EXPERIMENTALLY INDUCED OBESITY IN MICE AS A MODEL FOR THE EVALUATION OF ANOREXIGENIC AGENTS

by

Leonard T. Sigell

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APPROVED:

[redacted]

(Professor in Charge of Thesis)

[redacted]

(Chairman, Graduate Council)

DEDICATED

TO

MY PARENTS

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INTRODUCTION

One of the earliest and still most vexing socio-medical problems is the prevention and treatment of obesity. Only recently have scientists been able to approach this problem at a basic level. The observation that hypothalamic damage in animals could result in obesity provided a means for experimental investigation of the etiology and control mechanisms regulating hunger, satiety, and feeding behavior. To produce hypothalamic damage it is necessary to use stereotaxic techniques which limit the pharmacologic usefulness of this procedure. However, the recent observation that a single injection of auro thio-glucose (hereafter referred to as GTG) could induce massive obesity in mice was a suggestion for the large scale production of obese animals for research and assay purposes.

In order to be a valid model of human disease, an experimentally produced pathologic condition "must not only resemble the human disease and its symptoms, but also react comparably to drugs" (41). One must, therefore, evaluate the model itself in order to test its relationship to the human counterpart, or clinical obesity.

IN THE STUDIES DESCRIBED IN THIS THESIS THE OBJECTIVE HAS BEEN THE EVALUATION OF GTG INDUCED OBESITY AS A MODEL OF CLINICAL OBESITY. OBESITY HAS BEEN PRODUCED IN MICE BY THE INJECTION OF GTG. AN EVALUATION OF THIS EXPERIMENTAL OBESITY HAS INCLUDED STUDIES ON THE FOOD CONSUMPTION, FEEDING BEHAVIOR, LOCOMOTOR ACTIVITY, OXYGEN CONSUMPTION (REFERRED TO

TOTAL BODY WEIGHT AND LEAN BODY MASS), PHARMACOPATHOLOGY, AND THE RESPONSE TO ANOREXIGENIC AGENTS. ATTEMPTS TO RELATE THESE RESULTS TO THOSE OF OTHER INVESTIGATORS IN HUMAN OBESE SUBJECTS ARE DISCUSSED.

HISTORY

In order to appreciate the scope and limitations of this type of investigation, the following review of the historical development of knowledge concerning obesity is offered. Major emphasis has been placed on those factors which contribute to an understanding of the hypothalamic involvement in appetite regulation, and thereby to an appreciation of GTG obesity. In addition, a chronologic development of the pharmacologic principles and methods involved in the prophylaxis and treatment of obesity is reviewed.

Definition and Importance of Obesity

The term obesity is derived from the Latin "obesus" which means "eaten up" or "lean." This word gradually acquired the opposite meaning, that of being overweight. "Adiposity" is a synonym which describes the condition more adequately since it originates from the Latin "adeps" meaning "fat" (104).

Attempts to define obesity are fraught with difficulties. Since this problem has and is being studied by social-scientists as well as physiologists, pathologists, biochemists, anatomists, pharmacologists, and clinicians in most of the medical specialties, any attempt toward

a restricted definition would most likely encounter vigorous opposition. In contrast to most medical problems, obesity, by its very multi-origined nature, would appear to be one term which does not require limitations in definition in order to facilitate understanding.

It is generally agreed that whenever an abnormally large amount of adipose tissue is accumulated, the subject may be referred to as obese. The problem, then, is not the definition of obesity but of the abnormality. Whether the cause be psychiatric, pathophysiologic, or pathopharmacologic, the effect is always the same, i.e., deposition of fat above the defined normally acceptable limits. In most instances, a research pharmacologist can avoid a discussion of the borderline between obesity and normalcy. It is his responsibility to investigate means for decreasing the adiposity of patients who have previously been diagnosed by the clinician. He is also interested in etiological factors as a guidepost for the direction of researches aimed at treatment or reversal of the processes causing excess accumulation of adipose tissue.

The significance of obesity as a socio-economic problem is of ancient origin while the basis for medical concern about the effects of excess fat is only of recent origin. Man's primary nutritional problem always has been and still remains to be that of starvation. Nevertheless, the following quotation is food for thought:

> For the first time in history, the United States has produced a society in which less than one-tenth of the people turn out so much food that the Government's most embarrassing problem is how to dispose inconspicuously of 100 million tons of surplus farm produce. In this same society, the plain citizen can with an average of only one-fifth of his income buy more calories than he can consume. Refrigeration, automated processing and packaging conspire

> to defy season and banish spoilage. And in the wake of the new affluence and the new technics of processing comes a new American interest in how what people eat affects their health. To eat is human, the nation is learning to think, to survive divine (4).

A widely investigated aspect of obesity has been its statistical relationship to debilitating diseases. Kinsell (76) mentions that there is adequate evidence to indicate an increasing incidence of at least the following in obese individuals: diabetes, hypertension, atherosclerosis, hypothyroidism, Cushings Syndrome, gout, glycogen storage disease, eunochoidism, and hirsutism. To this list Thorn and Bondy (113) add degenerative arthritis and cancer and state that "indeed the only common cause of death which does not strike earlier in the obese than in the lean population is suicide!" Schwabe (106) has suggested that "50% of the American population over the age of 40 suffers from some degree of obesity." Briggs (30) has somewhat more conservatively estimated that the incidence is from 10 to 12 per cent in children and that about 15,000,000 adults in the United States are obese.

Even prior to the introduction of statistical information, it had long been considered desirable to prevent or reverse excess adiposity. Irrational methods of therapy long preceded any rational efforts to understand the mechanisms regulating feeding behavior and energy balance. Thus, for reasons of chronology, the treatment of obesity will be discussed prior to etiology.

Pharmacological Approaches to the Treatment of Obesity

Historically, the treatment of obesity has consisted of such measures as bleeding, leeches, blistering, vegetable diets with large quantities of vinegar, baths, induction of grief and anxiety, surgical removal of exuberant fatty tissue, emetics, digitalis, diuretics and preparations of halides (71). Waring's Practical Therapeutics, 1874, discusses the four most commonly used methods of treatment of obesity; i.e., ammonium bromide, Fucus Vesiculosus, Potassae Liquor (Solution of Potash - potassium bicarbonate), and alkalies (117). Concerning the widely used Fucus Vesiculosus (obtained from sea algae), Waring States:

> As a remedy for obesity, the decoction, or, which is preferable, the extract of this seaweed (in doses of grs. xl-lxxx daily, in divided doses) has been highly praised..... and its efficacy has been confirmed in his own person by Dr. Godefray.....in a period of thirty-four days, under the use of the extract, in doses of grs. ivss, thrice daily, taken at the commencement of each meal, he lost nearly 3¼ lbs. in weight.

Brunton (36), in 1889, in his A Textbook of Pharmacology, Therapeutics and Materia Medica listed remedies in common use for corpulence such as alkalies, alkaline waters, ammonium bromide, Banting's System (living on meat and green vegetables, and avoiding starch, sugars, and fats), Fucus Vesiculosus, potassium permanganate, saline, sodium chloride, sulphurous waters and vegetable acids.

In contemporary medical practice many of these or similar preparations are still used. Diuretics, thyroid extract (in place of iodine or Fucus Vesiculosus), digitalis (for "obesity" of cardiac origin), and starvation diets are widely employed, although with a somewhat greater

understanding of their pharmacologic mechanisms of action.

An original contribution of the twentieth century to this armamentarium is amphetamine. Although first prepared in 1887 (27), amphetamine was not used therapeutically until 1932 when it was introduced as an inhalant following Alles' (5) chemical and pharmacological investigations. Nathanson (96) and Davidoff et al (43) observed that weight reduction occurred as a side effect in patients using amphetamine as an inhalant:

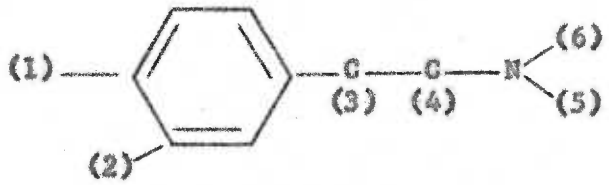
> Ten patients noticed a marked loss of appetite and with this a definite reduction in weight.....the loss of weight varied from seven to twenty pounds. A study of the basal metabolism in two patients who had lost weight indicated that the loss of weight was not due to an increase in the basal metabolic rate. The loss of weight can probably be explained by the lessened appetite and increased physical activity.

Today, nearly all of the more than 1500 American drug manufacturers prepare amphetamine containing anti-obesity agents for prescription purposes. Some combine the amphetamine with sedatives, thyroid, atropine, laxatives, or vitamins. Since amphetamine and its congeners are related to epinephrine, they possess pressor and bronchiodilatory characteristics. Clinically, and in the treatment of obesity, the amphetamines are used to produce central nervous system stimulation, reduction of fatigue, and suppression of appetite (80). This triad of actions is found not only in amphetamine but, also, in methamphetamine, phenmetrazine, phendimetrazine, benzphetamine, phenteramine, diethylpropion and other phenethyl amine derivatives. The structural similarities of these agents to each other and to epinephrine and ephedrine (the parent compounds) are shown in Figure 1.

Figure 1

STRUCTURAL RELATIONSHIPS OF SYMPATHOMIMETIC DRUGS

Figure 1



DRUG	1	2	3	4	5	6
Epinephrine	OH	OH	OH	H	CH_3	H
Ephedrine	H	H	OH	CH_3	CH_3	H
Amphetamine	H	H	H	CH_3	H	H
Methamphetamine (Desoxyephedrine)	H	H	H	CH_3	CH_3	H
Phenmetrazine	H	H	to 5	CH_3	-C-C-O-	H
Phendimetrazine	H	H	to 5	CH_3	-C-C-O-	CH_3
Benzphetamine	H	H	H	CH_3	-C-	CH_3
Phenteramine	H	H	H	$(CH_3)_2$	H	H
Diethylpropion	H	H	O	CH_3	CH_2-CH_3	CH_2-CH_3

Although numerous clinical investigations have elucidated the many pharmacologic actions and side effects of the amphetamine drugs, little is known about their mechanism of action in the treatment of obesity. Lack of knowledge concerning the mechanisms regulating energy balance, appetite, and feeding behavior has hindered the attainment of such information. During the past several years, Axelrod (21) has been studying the metabolism of the catecholamines and sympathomimetic amines and has contributed greatly to an understanding of the properties of these biologically active chemicals. He has shown that, unlike the catecholamines, the amphetamines are found in the brain and cerebrospinal fluid in high concentrations suggesting that there is probably little hindrance to their passage across the blood-brain barrier. This observation has been confirmed by the C^{14}-amphetamine studies of Young and Gordon (48). They found significant levels of radioactivity in brain tissue within ten minutes after intraperitoneal administration of 1.5 mg/kg of the labeled drug to rats indicating rapid localization of this drug in the brain. They also observed that increased motor activity was greatest when brain concentrations were greatest. Peak activity was observed at one half hour after injection while at four hours "the animals commenced to doze in their cages, and only traces of radioactivity could be detected."

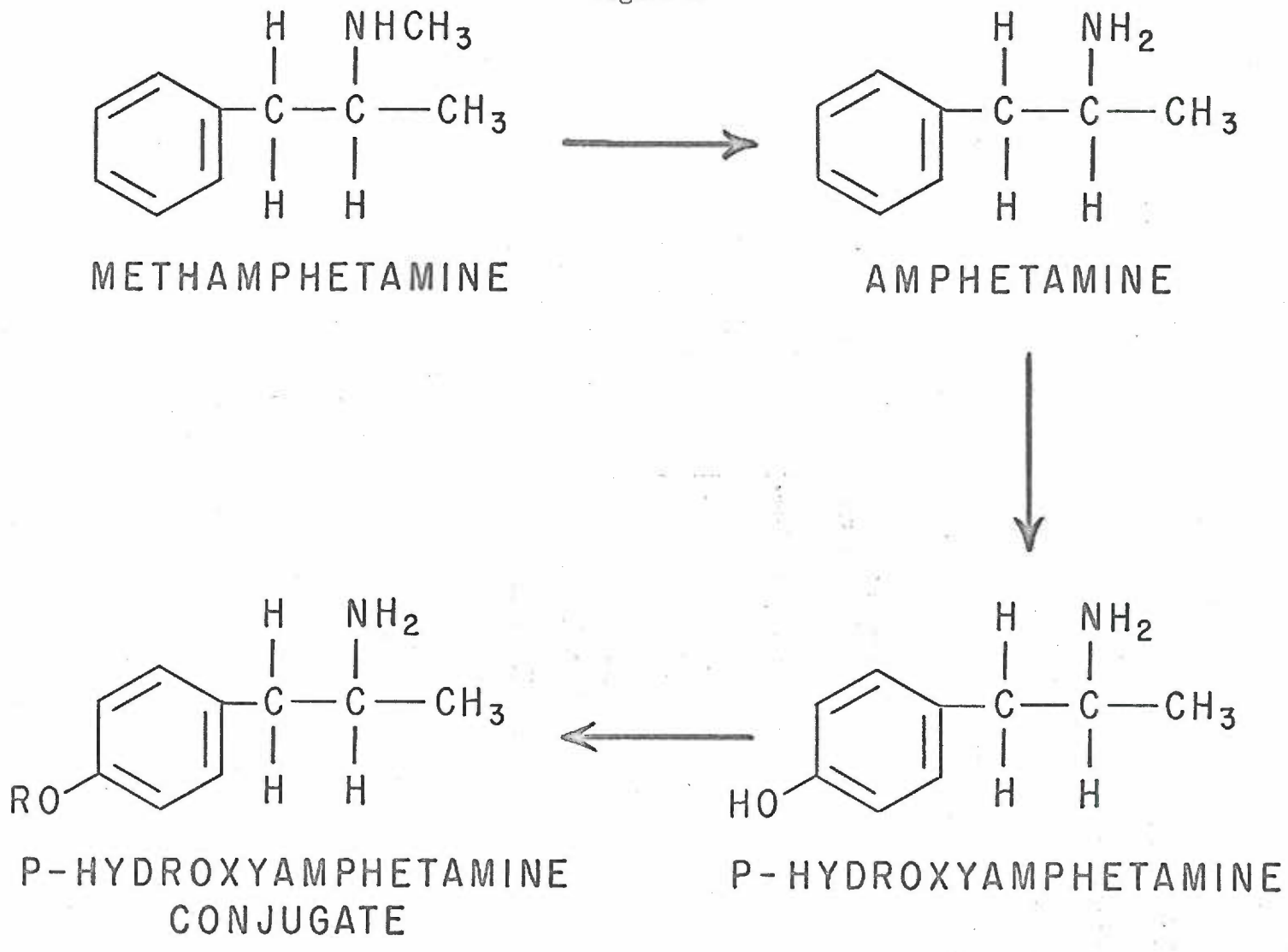
Axelrod (20)(21)(22)(23) has shown that the sympathomimetic amines are metabolized by a variety of pathways which include hydroxylation, conjugation, deamination, and N- and O- demethylation. Figure 2 shows the metabolism of amphetamine as proposed by Axelrod.

Figure 2

METABOLISM OF AMPHETAMINE

(After Axelrod)

Figure 2


METHAMPHETAMINE
AMPHETAMINE
P-HYDROXYAMPHETAMINE
P-HYDROXYAMPHETAMINE CONJUGATE

Smith (108) has shown that amphetamine-induced increases in locomotor activity are accompanied by a decrease in brain nor-epinephrine and an increase in brain 5-hydroxytryptamine (serotonin).

Early pharmacological and clinical studies indicated that there was probably a considerable margin of safety between therapeutic and toxic doses of amphetamines. Relative to the continued use of amphetamine, it is of interest that repeated sublethal doses in rats fail to produce detectable gross abnormalities (53). Some of the reported actions of the amphetamines are shown in Table 1.

It is necessary to recognize the great diversity of actions of the amphetamines in order to appreciate the difficulty of separating the anorexigenic effect from the other effects. Amphetamine, although far from being an ideal drug, has been the first pharmacologic agent to be introduced for the treatment of obesity as the result of rational scientific observation. Its usefulness in treating obesity was discovered by careful pharmacological investigation of the side effects resulting when amphetamine was being used for another therapeutic purpose. It is hoped that further studies on the biochemorphology of the amphetamines will lead to the development of more specific drugs which have the ability to produce reversible anorexia without the appearance of tolerance or adverse reactions.

TABLE 1

SELECTED ACTIONS OF THE AMPHETAMINES

1.	Cardiac Output	no change (6)
2.	Heart Rate	a) bradycardia (2)
		b) no effect (6)
3.	Electrocardiogram	no change (24)
4.	Blood Pressure	a) increase sufficient to contra-indicate in cardiacs (5)(109)
		b) increase in cats (103)
		c) below 30 mg no change above causes marked rise in systolic and diastolic pressures (3)(6)
		d) increase not sufficient to contra-indicate in cardiacs (80)(81)
		e) tachyphylaxis does occur (2)(80)
		f) inhibits nor-epinephrine induced increases in blood pressure in isolated heart preparations (98)
5.	Blood Sugar	does not influence carbohydrate utilization, concentration of sugar, lactate, or acetone bodies in the blood (3)
6.	Blood Lipids	increases rate of mobilization and blood levels of free fatty acids (97)(105)
7.	Pain	analgesic in chronic painful diseases (68)
8.	Addiction	does occur (1)(27)(50)

(continued on next page)

TABLE 1 (continued)

9.	Eye	mydriasis (2)(28)
10.	Gastrointestinal Tract	decreased motility (2) slows gastric emptying time (3)
11.	Respiration	a) respiratory dynamics, and vital capacity unchanged (6) b) analeptic in large doses (48) c) respiratory rate of animals increased (28)
12.	Basal Metabolic Rate	no change, few reports of increase attributable to increased psychomotor activity (3)(96)
13.	Uterus	increased tone, decreased amplitude of contractions (2)
14.	Kidney	no changes except in very large doses (2)(53)
15.	Electroencephalogram	a) abolishes abnormal discharges in children with mild epilepsy and behavioral disorders (28) b) increased arousal pattern at doses sufficient to produce psychomotor stimulation seen in all cortical regions and in leads from the medial and lateral thalamic nuclei, the hypothalamus, and the caudate nucleus (28) c) increased activity from medial hypothalamus (14)(32)(50)(119) d) EEG effects ended in 120 minutes in humans (60) e) EEG effects still present after 5 to 6 hours in conscious cat (28)

(concluded on next page)

TABLE 1 (concluded)

16.	Psychomotor Stimulation	does occur (28)(43)(50)(60)
17.	Locomotor Activity	increased (96)(108)
18.	Dose Response	smaller doses are needed to produce systemic than alerted EEG patterns and behavioral changes (28)
19.	Secretions	xerostomia (56% in one study on more than 400 cases) (3)(50)(95)
20.	Appetite	anorexia (3)(8)(9)(43)(47)(50)(96)

Psychological and Physiological Concepts of Feeding Behavior

Obesity is of multifactorial origin. To comprehend its etiology and nature, one must consider both the psychological and physiological components of the regulation of energy exchange. A discussion of current concepts related to these factors is offered here.

The complex of sensation that is peculiar to man and the higher animals which "urges and compels to ingestion of food" (38) is referred to as hunger and appetite. If hunger is defined in terms of the conditions leading to food intake rather than as a sensation complex, it is apparent that this urge is even more fundamental than the sexual urge. Feeding is, of course, a prerequisite for one's survival while reproduction is only essential for preservation of the species. All theories dealing with the regulation of hunger and appetite imply involvement of the nervous system as an integral part of the hunger mechanisms. But how this would explain the changes observed prior to and after feeding in those unicellular animals which have no specialized nervous systems is unknown:

> There are indications, then, that relative depletions of ingested food material, at least in some of the protozoa, results in increased motility (ameboid, ciliary) and increased avidity for food or rate of food intake. These phenomena probably indicate a condition of increased cell excitability; that is to say, a state of hunger in the protozoa is a state of increased excitability (38).

It is interesting to speculate that such a change in excitability may be an important factor in the regulation of food intake in man. Peripheral and central "receptor" cells may respond (as will be shown later) to environmental factors in such a way as to alter their

excitability, thereby triggering alterations in appetitive behavior (119). Another point of interest to ponder is that of the great eighteenth century physiologist, Haller, who stated, "Among animals, those having the shortest span of life, such as the insects, are the most voracious feeders. The caterpillar eats and defecates continuously" (70).

Prior to the twentieth century, three theories were proposed to account for this hunger phenomenon. The first suggested that hunger resulted from stimulation of sensory nerves in the digestive tract; i.e., the peripheral origin theory. The second has been termed the "central origin" theory which proposes direct stimulation of a hypothetical "hunger center" in the brain by the presence or absence of various substances in the blood or by changes in the metabolism of the center itself (alterations in cell excitability?). The third consisted of a compromise between the first two theories; i.e., a general sensation produced by central and peripheral alterations. Reviews and discussions of these theories are available (10)(83)(38)(84).

Later studies showed that total denervation or surgical removal of the stomach did not influence food intake regulation (64). These procedures do not eliminate hunger sensations nor even delay the onset of such sensations. It appears, therefore, that the site of food intake regulation is probably outside the gut. The observations that feeding per rectum as well as by the intravenous route appeased hunger (38), and that the taste, smell, perception, or proprioceptive feedback from oral food are not essential to the regulation of feeding (55), added

further support to this conclusion.

What, then, are the neural and psychic mechanisms regulating hunger and feeding behavior? If these were known and understood, it would appear that enlightenment would be forthcoming as to the pathophysiologic alterations causing obesity. Grossman (31) attempted to clarify understanding of the behavioral and psychic relations in depletion and repletion. He defines hunger as the complex of sensations evoked by depletion of body nutrient stores, appetite as the desire for food, fullness as the complex of sensations associated with repletion of body nutrient stores, and satiety as the corresponding effective state in repletion signifying a desire not to eat. He further suggests that at least a part of feeding behavior is learned:

> Before learning has occurred, as in the infant, depletion calls forth hunger sensations and unlearned feeding reflexes. The latter leads to repletion which, in turn, produces the sensation of fullness. Repetition of this cycle eventually leads to learned behavior in association with affective responses. Thus, the sensation of hunger evokes a desire for food or appetite, which, in turn, leads to appetitive behavior, learned food seeking and food taking activities which result in repletion and sensation of fullness. When conditioning has become established, the sensation of fullness is attended by the affective state of satiety which is reflected in a suppression of appetitive behavior.

One might conclude, then, that hunger is a bodily defense mechanism against depletion of nutrient stores. The sensation of hunger may result in an increased desire for food, appetite, and thereby appetitive behavior. Obesity would be classified in this regard as a psychopathologic state wherein an increase in appetite has produced an increase in appetitive or feeding behavior beyond the needs required to relieve the hunger sensation, and that this state results in a positive energy

balance. Unless this increase in feeding behavior can be balanced by a corresponding increase in energy expenditure, there will result an accumulation of potential energy in the form of fat.

What, then, are the physiologic mechanisms which regulate the psychic affective state of appetite? Since recent investigations have contributed much towards an answer to this question, they are reviewed in some detail at this time.

Fröhlich (58), in 1901, described a case of obesity, genital hypoplasia and progressive diminution in visual acuity in a 14-year old boy in whom operation revealed a craniopharyngioma. This syndrome (Fröhlich's syndrome) has been shown to be provoked by lesions of the hypothalamus. Bastrup-Madsen and Greisen (25) reported a case of obesity in a patient with acute leukemia where, at autopsy, it was found that the thalamus had been infiltrated by leukemic cells. These authors located the level of the hypothalamic infiltration in the region of the ventromedial nuclei. They noted in their paper that six other similar cases had been reported in the literature. Hetherington and Ranson (74) demonstrated that destruction of parts of the ventromedial nuclei of the hypothalamus leads to obesity. Anand and Brobeck (11) attempted to localize areas in the hypothalamus which would affect feeding behavior. They produced electrolytic lesions in different areas of the hypothalamus with a unipolar electrode. After surgery the rats were maintained in special controlled quarters and their feeding behavior observed. They found that (1) bilateral destruction of the ventromedial nuclei resulted in hyperphagia and obesity, (2) lateral hypothalamic lesions

yielded hypophagia and inanition, (3) when an animal had been made obese by ventromedial lesions, eating would cease upon later destruction of the lateral areas, and that (4) unilateral destruction was ineffective. These authors referred to the lateral area as a "feeding center", since they felt it may be the area responsible for the central hunger reaction or the urge to eat. Delgado and Anand (45) have produced hyperphagia by electrical stimulation of the lateral hypothalamus. The lateral hypothalamic area is herein referred to as the "feeding center" and the medial area as the "satiety center".

