TABLE III. Comparison of Complete and Incomplete Adjuvants in Their Ability to Induce Anaphylactic Sensitivity in Mice to Bovine Albumin.

Days between sensitization* and challenge†	Adjuvant in sensitizing emulsion*	Incidence of mortality	Survivors at 30 min.	Δt_{30}§
7	Complete	0/20 (0)‡	20	+ .2
7	Incomplete	" (0)	"	+ .6
10	Complete	1/25 (.04)	24‖	–3.3
"	Incomplete	0/25 (0)	25	–1.1
14	Complete	9/26 (.35)	18	–2.1
"	Incomplete	10/18 (.55)	9	–2.8
21	Complete	11/24 (.46)	16	–4.4
"	Incomplete	13/25 (.52)	15	–5.6
28	Complete	59/89 (.66)	38	–3.5
"	Incomplete	58/89 (.65)	39	–3.4
35	Complete	22/24 (.92)	11	–7.1
"	Incomplete	19/21 (.90)	5	–7.8

* Each mouse inj. on same day with a single sensitizing dose (0.2 ml) of bovine albumin-adjuvant emulsion containing 0.1 mg of bovine albumin.
† Challenging dose (0.2 ml) consisting of 1% bovine albumin in 0.85% NaCl inj. rapidly into tail vein.
‡ Numerator = No. of deaths recorded at 60 min.; denominator = No. of mice challenged; No. in parentheses is decimal fraction.
§ Δt_{30} = Mean change in rectal temperature (°C) of mice surviving 30 min. after challenge.
‖ Two of the 24 died within 3 hr following challenge.

of bovine albumin incorporated into water-in-oil emulsion containing *Mycobacterium butyricum* showed anaphylactic reactivity when challenged 28 days postsensitization with as little as 0.01 mg of bovine albumin. 2) Mice sensitized in a similar manner exhibited signs of anaphylaxis when challenged 196 days following sensitization. 3) Anaphylactogenicity in mice of bovine albumin in emulsions lacking *Mycobacterium butyricum* was comparable to that in emulsions containing acid-fast bacilli.

1. Morgan, P., Sherwood, N. P., Werder, A. A., *J. Immunol.*, 1957, v79, 46.
2. Morgan, P., Sherwood, N. P., Werder, A. A., Youngstrom, K., School of Avia. Med., USAF, Rep. No. 57-34, Feb. 1957.
3. Morgan, P., Sherwood, N. P., Werder, A. A., *ibid.*, Rep. No. 57-60, Feb. 1957.

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Anorexigenic Action of Methylphenidate (Ritalin) and Pipradrol (Meratran). (25178)

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Methylphenidate (Ritalin) and pipradrol (Meratran) are recently developed piperidine derivatives with central nervous system stimulant action. Meier *et al.*(1) and Brown *et al.* (2) suggested that the piperidine stimulants differ in certain respects from excitants of the amphetamine type, but they also demonstrated that these two classes of compounds share many pharmacologic properties. However, the newer agents have not been tested for possible anorexigenic action, so characteristic clinically and pharmacologically for drugs of the amphetamine class(3). Accordingly, an antiappetite assay was set up in experimental animals in order to compare piperidine stimulants with amphetamine-like agents.

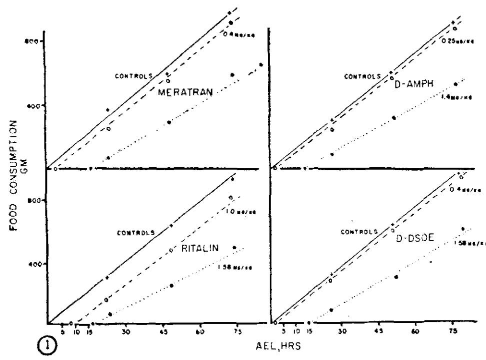
TABLE I. Anti-appetite Index (AAI) following Subcutaneous Administration of Piperidine- and Amphetamine-Class Agents.

Agent	AAI expressed in % — Dose, mg/kg .126	.25	.33	.40	.63	1.0	1.58
Methamphetamine HCl (d-desoxyephedrine)	9	15	20	29	56	61	
d-amphetamine SO_4	15	21	29	32	62	72	
Ritalin HCl (methylphenidate)		11		20	23	49	78
Pipradrol HCl (Meratran)	6	15	20	25	59		69

The results are reported here.

