A Screening Method for the Assessment of Appetite Suppressants

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A HOST of variable factors tend to make a precise analysis of the efficacy of anorexigenic agents in man difficult.[1] Despite the eventual necessity of proper clinical testing, it would be most desirable to have a simple screening procedure in animals for early evaluation of these agents. The method to be described is a simple bio-assay utilizing both aurothioglucose (ATG) obese and nonobese mice. The assay provides (1) a means of screening compounds with regard to appetite suppression in obese and nonobese animals, (2) an index for correlating locomotor activity (gross central nervous system responses) with appetite suppression, (3) a method for evaluating "tachyphylaxis" of sympathomimetic amines, and (4) a means of testing cumulative or delayed effects of appetite suppressants.

METHODS AND MATERIALS

Approximately 200 female Albino mice (Albino Farms) were used in this study. Prior to each experiment, and for a minimal period of two months following an injection of aurothioglucose, they were maintained at about 26 C and were permitted free access to water and Purina Dog Chow. All animals weighed between 17 and 25 gm. at the time they were given an injection of ATG. Aurothioglucose was used as a freshly prepared solution of a water soluble powder. Injections of ATG were made intraperitoneally in a dose of 0.8 mg. per gm. of body weight. During the assay procedure the animals being tested were individually housed in wire mesh cages and had free access to water. The diet employed during testing was a mixture of 2 parts Purina Laboratory Chow Meal and 1 part of melted lard. Food was dispensed in plastic feeding cups attached to cages by thin wire holders.

ATG-obese mice and litter mate nonobese mice given injections of ATG were trained to consume their daily food intake in an eight hour period. Daily eight hour food intake was charted for individual animals for four to six month periods. Each procedure was preceded by a four day control period. The animals were then given daily injections for a period of four to five days after which the period after drug administration was evaluated. At least two weeks elapsed before the same animals were used again for testing. Running activity was evaluated from five to fifteen minutes after injection. This activity was graded 1 to 4 plus.

d-Amphetamine sulfate, dl-amphetamine sulfate, phenmetrazine hydrochloride and chlorphentermine hydrochloride (formulas shown in Fig. 1) were used as freshly prepared solutions of water soluble powders at the dose levels indicated. All drugs were administered subcutaneously with clean but not sterile technic. Drugs were administered fifteen minutes before the presentation of food. Control injections of normal saline administered to forty mice produced no significant diminution in food intake in either obese or nonobese animals.

No animal was designated obese unless it had achieved a weight of at least 40 gm. prior to the onset of the experiment. All nonobese animals weighed between 25 and 30 gm. d-Amphetamine sulfate (5 mg. per kg.) was employed as the standard and depressed food intake 20 per cent in control animals and 30 per cent in ATG-obese mice with 4 plus locomotor activity patterns. Equipotent doses of other drugs were compared on the basis of their ability to produce equivalent suppression of food intake. Doses of the sympathomimetic amines were based upon the lean body mass of the mice. Obese animals received a dose comparable to the average weight of all the nonobese mice tested. Probabilities were estimated according to student's test of "t."[2]

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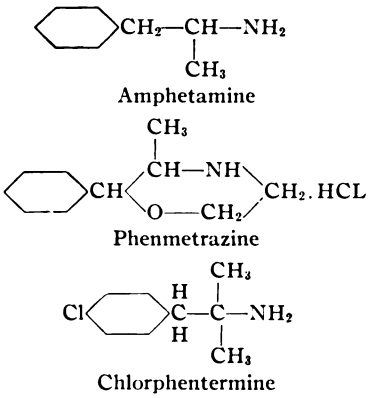

FIG. 1. Formulas of selected appetite suppressants.

TABLE I

Food Intake Data Comparing Twenty-Four Hour Ad Libitum Feeding with Eight Hour Dietary Adaptation

Animals	Food Intake (gm./day)	
	24 Hour Feeding	8 Hour Feeding
Nonobese.....	2.8 (10)	3.1 (10)
Obese.....	4.8 (10)	4.6 (10)

NOTE: Figures in parentheses represent number of animals.