Teitelbaum (111) suggests that the overeating which results from medial lesions could be considered a release phenomenon. That is, by destroying the ventromedial nuclei bilaterally, one destroys "an area of the nervous system which normally acts to inhibit feeding behavior." As a result, the lateral area is "released" from inhibitory control and increased neuronal activity of the feeding center will occur. Such a sequence might initiate enhanced hunger sensations which would trigger appetite (the desire for food) and appetitive behavior (food seeking). Thus, lesions produced in the lateral area (whether or not accompanied by medial damage) would result in starvation or lack of the "urge to eat". Andersson and Larsson (17) suggest, however, that medial lesions decrease satiety but do not influence hunger sensations. It is still unknown whether or not the satiety center has a direct inhibitory action upon the hunger center or whether these areas are activated by separate mechanisms.

Wyrwicha and Dobrzecha (119) implanted chronic stimulating

electrodes in the hypothalamus of goats in which an alimentary instrumental conditioned reflex (consisting of placing the left foreleg on the food tray in order to be rewarded with food) had been previously established. They found that stimulation of a number of points in the satiety area caused cessation of eating in hungry animals and frequently caused the animals to manifest fear and restlessness which would persist after stimulus withdrawal. Other points in the satiety center when stimulated would provoke cessation of eating and sudden ejection of food from the mouth without any defensive reaction. In two satiated goats, stimulation of the hunger center caused resumption of the conditioned movements and eating. When stimulation of the hunger center was accompanied by simultaneous stimulation of a medial area of the non-defensive type, eating became less intense or would cease and the trained movements would occur less frequently or not at all.

Anand, Dua, and Singh (13) have been able to successfully implant recording electrodes into these two regions as well as other hypothalamic and cerebrocortical areas. They recorded the normal electroencephalographic responses of these areas in satiated and fasted animals and, also, those responses occurring after experimentally-induced changes in levels of blood sugar, proteins, and lipids. They observed that hyperglycemia increased the electrical activity of the satiety center and decreased slightly that of the hunger center. Conversely, hypoglycemia decreased the activity of the satiety center and on occasion slightly increased that of the hunger center. No changes were observed in other areas of the hypothalamus nor in cortical areas. Alterations in blood proteins and lipids did not change the hypothalamic activity.

Baillie and Morrison (26) recently showed that bilateral lesions in the lateral hypothalamus of rats could completely suppress feeding "while still permitting activity which had been made characteristic of desire for food (lever-pressing)." They interpreted their results to mean that rats desired food but were unable to consume it; i.e., the lesion produced a true aphagia (inability to eat). "Our experiments show that rats in which the lateral hypothalamus has been destroyed continue to demonstrate appetite and a form of food seeking behavior, while showing complete failure to consume food by the orthodox route, to chew it adequately or to swallow it." These authors, then, consider the primary function of the lateral area as a seat for the control of motor feeding mechanisms although they grant that some "motivational" control of eating may also be centered here.

Bruthowski et al (37) have reported that aphagia occurred in a dog with bilateral complete denervation to the amygdaloid complex. Experimental and clinical reports suggest severe disturbances of feeding behavior following temporal lobe damage (59)(78). It should be apparent that much remains to be learned as to what areas regulate which components of feeding behavior. Simple ablation and stimulation studies cannot themselves supply the answers. There has, however, been some research which might indicate how central control mechanisms are activated; i.e., the nature of the receptors and the sensory stimuli.

Glucostatic Theory

Many theories have been proposed to suggest how the central nervous system is fed information about the energy balance of the body. The theory which has received the widest acceptance is the "glucostatic theory" of Mayer (87). He suggested that it would be improbable that the hypothalamic or other centers would be sensitive to alterations in the body content of fat or protein since the proportion of these substances lost to the total body stores during the interval between meals would be small. However, in the post-absorptive period, and in spite of gluconeogenesis, the glycogen stores become rapidly depleted. Gluconeogenesis and, as Mayer says, a "shifting of metabolic oxidation in non-nervous tissues from glucose to fat (as measured by the lowering of the respiratory quotient)" tend to minimize the drop in blood glucose. In this way, the body maintains levels of glucose necessary for the survival of the central nervous system. Feeding, however, can restore "full homeostasis" of the central nervous system. Mayer hypothesized that the central nervous system, which is dependent exclusively on a continued supply of glucose in blood, would possess glucoreceptor cells sensitive to fluctuations of available blood glucose. In order to test his hypothesis, he undertook a series of investigations. Hyperglycemia was produced by daily subcutaneous injections of glucose, fructose, or small doses of epinephrine. This resulted in a significant decrease in food intake. On the other hand, hypoglycemia was accompanied by a significant increase in food intake even if the doses used were small enough to maintain the blood glucose at physiological fasting levels.

Fat injections had no such effect on food intake. In animals specially treated so as to produce a long-lasting hyperglycemia, death occurred from inanition in spite of the presence of food in their cages. These results in themselves could not explain the paradox of diabetes mellitus wherein blood glucose levels are elevated (often for long periods of time) but the patient is frequently hyperphagic and/or obese. Diabetes mellitus, however, is characterized by an inability of cells to utilize glucose. Thorn and Bondy (113) point out that insulin acts directly on the cell membranes to facilitate the entry of glucose into insulin-responsive cells. "Liver has no demonstrable permeability barrier to glucose; yet the diabetic state in liver is characterized by inability to phosphorylate glucose, suggesting a direct effect of insulin on the phosphorylating mechanism in this tissue." If glucose is to influence the hypothalamic gluoreceptors, it must be able to enter these cells. In diabetes mellitus there is, therefore, an increased blood glucose but a decreased useable glucose.

Indirect confirmation of this hypothesis was obtained through tests on human subjects (84). Cooper and Dach (42) added further support to Mayer's hypothesis by recording electrical activity of the ventromedial and lateral nuclei in response to intravenous injections of insulin and dextrose. "Insulin sufficient to halve resting blood glucose levels and dextrose sufficient to double the resting values both caused an increase in amplitude of...activity in the ventromedial nuclei and the voltage of lateral hypothalamic activity was generally depressed." Food intake was depressed significantly by both agents. Similar studies were reported by Anand, Chhina and Singh (12). These results are especially

interesting when one considers that insulin does not affect the uptake of glucose by the brain (in toto) but only by specific cells (glucoreceptor cells) (114). Anand et al (16) added further support by studying oxygen consumption and glucose consumption of tissue slices of each hypothalamic center.

"It is concluded that the neurones in the region of the medial hypothalamic satiety center are 'glucoreceptors' as their activity (electrical) depends on the extent of glucose utilization" (15). When these receptors are activated, it is proposed that they initiate nervous messages which are sent to cortical areas to initiate the quest for food and control the amount eaten (84).

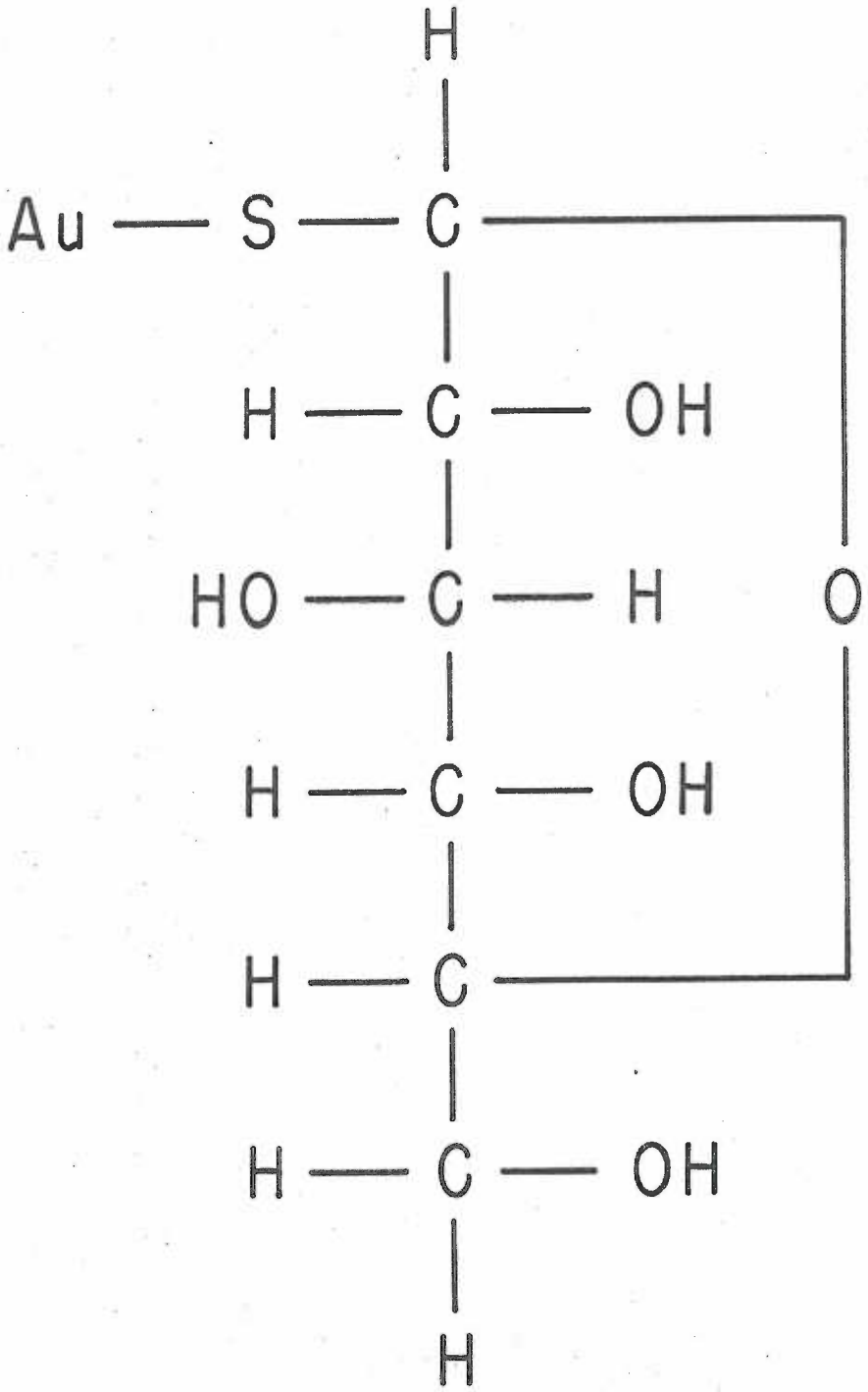
Effects of Gold Thioglucose

Further support for the glucostatic theory came with the discovery by Waxler and Brecher (118)(29) that gold thioglucose (GTG) (Figure 3) injections in mice could induce obesity. Following the injection of 35 mg doses of GTG (LD_{50}), about one third of the survivors became obese. These authors observed that the extra weight was due to an increase in adipose tissue and that except for some centrolobular fatty infiltration of the liver, no gross anatomic lesions were found in other organs. Marshall, Barrnett, and Mayer (91) described a similar obesity in mice, but upon histological examination they found extensive damage to the ventromedial nuclei of the hypothalamus. They observed "diffuse and punctate hemorrhages, widespread and marked edema involving more neural tissue than the hemorrhagic areas and pyknosis and degeneration

Figure 3

STRUCTURE OF GOLD THIOGLUCOSE

Figure 3



of nerve cells throughout areas of edema and hemorrhage." They point out, however, that the damage to the hypothalamus and other areas is extensive and not limited to the ventromedial area (although this area is always included). Gold thiomalate (91), and later, gold thioglycoanilide, and gold thiosulfate (90), which were used as controls did not cause obesity and did not lead to hypothalamic lesions. In animals that were autopsied three months after injection, the only lesion found was a deficit of neurons in the hypothalamus (especially the ventral portion). In some cases the pattern of the nuclei was lost altogether, whereas in other mice, the nuclei were recognized as clumps of cells containing fewer neurons. Friedman and Tepperman (56) studied the distribution of gold after intraperitoneal injections in mice. They observed that peak plasma levels occurred between 30 and 60 minutes after injection, and that the largest tissue concentrations are found in the kidneys and liver with very small quantities in the brain. In a study of animals that had been injected one, two, and three months prior to autopsy, the only organ with detectable gold, in very small quantities, was the liver. Mice that are fasted for 48 hours prior to GTG injection appear to be less vulnerable to the toxic effects of the drug (49) (56) (116). Higher liver and kidney gold concentrations in fasted animals were the only significant differences observed by Friedman and Tepperman. They hypothesized that fasting may permit the accumulation or "sequestration" of gold in indifferent tissues, thereby sparing vulnerable tissues from toxic concentrations. Wagner and DeGroot (116) studied rats injected with GTG and observed that kidney damage occurred even in rats that did not sustain detectable hypothalamic lesions.

Deter (46) observed that acute gastric ulcers developed in rats, but only in those where ventromedial damage had occurred from the GTG. Female rats incur more severe hypothalamic lesions than male rats following GTG (116). In radioautographic studies on mice, Debons et al (44) demonstrated gold accumulation in the ventral hypothalamus of all hyperphagic and obese animals studied (at the earliest after 6 hours). In non-obese survivors and mice injected with gold thiomalate, there were no radioautographic evidences of gold in the hypothalamus although gold was distributed diffusely throughout the brain:

> Therefore, it appears that although both gold thiomalate and gold thioglucose have access to brain tissue, it is probably the glucose moiety which is responsible for the focal accumulation of sufficient gold in the hypothalamus to produce a destructive lesion which can result in hyperphagia and obesity (44).

There is evidence (89) which even suggests that glucose competes with GTG for critical sites in the hypothalamus, thus adding even more support to the glucostatic theory. Perry and Liebelt (99) have observed extra-hypothalamic lesions associated with GTG obesity in regions which Debons (44) suggests might also function as glucoreceptive areas. Mayer (85) has further suggested that gastric hunger contractions may be, at least in part, under hypothalamic control. Waxler (91) has also demonstrated that GTG obese mice do not become pregnant and have an augmented susceptibility to tumor production.

Wagner and DeGroot (116) observed variable amounts (unrelated to dosage) of degranulation within the beta cells of the pancreatic islets, and gastric mucosal damage. No testicular damage was observed. Brookhart and Dey (34) reported that certain hypothalamic lesions in male

guinea pigs lead to reduction of sexual behavior without producing effects on spermatogenesis, seminiferous tubule and vesicle function, or gonadotropic function. It would seem possible that the extensive lesions from GTG could produce hypothalamic damage of the type necessary for the cessation of sexual behavior. This may be related to the conflicting reports as to whether or not GTG obese mice will reproduce.

Goodchild and Moore (61) report that even though GTG obese mice have small intestines one third longer than normal mice and that isolated intestinal parasites (Hymenolepsis diminuta - a tapeworm) would be expected to be larger in such cases, instead stunted and morphologically altered parasites are found in these mice. They suggest that GTG is slowly eliminated into the intestine via the bile, thus altering the biliary role in supporting tapeworm welfare. However, Mayer's data (39)(40)(86) suggests that blood glucose, blood lipids, hepatic lipogenesis, adipose tissue metabolism, fat mobilization, intestinal absorption, fasting levels of blood ketones, and retention of steroid hormones by GTG obese mice are all normal or altered in the same direction as in obese humans. Friedman and Tepperman (56) reported, as mentioned earlier, that the only organ in which gold could be detected at one, two or three months after injection was the liver. Perhaps the very presence of GTG in tissues produces many acute reversible pathologic lesions which are not apparent with the disappearance of GTG with the passage of time after injection.

It would seem that most of the results of the studies to date, utilizing GTG-produced obesity, appear to be consistent with Mayer's

glucostatic theory. Mayer (86) suggests that the satiety mechanisms possess a dual role, i.e., an inhibitory effect on the constantly activated lateral hypothalamic area, and a controlling function over gastric hunger contractions. The glucostatic component, however, is the primary mechanism whereby the metabolic state of the organisms can exercise a regulating role in the overall regulation of feeding homeostasis. In man, there are in addition to these physiological regulators, a number of conscious and subconscious factors which become integrated with or modify the effects of the entire regulatory process determining feeding behavior. Table 2 summarizes the studies on localisation and effects of GTG.

Intrahypothalamic Drug Injections

Grossman (65)(66) has developed a double cannula system which allows repeated application of crystalline chemicals to central structures. He has implanted the cannula into the lateral hypothalamic nuclei of rats by means of a stereotaxic instrument and administered crystalline drugs (one to five micrograms) into this region. He observed that epinephrine and norepinephrine, when placed into the lateral hypothalamus, induced "vigorous and prolonged eating in satiated animals." He has referred to this behavior as the result of adrenergic stimulation. Cholinergic drugs (acetylcholine plus physostigmine, and carbachol) caused a decreased food intake of normally hungry animals. Further tests (67) with autonomic blocking drugs suggested that the feeding center possesses neural elements sensitive to adrenergic stimulation,

TABLE 2

SELECTED ACTIONS OF GOLD THIOGLUCOSE

1.	Liver	a) centrolobular fatty infiltration (29)(118)
		b) only organ with detectable gold at one, two, and three months after injection (72)
2.	Adipose Tissue	increase quantities (29)(91)(118)
3.	Kidneys	gold localized therein (56)(116)
4.	Hypothalamus	a) ventromedial damage (91)
		b) gold localized in ventromedial area (44)
		c) capable of altering its sensitivity to GTG (52)
5.	Brain (extrahypothalamic)	a) extensive cellular or nuclear damage on acute observation (91)
		b) no damage observed at three months after injection (91)
		c) localization of extrahypothalamic lesions (99)
6.	Stomach	a) gastric ulcers in animals with ventromedial lesions (46)
		b) gastric mucosa damage (116)
7.	Reproduction	animals fail to become pregnant (91)
8.	Tumors	increased susceptibility (91)
9.	Pancreas	beta cell degranulation (116)

and that the results of the intrahypothalamic injections were not due to side actions of the drugs. Grossman has proposed that feeding behavior is an adrenergic action and that drinking behavior is a cholinergic mechanism with controls very similar to and anatomically adjacent to the feeding mechanisms.

Stein and Seifter (110) confirmed these results and have suggested that the cholinergic drinking mechanism is muscarinic and not nicotinic. This conclusion was based on evidence that implanted muscarine (an alkaloid from mushrooms) would produce an effect similar to that of carbachol (a parasympathomimetic) while nicotine (a ganglionic blocker) was no more effective than sodium chloride. Wagner and DeGroot (115) have studied the effects of intra-hypothalamic drugs (solutions) injected into the ventromedial and lateral hypothalamus, the lateral amygdaloid complex, and the globus pallidus. These authors state that diffusion of soluble drugs can be controlled and that these drugs, in contrast to crystalline agents, do not produce cellular brain damage at the site of deposition. Selected results of Wagner and DeGroot are summarized in Table 3.

Wagner and DeGroot's results are consistent with those of Grossman. Together, they suggest that adrenergic agents may affect feeding behavior through actions on both the ventromedial and lateral mechanisms. Such an assumption suggests that adrenergic drugs reach these hypothalamic areas following oral or systemic administration. However, no direct evidence to substantiate this assumption is presently available.

TABLE 3

EFFECTS OF INTRAHYPOTHALAMIC DRUG INJECTIONS

(Based on results of Wagner and DeGroot)

TABLE 3

Cannulated Area	State of Animal	Epinephrine	Norepinephrine
Ventromedial Nuclei	Satiated	Increase feeding	———
Ventromedial Nuclei	Fasted	Increase feeding	Decrease feeding
Lateral Hypothalamus	Satiated	Increase feeding	———
Lateral Hypothalamus	Fasted	———	Increase feeding
Lateral Amygdala	Either	No effect	No effect
Globus pallidus	Fasted	Increase feeding	Increase feeding

Amphetamines and the Hypothalamic Feeding Mechanism

Brobeck et al (32) have observed an increase in frequency and amplitude of electrical activity from the satiety mechanism following injections of amphetamine. These changes, lasting two hours in anesthetized cats, were not accompanied by any response from the lateral hypothalamus. Emphasizing that this type of experiment is not sufficient to establish the site of action of a drug, the authors suggest that amphetamine directly or indirectly excites the ventromedial satiety mechanism. Anand et al (14) administered phenmetrazine (an amphetamine congener) intravenously to unanesthetized cats and monkeys with electrodes in the medial, lateral, and "other" areas of the hypothalamus. They observed an increase in frequency, but a fall in amplitude, of the electrical activity of the satiety mechanisms. However, no change was observed in other hypothalamic and cortical regions tested. Sharp et al (107) produced electrolytic lesions of the satiety center in four cats and administered amphetamine to these animals once every twelve hours. The hyperphagic animals, allowed unlimited access to food, were found to be "relatively refractory" to this schedule of amphetamine administration. These authors suggest that their results confirm the hypothesis of Brobeck, Larsson, and Reyes (32) that amphetamine acts on the ventromedial area. However, the latter workers pointed out that the electrical effects only lasted two hours. It would seem probable, then, that the hyperphagic animals would eat rapidly to compensate for the short-lived drug-induced hypophagia.

It has been shown that the anorexigenic effect of amphetamine persists after vagotomy and splanchnolumbar sympathetectomy, thus suggesting that the action of amphetamine is not due to any effect on gastrointestinal sensations. Andersson and Larsson (17) observed that the anorexigenic effect of amphetamine is markedly reduced by prefrontal lobotomy in the dog, indicating that its action is an indirect one mediated by the prefrontal and perhaps other association areas of the cerebral cortex.

Brodie (33) has shown that the sympathetic nervous system is essential to supply fuel for energy processes such as the breakdown of adipose tissue to free fatty acid and of glycogen to glucose. As a sympathomimetic agent, it is possible that at least a part of the action of amphetamine in reducing obesity could be through its stimulation of energy expenditure (as manifested in the increased activity of the animals). Brodie states that:

> The sympathetic nervous system increases the output of FFA energy substrates through the rapid activation of adipose tissue lipase and liver phosphorylase. These enzymes can be activated by catecholamines, a ganglionic stimulant, and by cold exposure. Chemical sympathectomy prevents activation of these enzymes by cold exposures and ganglionic stimulation..... The organism has an absolute requirement for the sympathetic system in order to mobilize energy substrates required to express behavior.

To what extent the action of amphetamine can be attributed to the above mechanisms remains to be established.

One might assume, at this point, that the ideal agent for the reduction or prophylaxis of obesity would possess specific lateral hypothalamic depressing or medial excitatory effects without producing

any effects elsewhere except in areas that enhance the total response. In pharmacological terms, the ideal drug would possess properties which allow it to react specifically and reversibly with hypothalamic and selected peripheral receptors (such as those responsible for mobilization of stored fat), the other sites of drug contact functioning as "acceptors" only. (The term "acceptor" as used here implies any site of drug-cell interaction producing no demonstrable pharmacological action). As more knowledge is gained concerning the physical-chemical nature for these central areas, the possibility of being able to synthesize such a drug may become more of a probability.

Resume

An attempt has been made to present pertinent studies leading to the present state of understanding about the mechanisms controlling food intake (and thereby obesity). It has been suggested that GTG may be a useful tool for the evaluation of drugs to be used in the treatment or prophylaxis of obesity. Studies designed to evaluate such an experimental model as regards its resemblance to clinical obesity and its response to drugs appear to be an essential step for the establishment of the technique. The report and discussion of such investigations will occupy the remainder of this thesis.

METHODS AND MATERIALS

General Procedures

The most important limiting factor in GTG research is the number of obese animals that can be produced during the experimental period. Large numbers of mice are required in order to produce an adequate quantity of obese mice. For these experiments, twenty pregnant Swiss mice were procured and placed in separate plastic cages with ad lib food and water. Their offsprings were allowed to mate freely. Then, six weeks after birth of the second generation, each mouse was transferred to an individual metal cage of dimensions of 20 by 20 by 12 centimeters which had heavy screen on the bottom to allow for removal of waste materials to a tray below the cage. Calibrated four-ounce water bottles were used for measurement of water consumption. Preweighed standard Purina laboratory chow pellets were placed in the cages for varying periods of time (depending on the individual experiment), and then re-weighed with the collected "escaped" food droppings (exclusive of feces) to allow for measurement of total food consumption. The animals were kept in a semi-controlled environment, i.e., room temperature varied between 23° and 27° C and was maintained during the summer months at this level by means of an air conditioning unit. The room was lighted between 7 a.m. and 6 p.m. daily throughout the seventeen months of the experiment.