Methods. Anorexigenic testing was carried out in cats selected and trained to develop constancy of eating habits. Each drug was administered subcutaneously at not less than 4 logarithmically spaced dose levels; 5 cats were employed per dose level. Each dose was given 3 times, 45 minutes prior to and 6 and 16 hours following feeding time: medication was discontinued thereafter. Time elapsed between presentation of food and first food consumption was measured and averaged in each group of 5 cats. This average was referred to as average eating latency (AEL). Control animals eat as soon as the food is presented: in these, AEL is zero. Food consumption was recorded over 3-day periods in treated and untreated animals. In 150 controls variability of food consumption was not more than 10%; similar variability was found in the case of medicated animals. The difference between average food consumption of experimental and control cats was expressed in per cent of control value and referred to as average anti-appetite index (AAI). Minimal effective dose (MED) was the average dose capable of producing an AEL of 30 minutes or/and AAI of at least 20%. In some instances the method of Estes and Skinner(4) involving operant behavior of rats was adopted to anorexigenic screening. Rats were trained either solely in bar pressing for aperiodic food reward, or conditioned additionally to interrupt their bar pressing behavior at the onset of a click-shock sequence. These latter animals are said to develop conditioned emotional response (CER) or "anxiety"(5). After a suitable training period the drugs were administered in single doses at 2 dose levels *via* subcutaneous route, 2 to 3 rats per dose. Effect on coordinated motor activity was measured in rats by the method of Schulte *et al.*(6). Each agent was given at 3 to 4 logarithmically spaced dose levels, 5 to 6 rats per dose. The MED values were estimated from the dose-effect curves as the points of occurrence of a 30% increase in activity. LD_{50} values were obtained in mice by the method of Miller and Tainter(7); in cats, approximate toxicities (ALD_{50} values) were estimated according to the method of Wright(8).

Results. The anti-appetite action of methylphenidate (Ritalin), pipradrol (Meratran), d-desoxyephedrine (methamphetamine) and d-amphetamine was studied first in cats. AAI values obtained with various doses of these compounds are shown in Table I. Food consumption data for all 4 compounds are shown in Fig. 1. It is at once apparent that the piperidine stimulants are, in cats, potent anorexiants. Moreover, the anti-appetite effect is both quantitatively and qualitatively quite similar for piperidine stimulants on one hand and sympathetic amines on the other. In the case of either of the 4 agents listed doses as small as 0.25-0.40 mg/kg delayed the eating response by half an hour or more; however, cats started eating again before the second administration of the compound approximately 7 hours later. With larger doses AEL increased until, with doses of about 1 mg/kg, cats did not touch the food for at least 8 hours so that the compound was given the second or sometimes the third time before the animal started eating. Within the lower dose range (0.25-0.63 mg/kg) the drug effect seemed confined to delaying the first food intake; upon termination of medication food consumption was resumed at normal rate. This is indicated by the fact that the slopes of control and experimental food consumption curves were parallel for these doses (Fig. 1). At higher dose levels not only did the cats refrain from eating during as well as for some time after



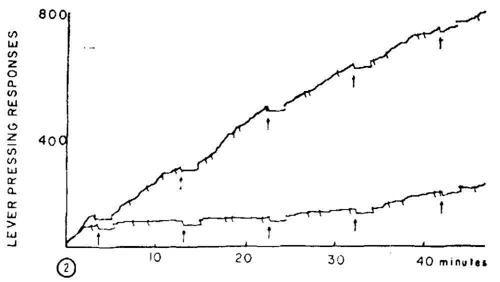


FIG. 1. Anti-appetite effects of d-desoxyephedrine (D-DSOE), d-amphetamine (D-AMPH), pipradrol (Meratran) and methylphenidate (Ritalin). Abscissa: cumulative food consumption in g. Ordinate: Average Eating Latency (AEL) in hr. Figure represents data obtained for 2 dose levels/agent, 5 cats/dose. Controls represent average food consumptions, 15 cats for each curve, recorded during week preceding experiments. Other legends in the figure.

FIG. 2. Effects of pipradrol (Meratran) on conditioned emotional response (CER) of a rat. Upper curve—control CER. Lower curve—CER following pipradrol, 2 mg/kg, s.c. Abscissa—cumulative index of lever pressing; slope of the record indicates rate of lever pressing. Ordinate—time in min. Short strokes—aperiodic positive reinforcement (food pellet). Depression in the record indicated conditioned stimulus in form of a clicking noise introduced at the arrow and terminated 2 minutes later by electric shock.

termination of the drug treatment, but the slope of the curve for experimental food consumption was considerably flatter than that for controls (Fig. 1). This was also reflected by a drastic increase of the anti-appetite index (AAI) at higher dose levels (Table I). At these dose levels average food consumption was lowered from the average control value of about 350 g per day to 175 g or less.

