few of the drugs are sufficiently different to warrant further comment. The convulsions and deaths produced by high doses of hyoscine could not be antagonized by phenobarbitone (Table 2). The animals which convulsed invariably died and before death showed several signs of respiratory difficulties. The picture presented is very similar to the one presented by tetrodotoxin (Frank & Pinsky, 1966) and as in the case of the latter drug, we would suggest that in higher doses hyoscine produces anoxic convulsions and death due to an action of the drug at a site other than the central nervous system.

TABLE 4. *Classification of centrally acting drugs based on their action in intact mice*

I General depressants Shift phenobarbitone dose-response curve to left on the dose axis.	II General stimulants Convulsants when given alone. Do *not* shift phenobarbitone dose-response curve to the left on the dose axis.
Group A Convulsions in animals when given alone in high doses. *Class* 1. Produce a large shift of phenobarbitone curve to left in a convulsant dose. e.g. Diphenhydramine Promethazine Gammahydroxybutyrate Gammabutyrolactone Local anaesthetics* *Class* 2. Shift phenobarbitone curve to left with small doses. Produce anoxic convulsions and death with higher doses. e.g. Hyoscine Tetrodotoxin* *Class* 3. Produce a smaller shift in phenobarbitone curve in convulsant dose than in non-convulsant dose. e.g. Pethidine	*Group A* Antagonize effect of phenobarbitone (shift the dose-response curve to higher doses) in convulsant doses. e.g. Bemegride Picrotoxin Leptazol*
Group B Produce loss of righting reflex when given alone. No convulsions when given in a high dose. e.g. Meprobamate Diazepam Barbiturates*	*Group B* Do not antagonize phenobarbitone in convulsant doses and do not potentiate it (no shift in dose-response curve). e.g. Strychnine Caffeine Nikethamide
Group C Produce loss of righting reflex when given alone, and convulsions in high doses. Shift phenobarbitone curve to smaller doses in convulsant and non-convulsant doses. e.g. Chlorpromazine	

* Indicates results from previous work (See **Discussion**).

Chlorpromazine produced loss of righting reflex at low doses but in higher doses convulsions. This pattern is so different from that produced by all anaesthetic agents as to suggest that this central stimulation is due to a mechanism other than an exaggeration of stage II of general anaesthesia.

Pethidine produced a very odd effect in that although it shifted the phenobarbitone dose-response curve to the left, smaller doses of pethidine produced a greater shift than did larger doses. It has been suggested that it is not pethidine itself but its metabolite norpethidine which is responsible for the central nervous system stimulation observed on administering large doses of the former (Deneau & Nakai, 1961). Thus it is possible that in the present studies when pethidine is given in high doses norpethidine is produced in sufficient quantities to antagonize the anaesthetic effect of pethidine plus phenobarbitone. Thus norpethidine would have an effect similar to some of the general stimulants. This possibility will be discussed further in a later paper (Frank & Jhamandas, 1970).

On the basis of the results obtained in this and previous studies (Frank & Sanders, 1963 ; Frank & Pinsky, 1966) we have set up a classification of centrally acting drugs based on the effects we have observed using intact white mice (Table 4). There is no doubt that the differences used in setting up the various groups and classes exist. However, it is not clear at this time if these differences represent important differences in the mechanisms of action of the various drugs on the central nervous system or are merely by-products of differences in the absorption fate or distributions of these drugs. It also might be argued that some of the factors we ignored in setting up this classification, for example the potent spinal reflex depression of meprobamate and diazepam, are important enough to warrant the setting up of separate classes.

Such difficulties can be cleared up only by further study and experimentation. On the other hand, this classification (Table 4) does represent at least a preliminary attempt to classify drugs modifying central nervous function based on their effects on the central nervous system rather than on their clinical use or chemical structure. Further we would suggest that our main division of these drugs into general depressants and general stimulants represents an important distinction based on their mechanisms of action.

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