Since most of the experiments involved repeated drug administrations of small dosages, rigid control procedures for injections were employed. All syringes were soaked in both Hemosol[R] and sodium dichromate-sulfuric acid cleaning solutions prior to washing and autoclaving. Freshly sharpened small (27 gauge 3/8 inch) stainless needles were used to minimize trauma or excitation from the injection procedure. Drug solutions were prepared by weighing the powdered or crystalline substances on an analytical balance and dissolving them in normal sterile saline (NSS) solution. However, no attempt was made to prepare the site of injection, although no single syringe was used on more than one animal. Essentially, then, near-aseptic technique was employed for all chronic procedures.

A total of 528 mice were used, 385 of which were injected with varying dosages of GTG and 143 were kept as uninjected controls. For most of the experiments each animal served as its own control. Following injection, regular records of total body weight were taken.

Fat-Free Body Mass Methods

To select drug dosages and, as a reference standard for all the metabolic experiments, it was necessary to determine first the fat-free body mass. Because of the large number of animals to be used, it appeared desirable to establish a rapid means for determining this measure.

Twelve mice varying in weight from 20 to 46 grams were sacrificed and individually shaved (to avoid trapped air), weighed in air, and weighed under water after attempting to remove residual air. When a constant submerged weight (no change in weight upon repeated treatment) was reached, the total body specific gravity was determined by the formula:

$$\text{Sp. gr.} = \frac{Aw}{Aw - Sw}$$

where Sp. gr. = specific gravity

Aw = weight in air after shaving

Sw = weight submerged in water

Each animal was then placed in a syrup of dry ice and ethanol and quickly frozen. In the frozen state the animal was homogenized on a porcelain pill-tile utilizing a sharp cleaver and rubber-policeman. The entire preparation was transferred to a 250 ml pyrex ball flask and partially dried under reduced pressure in a Rinco rotary evaporator. No attempt was made to remove all water by this procedure. The contents of the flask were wrapped in a small surgical gauze bag and placed in an extraction thimble. This was then placed in a Soxhlet apparatus and

extracted with low boiling point (30 - 60°C) petroleum ether (ligroine). A water bath under the boiling flask was maintained at 57°C and the siphoning cycle varied between four and six minutes in all determinations. Extraction was continued for eight hours at which time the flask was again placed on the rotary evaporator for two hours to insure removal of water and petroleum ether. The pre-weighed flask was again weighed to determine the amount of fat extracted. It was then refilled and the extraction procedure repeated for four more hours until all weight readings agreed within 1.0 per cent. This usually occurred on the first two readings, but on one occasion required one extra extraction.

The contents of the extraction thimble were dried overnight and then placed in another flask and further dried under reduced pressure to constant weight. The residual material always consisted of a brittle dry mass equivalent to fat-free body mass. No attempt had been made to empty the intestinal tracts of these animals prior to extraction.

Feeding Behavior Equipment

In order to investigate the feeding behavior and activity of mice with and without long term drug administration, special cages were designed. Each cage consisted of a sheet metal box 20½ cm high, 20½ cm wide, and 25½ cm long with a floor of heavy screen. An individual cage was supported by four metal legs varying in length so that when placed side by side each successive cage would be about 3½ cm higher than the previous one. One end of the cage was attached to a plywood wall, 2½ cm thick, which acted as a support for the food cups, water supply, and "T" bar. A hole was drilled through the wood and through the sheet metal face at a height of 12 centimeters above the floor of the cage which allowed for placement of the food cups. To reach the food, the animal must climb onto a "T" bar lever, the external extension of which allowed recording of bar pressing on a smoke-drum kymograph. A special recording lever assembly prevented sway and allowed adjustment of excursion from the "T" bar. The water supply was so placed as to allow for free access at all times. The food cups consisted of seven dram prescription vials cut to such a length as to allow the animal to freely approach the food. Two Purina lab chow pellets were placed in each cup, a hole drilled through the base of the cup and pellets, and the food was held in place by a wire paper clip through the entire assembly which allowed for bracing of the food cup in the feeding assembly. The cup was replaced by an empty, otherwise identical, vial at the end of each feeding period.

To test locomotor activity, a support plate supplied with five series connected silicon solar-cells mounted in plastic cases (output 0.3 to

0.45 volts and 10 - 16 milliamperes) was attached to the underside of the cage and connected directly to the D.C. driver amplifier of a Grass Model 5A Polygraph. Whenever the animal was positioned over a cell, a light beam would be interrupted resulting in a marking deviating from the baseline. Four such plates were used in these experiments.

Sixteen feeding cages were made and placed side by side in two groups of eight each. Each setup was connected to a long smoke-drum kymograph and two cages in each group connected to the polygraphs. Photographs of the cages and of the entire recording mechanism are presented in Figures 4 and 5.

Metabolism Experiments

In these experiments, oxygen consumption and carbon dioxide production were determined independently.

For the investigations requiring measurement of oxygen consumption, a modified Haldi spirometer was used. Haldi et al (69) designed an apparatus consisting of a battery of six spirometers each in communication with a chamber housing a single animal. Each spirometer is suspended in water in a housing made of plastic tubing 12 cm in diameter and attached to a counterweight by a string over a graduated ball-bearing pulley. The counterweight bucket (containing birdshot) rides in a plastic shaft 2.5 cm in diameter. A fine wire pointer attached to the top of the spirometer allows recording of descent. A hole in the center of the bottom of each plastic housing for the spirometers is fitted with an L-shaped tube, the upper arm of which is attached by a rubber tube to a manifold. The lower arm communicates via a plastic tube with the animal chamber. Haldi connected the inlet tube directly to a tank

Figure 4

TYPICAL FEEDING CAGE

Figure 5

FEEDING CAGES, PHOTO-CELL PLATES

AND RECORDING MECHANISMS IN USE

Figure 4

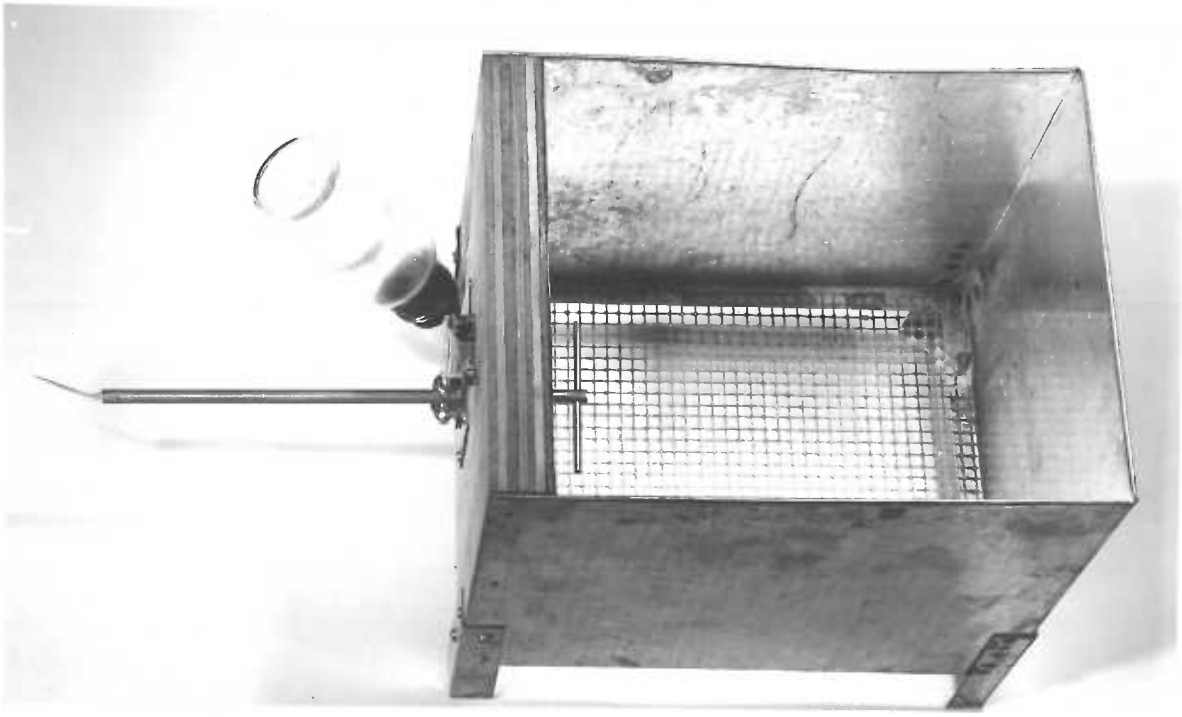
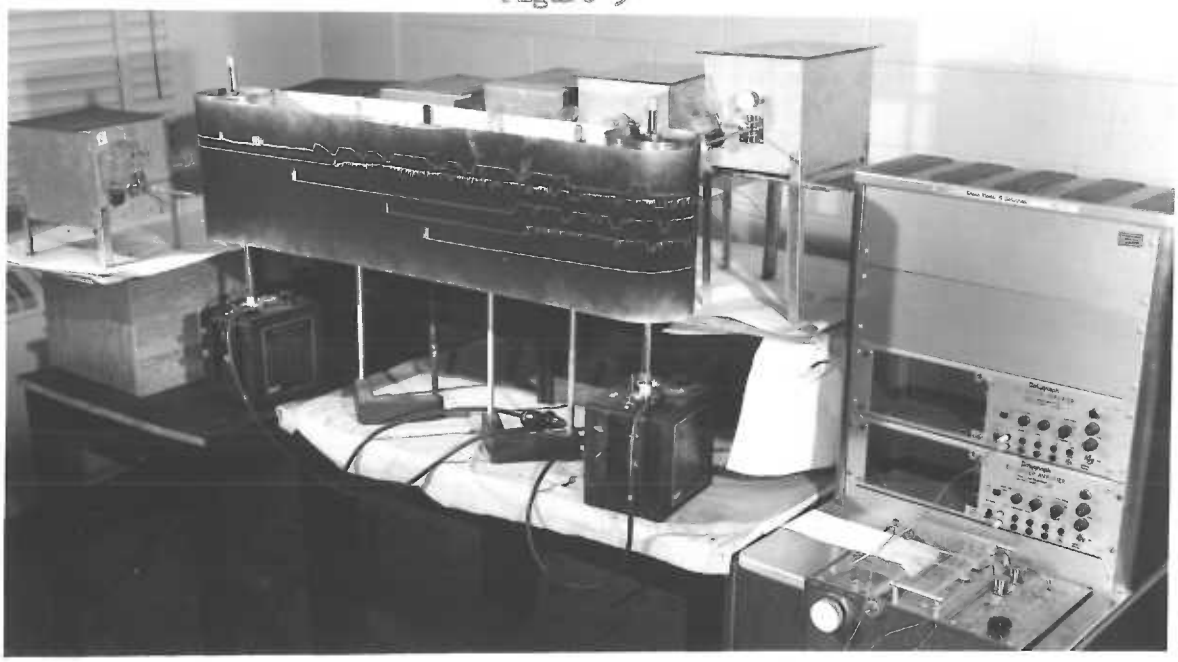


Figure 5



of oxygen. In these experiments the oxygen was supplied to the apparatus by first being passed through a Foregger pediatric anesthesia machine. This allowed fine adjustment of flow rates and direction of gas flow since the machine is fitted with a unidirectional diaphragm flow valve. Oxygen passes through a chamber of soda lime en route to the spirometers. A small wire mesh bucket of soda lime is inserted in the inner tube of the spirometer and a wire screen is filled with soda lime and placed under the animal in the chamber. Each spirometer was calibrated by withdrawing air from the chamber with a graduated syringe.

Since all determinations involved the mouse being restricted to the chamber for fifteen minutes in a large mesh restraining cage, it was felt necessary to see how temperature and carbon dioxide concentration would vary during the procedure.

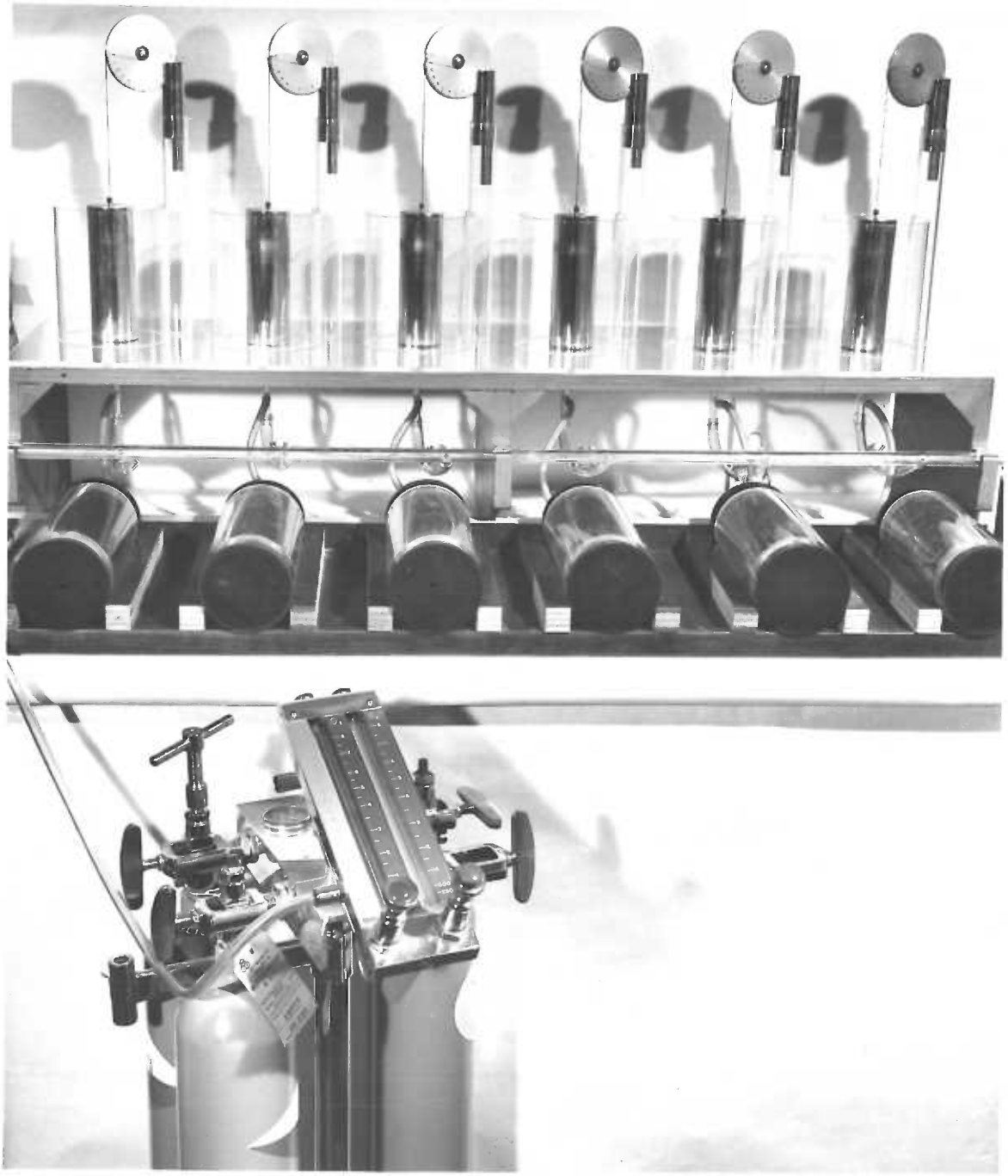
Samples of air removed from the chamber after fifteen minute determinations did not show detectable differences from room air as regards carbon dioxide concentration. (These determinations were based on titrations of 50 ml air samples mixed with 1/N sodium hydroxide titrated against hydrochloric acid to phenolphthalein and methyl orange end points). However, it was observed that if animals were left in the chambers longer than 22 minutes, an increase in excitability could be observed. Samples of air indicated a carbon dioxide concentration of 2.3 volumes per cent. Therefore, all observations were limited to fifteen minute periods.

Temperature in the chambers was found to rise from 26.5 $\pm$ 0.5° to 28 $\pm$ 0.5° during the tests. Since all determinations were performed

Figure 6

MODIFIED HALDI SPIROMETER

Figure 6



under the same conditions and since this variation was constant, no attempt was made to maintain a controlled temperature by such means as submersion in constant temperature water baths. These conclusions are in agreement with those of Haldi.

A picture of the apparatus (constructed by the Research Instrument Service of the University of Oregon Medical School, according to the specifications of Dr. A. J. Rampone, Associate Professor of Physiology) is shown along with the Foregger Anesthesia machine in Figure 6.

For the determination of carbon dioxide production a titrametric technique was used. Mice were placed in clear plastic cylinders sealed on each end by rubber stoppers fitted with small plastic funnels. Carbon dioxide free oxygen was supplied to the chamber and removed by an aspirator connected in series with a sintered glass bubbling tube placed in a container of known strength sodium hydroxide solution. After titration against standard hydrochloric acid to phenolphthalein and methyl orange end points, the carbon dioxide production was calculated.

RESULTS

Preliminary Study of the Incidence of Gold Thioglucose Obesity

Forty-two animals were used in a preliminary experiment designed to show the food and water consumption characteristics of mice before and after injection of GTG. Three weeks were allowed for the control period during which time the weekly averages for each of these values was determined. Three groups of ten animals each were then injected with 15, 20, and 25 mg of GTG in sesame oil, respectively. The data for these 30 animals prior to the GTG injection is shown in Table 4. Only eight of the original 30 mice were still alive three days after injection. After allowing three weeks for total recovery from the effects of the injection, the food and water intake and body weight was followed for a few weeks thereafter. The data for these animals are shown in Tables 5 and 6. One mouse (number 10) survived the injection but ate and drank only sparingly and died after four weeks. Four of the seven survivors showed marked hyperphagia and became obese. Their weekly food consumption almost doubled the control values. Water intake also increased, but not in proportion to the increase in food intake. Only one mouse (number 24) survived the 25 mg dosage. It showed a weight gain from 23 to 73 grams within fourteen weeks of the injection. It should be noticed that hyperphagia was evident in those animals destined to become obese by the first recording period after injection (week six).

These results cannot be regarded as representative of the incidence of obesity at varying doses of GTG. Succeeding groups, injected with the same dosages of GTG, showed wide variations in the incidence of

TABLE 4

RESULTS OF PRELIMINARY STUDY OF FOOD
AND WATER CONSUMPTION IN MICE
PRIOR TO INJECTION OF GOLD THIOGLUCOSE

TABLE 4

Animal No.	Food Intake (Gm)			Water Intake (cc)			Body Weight (Gm)			Dose GTG (mg)
	Wk 1	Wk 2	Wk 3	Wk 1	Wk 2	Wk 3	Wk 1	Wk 2	Wk 3	
1	25.8	24.0	23.5	45	43	34	20	21	21	15
2	23.8	25.5	24.0	40	43	40	23	21	21	15*
3	20.0	30.0	29.5	52	56	40	22	23	24	15
4	21.8	28.0	31.0	39	47	40	23	24	25	15
5	29.9	30.5	22.6	48	46	42	24	24	26	15
6	35.6	29.5	29.5	40	39	43	26	26	27	15*
7	27.2	30.0	30.2	43	44	42	22	25	25	15
8	23.0	25.0	15.0	40	42	44	22	21	22	15*
9	27.4	29.5	32.0	44	45	40	23	23	25	15
10	24.1	27.5	31.0	43	45	41	22	22	22	15*
11	30.3	31.5	33.0	43	62	42	27	28	28	20
12	23.1	23.5	27.0	28	33	34	22	22	23	20
13	22.8	25.5	27.5	42	48	42	21	21	22	20*
14	27.5	30.0	28.5	50	50	48	27	25	26	20
15	34.8	39.0	31.4	63	93	56	25	25	26	20*
16	23.1	40.0	35.5	38	58	43	26	22	22	20*
17	23.2	28.0	35.0	59	39	42	24	22	21	20
18	19.6	23.5	32.0	48	59	45	26	22	24	20
19	24.8	32.0	35.2	37	43	35	26	23	25	20
20	35.0	30.0	29.5	48	64	38	27	23	23	20
21	25.6	24.5	26.2	32	65	37	27	25	24	25
22	26.0	26.5	30.0	60	55	47	28	22	24	25
23	26.0	30.0	32.0	38	56	38	24	23	25	25
24	24.3	29.5	32.0	48	37	52	23	22	23	25*
25	18.0	28.5	31.5	37	80	46	24	19	22	25
26	20.8	29.5	30.5	36	50	40	26	24	23	25
27	24.7	30.5	29.0	39	70	15	26	26	25	25
28	25.7	22.5	20.0	21	17	14	20	20	21	25
29	33.2	35.5	33.8	51	49	49	26	28	28	25
30	17.1	29.0	26.5	25	53	52	21	21	20	25
Mean	25.47	28.95	29.15	42.6	51.0	40.7	24.1	23.1	23.8	

* Survived the acute effects of GTG (all others died within three days).

TABLE 5

FOOD AND WATER INTAKE AND BODY WEIGHT MEASUREMENTS
FOR MICE SURVIVING THE ACUTE EFFECTS OF
GOLD THIOGLUCOSE

TABLE 6

FOOD AND WATER INTAKE AND BODY WEIGHT MEASUREMENTS
FOR SURVIVING MICE
THAT DEVELOPED HYPERPHAGIA AND OBESITY

TABLE 5

Animal No.	Food Intake			Water Intake			Body Weight			
	Wk 6	Wk 7	Wk 8	Wk 6	Wk 7	Wk 8	Wk 6	Wk 7	Wk 8	Wk 17
2	38.0	42.0	54.0	64	50	44	28	34	29	56
6	43.0	50.1	45.1	55	60	60	34	38	38	58
8	38.0	39.0	38.0	34	48	54	30	34	38	50
10	15.5	6.0*		18	8*		15	11*		
13	34.1	41.0	63.1	40	43	37	21	28	37	36
15	21.0	48.8	59.3	46	60	36	30	34	39	40
16	28.0	42.8	63.0	25	50	52	22	30	35	30
24	64.0	56.0	61.0	58	60	73	37	40	47	73
Mean	33.0	45.7	56.2	43.9	54.4	50.9	27.1	34.0	38.7	49.0

*Animal died during following week.

TABLE 6

Animal No.	Mean Food Prior (Gm)	Food Intake Week 8	Mean Water Prior (ml)	Water Mean Week 8	Mean Wt. Prior (Gm)	Weight Week 8
2	24.4	54.0	41.0	44.0	21.7	56
6	31.5	45.1	40.7	60.0	26.3	58
8	21.0	48.0	42.0	54.0	21.7	50
24	28.6	61.0	49.0	73.0	22.7	73
Mean	26.4	52.0	43.2	57.8	23.1	59.3

obesity and in the occurrence of acute toxicity. In all experiments animals whose total body weight was in excess of 45 Gm were designated as obese. A major difficulty incurred with this technique, then, is the inability to predict the incidence of obesity.

Fat-Free Body Mass Determinations

In order to determine the total body fat content of mice for calculations of the lean body mass (fat-free body mass), chemical extraction procedures are usually required. However, by utilizing the methods described herein for comparing the total body specific gravity to the chemically extracted fat values of the same animals, it was observed that these two measures are linearly ($r = 0.99$) related. The results obtained from such studies on nine GTG injected and three control mice are presented in Table 7, and the relationship of per cent body fat to total body specific gravity is shown in Figure 7. Table 7 further shows that there is no direct relationship between total body weight and the per cent body fat. There is some suggestion here that the fat-free body weight of obese mice may be greater than that of non-obese. Included in this table for purposes of comparison are the actual extracted and calculated per cent fat values from these 12 mice and the values obtained by extending and utilizing for mice the guinea pig and human formulas calculated by Rathbun and Pace (102). Their formulas were based on similar observations. It can be seen from the table that extension of these formulas for use in mice would result in high estimates of body fat content.