RESULTS

Comparative Food Intake Data of Obese and Nonobese Mice

A comparison of food intake of obese and nonobese mice on twenty-four hour *ad libitum* feeding revealed the intake of nonobese mice to be 2.8 gm. (average of twenty mice) per day, while the obese mice averaged 4.8 gm. (average of twenty mice) per day. Mice were trained to ingest their daily food complement in eight hours in an effort to employ a time interval covering the expected duration of action of a single subcutaneous injection. Nonobese mice readily adapted to the restricted eating time achieving their twenty-four hour intake level in several days, whereas obese mice required several weeks to achieve a similar adaptation. Table I demonstrates the ability of both groups of mice to adapt their twenty-four hour food intake to an eight hour period.

The Comparative Efficacy of Selected Sympathomimetic Amines

d-Amphetamine Sulfate. d-Amphetamine sulfate was employed as the standard in a dose of 5 mg. per kg. of body weight in nonobese mice and an equivalent dose based upon the lean body mass of the obese animals. Figure 2 reveals the food intake suppression to be 20 per

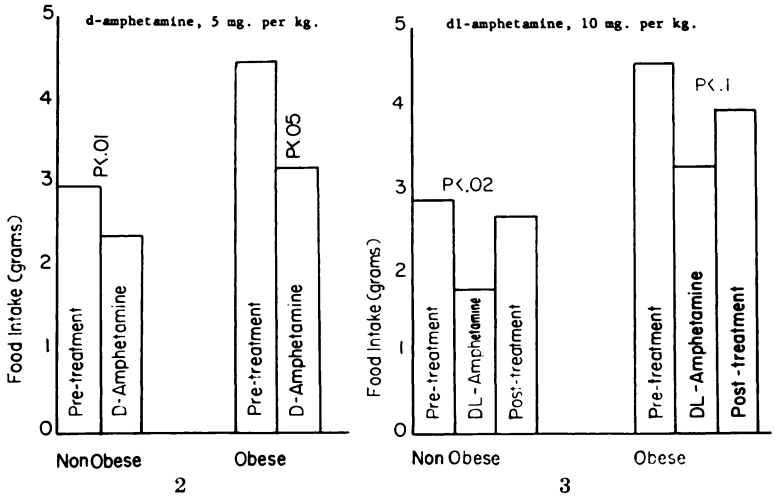

FIG. 2. Food intake suppression in obese and nonobese mice following injection of d-amphetamine sulfate 5 mg. per kg.

FIG. 3. Food intake suppression in obese and nonobese mice following injection of dl-amphetamine sulfate 10 mg. per kg.

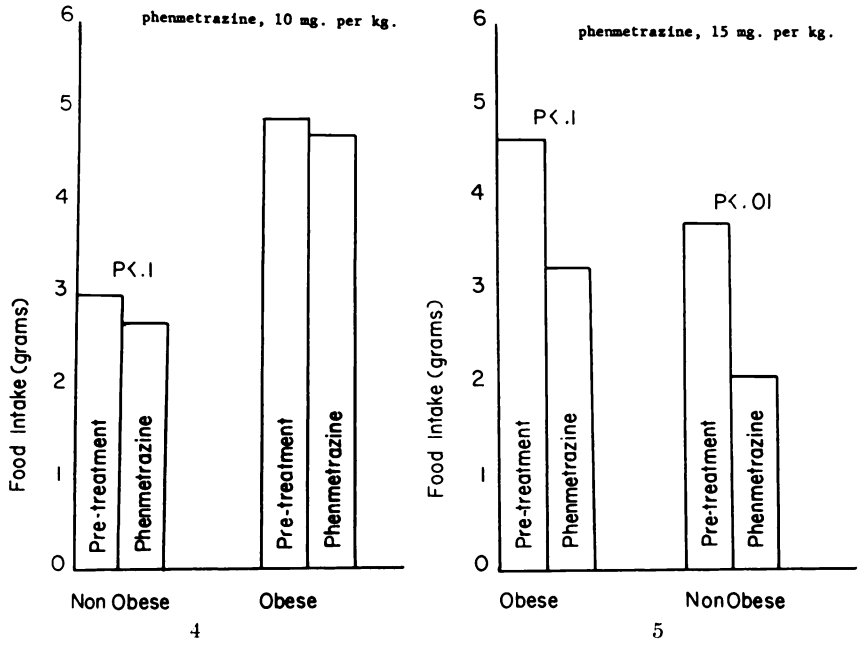

FIG. 4. Food intake suppression in obese and nonobese mice following injection of phenmetrazine hydrochloride 10 mg. per kg.