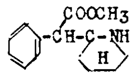
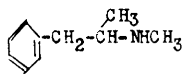
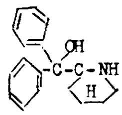
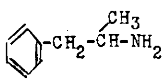
It can be said that at lower dose levels eating latency expresses duration of drug action since, when the latent period is over, normal eating habits are resumed. Contrarily, with larger doses there is a change in rate of food consumption which could last for several days (Fig. 1). That relatively large doses of piperidine and amphetamine-like stimulants seriously impair feeding was borne out also in a separate experiment in which particularly well nourished cats were given single doses of 1.58 mg/kg of methylphenidate, pipradrol, d-amphetamine and methamphetamine, 3 cats per agent, on 4 consecutive days. These cats ate from 75 to 100 g of food per day and lost between 10 and 15% of their body weight during this time period.

Anorexigenic activity of the compounds in question was further tested in rats trained in bar pressing for food reward. Since piperidine stimulants are referred to as ataractics, *i.e.* tension-reducing agents(9) it was of interest to test also whether or not these agents would affect bar pressing in rats with conditioned emotional response (CER) superimposed over their operant behavior. Doses of 1.0 and 2.0 mg/kg of methylphenidate, pipradrol, methamphetamine and d-amphetamine decreased significantly operant behavior in both unconditioned and CER rats (*cf.* Fig. 2 for effect of pipradrol on a CER rat). Similar findings were reported for d-amphetamine by Dews (10) in certain conditions of pigeon operant behavior; to the contrary, Brady(5,11) found with the same compound an increase of operant behavior in CER rats.

To complete the comparison between piperidine excitants and amphetamine-like compounds, the potency of these compounds to stimulate coordinated motor activity was measured in rats, and their toxicity in mice and cats. The results together with the data on cat anorexia are shown in Table II. Some of the experiments on motor activity and toxicity reported here were carried out earlier in separate investigations(1,2,6) dealing with single compounds of the present series; agreement between various sets of data is close. There appears to be little difference between the compounds in question with regard to both their pharmacologic activity and toxicity.

Discussion. This study demonstrates potent anorexiant action in experimental animals of 2 piperidine excitants, methylphenidate (Ritalin) and pipradrol (Meratran). It also provides data for a comparison of this action as exhibited by Ritalin and Meratran on one

TABLE II. Anti-appetite, Motor Stimulant and Toxic Actions of Methylphenidate, d-desoxyphedrine, Pipradrol and d-amphetamine. Chemical structures represented so as to emphasize similarity of compounds in question.

Compound	Structure	Anti-appetite action MED, mg/kg	ALD_{50}, mg/kg cats	Toxicity LD_{50}, mg/kg mice	Motor activity MED, mg/kg
		p.o.	p.o.	p.o.	p.o.
Methylphenidate HCl (Ritalin)		.33	85	285 ± 25	1.6
Methamphetamine HCl (d-desoxyephedrine)		.33	50	180 ± 35	1.0
Pipradrol HCl (Meratran)		.40	45	168 ± 18	1.5
d-amphetamine SO_4		.25	43	83.5 ± 6	.7

hand and amphetamine and methamphetamine on the other, on the basis of which some suggestions may be made with reference to the mechanism and site of action of the older amphetamine-like agents on which there is no unanimous agreement(3). Meratran and Ritalin are probably devoid of peripheral smooth muscle activity(1,2); their anorexigenic action therefore should be central. The close qualitative and quantitative resemblance between anorexigenic action of piperidine stimulants on one hand and that of the 2 sympathetic amines on the other strengthens the suggestion that amphetamine and methamphetamine are also central anorexiants by virtue of their action on the hypothalamus, either directly or *via* the cortex(3,12,13).

Meratran and Ritalin were not previously reported as anorexiants in experimental animals. However, their anorexigenic action is mentioned in clinical literature(14,15). That this is infrequent may be due to the fact that these agents are usually employed in emotionally unstable patients; since appetite of such patients is notoriously variable, the anorexigenic action of piperidine stimulants may easily escape notice. Although it is not our intention to suggest a resemblance between psychotics and the rats with "conditioned emotional response" which were rendered anorexic in this investigation, our data may suggest that anorexia could be a side action of piperidine stimulants in regressed schizophrenics or senile patients in whom their use is indicated. On the other hand, it may suggest their trial as anorexiants in cases of non-psychiatric obesity.