A regression formula was calculated to best describe the data shown in Figure 7. By simply determining the total body specific gravity, one can calculate from the revised regression formula the per cent body fat and, therefrom, the fat-free body mass. This formula was used in the

TABLE 7

DATA FROM SPECIFIC GRAVITY AND TOTAL BODY FAT DETERMINATIONS ON NINE GOLD THIOGLUCOSE INJECTED AND THREE SIMILARLY STUDIED CONTROL MICE

TABLE 7

Sex	Dose GTG (mg)	Wt. in air (Gm)	Wt. in Water (Gm)	Sp.Gr.	Dry Wt. (Gm)	Solids Wt. (Gm)	Fats Wt. (Gm.)	% Fat Based on Dry Wt.	% Fat by House Formula	% Fat by Guinea Pig Formula	% Fat by Human Formula	Fat Free Body Mass (Gm)
F	0	30.10	1.30	1.045	15.014	10.080	4.934	32.26	16.39	25.10	26.50	25.17
F	0	33.65	1.15	1.035	16.301	8.949	7.352	45.10	21.85	30.10	31.60	26.30
F	0	23.20	1.20	1.055	9.791	8.211	2.580	26.35	11.12	20.20	21.50	20.62
F	10	26.60	1.00	1.040	10.950	6.577	4.373	39.95	17.18	27.60	29.06	22.03
M	10	45.75	0.75	1.017	22.381	9.113	13.238	59.28	29.03	39.24	41.13	32.45
M	10	36.80	0.95	1.026	21.626	11.846	9.780	45.22	26.59	34.60	36.30	27.02
F	10	20.55	0.90	1.046	11.184	8.340	2.844	25.44	13.84	24.60	26.00	17.71
F	10	30.90	1.35	1.046	13.214	9.194	4.020	30.42	13.01	24.60	26.00	26.88
M	10	35.00	0.95	1.028	16.817	7.941	8.876	52.78	25.36	33.60	35.30	26.12
M	10	39.70	1.05	1.027	20.795	10.193	10.602	50.98	26.71	34.10	35.80	29.10
M	10	46.20	0.40	1.009	27.405	11.100	16.305	59.50	35.29	43.40	45.50	29.90
F	10	33.60	1.12	1.034	16.243	9.006	7.237	44.55	21.54	30.60	32.20	26.36

Per cent Fat Formulas: 1. House Formula derived herein.
2. Guinea Pig and Human Formulas derived by Rathbun and Pace (102).

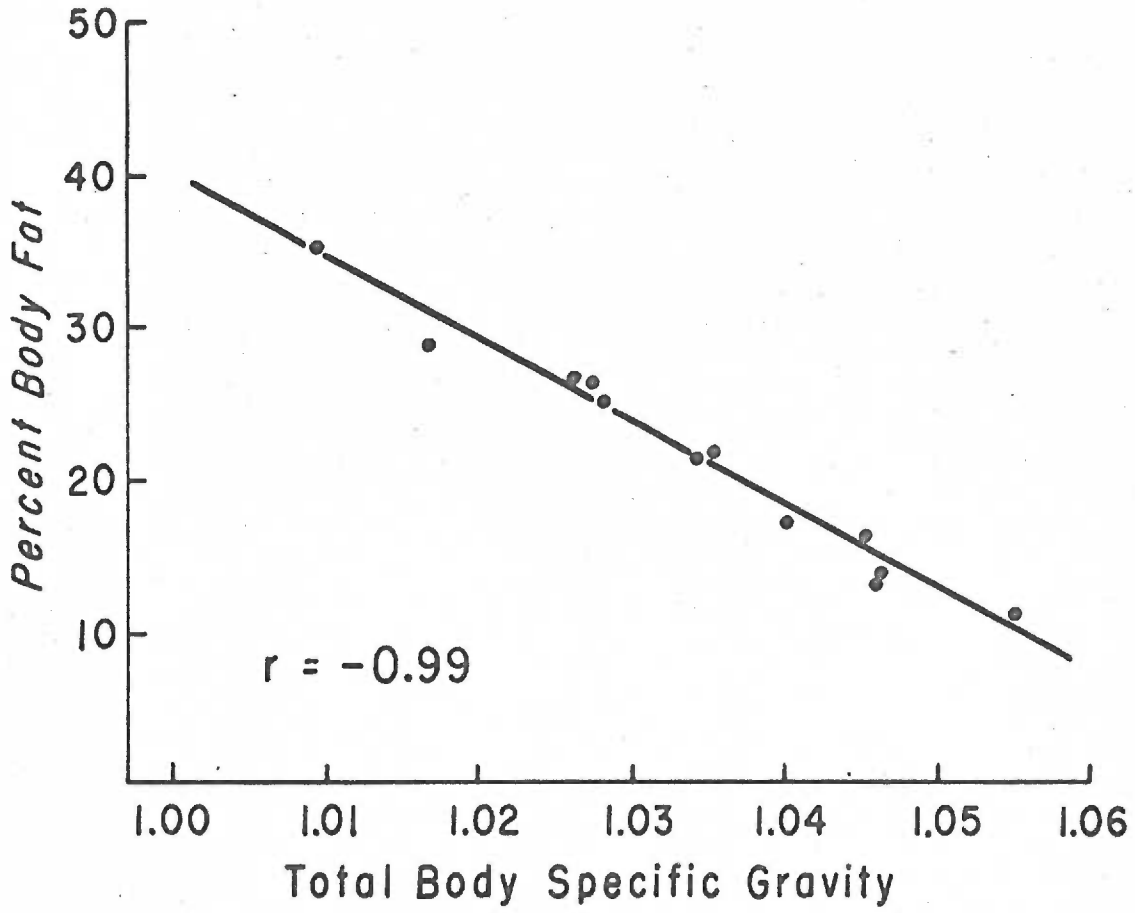
Figure 7

PLOT OF DATA FROM TABLE 7 SHOWING

THE RELATIONSHIP OF TOTAL BODY SPECIFIC GRAVITY

TO PER CENT BODY FAT

Figure 7



% BODY FAT = 588.4 - 548.3 (Sp. Gr.)

metabolic experiments which follow as an indirect means of estimating the fat-free body weight of mice:

$$\text{Per cent Fat} = 588.4 - 548.3\ (\text{Sp. Gr.})$$

Basal Metabolism Studies

Information about the basal metabolism of GTG obese mice is a prerequisite to the understanding of the dynamics and mechanisms concerned in this pathologic alteration. Such information is also essential for studies on the effects of pharmacotherapy of obesity.

Utilizing the spirometer described in the "Methods and Materials" section of this thesis, the objective of this portion of the study has been to observe the oxygen consumption of GTG obese animals as a function of activity, body weight, fat-free body mass, and restricted food intake. A total of 1450 fifteen-minute readings on obese and normal mice have been performed in these studies.

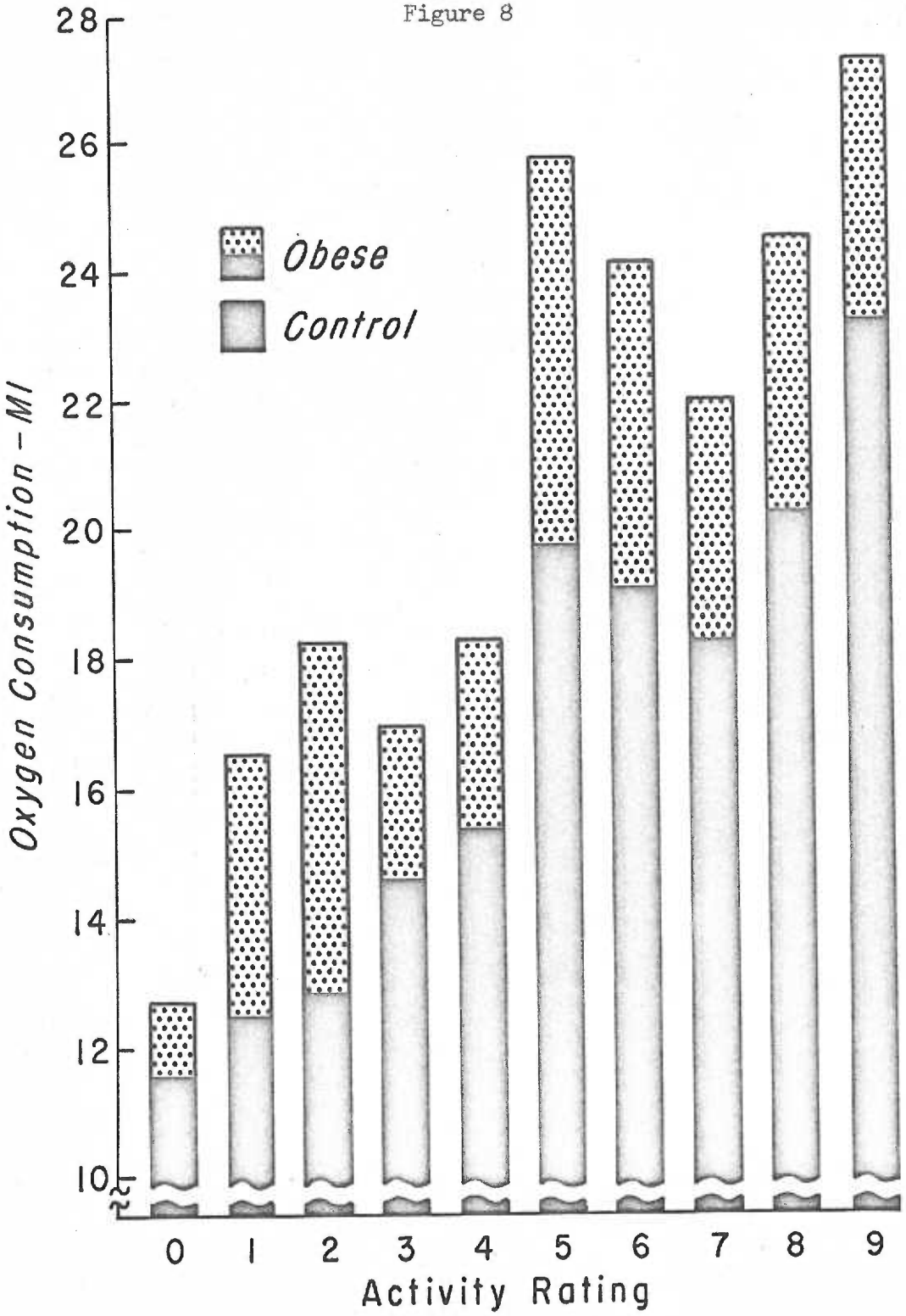
The first experiment was designed to determine the basal oxygen consumption of obese and normal mice and to study how activity influenced these values. After the mice were placed in their individual restraining cages and allowed to become accustomed to the chamber, six successive fifteen-minute readings were taken. Activity for each animal was adjudged on a zero to 4+ judgment scale at 3, 7, 11 and 14 minutes of each experimental period. These scores were totaled and used as a measure of the overall activity and plotted against oxygen consumption at each activity level. Figure 8 represents a plot of the mean values from 311 determinations. Fifty-seven and 59 basal (zero activity) measures were obtained for 22 obese and 24 control animals, respectively (mice were not fasted prior to testing). After all studies were

Figure 8

EFFECT OF INCREASING LEVELS OF ACTIVITY ON OXYGEN CONSUMPTION IN OBESE AND CONTROL MICE

Oxygen consumption represents the average volume in milliliters consumed during each 15-minute test period.

Figure 8


28
26
24
22
20
18
16
14
12
10
Oxygen Consumption - Ml
Obese
Control
0
1
2
3
4
5
6
7
8
9
Activity Rating

completed the mice were sacrificed and total body specific gravity and fat-free body weight determined. No attempt was made to determine body surface area. All values of oxygen consumed were converted to standard temperature and pressure and calculated as a function of total body weight, per gram body weight, and per gram fat-free body weight. These values and the results of group statistical comparisons are shown in Table 8.

The obese animals demonstrated a total oxygen consumption significantly greater than that of the controls. However, when these values are referred to per gram total body weight, the controls consumed more oxygen than the obese mice ($p = 0.0001$). When referred to the fat-free body weight there no longer existed any statistical difference between the two groups. In other words, the oxygen consumption of the obese and the normal mice is essentially identical (on a unit weight basis) if the body fat content is ignored.

To test the hypothesis that this difference in oxygen consumption is indeed due to differences in fat content and not due to drug idiosyncrasy, measurements of the oxygen consumption of GTG injected mice six days after injection (sufficient time to recover from drug action but prior to development of obesity), and studies on the effects of restricted food intake and resultant weight (fat) loss on oxygen consumption were performed.

In the first experiment, twenty basal readings on GTG injected mice six days after injection showed a mean value of 10.64 ± 3.20 ml. This value is even lower than that for the control mice of the previous experiment which was 11.54 ± 2.88 ml. The injection of GTG did not cause

TABLE 8

COMPARATIVE BASAL OXYGEN CONSUMPTION OF OBESE AND CONTROL MICE AS A FUNCTION OF TOTAL BODY WEIGHT, PER GRAM OF BODY WEIGHT, AND PER GRAM OF FAT-FREE BODY WEIGHT

TABLE 8

Group	Total O_2 Consumption Per Mouse (ml) *	O_2 Consumed Per Gram Body Weight **	O_2 Consumed Per Gram of Fat-Free Body Weight ***	Fat-Free Body Weight ****	Number of Detn's.
Obese	12.76 ± 1.45	0.301 ± 0.063	0.452 ± 0.100	28.30 ± 1.76	57
Control	11.54 ± 2.88	0.406 ± 0.110	0.481 ± 0.125	24.16 ± 2.34	59

Values are Means ± Standard Deviations

* $p =< 0.01$

** $p =< 0.0001$

*** $p =< 0.49$ (not significant)

**** $p =< 0.0001$

"p" values derived from critical ratio test (large sample significance test)

an increase in oxygen consumption in these animals.

The second experiment (restricted food intake) was conducted as follows. Six obese mice were selected because of their highly elevated total oxygen consumption values (17.61 $\pm$ 5.22 ml). On two adjacent days a total of eighty-four readings (fourteen on each mouse) were taken in order to establish baseline values for each animal.

During the ensuing weeks, they were fed approximately one half of their normal daily quantity of food and repetition of the two-day readings occurred each week for six weeks. The results from these experiments are shown in Table 9.

One observes a decreasing oxygen consumption as the total body weight decreases. The oxygen consumption and activity continued to drop as is indicated by the ease of obtaining a large number of basal determinations during weeks five and six. However, at the end of the experiment, the animals were given free access to food for six weeks prior to determination of their lean body mass which was then found to be 31.34 $\pm$ 1.02 Gm. It should be observed that by the fifth week of the test period, the mean weight of the animals was less than the subsequent figure for the lean body mass. One animal died during the final reading of the last week. Its oxygen consumption for the final basal readings on this same day was 2.71 $\pm$ 1.18 ml.

It is interesting to note that in all these experiments the fat-free body weight of the obese animals is very significantly greater than that of the control animals (Table 8).

The determinations of carbon dioxide by the method previously described were inconclusive. Wide variations were observed not only for

TABLE 9

EFFECTS OF RESTRICTED FOOD INTAKE

AND RESULTANT WEIGHT LOSS ON THE OXYGEN CONSUMPTION

OF OBESE MICE

TABLE 9

Week	Mean Weight	Mean Total Body O_2 Consumption (ml)	Number of Basal Determinations
Control	46.17	17.61 ± 5.22	40
1	41.17	12.26 ± 3.48	45
2	41.58	16.06 ± 5.22	31
3	40.83	12.00 ± 3.10	34
4	35.83	9.48 ± 1.94	30
5	30.75	6.77 ± 2.13	54
6	27.45	6.97 ± 3.48	48*

* One animal died during the test

separate animals, but for the same animal. The inconsistency of the results prevented calculation of a dependable respiratory quotient.

Chronic Amphetamine Administration

Recent attempts to elucidate a hypothalamic mechanism of action for the amphetamine drugs have shown that animals with damaged or destroyed ventromedial nuclei will respond differently to short-term amphetamine administration than will normal animals (75)(107). Since clinical use of the amphetamines is generally over a long period of time, it was believed that a chronic study such as the one to be described here would contribute to an understanding of changes in feeding behavior as a function of the treatment of obesity with amphetamine.

Sixteen animals (eight obese, eight controls) were randomly selected from the total population of GTG treated and untreated mice and placed in individual feeding cages of the type described in the "Methods" section. All but one were female and all were full-grown (five months) adult litter-mate mice. The controls ranged in weights from 22 to 34 grams, and the obese from 46 to 73 grams. Individual records of food intake were made daily and the mean values recorded during the "acquaintance" period. The animals required training in order to climb on the bar to get food and to learn to consume their total daily diet within a period of seven hours. Figure 9 shows the mean daily food intake for each group of eight animals for the first 80 days of the experiment.

It can be seen that the obese animals required a longer period of time to establish a consistent food intake level which, however, never did reach the amount of food consumed by these animals in the 24-hour free-feeding situation. The control animals had stabilized at their

Figure 9

EIGHTY-DAY RECORDS OF MEAN DAILY FOOD INTAKE

FOR OBESE AND CONTROL MICE

Food intake is expressed in terms of the mean values of food consumed in grams during each daily seven-hour feeding period.

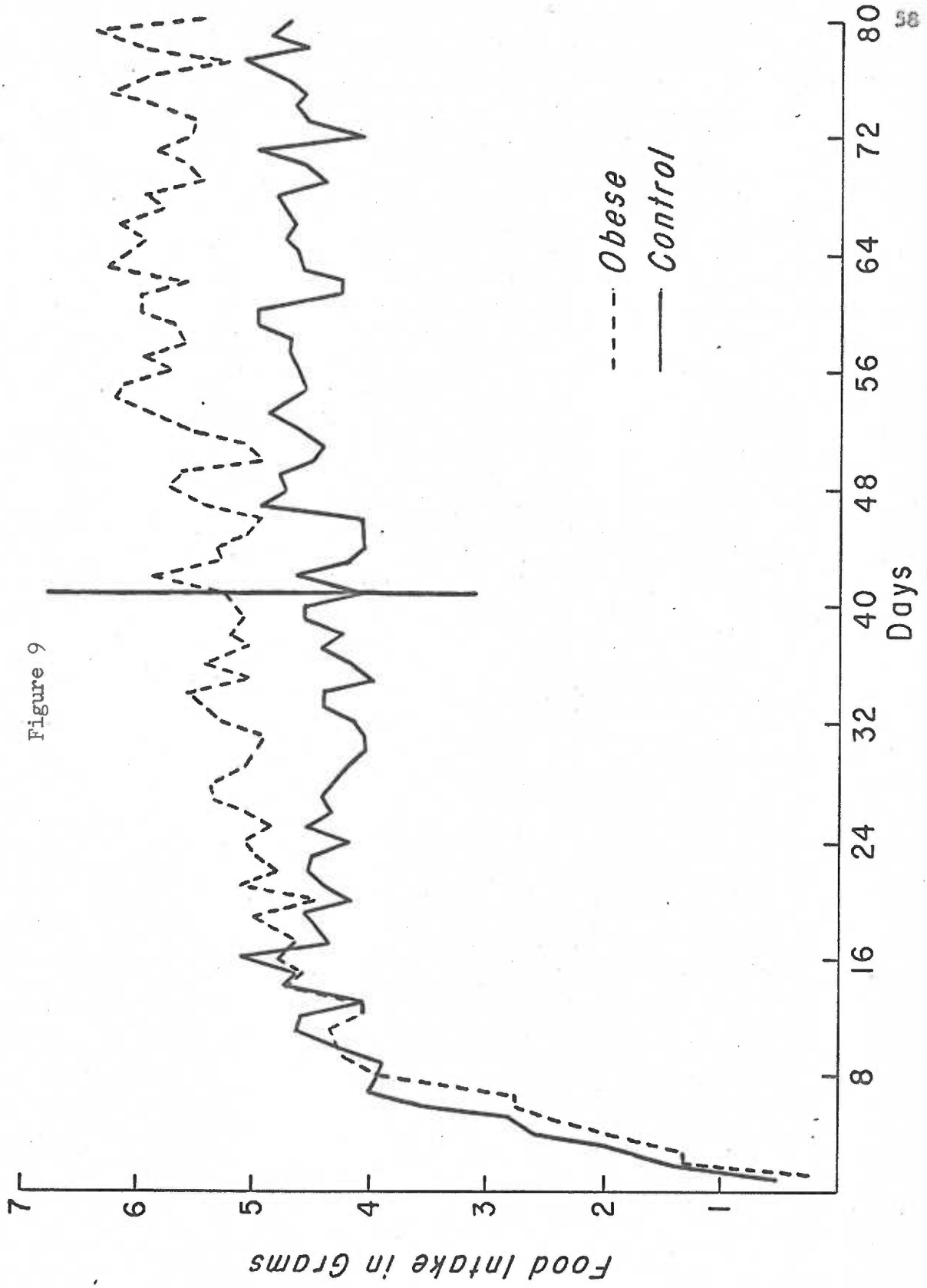

Food intake in Grams
7
6
5
4
3
2
1
8
16
24
32
40
48
56
64
72
80
Days
Obese
Control

Figure 9

24-hour free-feeding levels by the fourteenth day of the experiment. However, after the seventh day, the obese animals consistently consumed more food than the controls.

By the forty-first day, the feeding pattern appeared to be fairly well established. During the next 40 days (640 individual readings), the individual and group food intake values were tabulated and mean values and standard deviations calculated. This procedure allowed for the quantitative evaluation and expression of alterations in feeding behavior as being significant or not significant. Thus, any single daily value outside of the 95 per cent (± 2 standard deviations) confidence interval was designated as significant and any value outside of the 99 per cent (± 3 standard deviations) confidence interval was regarded as very significant. The meaning of such an expression will vary from animal to animal since the daily food intake varied widely in some and assumed narrow limits in other mice. The individual and group mean values and standard deviations for the first forty days of observations are given in Table 10.

In order to establish a quantitative method for the evaluation of locomotor activity in each group of eight mice, the solar-cell system described under "Methods" was used. During the forty-day control period, four animals (the first two in each group) were each given 13 intraperitoneal injections of normal sterile saline and were monitored for locomotor activity after each injection. The results were pooled and statistically analyzed. Each time an animal crossed a solar-cell, the beam would be broken resulting in a marking varying from the baseline. All such markings during any single 20-minute interval for a test period

TABLE 10

CALCULATIONS USED TO DEFINE

NORMAL AND SIGNIFICANTLY ALTERED LEVELS OF DAILY FOOD INTAKE

IN OBESE AND CONTROL MICE

Values expressed in grams.

TABLE 10

Animal	Mean Food Intake	SD	$\bar{X}$+2 SD*	$\bar{X}$-2 SD	$\bar{X}$+3 SD**	$\bar{X}$-3 SD	Description
1	4.35	0.38	5.11	3.59	5.49	3.21	Control
2	4.57	0.50	5.57	3.57	6.07	3.07	Control
3	4.70	0.29	5.28	4.12	5.57	3.83	Control
4	Died during control period.						
5	4.76	0.43	5.62	3.90	6.05	3.47	Control
6	4.69	0.54	5.77	3.61	5.31	3.07	Control
7	4.89	0.79	6.47	3.31	7.26	2.52	Control
8	4.48	0.74	5.96	3.00	6.70	2.26	Control
MEAN	4.62	0.26	5.14	4.10	5.40	3.84	Control
9	4.52	1.08	6.68	2.36	7.76	1.28	GTG
10	5.15	0.80	6.75	3.55	7.55	2.75	GTG
11	5.13	0.38	5.89	4.37	6.27	3.99	GTG
12	6.22	0.51	7.24	5.20	7.75	4.69	GTG
13	5.80	0.83	7.46	4.14	8.29	3.31	GTG
14	6.21	0.54	7.29	5.13	7.83	4.59	GTG
15	5.81	0.70	7.21	4.41	7.91	3.71	GTG
16	6.17	0.68	7.53	4.81	8.21	4.13	GTG
MEAN	5.72	0.36	6.44	5.00	6.80	4.64	GTG

* $\bar{X} \pm 2$ SD = Mean $\pm$ 2 Standard Deviations

** $\bar{X} \pm 3$ SD = Mean $\pm$ 3 Standard Deviations

TABLE 11

CALCULATIONS USED TO DEFINE NORMAL AND

SIGNIFICANTLY ALTERED LEVELS OF LOCOMOTOR ACTIVITY

These values represent the mean number of times that saline injected mice break the photo-cell beam during successive twenty-minute periods for seven hours. Any score in excess of two or three standard deviations from a given mean score is regarded as significantly or very significantly increased locomotor activity respectively. These scores are based on the results of 42 to 52 records at each time interval.