FIG. 5. Food intake suppression in obese and nonobese mice following injection of phenmetrazine hydrochloride 15 mg.per kg.

cent in nonobese mice and 30 per cent in ATG-obese mice over a four day period. At this dose level, 4 plus locomotor activity was present.

dl-Amphetamine Sulfate. Racemic amphetamine sulfate produced a result comparable to d-amphetamine at a dose level of 10 mg. per kg. Figure 3 graphically demonstrates food intake suppression during treatment and shows an effect of the drug after administration in the four day period following discontinuance of the drug. This dose level was associated with a 33 per cent reduction in food intake of nonobese mice, a 23 per cent reduction in food intake of obese mice and a 3 plus locomotor activity.

Phenmetrazine Hydrochloride. In doses of 10 mg. per kg. phenmetrazine did not produce a greater than 20 per cent food intake suppression in either obese or nonobese animals. However, 15 mg. per kg. produced a 43 per cent reduction of food intake in the nonobese mice and a 30 per cent reduction of food intake in obese mice in association with 4 plus locomotor activity. These results are depicted in Figures 4 and 5.

Chlorphentermine Hydrochloride. Previous experiments indicated that a single dose of d-amphetamine failed to produce significant food intake suppression when animals were allowed access to food for a twenty-four hour period. Recently published data[3] suggest that chlorphentermine has a delayed onset of action, a prolonged duration of action and minimal locomotor activity. Chlorphentermine was assayed in both animals on an eight hour feeding schedule and on a twenty-four hour feeding schedule. The results in Figure 6 indicate no significant suppression of food intake at eight hours with chlorphentermine in either obese or nonobese mice. At twenty-four hours, however, a significant suppression of food intake was demonstrated in both groups of mice (Fig. 7). Of some interest was the complete lack of enhanced locomotor activity seen with the other agents tested.

Tachyphylaxis. In an effort to determine

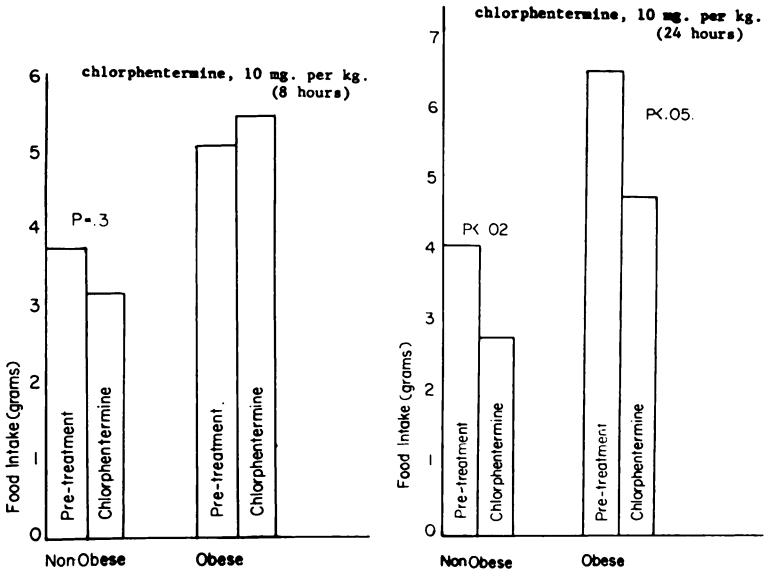

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FIG. 6. Food intake suppression in obese and nonobese mice following injection of chlorphentermine hydrochloride 10 mg. per kg. (eight hour feeding).

