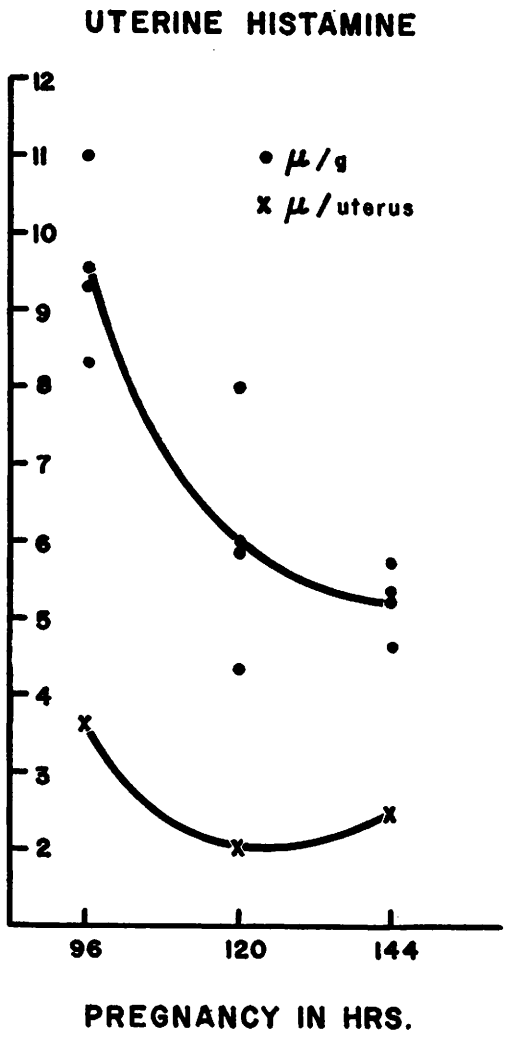

17. Studies recently completed in our laboratory show estradiol, estrone and estriol equally effective on a molar basis in releasing histamine.

Summary. Bioassay of uteri of pregnant rats reveal a significant reduction of concentration and content of histamine during 24 hr prior to actual implantation of blastocyst.

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FIG. 1. Histamine concentration (μ/g) and content (μ/uterus) during early pregnancy. Curves connect means of 3 samples, each sample pool of 5 rats.

Detection of Depressant Components of Drug Activity. (24635)

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Seeking a rapid means of detecting subtle central depressant effects of drugs, we administered substances to mice followed by a subhypnotic dose of hexobarbital. In this, and in similar procedures(1,2,3), it is assumed that depressants will add to the effect of the barbiturate and induce loss of the righting reflex. To determine whether central depressant activity could be detected among drugs not ordinarily classified as CNS depressants, widely different types of compounds have been assayed with the results set forth below.

Method. Drugs were administered orally or intraperitoneally when necessary to unstarved, adult male albino mice (CF #1) in groups of 10, at 3 or more doses. Thirty minutes later, (or more, depending on time of peak activity of test drugs) a subhypnotic

TABLE I. Potentiation of Subhypnotic Dose of Hexobarbital.

Drug	ED_{50} (95% limits), mg/kg p.o.
Hydroxazine	250 (167– 375)
Meprobamate	123 (108– 140)
Prochlorperazine	21 (15– 30)
Chlorpromazine	4 (2– 6)
Phenaglycodol	62 (41– 94)
Reserpine*	5 (3– 8)
Perphenazine	7 (6– 8)
Thiopropazate	5 (3– 7)
Phenobarbital†	28 (18– 43)
Glutethimide	43 (33– 56)
Diphenylhydantoin*	23 (15– 36)
Trimethadione†	370 (255– 537)
Acetylsalicylic acid	900 (643–1260)
Morphine	14 (10– 19)
S.K.F. 525-A	No effect
Nikethamide	*Idem*
Ethopropazine	73 (50– 106)
Sulfanilamide	160 (103– 248)
Iproniazid	185 (137– 250)
Diphenhydramine	200 (149– 238)
Tripelennamine	Marked excitation

* Given 3 hr before hexobarbital.
† " 2 " " " .

dose of hexobarbital sodium (50 mg/kg) was injected intraperitoneally. Animals were placed on their backs and those unable to right within 2 minutes were counted. The dose required to induce loss of the righting reflex in 50% of the animals (ED_{50} with 95% fiducial limits) was computed by the method of Litchfield and Wilcoxon(4) with results summarized in Tables I and II. In addition, the time interval between loss and spontaneous return of the righting reflex was recorded in minutes for each dose. The interval corresponding to the ED_{50} was then estimated graphically. These results are not recorded.