TABLE 11

Time-Period after injection	1	2	3	4	5	6	7	8	9	10	11	12	15	18	21
Mean number of beam interruptions	48	42	44	33	37	42	36	31	28	29	30	21	22	17	18
Standard deviation	31	28	37	22	27	38	28	31	30	27	24	19	18	22	14
Mean + 2 standard deviations (Significant)	110	98	118	77	91	118	92	93	88	83	78	59	58	61	46
Mean +3 standard deviations (Very Significant)	141	126	155	99	118	156	120	124	118	110	102	78	76	83	60

TABLE 11

Successive 20 Minute Periods after Injection	1	2	3	4	5	6	7	8	9	10	11	12	15	18	21
Total Number of Injections	52	52	51	51	51	51	50	51	50	50	50	50	50	49	42
Mean Number of Deviations from Baseline	48	42	44	33	37	42	36	31	28	29	30	21	22	17	18
Standard Deviation of the Mean Deviations	31	28	37	22	27	38	28	31	30	27	24	19	18	22	14
Mean + 2 Standard Deviations (Significant)	110	98	118	77	91	118	92	93	88	83	78	59	58	61	46
Mean + 3 Standard Deviations (Very Significant)	141	126	155	99	118	156	120	124	118	110	102	78	76	83	60

of seven hours were totaled. Significant increases in activity were defined on the same basis as alterations in food intake and the calculations on which these definitions are based are shown in Table 11. It can be seen from the table that activity was greater at the beginning of the feeding period (twenty minutes after injection) than at the end. Since no differences were observed between the individual values obtained on obese and on control mice, no attempt was made to establish separate control values for each group.

After injection of d-amphetamine it was observed that locomotor activity always followed a characteristic pattern. Instead of the normal first period activity of 48 $\pm$ 31 baseline deviations, d-amphetamine would produce an immediate (onset less than one minute) increase to over 500 per 20-minute period. The animals could be observed to perform unidirectional circling movements in the cages. They would often press the feeding bar and release it in rapid succession, but they would never climb onto it. Occasionally, they would stand in one place grooming themselves, but this behavior never persisted for more than two minutes per occurrence. Although activity measurements were those of only two animals in each group, by observing the other animals, one could see that the results were characteristic for the entire group. Using this technique it was possible to obtain a measure of the relationship between duration of central nervous system stimulation and duration of food intake suppression produced by d-amphetamine.

The third parameter of this experiment consisted of a method to determine the length of time after drug injection before normal feeding would recur. The bar-pressing kymograph records described in the "Methods"

section were used for this purpose. Each twenty-minute feeding period required a 10 cm length of the kymograph record. A measure of feeding behavior could be determined from these records since normal feeding followed a characteristic pattern over the 7-hour feeding period. To describe this pattern, the total bar-pressing time (based on six saline injection records) during each twenty-minute interval was expressed in centimeters for each animal, and the average values for the two obese and two controls were plotted against time. Figure 10 (based on Table 12) represents a graphic presentation of these results and of those occurring after administration of d-amphetamine sulfate (also six injections). The point at which the post-drug bar-pressing line crossed over the saline injection line was interpreted as the time required for recovery from the food intake suppressing effects of d-amphetamine. (No animal was observed to press the bar during the 20-minute post-injection period prior to presentation of food.) It would appear from Figure 10 that the obese animals pressed the bar consistently longer after recovery from the drug. Comparison of the mean values for each of these records indicates that this is indeed the case. However, individual "t" tests on each point after the second hour show that at one point only was there a significant difference in bar-pressing. The d-amphetamine appears to suppress feeding behavior for about two hours in mice after which time a return to normal (not significantly augmented) feeding behavior reoccurs. Typical locomotor activity and bar-pressing records before and after d-amphetamine are shown in Figure 11.

The standard for success of drug treatment was weight loss. Animals were weighed on each injection day. In contrast to classical dosage

TABLE 12

TABULATED BAR PRESSING RECORDS OF OBESE AND CONTROL MICE

AS A FUNCTION OF TIME

FOLLOWING d-AMPHETAMINE INJECTIONS

TABLE 12

Time hr	Time min	CONTROL Saline Group (cm)*	CONTROL Saline S.D.	CONTROL Drug Mean (cm)	CONTROL Drug S.D.	OBESE Saline Mean (cm)	OBESE Saline S.D.	OBESE Drug Mean (cm)	OBESE Drug S.D.
0	20-40	4.74	0.71	0	0	5.58	1.49	0	0
1		2.11	1.28	0.047	0.004	3.71	1.20	0.085	0.063
1	20	2.38	1.03	0.033	0.36	3.87	1.28	0.50	0.25
1	40	3.82	0.78	1.44	0.86	4.90	1.29	2.69	1.70
2		2.71	1.15	3.25	0.36	4.99	1.00	4.05	1.40
2	20	3.51	0.99	4.16	0.68	4.66	1.14	5.20	0.73
2	40	3.86	0.85	5.44	1.35	4.56	1.34	5.61	0.75
3		4.72	0.92	5.76	0.97	4.15	1.49	5.96	0.57
3	20	3.34	0.64	6.15	0.87	3.81	1.75	6.01	1.02
3	40	3.15	0.84	5.92	0.97	3.70	0.86	6.34	0.75
4		3.32	1.37	5.63	0.49	4.13	0.86	5.44	0.94
4	20	4.09	1.14	4.71	1.55	2.98	0.97	5.45	1.30
4	40	4.81	1.31	3.62	1.45	2.73	0.42	5.87	0.74
5		3.37	1.14	3.10	1.25	2.79	0.70	4.63	0.80
5	20	2.92	1.07	2.88	1.35	2.03	1.03	5.34	1.27
5	40	2.36	0.97	2.53	1.28	2.84	1.03	3.89	1.47
6		2.68	1.17	2.75	1.72	2.98	1.24	3.67	0.57
6	20	3.47	2.12	2.90	0.34	2.20	0.94	3.04	0.70
6	40	3.10	1.35	3.83	1.00	2.60	0.77	3.38	1.26
7		2.65	1.76	3.50	0.69	3.35	0.73	3.57	1.57
7	20	2.78	1.61	2.78	1.27	2.80	0.37	2.23	1.10

*cm = centimeters of record. A 10 cm value represents 20 minutes of bar-pressing.

Figure 10

BAR PRESSING RECORDS FOR OBESE AND CONTROL MICE

AFTER SALINE OR d-AMPHETAMINE INJECTIONS

Figure 10

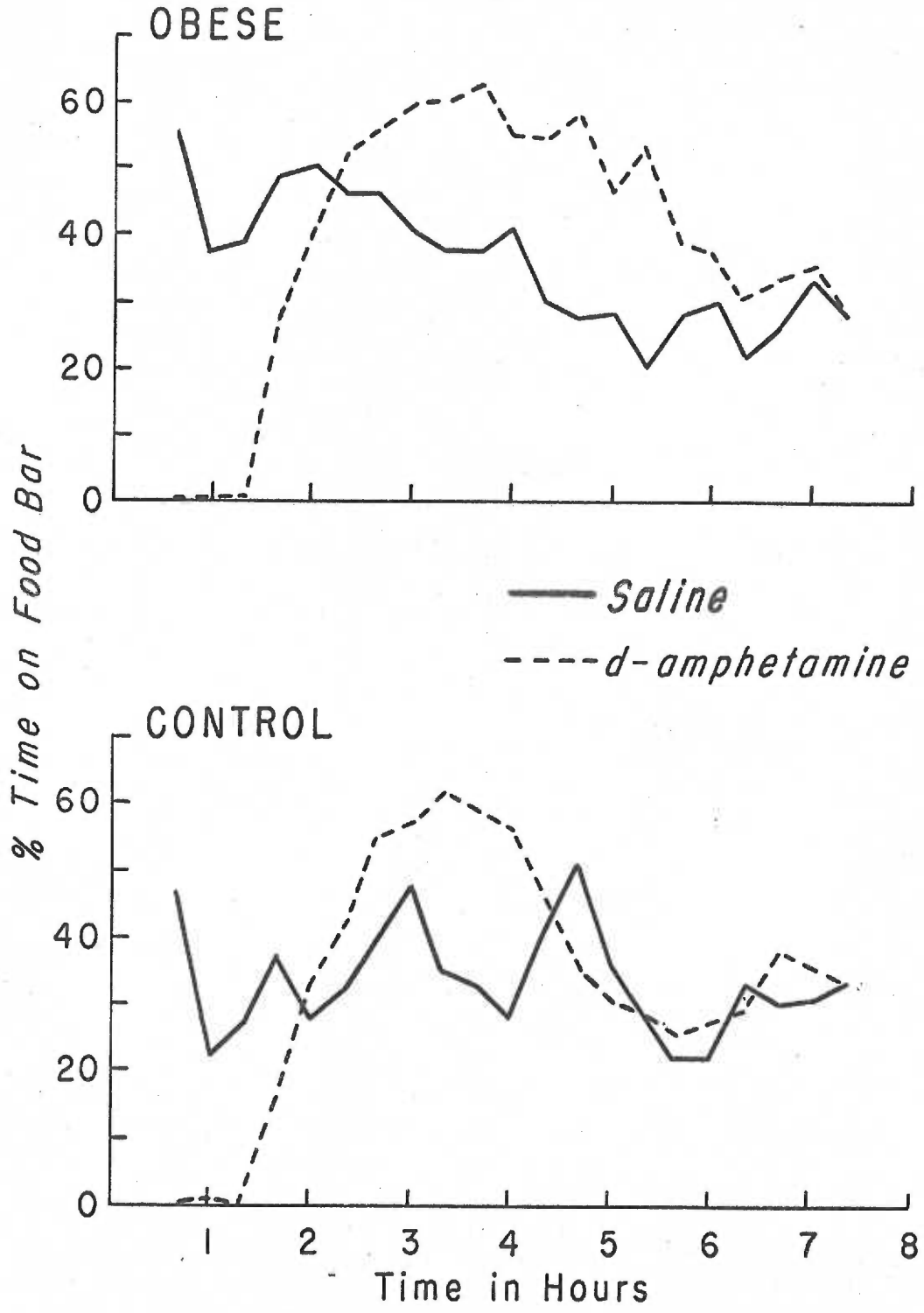
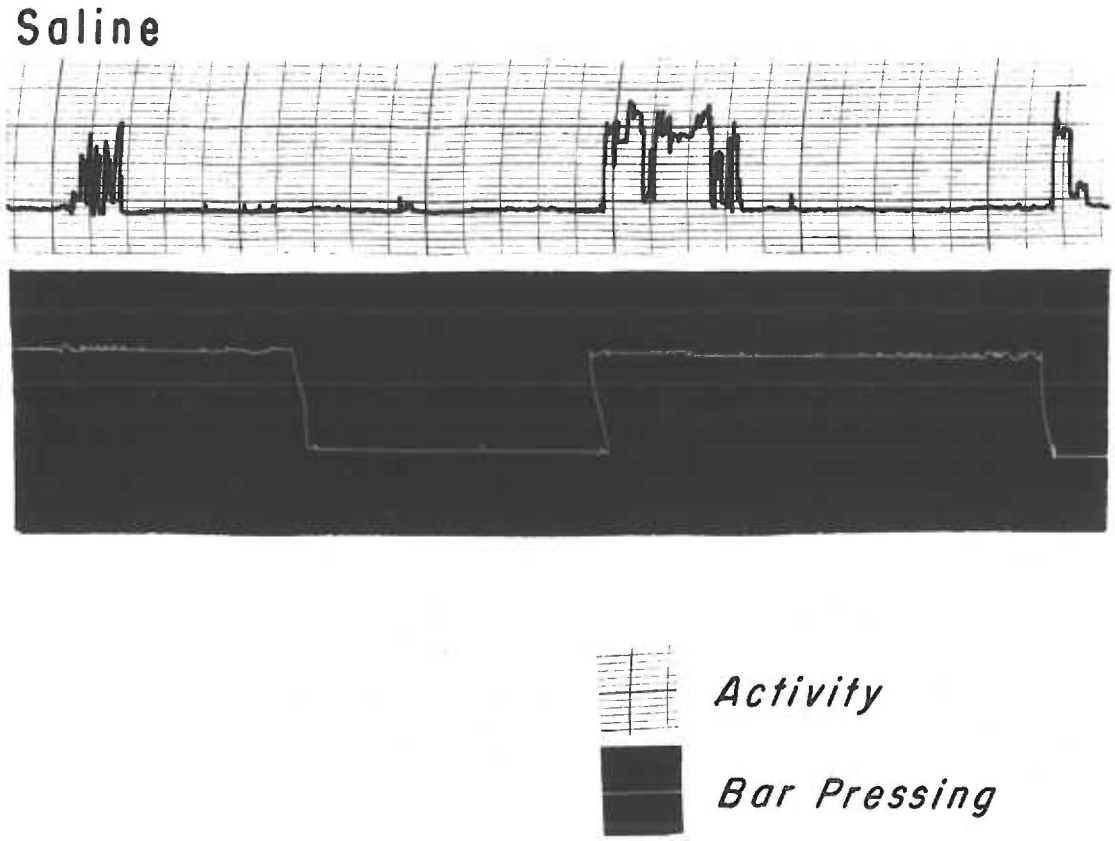

OBESE
60
40
20
0
Saline
d-amphetamine
CONTROL
% Time on Food Bar
60
40
20
0
1
2
3
4
5
6
7
8
Time in Hours

Figure 11

TYPICAL POLYGRAPH AND KYMOGRAPH RECORDS

AFTER SALINE OR d-AMPHETAMINE INJECTIONS

Figure 11



procedures, all obese and control mice received the same drug dosage. This was done to allow for comparison of the two groups under the same treatment. The drugs used in this experiment were not administered on a body weight basis since the obese and normal animals differ principally in fat content, and these drugs have not been demonstrated to become distributed in body fat. Since all animals were to receive the same dosage of each drug, an arbitrary weight of 29 grams was selected as a base for dosage calculations. All animals received, therefore, 0.145 mg (equivalent to 5 mg/kg in a 29 Gram mouse) of d-amphetamine in 0.2 cc of normal sterile saline every Tuesday, Thursday, and Saturday. Food intake on the day after drug administration was always recorded.

Figure 12 shows the results of 10 such administrations of d-amphetamine sulfate (5 mg/kg) on food intake and body weight. At this dosage level the obese mice were essentially unaffected, whereas there were three days of significant food intake suppression in the controls. After 10 such administrations, the dosage of the drug was doubled (Figure 13). On five of the 10 test days, the control mice showed a significant decrease in food intake. Such a response was observed on two days for the obese. It should be noticed, however, that both groups showed weight loss at this dosage level. The dosage was then increased to the equivalent of 15 mg/kg (Figures 14 and 15) and maintained at that level to see if tolerance would occur requiring an elevation of dose in order to continue drug response. Tolerance did not occur during the 35 consecutive administrations. Weight loss was not consistent in the control group but in the obese group decreased by as much as 6½ grams (about a 17 per cent loss from the starting weight at this dosage level)

Figure 12

MEAN PER CENT FOOD CONSUMPTION AND MEAN BODY WEIGHT (IN GRAMS) OF 8 OBESE AND 8 CONTROL MICE GIVEN 5 MG/KB OF d-AMPHETAMINE ON SUCCESSIVE TUESDAY, THURSDAYS AND SATURDAYS

Each spot represents the group mean body weight on the respective day of injection and each bar represents the group mean per cent of saline control food intake.

Figure 12

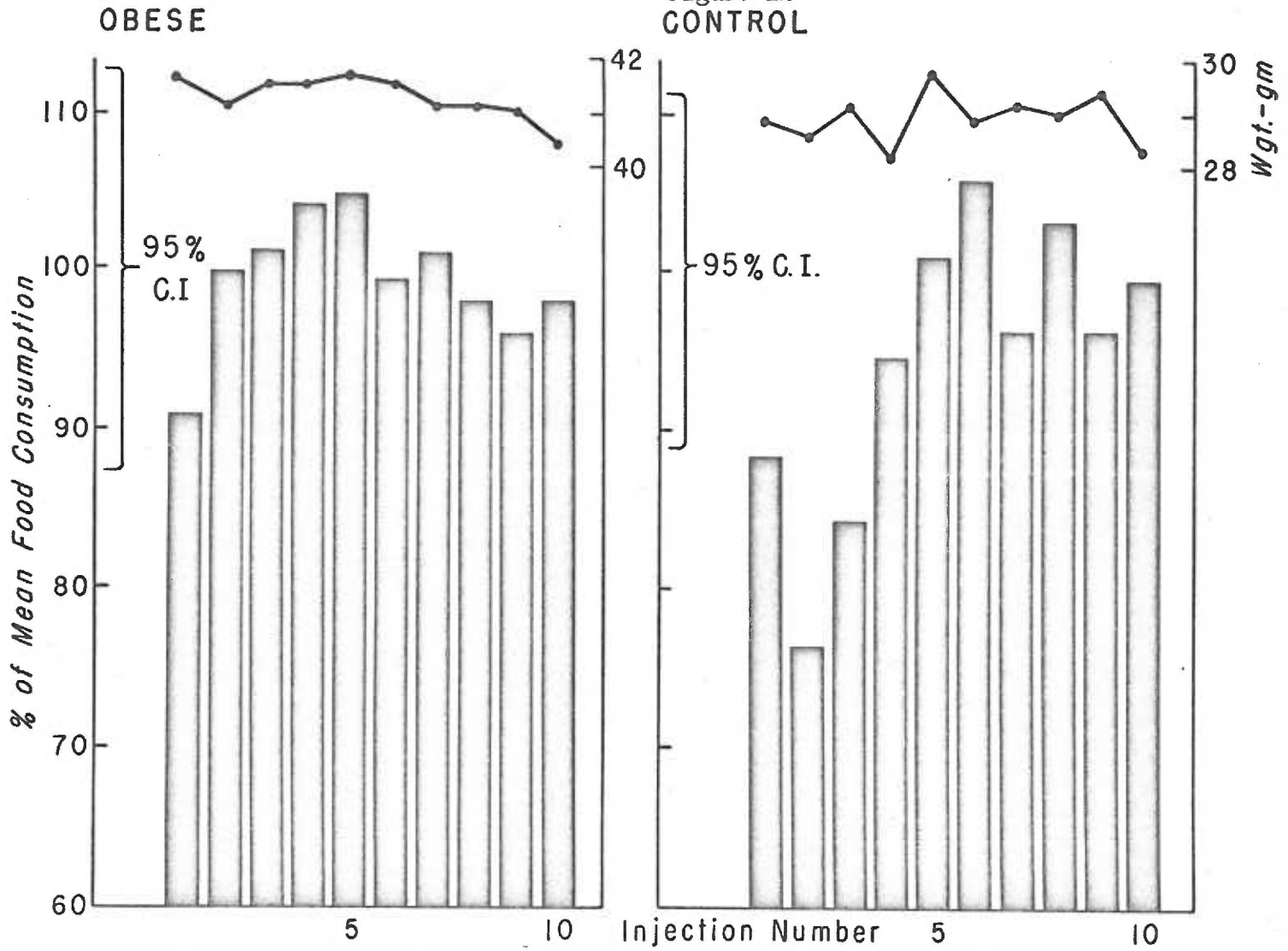

OBESE
CONTROL
110
100
90
80
70
60
95%
C.I
95% C.I.
42
40
30
28
Wgt.-gm
% of Mean Food Consumption
5
10
Injection Number
5
10

Figure 13

MEAN PER CENT FOOD CONSUMPTION AND MEAN BODY WEIGHT (IN GRAMS) OF 8 OBESE AND 8 CONTROL MICE GIVEN 10 MG/KG OF d-AMPHETAMINE ON SUCCESSIVE TUESDAYS, THURSDAYS AND SATURDAYS

Each spot represents the group mean body weight on the respective day of injection, and each bar represents the group mean per cent of saline control food intake.

Figure 13

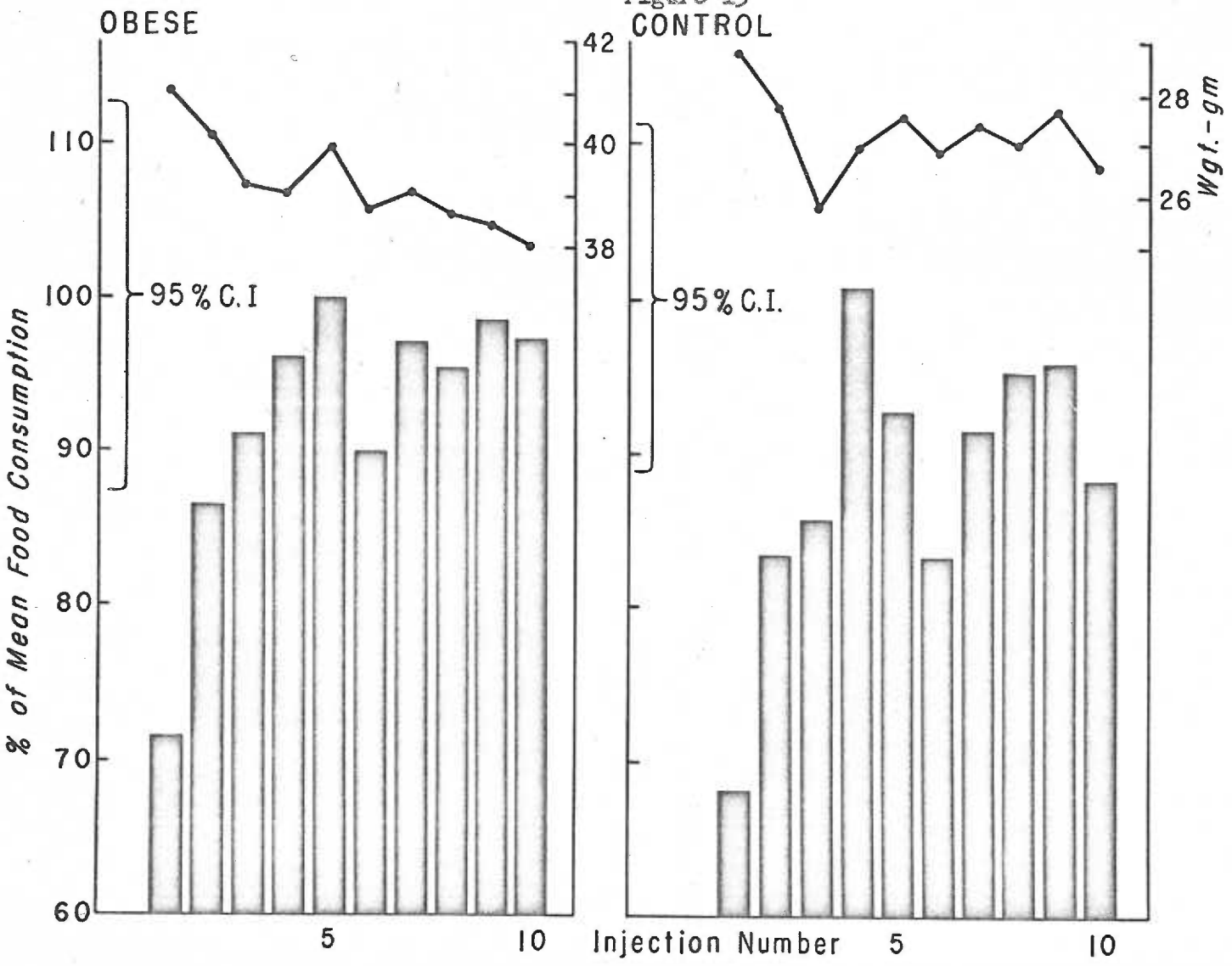

OBESE
CONTROL
110
100
90
80
70
60
42
40
38
28
26
95% C.I
95% C.I.
% of Mean Food Consumption
Wgt.-gm
5
10
Injection Number
5
10

Figure 14

MEAN PER CENT FOOD CONSUMPTION AND MEAN BODY WEIGHT (IN GRAMS) OF 8 OBESE MICE GIVEN 15 MG/KG OF d-AMPHETAMINE ON SUCCESSIVE TUESDAYS, THURSDAYS AND SATURDAYS

Each spot represents the group mean body weight on the respective day of injection.