FIG. 7. Food intake suppression in obese and nonobese mice following injection of chlorphentermine hydrochloride 0 mg. per kg. (twenty-four hour feeding).

tachyphylaxis to food intake suppression, obese and nonobese mice were given daily injections of 5 mg. per kg. of d-amphetamine subcutaneously for a period of twenty-five days. Tachyphylaxis did occur as demonstrated by the block graph in Figure 8. A significant suppression of food intake occurred during the first two weeks, and gradually diminishing effects were noted during the ensuing weeks. It is of interest to note that two "breakthrough" periods were present following which suppression of food intake was noted again.

COMMENTS

Previously published assay procedures have employed rats,[4] cats,[5] or dogs[6] as test animals. The latter methods suffer the limitations of requiring either multiple injections or a relatively brief exposure to food. The present method combines the economy of using small animals yet offering quantitative data with a single daily injection and constant exposure to food over an eight hour period. While the relative potencies of appetite suppressants generally are consistent no matter which animal species is employed, there appears to be some species difference as regard sensitivity to the appetite suppressive properties of sympathomimetic amines. For example in man, dogs and cats appetite suppression may be obtained with doses of d-amphetamine from 0.25 to 0.625 mg. per kg. of body weight, while in rats and mice a comparable result is achieved with 2.5 to 5 mg. per kg. of body weight.

To our knowledge heretofore no previous assay method has employed obese animals as test objects. Aurothioglucose obese mice are hyperphagic, consuming nearly twice the amount of food ingested by their nonobese counterparts. There appears to be no doubt that these animals are quite sensitive to the appetite suppressant effects of sympathomimetic amines despite the "chemical ablation" of the satiety center (ventromedial areas). A comparable sensitivity exists for rats made hyperphagic following bilateral destruction of the ventromedial nuclei.[7,8] The greater variation in daily food intake among obese mice is to be expected in view of the greater

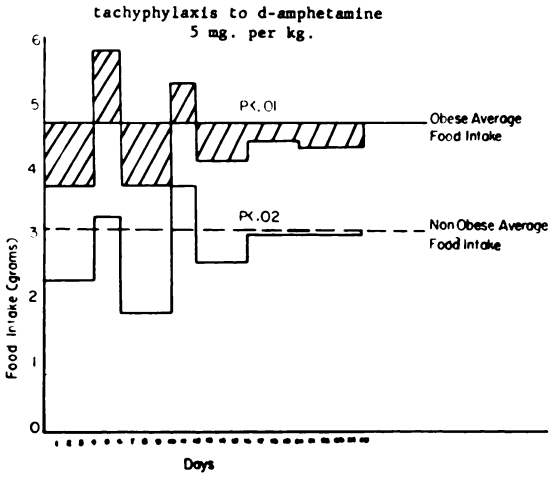

FIG. 8. Tachyphylaxis to d-amphetamine sulfate 5 mg. per kg.

differences in weight among these animals. The weights of obese animals in this study varied from 40 to 65 gm. Since a standard test dose of sympathomimetic amine based upon the average weight of nonobese mice was employed, statistically significant differences in food intake were not always demonstrable in obese animals as compared to the less variable nonobese mice. If drug dosages were based upon body weight, the sensitivity of these animals would have been markedly enhanced.

The increased locomotor activity seen following the administration of sympathomimetic amines offers a gross measure of central nervous excitation in rats and mice. Quantification of spontaneous activity in rats[9] revealed that 5 mg. per kg. of d-amphetamine produced a maximum total effect lasting approximately seven hours. The maximum total effect of dl-amphetamine was produced with a dose of 20 mg. per kg. In general, a dose of sympathomimetic amine producing a greater than 20 per cent reduction in food intake is accompanied by 3 to 4 plus locomotor activity. Among the compounds tested a notable exception to the gross correlation of enhanced locomotor activity and appetite suppression is chlorphentermine. Since electroencephalographic data and behavioral studies have demonstrated many qualitative similarities between chlorphentermine and other members of the amphetamine family,[3] the absence of increased locomotor activity in the first thirty minutes of observation may be partly related to the delayed onset of action and protracted duration of activity of this compound.