Results. The first 14 compounds listed in Table I, generally regarded as central depressants, include analgesics, ataraxics, anticonvulsants, and sedative-hypnotics. Their effects in this test procedure involve, essentially, conversion of a subhypnotic dose of hexobarbital to a hypnotic one. Thus, at ED_{50} dose levels their effectiveness may be equated roughly to 20 mg/kg of hexobarbital intraperitoneally. The method is sufficiently sensitive to detect mild depression elicited by analgesics and ataraxics. More potent depressants are detected at small fractions of dose required to produce overt signs of depression. The ED_{50}'s, being inversely proportional to potency, provide a relative measure of CNS-depressant activity.

The next compound, β-diethylaminoethyl-diphenylpropyl acetate (S.K.F. 525-A), gave negative results, although it prolongs the action of a hypnotic dose of hexobarbital(5) by inhibiting enzymes which oxidize the side-chain of hexobarbital(6). A similar mechanism cannot obtain in the present test because a hypnotic dose of the barbiturate is not given. Nikethamide prolongs the effect of a full hypnotic dose of hexobarbital in mice (unpublished observation) but, in agreement with its classification as a central stimulant, it was completely inactive in our test.

The remaining drugs listed in Tables I and II are not classed as CNS-depressants, yet loss of the righting reflex was induced by all of them, and in some instances (Compounds 17, 18, 19 and 20) dose-response relationship was linear. The antiparkinson drug (Ethopropazine) and sulfanilamide, both of which produce drowsiness in patients, potentiated the action of hexobarbital. Iproniazid induces convulsions and is used clinically to counteract depression. It inhibits monoamine oxidase which inactivates norepinephrine and serotonin in the brain(7), and this action, too, tends to support its classification as a stimulant. Nevertheless, in our study iproniazid exhibits depressant effects in the mouse. The antihistamines diphenhydramine and tripelen-

TABLE II. Non-Linear Potentiation of Subhypnotic Dose of Hexobarbital.

Drug	Dose and route of admin.	Loss of righting reflex (%)
Acetazolamide*	50 mg/kg, p.o.	20
	100	20
	200	40
	400	40
Epinephrine	.04 i.p.	20
	.08	20
Insulin	.05 U, i.p.	50
Atropine	150 mg/kg, p.o.	20
	200	60
	400	10
Adiphenine	200	30
	400	20
Homatropine methyl bromide	200	30
	400	30
	800	20

* Given 6 hr before hexobarbital.

namine are markedly excitant for mice, yet within a selected dose range diphenhydramine potentiated hexobarbital while tripelennamine was totally inactive. However, both drugs significantly prolong the effect of a full hypnotic dose of barbiturate(8).

Recording duration of loss of righting reflex is a time consuming procedure, but it discloses unusual effects. For example, with all drugs the interval averaged 30 (range 10 to 50) minutes, except with diphenhydramine which prolonged the duration beyond 3 hours. The mechanism of this reaction has not been investigated.

The 6 agents listed in Table II induced loss of the righting reflex, but dose-response relationship was not linear. Acetazoleamide, a carbonic anhydrase inhibitor, is effective in blocking epileptic seizures. Koch and Woodbury(9) reported that this compound decreases brain excitability, and concluded that its anticonvulsant properties stem from an increased concentration of carbonic acid within brain cells. This type of depression is readily detected. Epinephrine and other phenethylamines are known to cause some central depression. Thus, Quastel and Wheatley(10) suggested that epinephrine retards brain metabolism, and Ivy *et al.*(11) reported that it produced analgesia in man and animals. Later the hormone was shown to prolong the action of hypnotic doses of hexobarbital(12) and chloral hydrate(13). In agreement with earlier work, our experiments reveal a component of depressant activity. Insulin has been reported to depress brain metabolism (14) and to prolong the effect of an hypnotic dose of hexobarbital in mice(12). Similar results were obtained in the present test. Higher doses produce convulsions. The last 3 drugs listed in Table II are antispasmodics. They are capable of producing convulsions and are recognized central excitants. Nevertheless, they potentiated hexobarbital. Thus, it appears that these drugs possess depressant properties. The number of animals losing the righting reflex, however, decreased with increasing dosage, presumably because stimulation supervenes at higher doses.

Discussion. In our test, loss of the righting reflex is equated to central depression. No opinion can be offered as to mechanisms whereby this depression is brought about. The operator will have to discriminate between central depressants and drugs like succinylcholine which act peripherally. Distal effects which reduce the number of impulses reaching the sensorium and prepare the animal for sleep will be more subtle and more difficult to assess.

Summary. A method based upon potentiation of a subhypnotic dose of hexobarbital has been employed to detect depressant activity of drugs. It is rapid, and gives reproducible results with known CNS depressants. It appears to differentiate depressant drugs from those which prolong the action of a hypnotic dose of hexobarbital by interference with biotransformation mechanisms. It affords a rapid means of detecting depressant activity of drugs not ordinarily classified as central depressants.

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