Figure 14

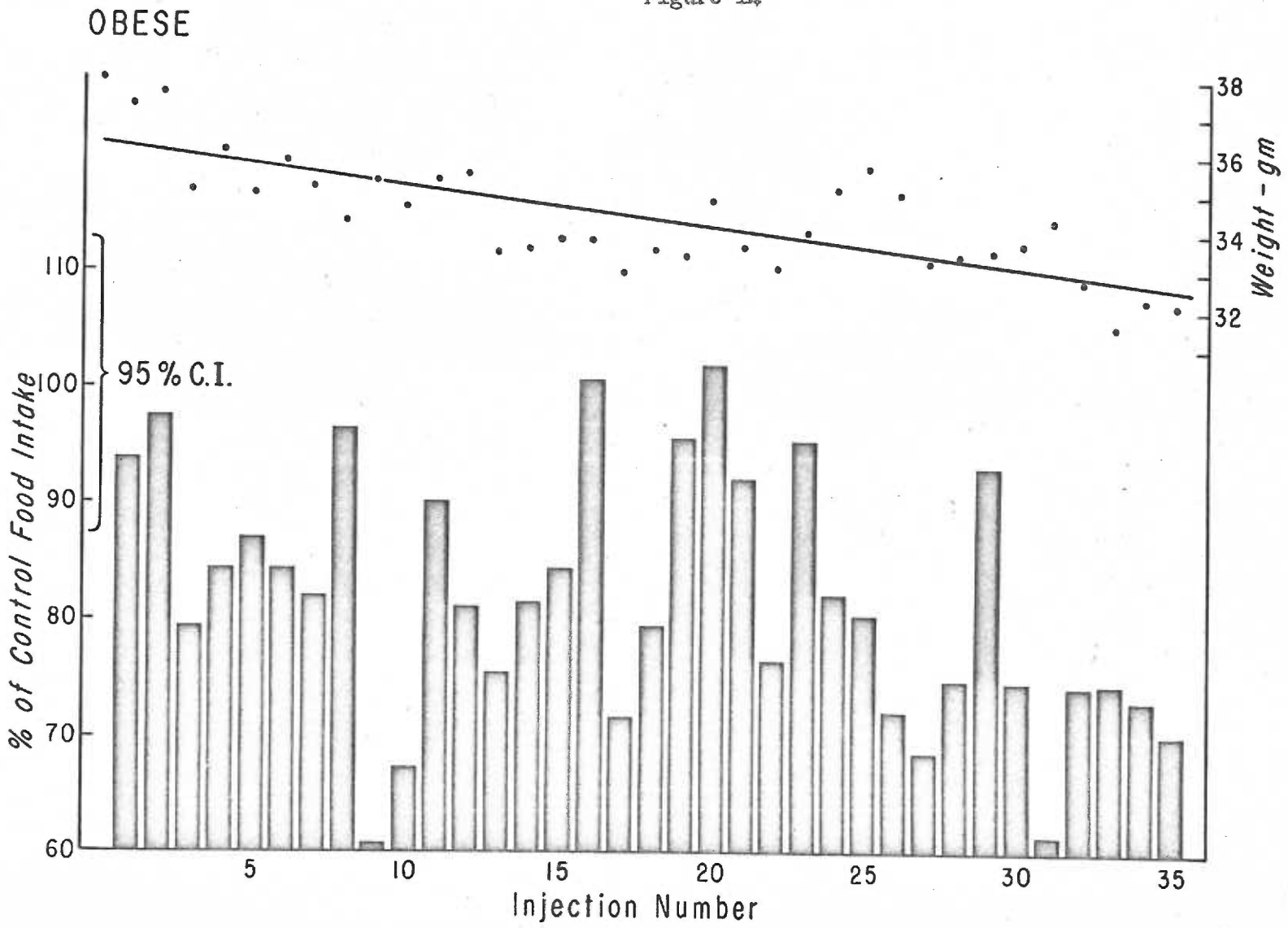

OBESE
38
36
34
32
Weight - gm
110
100
90
80
70
60
95 % C.I.
% of Control Food Intake
5
10
15
20
25
30
35
Injection Number

Figure 15

MEAN PER CENT FOOD CONSUMPTION AND MEAN BODY WEIGHT (IN GRAMS) OF 8 CONTROL MICE GIVEN 15 MG/KG OF d-AMPHETAMINE ON SUCCESSIVE TUESDAYS, THURSDAYS AND SATURDAYS

Each spot represents the group mean body weight on the respective day of injection.

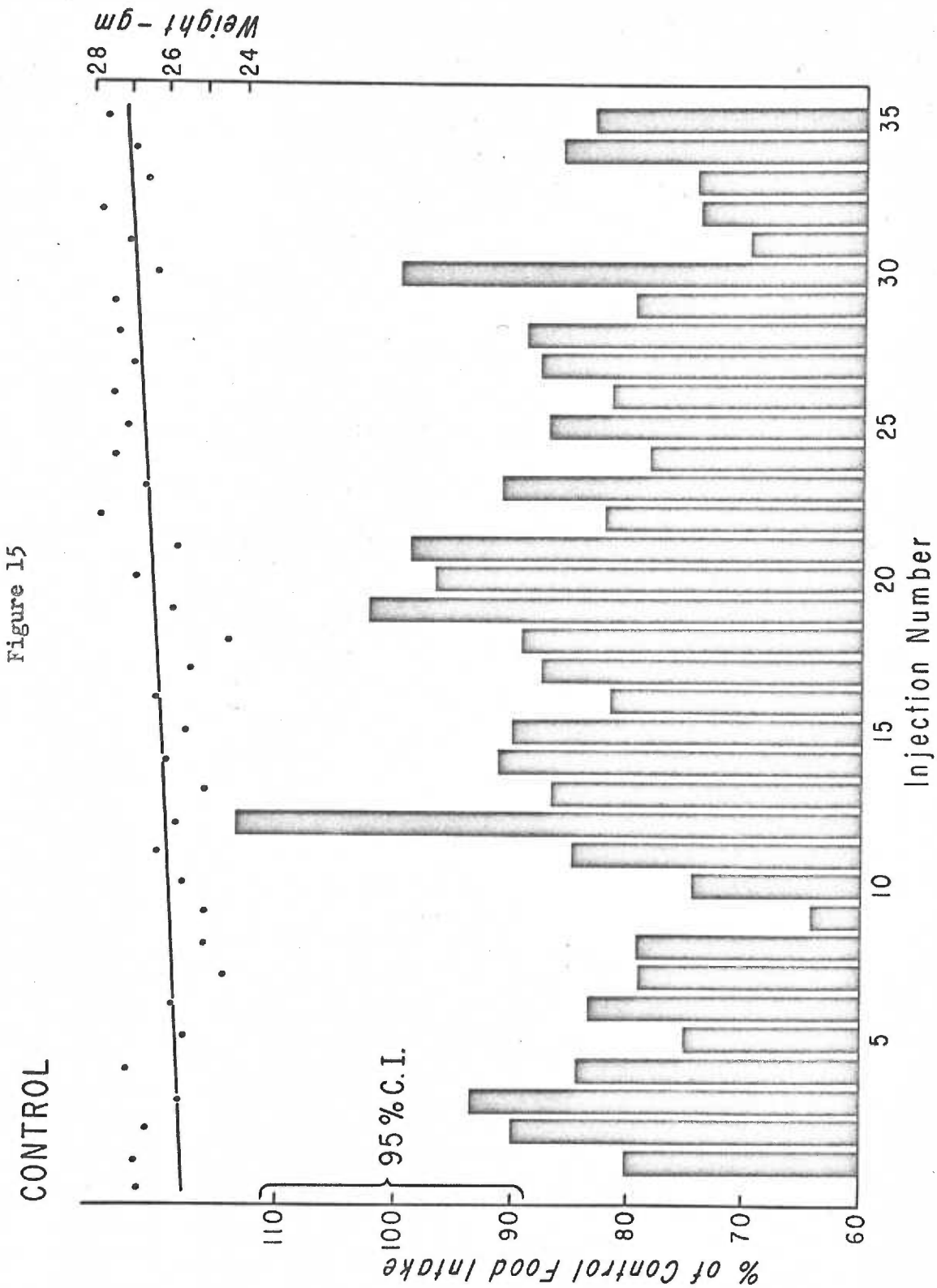

CONTROL
Weight - gm
28
26
24
% of Control Food Intake
110
100
90
80
70
60
95% C.I.
5
10
15
20
25
30
35
Injection Number

Figure 15

during the test.

On 23 of the 35 test days there occurred a significant decrease in food intake for the control animals and on all but three days the intake was less than the pre-drug levels. For the obese animals, significant decreases occurred on 25 of the 35 days and on all but two days the intake was below the pre-drug level. The mean readings for each day at all three dosages are given in Table 13 along with the corresponding readings for the day after injection. It can be seen that in most cases the food intake on the day after injection was greater than 100 per cent of the pre-drug levels. The obese and control mice showed significantly elevated total daily food consumption on three and eight days, respectively. (The individual animal data used in the calculations shown herein are included in the "Appendix" section of this thesis.)

The question frequently arises as to whether or not the food intake suppressant and the central stimulation activities of d-amphetamine are independent or interrelated. By measuring the length of time required after injection to return to non-significantly altered bar-pressing and locomotor activity, one can obtain a measure of this relationship. Figures 16, 17 and 18 represent a graphic presentation of this information. The obese and the control mice showed about the same duration of both responses at 5 and 10 mg/kg of d-amphetamine. The intensity and duration of locomotor activity stimulation in the control mice was maintained throughout the 35 tests as was bar-pressing behavior. The animals ate while still showing significantly increased activity. In contrast, the obese animals showed progressively less locomotor activity stimulation which, after the twentieth dose, was significant for a shorter

TABLE 13

FOOD INTAKE RECORDS

ON THE DAY OF AND ON THE DAY AFTER

d-AMPHETAMINE INJECTIONS

TABLE 13

Injection No.	Injection Day Food Intake of Controls	Food Intake Day After Injection (Controls)	Injection Day Food Intake of GTG Obese	Food Intake Day After Injection (Obese)	Dose (mg/kg)
1	4.08	4.12	5.20	5.90	5
2	3.52	4.34	5.72	5.99	5
3	3.88	4.33	5.79	5.62	5
4	4.36	4.64	5.96	6.32	5
5	4.67	4.53	5.98	6.03	5
6	4.89	4.79	5.68	5.95	5
7	4.44	4.67	5.78	6.20	5
8	4.76	4.85	5.60	5.98	5
9	4.44	4.60	5.47	5.60	5
10	4.83	5.02	6.22	5.89	5
11	3.15	4.20	4.10	5.30	10
12	3.86	4.72	4.95	6.14	10
13	3.96	4.91	5.22	6.29	10
14	4.66	5.57*	5.50	6.40	10
15	4.28	4.33	5.73	5.57	10
16	3.84	4.49	5.15	5.83	10
17	4.23	5.04	5.57	6.33	10
18	4.40	4.88	5.47	5.93	10
19	4.43	4.76	5.65	5.91	10
20	4.07	5.08	5.58	6.49*	10
21	3.70	4.70	5.37	5.25	15
22	4.17	4.72	5.57	6.09	15
23	4.34	4.93	4.53	5.59	15
24	3.90	4.98	4.83	5.99	15
25	3.47	4.36	4.99	5.75	15
26	3.87	4.47	4.83	4.96	15
27	3.66	4.65	4.70	5.08	15
28	3.67	4.58	5.54	4.82	15
29	2.97	4.10	3.48	5.64	15
30	3.45	4.92	3.85	5.97	15
31	3.93	5.52*	5.17	6.61*	15
32	5.28	5.87*	4.66	5.97	15
33	4.02	5.04	4.33	5.68	15
34	4.23	4.78	4.67	5.76	15
35	4.17	4.70	4.85	5.76	15
36	3.78	4.50	5.77	5.86	15
37	4.06	4.54	4.11	5.80	15
38	4.13	5.77*	4.57	6.26	15
39	4.75	5.46*	5.49	6.71*	15
40	4.48	4.95	5.85	6.14	15

(concluded on next page)

TABLE 13

(Concluded)

Injection No.	Injection Day Food Intake of Controls	Food Intake Day After Injection (Controls)	Injection Day Food Intake of GTG Obese	Food Intake Day After Injection (Obese)	Dose (mg/kg)
41	4.57	4.85	5.28	6.37	15
42	3.79	5.17*	4.38	5.51	15
43	4.22	4.66	5.46	5.76	15
44	3.62	4.61	4.70	5.53	15
45	4.02	4.87	4.61	6.02	15
46	3.78	4.80	4.12	5.87	15
47	4.06	5.14*	3.94	4.60	15
48	4.12	4.63	4.29	5.80	15
49	3.68	4.92	5.34	5.36	15
50	4.62	6.09*	4.28	5.27	15
51	3.22	4.50	3.52	4.62	15
52	3.43	4.88	4.26	4.77	15
53	3.45	5.12*	4.27	4.85	15
54	3.98		4.19		15
55	3.85	4.48	4.03	5.43	15

* = Significantly elevated food intake

Figure 16

DURATION OF SIGNIFICANTLY ALTERED LOCOMOTOR ACTIVITY

AND BAR PRESSING AFTER d-AMPHETAMINE (5 MG/KG) INJECTIONS

Figure 16

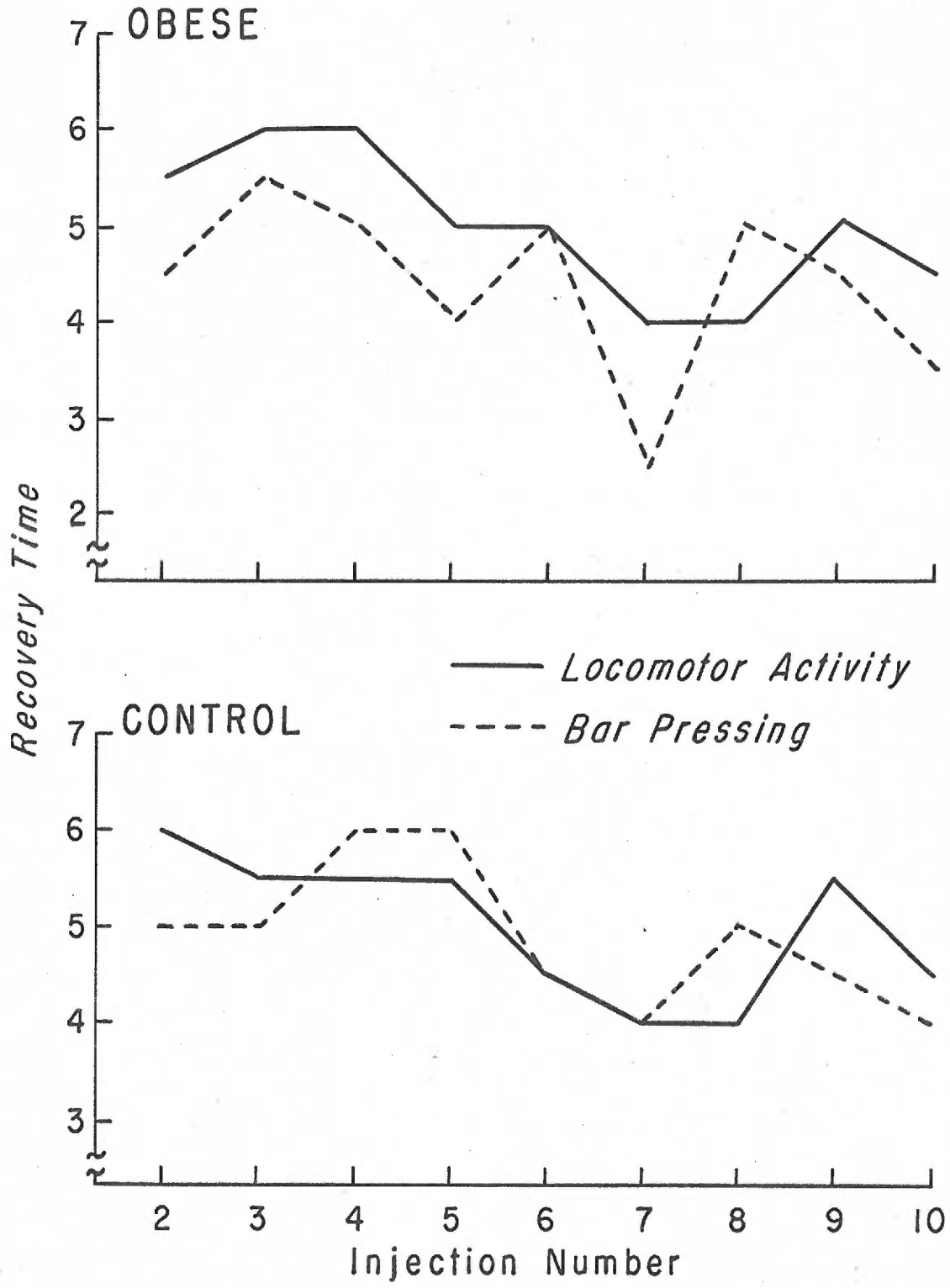

OBESE
CONTROL
Recovery Time
Locomotor Activity
Bar Pressing
7
6
5
4
3
2
2
3
4
5
6
7
8
9
10
Injection Number

Figure 17

DURATION OF SIGNIFICANTLY ALTERED LOCOMOTOR ACTIVITY

AND BAR PRESSING AFTER d-AMPHETAMINE (10 MG/KG) INJECTIONS

Figure 17

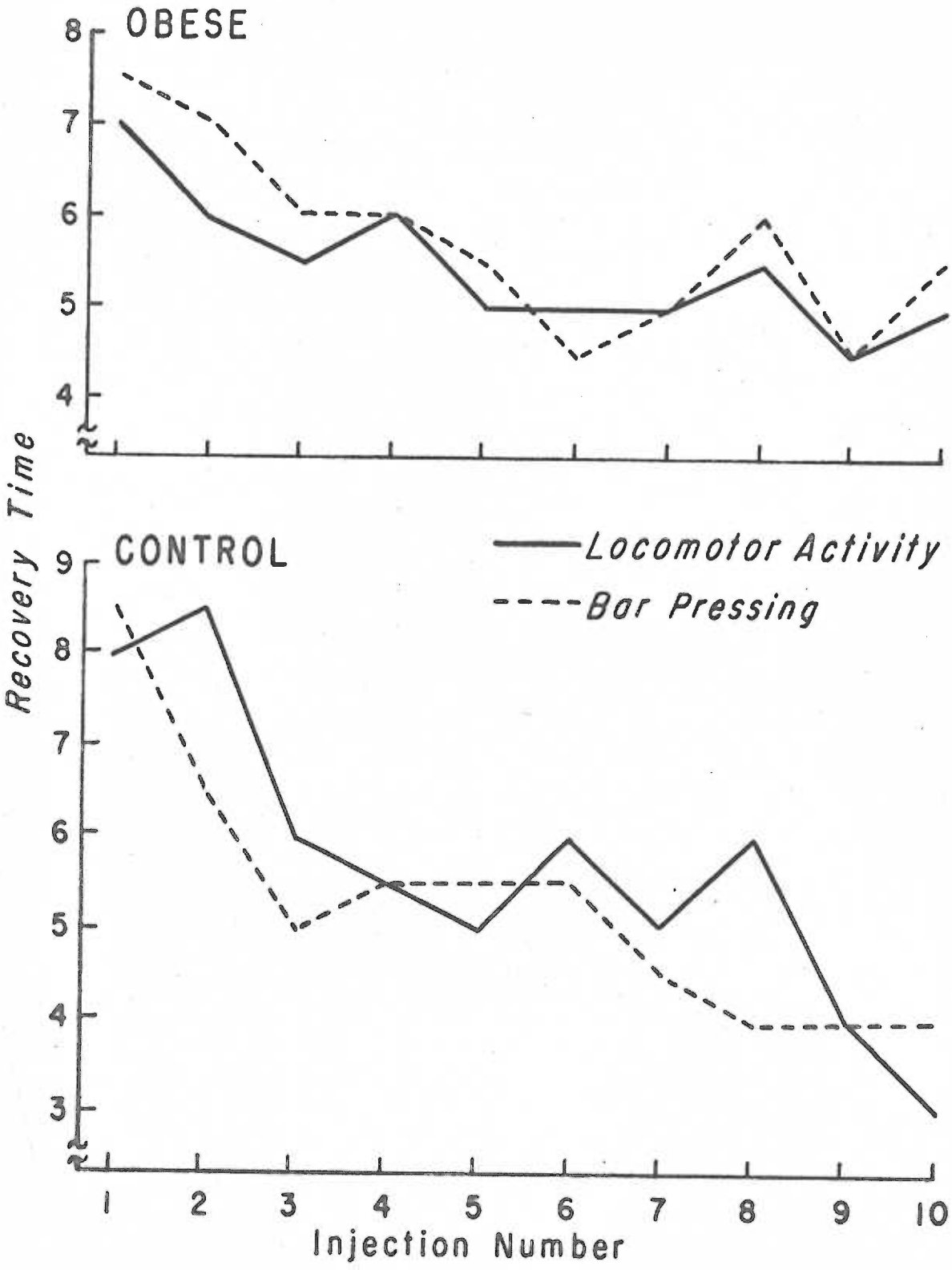
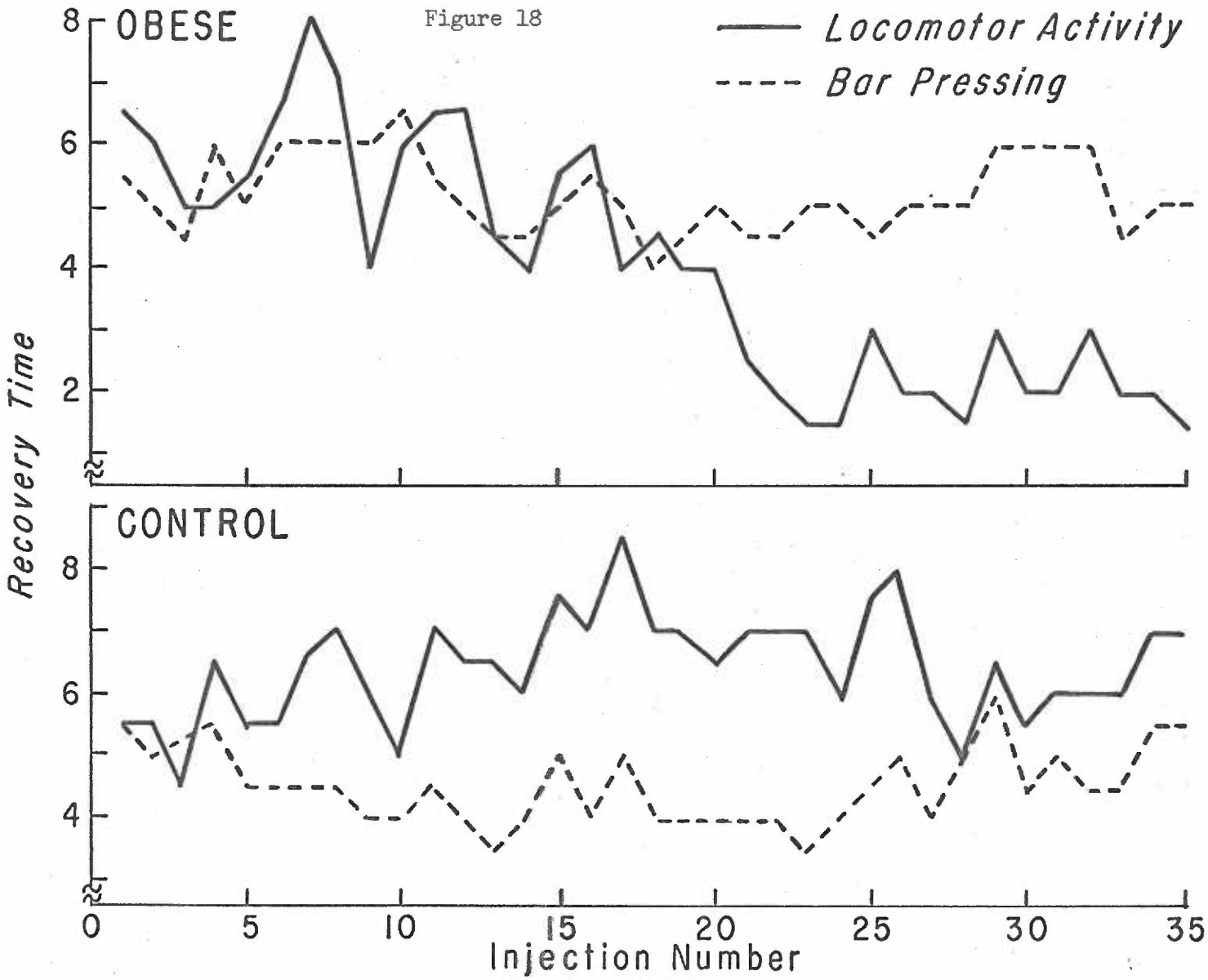

OBESE
CONTROL
Recovery Time
Locomotor Activity
Bar Pressing
Injection Number
8
7
6
5
4
9
3
1
2
10

Figure 18

DURATION OF SIGNIFICANTLY ALTERED LOCOMOTOR ACTIVITY

AND BAR PRESSING AFTER d-AMPHETAMINE (1.5 MG/KG) INJECTIONS

Figure 18


OBESE
CONTROL
Locomotor Activity
Bar Pressing
Recovery Time
8
6
4
2
0
5
10
15
20
25
30
35
Injection Number

duration of time. The animals were observed to be much more calm than the controls at the same observation time. Although they demonstrated less activity, they did not seek food (bar-press). The results of both groups showing that tolerance to food intake suppression did not occur at the 15 mg/kg dose agree with the data shown in Table 13.