Anorexigenic action of Ritalin and Meratran constitutes one more pharmacologic property that these compounds share with amphetamine stimulants, the other being their potent motor stimulant (Table II) and their weak analeptic and cardiovascular actions(1, 2). An additional interesting similarity is that both Ritalin and centrally active sympathetic amines are effective antagonists of reserpine depression(1,16,17). It has been hypothesized by Everett and Toman(18) that desoxyephedrine might have replaced in these experiments norephinephrine or related compounds which are depleted in central neurones following administration of reserpine(19). It may be suggested that all 4 compounds studied at present could act in this fashion since they are structurally related having a secondary nitrogen 2 carbons removed from the phenyl ring (Table II).

Expectation was voiced on several occasions (14,15) that the clinical effects of piperidine excitants should differ from those of the amphetamine-like agents. This may have been based on the somewhat subjective impression that piperidine compounds, contrary to amphetamine, do not "irritate" or "aggravate" experimental animals(2). On the other hand, a recent editorial(20) warned against undue optimism in expecting distinct clinical differences between piperidine and amphetamine stimulants. The data adduced here support this view entirely since they clearly indicate that there is little pharmacological basis for differentiating between these two classes of compounds.

Summary. 1. Anti-appetite action of d-amphetamine, d-desoxyephedrine, pipradrol (Meratran) and methylphenidate (Ritalin) was tested in selected trained cats. The agents were given s.c., once prior to, and twice following presentation of food. 2. A delay in eating response of half hour or more appears at dose levels of 0.25 to 0.4 mg/kg with all 4 agents. At doses of 0.63-1.58 mg/kg food consumption was reduced by more than half and eating response delayed 10-30 hours. 3. The compounds inhibited also at 1 to 2 mg/kg dose levels the operant behavior (bar pressing for food reward) of unconditioned and conditioned rats. 4. No qualitative or quantitative differences were found among the 4 agents studied with regard to stimulation of motor activity, toxicity and anorexigenic action. 5. Site of action of the anorexigenic effect of the compounds is discussed and similarity of this and other effects of piperidine stimulants on one hand and of amphetamine excitants is stressed.

1. Meier, R., Gross, F., Tripod, J., *Klin. Wochen-Schr.,* 1954, v32, 445.

2. Brown, B. B., Werner, H. W., *J. Pharmacol. and Exp. Therap.,* 1954, v110, 180.

3. Harris, S. C., Ivy, A. C., Searle, L. M., *J.A.M.A.,* 1947, v134, 1469.

4. Estes, W. K., Skinner, B. F., *J. Exp. Psychol.,* 1941, v29, 390.

5. Brady, J. V., *Fed. Proc.,* 1958, v17, 1031.

6. Schulte, J. W., Reif, E. C., Bacher, J. A., Jr., Lawrence, W. S., Tainter, M. L., *J. Pharmacol. and Exp. Therap.,* 1941, v71, 62.

7. Miller, L. C., Tainter, M. L., PROC. SOC. EXP. BIOL. AND MED., 1944, v57, 261.

8. Wright, H. N., *J. Am. Pharm. Assn.,* (Sci. Ed.), 1941, v30, 177.

9. Fabing, H. D., *Disesases of Nervous System,* 1955, v16, 5.

10. Dews, P. B., *Fed. Proc.,* 1958, v17, 1024.

11. Brady, J. V., *Science,* 1956, v123, 1033.

12. Sangster, W., Grossman, M. T., Ivy, A. C., *Am. J. Physiol.,* 1948, v153, 259.

13. Anand, B. K., Brobeck, J. R., *Yale J. Biol. & Med.,* 1951, v24, 123.

14. Hoch, P. H., Discussion of paper by H. D., Fabing, J. Robert Hawkins, J. A. L. Moulton, *Am. J. Psychiat.,* 1955, v3, 832.

15. Daly D., Yoss, R. E., *Proc. Staff Meet. May Clin.,* 1956, v31, 620.

16. Carlsson, A., Linquist, M., Magnusson, T., *Nature,* 1957, v180, 1200.

17. Everett, G. M., Smith, A. H., Toman, J. E. P., *Abst., Am. Soc. Pharmacol. and Exp. Therap.,* p. 11, Aug. 1958.

18. Everett, G. M., Toman, J. E. P., Chap 6, *Proc. Soc. Biol. Psych.,* 1958, Grune & Stratton, New York, 1959.

19. Holzbauer, M., Vogt, M., *J. Neurochem.,* 1956, v1, 8.

20. Psychotherapeutic Drugs, Report of AMA Council, J.A.M.A., 1958, v166, 1040.

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