The chemical formulas of the compounds employed in this study are given in Figure 1. Previous analysis of structure activity relationships have demonstrated the basic importance of the phenylethylamine nucleus for appetite suppression. Maximal sympathomimetic potency is obtained when 2 carbons are interposed between the benzene ring and amine group. The addition of a hydroxyl group on the beta carbon generally results in increased cardiac activity, increased hyperglycemia and diminished central activity. Such a substitution is present in phenmetrazine and undoubtedly contributes to the decreased dose-related response as compared with d-amphetamine. The fact that appetite suppresion with d-amphetamine is about twice as great as with dl-amphetamine is not surprising, since current pharmacologic evidence suggests the l-form to have minimal central nervous system stimulating properties.[10] The delayed onset of action and protracted duration of action of chlorphentermine are possibly related to the slower rate of detoxication of this compound in the body. The para chloro substitution coupled with the dimethyl addition to the alpha carbon tend to delay deamination and para-hydroxylation of the drug. If animal studies are confirmed in man, a single dose of this compound may provide twenty-four hour appetite suppression with minimal excitation of the central nervous system.

Clinical observations have suggested that tolerance to amphetamines develops following long-term usage. Some controversy exists as to whether this tolerance or tachyphylaxis is related to psychologic or pharmacologic factors. There appears to be no doubt that sensitivity to the appetite suppression and locomotor activity decreases over a twenty-five day period until no significant effect is obtained. This appears to be true tachyphylaxis, a factor which should be considered when amphetamines and their derivatives are em-

ployed clinically as adjuncts in weight reduction regimens.

SUMMARY

A simplified method for screening appetite suppressants in obese and nonobese animals is presented. In addition to evaluating appetite suppression, the method provides an index for estimating central nervous system activity (locomotor activity), a means of evaluating tachyphylaxis and a method for testing cumulative or delayed effects of these compounds.

d-Amphetamine (5 mg. per kg.) was employed as the standard and depressed food intake 20 per cent in control animals and 30 per cent in ATG-obese mice, with 4 plus locomotor activity patterns. dl-Amphetamine produced similar food intake suppression and activity patterns at a dose of 10 mg. per kg., whereas phenmetrazine produced equivalent results with 15 mg. per kg. Chlorphentermine (10 mg. per kg.) appeared to be the only agent tested which had a delayed onset and protracted duration of action, producing depression of food intake over a twenty-four hour period without evidence of increased locomotor activity.

Tachyphylaxis to daily injections of d-amphetamine was demonstrated over a twenty-five day period.

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REFERENCES

1. Friedman, G. Efficacy and pharmacology of anorexigenic agents. *New York J. Med.*, 60: 2277, 1960.
2. Fisher, R. A. Statistical Methods for Research Workers, 10th ed. New York, 1946. Hafner Publishing Co.
3. Gylys, J. A., Hart, J. J. D. and Warren, M. R. Chlorphentermine hydrochloride, p-chloro-alpha, alpha dimethylphenethylamine hydrochloride, a new anorexigenic agent. *Fed. Proc.*, 20: 328, 1961.
4. Tainter, M. L. Actions of benzedrine and propadrine in the control of obesity. *J. Nutrtion*, 27: 89, 1944.
5. Karczmar, A. G. and Howard, J. H., Jr. Anorexigenic action of methylphenidate (Ritalin) and pipradrol (Meratran). *Proc. Soc. Exper. Biol. & Med.*, 102: 163, 1959.
6. Harris, S. C., Ivy, A. C. and Searle, L. M. The mechanism of amphetamine-induced loss of weight. *J.A.M.A.*, 134: 1468, 1947.
7. Epstein, A. N. Suppression of eating and drinking by amphetamine and other drugs in normal and hyperphagic rats. *J. Comp. & Physiol. Psychol.*, 52: 37, 1959.
8. Stowe, F. R., Jr. and Miller, A. T., Jr. The effect of amphetamine on food intake in rats with hypothalamic hyperphagia. *Experientia*, 13: 114, 1957.
9. Schulte, J. W., Reif, E. C., Bacher, J., Lawrence, W. S. and Tainter, M. L. Further study of central stimulation from symptho-mimetic amines. *J. Pharmacol. & Exper. Therap.*, 71: 72, 1941.
10. Elkes, J. Neurological Basis of Behaviour, Ciba Foundation Symposium. Little, Brown & Co.