Acute Drug Responses

It was stated earlier that one of the criteria for an experimental disease model was that it must respond to drug treatment in a manner analogous to the human counterpart. This section was designed to further test if GTG obesity meets this requirement. It consists of a series of experiments with d-amphetamine, phenmetrazine, phendimetrazine, and methyphenidate. The recommended human oral dosages of each of these agents may be used to compare their relative potency as anorexigenic agents.

d-amphetamine	5 mg	(given 3 times a day)
phenmetrazine	25 mg	(given 3 times a day)
phendimetrazine	70 mg	(given 3 times a day)
methylphenidate*	10 mg	(given 3 times a day)

*A mild central stimulant which is claimed by the manufacturer not to be anorexigenic at the usual clinical dose.

Phendimetrazine differs from phenmetrazine only by the inclusion into the molecule of an extra methyl group. Thus, at first glance, one might not expect to see the wide variation in recommended dosage shown above. However, phenmetrazine is supplied as the hydrochloride salt and phendimetrazine as the bitartrate. Therefore, each 25 mg of phenmetrazine base is equivalent to 32.85 mg of phendimetrazine base.

The first experiment consisted of an attempt to establish a dose-response curve of anorexigenic efficiency for phenmetrazine. Thirty

moderately obese (40 - 48 Gm) mice whose seven-hour food intake had reached stabile levels were used in five groups of six animals each. They were injected with normal saline solution and their seven-hour food intake measured. On the following day, the groups were injected with 30, 60, 75, 90 and 105 mg/kg of drug, respectively. Results were calculated as a percentage of the saline food intake levels and are shown in Figure 19. Individual "t" tests and an analysis of variance showed these means to differ significantly from their pre-test control values, in all cases except for methylphenidate. Four animals were injected on the day prior to this test with 150 mg/kg. Three of these animals convulsed and died within two hours after the injection. The fourth recovered but was hyperexcitable for over 24 hours.

In an experiment of similar design, d-amphetamine was administered to moderately obese and control mice and phenmetrazine and phendimetrazine to moderately obese mice in doses of 5, 10, 15, and 20 mg/kg of each drug, respectively. Each point on Figure 20 represents results for six animals. As can be seen, the responses of both the moderately obese and control mice to d-amphetamine were essentially identical and no significant response to either of the drugs was observed at these dosages.

In the third and fourth experiments, moderately obese mice were used to determine dose-response curves for phendimetrazine and for methylphenidate. Here again, six animals were used at each dosage level. In all cases no animal was used which had received any of the amphetamines prior to this test. The results are shown in Figures 21 and 22. Methylphenidate was the only drug which did not produce a consistent

Figure 19

DOSE RESPONSE CURVE FOR PHENMETRAZINE

IN MODERATELY OBESE MICE

(response = food intake suppression)

Figure 19

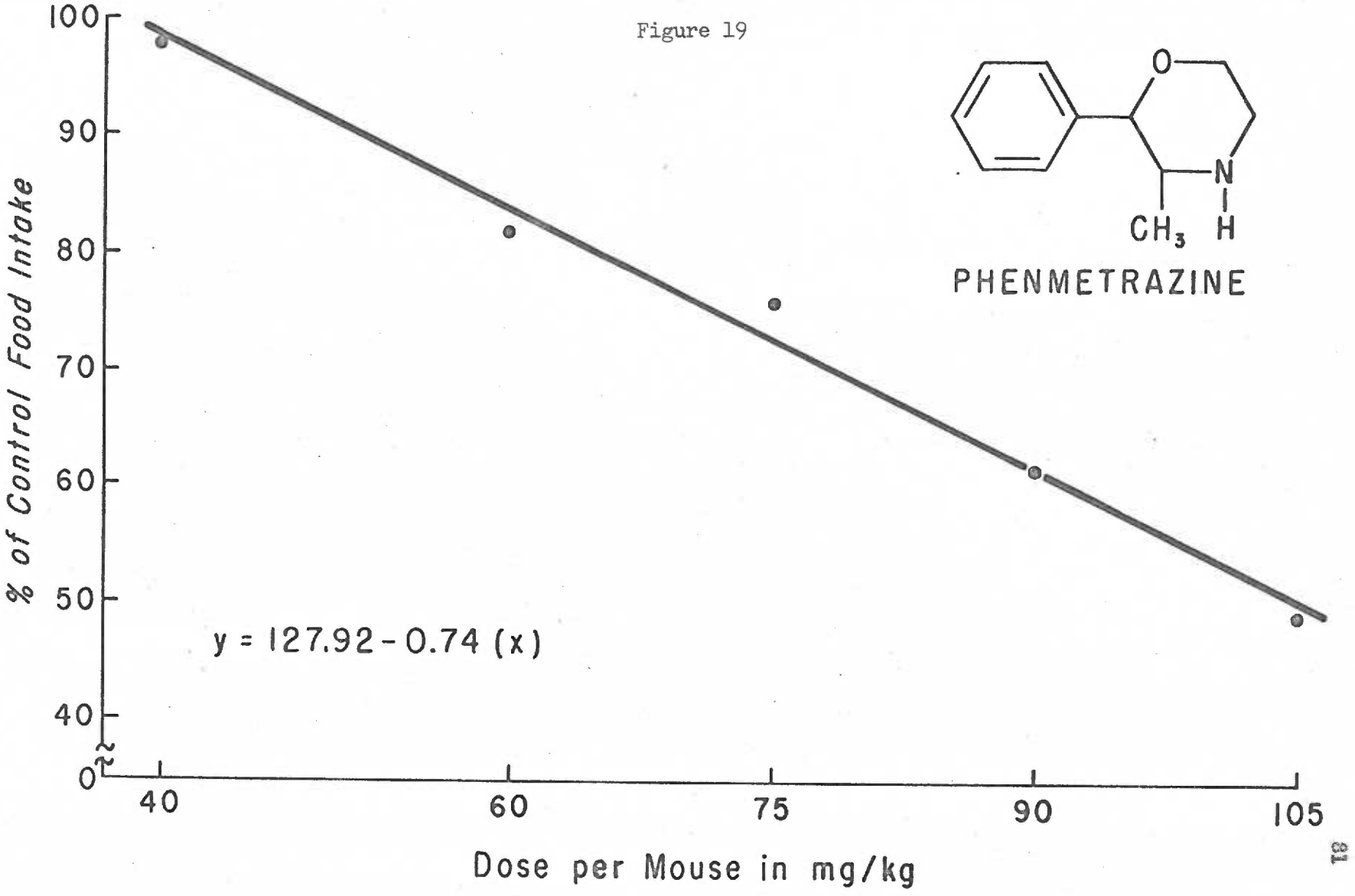

100
90
80
70
60
50
40
0
% of Control Food Intake
y = 127.92 - 0.74 (x)
40
60
75
90
105
Dose per Mouse in mg/kg
O
N
CH3 H
PHENMETRAZINE

Figure 20

DOSE RESPONSE CURVE FOR LOW DOSES OF PHENMETRAZINE AND PHENDIMETRAZINE

IN MODERATELY OBESE

AND d-AMPHETAMINE IN NORMAL AND MODERATELY OBESE MICE

(response = food intake suppression)

Figure 20

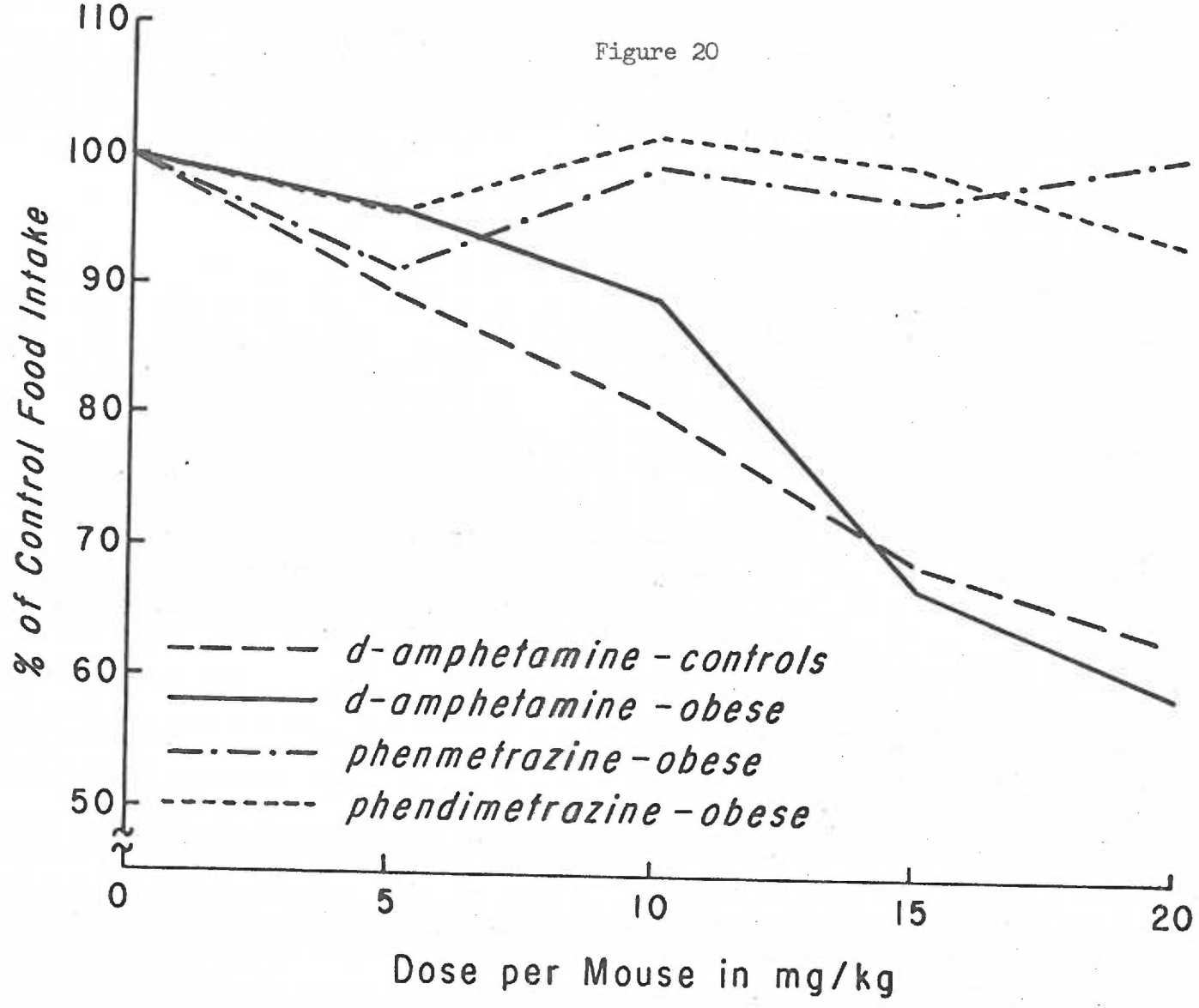

110
100
90
80
70
60
50
% of Control Food Intake
d-amphetamine - controls
d-amphetamine - obese
phenmetrazine - obese
phendimetrazine - obese
0
5
10
15
20
Dose per Mouse in mg/kg

Figure 21

DOSE RESPONSE CURVE FOR PHENDIMETRAZINE

IN MODERATELY OBESE MICE

(response = food intake suppression)

Figure 21

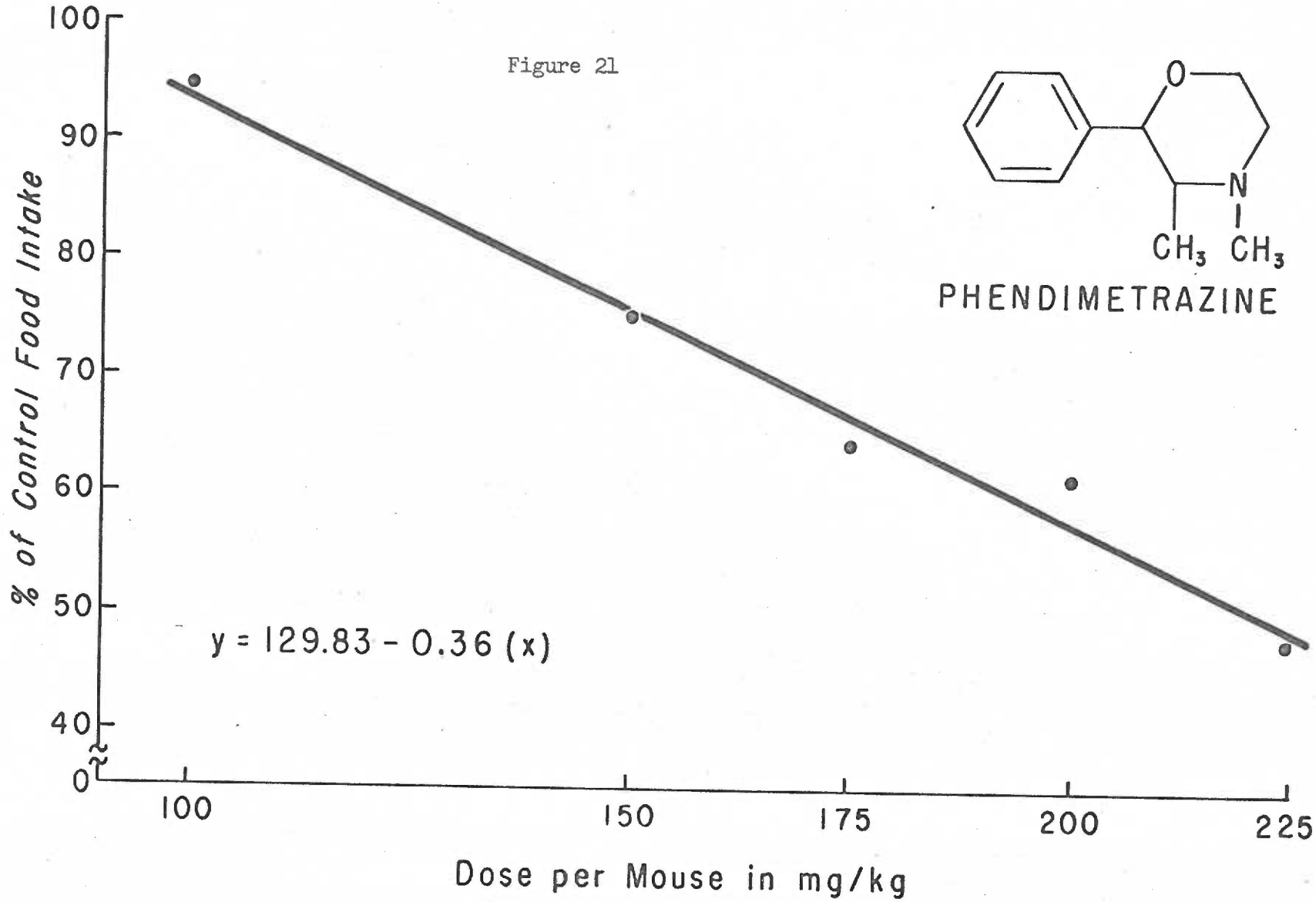

100
90
80
70
60
50
40
0
% of Control Food Intake
y = 129.83 - 0.36 (x)
100
150
175
200
225
Dose per Mouse in mg/kg
O
N
CH3 CH3
PHENDIMETRAZINE

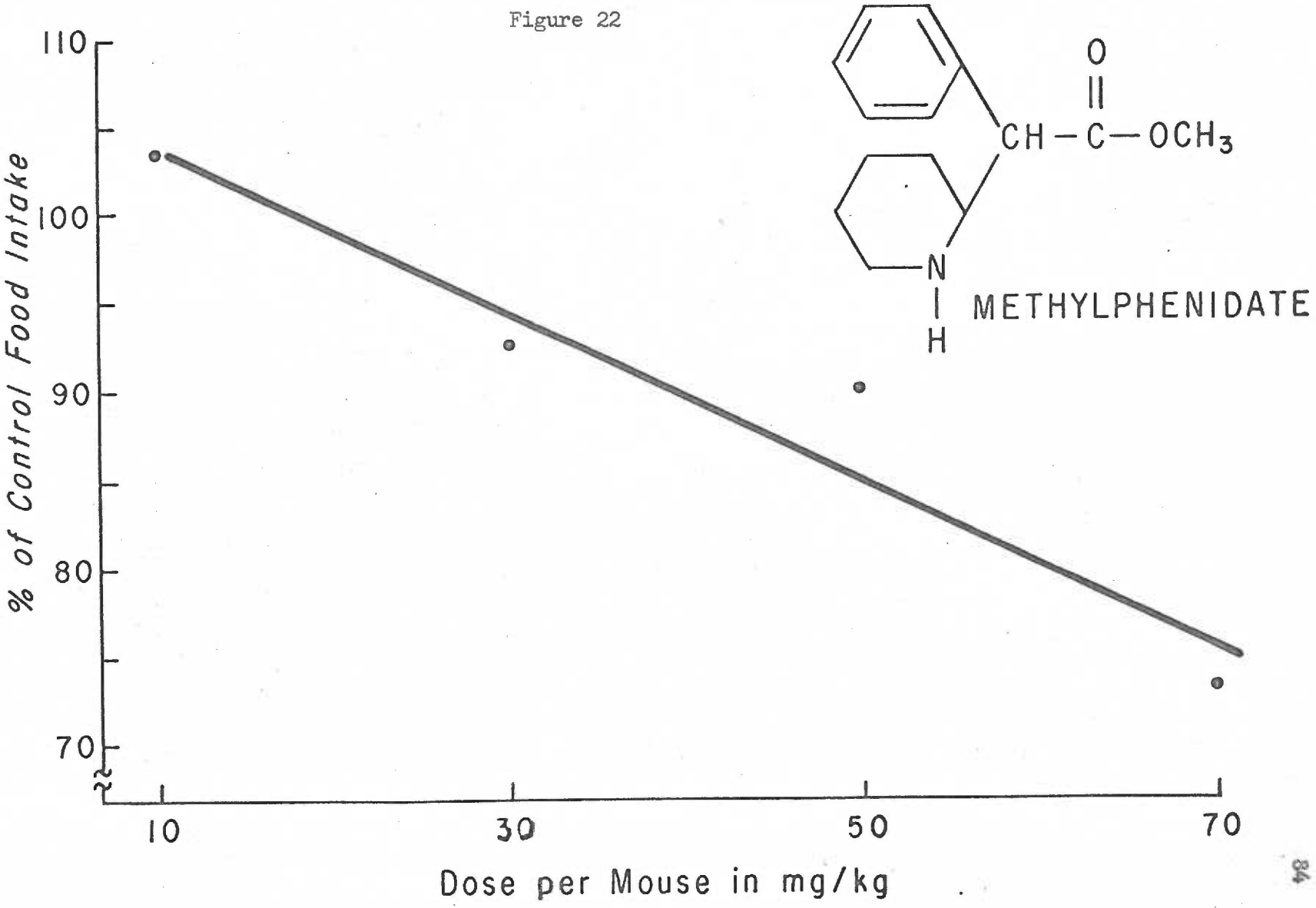
Figure 22

DOSE RESPONSE CURVE FOR METHYLPHENIDATE

IN MODERATELY OBESE MICE

(response = food intake suppression)

Figure 22


110
100
90
80
70
% of Control Food Intake
10
30
50
70
Dose per Mouse in mg/kg
O
CH – C – OCH3
N
H
METHYLPHENIDATE

food intake suppression although there were sufficient effects in some animals (increasing incidence with higher doses) to produce a dose-dependent decrease in mean food consumption for the entire group.

Comparison of the animal dosage of each drug necessary for a 25 per cent decrease in food intake to the human clinical doses, supplies a means to evaluate drug responsiveness of GTG obesity as a model of its clinical counterpart. Table 14 presents such a comparison and employs d-amphetamine as a reference for comparing the potency of the other agents. (For purposes of biochemorphological comparison, it is interesting to note that methylphenidate is structurally related to the phenylethylamines (amphetamines) and, especially, to the two oxazine compounds.)

The objective of the fifth experiment was to establish the LD_{50} of d-amphetamine for normal and GTG obese mice. Eighty-eight normal (22 to 26 gram) mice were injected in groups of eight with d-amphetamine at 5 mg/kg increments from 10 to 60 mg/kg. All dosages produced increased activity and doses over 25 mg/kg caused convulsive behavior and persistent preening which often led to scratching and bleeding of the face and ventral surfaces. This activity reached a peak at about 45 minutes after injection and was sustained for three hours. After this time the mice were less active (often lethargic), but low intensity auditory stimuli could elicit convulsive behavior. Deaths occurred between two and four hours after injection. The number of deaths for each group of eight mice injected with various doses of d-amphetamine is shown below:

TABLE 14

COMPARISON OF DRUG DOSAGES REQUIRED

TO PRODUCE A 25 PER CENT DECREASE IN FOOD INTAKE

IN OBESE MICE TO THE USUAL HUMAN CLINICAL DOSAGE

Table 14

Drug	Total Single Clinical Dose	Ratio to d-Amphetamine Dose	Animal Single Dose	Ratio to d-Amphetamine Dose
d-Amphetamine	5 mg	1	13 mg/kg	1
Phenmetrazine	25 mg	5	72 mg/kg	5.5
Phendimetrazine	70 mg	14	152 mg/kg	11.7
Methylphenidate	20 mg[a]	4	72 mg/kg	5.5

a. The manufacturer's high suggested oral dose is used here as this drug is described as possessing a potency between that of caffeine and d-amphetamine (63).

Dose (mg/kg)	10	15	20	25	30	35	40	45	50	55	60
Number of Deaths	0	2	0	1	0	2	1	1	2	5	8

Thus, the LD_{50} for normal mice is between 50 and 55 mg/kg.

To establish the LD_{50} for obese animals, the same procedure was used. All animals weighed between 43 and 60 grams and in all cases 4 were females and 4 males. No sex difference was observed in the quantitative or qualitative responses to the d-amphetamine. The number of deaths for these animals was as follows:

Dose (mg/kg)	10	15	20	25	30	35	40
Number of Deaths	0	1	1	2	2	5	7

Thus, the LD_{50} for the obese mice was considerably lower than that for the control mice; i.e., between 30 and 35 mg/kg.

Pharmacopathology

Acute Toxicity of Gold Thioglucose

In the first experiments, the GTG was administered in the form of a sesame oil suspension. Later experiments involved use of a water solution of the yellow powder. The responses to both dosage forms were the same. After injection of GTG to mice fasted for 48 hours, no signs of toxicity could be observed during the first two or three hours. In animals that were usually destined to die, one would then observe a mixture of signs and symptoms often including ataxia, tremors, paraplegia, profuse salivation, tendency to reel to one side (usually to the left), irregular rate of eye blinking, and an externally palpable dilated stomach. The respiration was usually deep and slow but seldom noticeably labored. Autopsy and macroscopic observations showed no abnormalities other than the swollen stomachs and occasional ruptured stomachs often associated with ascites. Although all the animals had been offered unlimited food immediately after injection, the intestinal tract was always found to be empty (except for an occasional hard bolus in the distal colon) on autopsy. In all cases the stomachs were full of food material of a semisolid and rather homogeneous consistency. The stomach and contents of one 19-gram mouse weighed 4.19 grams. Saline injected controls never showed stomachs and contents weighing over 1.5 grams.

In animals that died from the toxic action of GTG, the tongue was frequently extended and it was not uncommon to observe bleeding from the nose. Prior to death these animals grasped the floor of the cage

securely with their teeth.

Chronic Effects of Gold Thioglucose

After recovery from the injection (no observable symptoms after four days), weight gains would occur reaching a maximum at about 60 days. Not all animals demonstrated hyperphagia or obesity. However, those that did become obese often weighed between 50 and 75 grams (normal mature weight for these mice was between 23 and 33 grams).

The fur on these very obese animals was somewhat sparse and more coarse than that of the controls. Figures 23 and 24 show a sixty gram obese mouse and a thirty gram control mouse both intact in a dorsal view and following laparotomy in a ventral view. The obese mice always possessed large quantities of subcutaneous and intra-abdominal fat.

It was interesting to note that the total dry weight of cleaned intestine from GTG obese mice was greater than that of controls. Table 15 shows the results obtained on eight mice (four moderately obese and four non-obese but GTG injected controls) comparing lengths and weights of intestine after pre-cleaning and drying. Histological cross-sections taken from identical levels (seven centimeters distal to the pyloris) show that the obese intestine (Figure 25) differed from the control (Figure 26) by having a greater cross-sectional diameter, an enlarged lumen, and a greater mass and size of villi. It would be interesting to know if this general increase in intestinal area involves cellular hypertrophy or hyperplasia.

Figure 23

DORSAL VIEW OF AN OBESE AND A CONTROL MOUSE

Figure 24

VENTRAL VIEW OF AN OBESE AND A CONTROL MOUSE AFTER LAPAROTOMY SHOWING VARIATIONS IN SUBCUTANEOUS AND INTRA-ABDOMINAL FAT

Figure 23

Figure 24

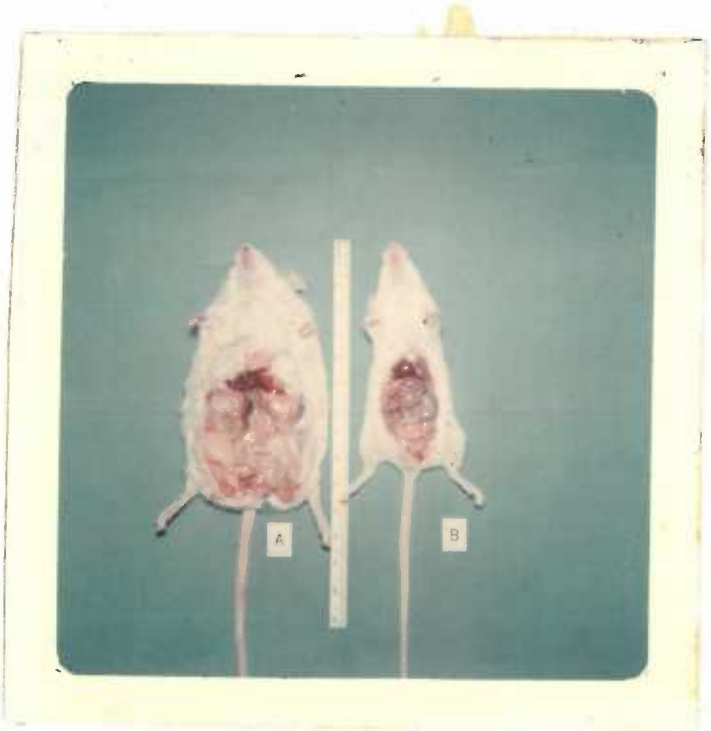


TABLE 15

COMPARATIVE LENGTHS AND WEIGHTS
OF INTESTINE FROM OBESE AND CONTROL MICE

(These were litter-mate mice injected with GTG five months prior to this study. The term, control mice, here refers to GTG injected non-obese mice.)

TABLE 15

Animal Number	GTC Dose	Gross Weight	Intestinal Length	Intestinal Dry Weight (Gm)	Weight/ Unit Length (mg)
1	0	30 Gm	54 cm	0.5306	9.83
2	0	34 Gm	56 cm	0.5857	10.46
3	0	30 Gm	58 cm	0.6663	11.49
4	0	31 Gm	53 cm	0.5252	9.91
5	10	53 Gm	65 cm	0.8087	12.44
6	10	44 Gm	61 cm	0.8462	13.87
7	10	52 Gm	67 cm	0.8661	12.93
8	20	51 Gm	57 cm	0.8534	12.44

Preliminary histological studies of the brain were performed following the termination of the experiments. Two methods were employed: a) frozen sections were prepared on a cryostat and stained with thionin, and b) paraffin sections were prepared and stained with hematoxylin and eosin. As previously mentioned, various investigators have reported ventromedial hypothalamic lesions following the administration of GTG. The present histological studies have not substantiated these reports. Neither cellular nor nuclear alterations are evident, as shown in Figure 27. However, it must be emphasized that these are the results of studies on only three obese and one control animal.

Figure 25

CROSS-SECTION OF INTESTINE FROM AN OBESE MOUSE

(X165)

Figure 26

CROSS-SECTION OF INTESTINE FROM A CONTROL MOUSE

(X165)

Figure 27

THIONIN STAINED FROZEN BRAIN SECTION

FROM AN OBESE MOUSE

SHOWING THE VENTRAL CANAL AND THE VENTROMEDIAL NUCLEI

(X330)

Figure 25

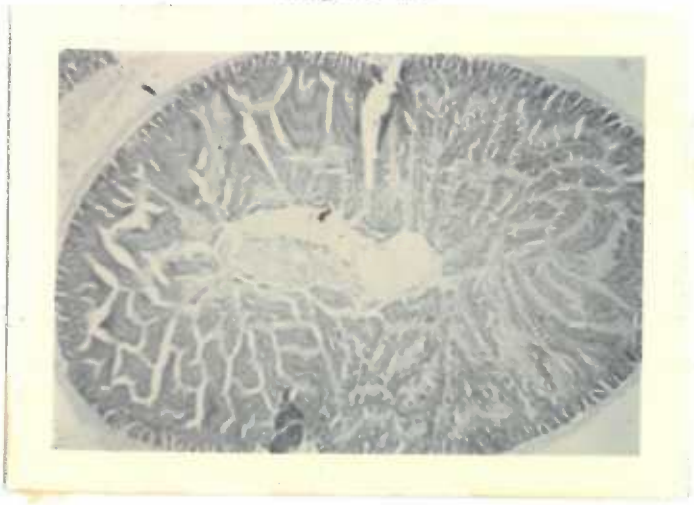
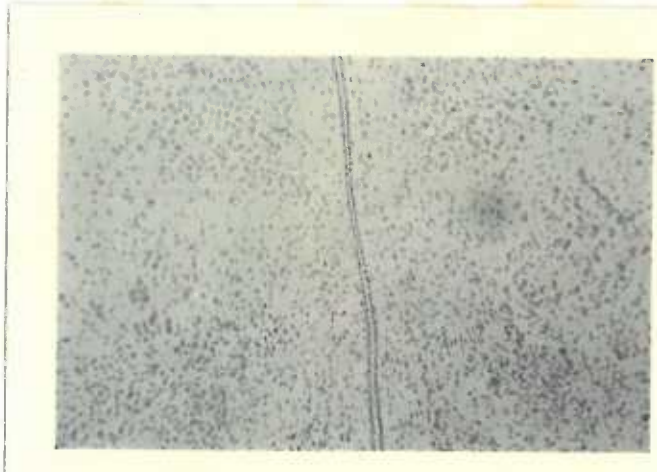


Figure 26

Figure 27



DISCUSSION

Surface Area as a Metabolic Reference Standard

Mention of surface area as a reference standard is conspicuously absent in the metabolic experiments described here. This is not the result of oversight. Surface area is probably the most widely used, abused, and misunderstood standard in clinical medicine and in biological research.

The choice of a suitable reference standard is essential to the interpretation of energy metabolism data and for comparing intra and interspecific metabolic rates. The standard used will often bias the interpretation of the experimental results. For example, the oxygen consumption of an obese animal will appear to be subnormal if expressed as a function of units of body weight.

Kleiber (77) has reviewed the numerous attempts to establish and justify methods to compute the body surface area. He expresses his alarm at the apparent lack of uniformity in methods of computing surface area as follows:

> In 10 papers (from 8 laboratories) studied for this review, metabolic rates of rats are expressed per unit of surface area. Four of the ten authors did not state how they measured or calculated this area. One multiplied the 2/3 power of body weight (in kg) by 7.42, another by 9.1, a third by 10, to calculate the surface area in square decimeters. One author multiplied the 3/5 power of body weight by 12.44, and two have calculated a new surface-weight relationship presumably by an intricate logarithmic interpolation between three older ones $7.42 \times W^{2/3}$, $11.36\ W^{2/3}$ and $12.44\ W^{3/5}$. That - for rat metabolism only - is this year's result of a century of surface law.

He further points out that the general acceptance of the formula of DuBois (51) for humans is probably related to the clinician's lack of time to develop new surface formulas for every new paper published. Miller (93) suggests that the concept of surface area is based on naive interpretations of the mechanisms of body heat loss and should be considered a meaningless reference standard. Miller and Blyth (94) point out an important inconsistency of the surface law in comparing the influence of altered body composition on basal metabolism. They hypothesized that the addition of fat should influence total oxygen consumption less than the addition of an equivalent mass of muscle although the increase in calculated surface area in the two cases would be the same. These authors considered a large part of the intraspecific variation in body composition to be due to differences in fat content. If this were the case, then the lean body mass (fat-free body mass) should be a more appropriate metabolic reference standard than either body weight or surface area.

They tested this hypothesis in humans by plotting the basal oxygen consumption as a function of body weight, surface area, and lean body mass. They calculated simple correlation, regression, and ninety per cent confidence intervals for each set of measures. Their results allowed for prediction of oxygen consumption from (1) lean body mass within $\pm$ 22 ml ($r = 0.92$), (2) from body weight within $\pm$ 31 ml ($r = 0.85$), and (3) from surface area within $\pm$ 32 ml ($r = 0.84$). They also showed that much of the validity of surface area and body weight is due to their high degree of correlation with lean body mass since these measures are not independent variables.

For these reasons the surface area has not been used as a metabolic reference standard in the research reported here. In its place the more meaningful lean body mass is used.

Lean Body Mass Determinations

The measurements of lean body mass which were used herein were based on the following observations.

Rathbun and Pace (102) found that a linear relationship between total body fat and body specific gravity existed for guinea pigs. They postulated such a relationship to exist also for humans and calculated equations for both guinea pig and man. The guinea pig equation is

$$\text{Per cent fat} = 100\left(\frac{5.135}{\text{sp.gr.}} - 4.694\right)$$

and that for the human was estimated to be

$$\text{Per cent fat} = 100\left(\frac{5.548}{\text{sp.gr.}} - 5.044\right)$$

Direct substitution into these equations of values for body specific gravity of guinea pigs and man obtained by special techniques, would yield values for total body fat. Pitts (100) has further extended these studies and investigated the composition and constancy of the various body compartments. Pitts and Hollifield (101) described a technique for estimating the fatness of the total body from measurements on the eviscerated carcasses of genetically obese mice and normal littermates. Methods for predicting the total body fat from specific gravity of normal or of GTG obese mice have not been reported in the literature.

Such a method has been developed and utilized in this investigation.

A linear relationship between total body specific gravity and per cent body fat has been demonstrated ($r = -0.99$) and a regression formula derived which allows for the calculation of fat-free body mass if the total body specific gravity is known (Table 7 and Figure 7). This information was used in the calculation of the drug dosages and for the studies on oxygen consumption.

It is interesting to note that the values for fat-free body mass obtained by direct extraction or by the formula derived here disagree with those obtained by using the guinea pig and human formulas of Rathbun and Pace (102). Use of the guinea pig formula for estimating per cent fat in mice would have resulted in an error of 9.16 per cent (based on the group means), and the human formula would have caused a 10.75 per cent error of estimation since their human formula was derived from extrapolation of their results in guinea pigs.

Metabolism Experiments

It has been shown herein that the fat-free body weight of obese mice is greater than that for non-obese (Table 8). In addition, the obese mice demonstrated an elevated total oxygen consumption. If, however, the oxygen consumption is recalculated in terms of equal units (grams) of fat-free body weight, then the variation disappears. This suggests that the obese mice consume more oxygen because they possess a greater amount of metabolically active tissue, such as muscle and adipose tissue, than the control mice. It does not, however, rule out the possibility that a part of the effect could be caused by an increased rate of oxygen utilization in the obese mice. However, no reports suggesting such metabolic abnormalities could be found in the literature. On the contrary, numerous reports (39)(40)(86)(118) suggest metabolic normalcy. The results obtained for GTG obese mice agree with those of Brooks and Marine (35) for rats with hypothalamic electrolytic lesions. Their rats showed a decrease in rate of oxygen utilization but "as they became obese the oxygen use per gram of body weight fell even farther below normal but the total oxygen consumption gradually rose and eventually exceeded the normal."

Further support for this suggestion of increased metabolically active tissue is found in the histological and gross anatomical observations reported here. It was found that the intestinal tracts of the obese mice were longer and heavier (Table 15) than those of the normal mice. Mayer (86) has also reported enlarged livers, kidneys, ovaries, and uteri in GTG obese mice.

Any attempt to compare the results of the metabolic studies in GTG mice to human measurements is difficult since much of the clinical data on metabolic rate associated with obesity are inconclusive. Results are usually expressed as a function of the calculated surface area and both decreases and increases in metabolic rate have been observed in obesity. Teppeman (112) states that the absolute oxygen consumption of a resting obese subject is greater than that of his lean control. Such was also the case in the GTG obese mice and their lean controls. Since GTG treated non-obese mice did not show the elevated oxygen consumption and since fasting, with resultant fat loss, resulted in a lowering of the basal oxygen consumption, it was concluded that the effect was the result of the obesity and not due to a drug idiosyncrasy. These animals, therefore, resemble clinical obesity in respect to comparative oxygen consumption between obese and non-obese subjects. Contrarily, genetically obese mice show a decreased total oxygen consumption (92).

It would have been desirable to know the respiratory quotient of these animals in order to evaluate in greater detail the metabolic aspects of GTG obesity. However, it is difficult to measure the small volumes of gases utilized and produced by these animals with the accuracy required for respiratory quotient determinations. The technique described herein was based on titrimetry. Mayer et al (82)(88) used "very elaborate" open-circuit trains for the simultaneous measurement of both oxygen utilization and carbon dioxide production in hereditarily obese and diabetic mice. They reported only their oxygen consumption results and

did not extend the technique to their GTG animals. When adequate techniques for simultaneous micro-determinations of both oxygen and carbon dioxide measurements become available, a precise respiratory quotient measurement will be possible.

Amphetamine Tolerance

It is interesting to note that Friedman, Weingarten, and Janowitz (57) observed tolerance to food intake suppression during 25 daily administrations of d-amphetamine (5 mg/kg) to mice. During the first two weeks they observed significant decreases in food intake with two "breakthrough" periods of enhanced feeding in both obese and control mice. After this time, the drug had little effect in either group. In contrast, the 15 mg/kg dosage used herein and administered every other day produced a consistent decrease in food intake in both groups (Figures 14 and 15), while only the obese showed a decreasing body weight over the three-month period during which this dose was given. The only suggestion of possible tolerance emanated from the observation that the 15 mg/kg dose produced an 18 per cent mean suppression of food intake in the chronic experiment whereas a 13 mg/kg dose caused a 25 per cent suppression in the acute experiment. The suggestion that frequency of administration may be involved in the development of tolerance is supported by the preceeding results and strengthened by Epsteins' (54) observation that rats with electrolytic lesions in the ventromedial hypothalamus and given amphetamine on alternate days did not acquire drug tolerance.

enhanced susceptibility is derived from the observation that weight loss occurred in the obese but not in the normal mice (Figures 14 and 15). Results reported by Altschuler and Lieberson (7) suggest that mature obese animals are capable of mobilizing free fatty acids more readily than non-obese animals. An explanation for this phenomenon remains to be determined.

The observation that tolerance occurred to locomotor activity but not to the anorexic and fat mobilizing (weight loss) response (Figure 18) suggests that these two actions of d-amphetamine are not necessarily produced by drug action at a single common receptor site. Table 1 shows that this drug is capable of producing a large number of responses. In the treatment of obesity, four actions are considered desirable; i.e., (1) suppression of appetite, (2) depression of hunger, (3) promotion of enhanced energy expenditure, and (4) mobilization of depot fats. All other responses would be regarded as "side-effects". The first three factors have been examined in the historical review. Regarding the fourth factor, recent studies have shown that pituitary hormones are capable of causing the mobilization of depot fats (18)(19). Astwood (18) has suggested that fatty acid release from and depletion of fat depots is promoted by the hormones of the adrenal medulla and by pituitary hormones such as corticotropin, the intermedins, thyrotropin, and growth hormone. Astwood, Barrett, and Friesen (19) have also identified other pituitary peptides which have been shown to be active in fat mobilization.* Whether or not the fat mobilizing property of

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*Li has also reported identifying a pituitary hormone with powerful fat-mobilizing properties. He calls it "lipotropin" and states that it consists of a single chain of 59 amino acids. This report was abstracted in Medical World News, April 10, 1964. 5, 104.

d-amphetamine is related to its ability to stimulate the release of one of these hormones directly or by a hypothalamo-hypophyseal mechanism, or by direct peripheral actions remains to be determined. It would seem probable that more than one site of action would be required to accomplish stimulation of energy expenditure, mobilization of depot fats, and depression of feeding behavior.

In the preliminary experiment on food and water consumption, it was observed that increased feeding was accompanied by increased drinking (Tables 5 and 6). Greer (62) showed that the hypothalamus possesses mechanisms for the regulation of drinking behavior in rats. However, the mechanism by which the interaction of feeding and drinking is accomplished is still unknown. Explanation of this phenomenon is made more complex by the observations of Grossman (65)(66) that the hypothalamic feeding mechanisms respond to locally injected adrenergic drugs while the drinking mechanisms are affected by cholinergic drugs. These observations are strengthened by the results of studies using adrenergic or cholinergic blocking agents (67).

Mechanism of Action of GTG

The preliminary observations reported herein that the hypothalamic ventromedial region appears to be normal in mice studied seven months after injection (Figure 27) suggests that further investigations into the mechanism of action of GTG in producing its hypothalamic effects are indicated. Possible support for this suggestion is derived from the recent investigations of Edelman, Livingston, and Schwartz (52) which indicate that the ventromedial area "does not appear to contain a dense population of specific high affinity glucose receptors for which glucose and gold thioglucose compete for attachment." Substances besides glucose appear to affect the hypothalamic mechanisms. Hervey (73) observed that experimentally produced hypothalamic hyperphagia in one rat of a parabiotic set is accompanied by inanition in the other animal. Subsequent lesions in the thin animal resulted in hyperphagia. Since glucose was not a variable in these experiments, Hervey suggested that the animals with normal hypothalami ate less due to a factor produced in response to the operated animals overfeeding, thus suggesting a feedback mechanism in the control of food intake. It will be interesting to see how these and future observations may affect the status of Mayer's glucostatic theory.

Evaluation of the Model

The title of this thesis would indicate that the researches reported herein are an attempt to compare GTG obesity to clinical obesity. In only a few instances could this comparison actually be made. It is a less difficult task to study the physiological, biochemical, and behavioral characteristics of obesity in mice than in man. Human investigations are complicated by a very difficult to control variable - the psyche. This factor by itself has precluded the treatment of many obese patients. It can markedly affect the interpretation of metabolic data and grossly alter feeding patterns. Until adequately controlled, it will be difficult to perform experiments designed to contribute to an understanding of the basic alterations of normal function resulting in obesity. However, it has been shown here that GTG obesity possesses metabolic and behavioral characteristics which are similar to those currently available from studies on human obesity. For these reasons, it would seem justifiable to propose that in spite of the lacking human psychic component, GTG-induced experimental obesity would be a useful experimental tool for the screening and evaluation of new drugs for the prophylaxis and treatment of clinical obesity.

SUMMARY AND CONCLUSIONS

1. Obesity has been produced in mice by the injection of gold thioglucose (GTG) and the resultant alterations in feeding and drinking behavior have been studied. These animals have demonstrated a marked elevation in quantities of food consumed and an associated, but not proportional, rise in water consumption.

2. A method for the indirect determination of fat-free body mass has been established and used for metabolic studies on normal and obese mice.

3. A decreased total food consumption, a two-hour depression of normal feeding behavior, a very significant (specifically defined) elevation in locomotor activity, and a decreased body weight (in obese animals only) have been observed following chronic administration of d-amphetamine to mice housed in controlled feeding cages. When the drug was administered on alternate days for 55 injections, no signs of drug tolerance were observed.

4. When compared to the effects of human treatment, the experimental animals responded similarly in regard to feeding behavior when injected acutely with d-amphetamine, phenmetrazine, phendimetrazine, and methylphenidate.

5. An elevated total basal oxygen consumption has been demonstrated for GTG obese mice. This variation disappears upon recalculation in terms of units of fat-free body weight.

6. GTG obese mice were observed to have slightly longer and much heavier (histologically examined) intestinal tracts.

7. The lean body mass (fat-free body mass) of GTG obese mice was found to be significantly greater than that of non-obese.

8. It is proposed that this type of experimental obesity may be a useful tool for the screening and evaluation of the properties of new anti-obesity drugs.

APPENDIX

TABLE 16

FOOD INTAKE RECORDS FOR INDIVIDUAL ANIMALS AFTER d-AMPHETAMINE (5 mg/kg)

Injection No.	1	2	3	4	5	6	7	8	9	10
Animal No.										
1A	3.80	3.08	3.98	3.83	4.52	5.81	4.83	3.79	3.47	4.08
2A	3.59	3.85	3.46	4.36	4.18	4.30	3.96	4.51	3.89	5.47
3A	4.23	3.33	4.18	4.38	4.19	4.81	4.43	4.96	4.77	4.77
5A	3.98	3.44	3.89	4.47	4.90	5.22	3.03	4.93	3.81	5.46
6A	4.22	3.23	4.36	4.50	5.57	5.49	4.73	6.13	5.43	4.38
7A	5.21	4.26	4.09	5.77	5.39	4.41	5.24	4.37	4.70	5.35
8A	3.56	3.45	3.18	3.24	3.97	4.16	4.83	4.66	5.00	4.32
1B	5.62	6.03	5.30	6.25	6.00	6.40	5.53	5.31	5.23	6.24
2B	4.39	3.79	4.87	6.00	5.78	5.53	5.59	5.39	5.29	6.26
3B	5.25	4.43	4.40	5.17	5.21	5.08	5.69		4.40	5.53
4B	5.90	6.16	5.58	6.04	5.92	6.61	6.75	6.14	6.71	6.16
5B	4.64	6.33	6.43	4.77	6.00	5.49	5.88	4.69	5.93	6.27
6B	5.31	5.41	6.80	5.94	5.97	2.94	3.52	3.38	3.90	6.41
7B	4.44	6.08	5.78	5.94	5.83	6.41	4.89	6.75	5.03	6.05
8B	6.05	7.52	7.17	7.55	7.09	6.97	7.39	7.53	7.23	6.81

TABLE 17

FOOD INTAKE RECORDS FOR INDIVIDUAL ANIMALS AFTER d-AMPHETAMINE (10 mg/kg)

Injection No.	1	2	3	4	5	6	7	8	9	10
Animal No.										
1A	2.68	3.36	2.94	4.25	3.35	3.23	3.02	3.79	4.21	3.82
2A	3.82	4.06	4.43	4.04	4.21	3.71	4.09	3.88	3.18	3.15
3A	1.80	3.27	4.49	4.67	4.09	4.12	5.19	4.39	4.38	3.56
5A	2.91	4.23	3.89	4.19	4.29	4.40	3.34	3.64	4.40	4.45
6A	4.20	3.95	3.91	4.78	4.37	4.39	5.33	5.21	5.18	3.87
7A	5.37	4.96	5.22	5.92	5.42	3.61	4.86	5.99	5.21	5.37
8A	1.29	3.16	2.85	4.80	4.25	3.44	3.75	3.90	4.45	4.27
1B	4.19	2.65	5.34	5.43	5.66	5.54	5.06	6.01	4.88	5.31
2B	3.67	4.79	4.81	4.86	5.52	5.27	5.59			
3B	3.61	4.22	4.68	5.11	5.69	4.61	5.26	4.96	5.78	4.51
4B	4.61	6.20	5.47	5.78	6.06	5.02	4.57	5.82	6.00	4.79
5B	2.37	4.75	4.81	5.32	4.29	3.23	6.27	5.77	5.45	5.51
6B	4.76	5.57	7.04	5.52	5.79	5.32	4.65	4.91	5.43	5.89
7B	4.02	5.29	2.72	4.65	6.59	5.93	6.02	5.13	6.03	6.31
8B	5.59	6.15	6.90	7.30	6.22	6.22	7.16	5.70	5.98	6.74

TABLE 18

FOOD INTAKE RECORDS FOR INDIVIDUAL ANIMALS AFTER d-AMPHETAMINE (15 mg/kg)

Injection No.	1	2	3	4	5	6	7	8	9	10
Animal No.										
1A	2.60	3.24	3.57	2.01	3.23	4.72	2.35	3.67	2.42	2.46
2A	3.81	3.83	3.34	2.97	2.57	2.77	2.36	3.35	2.52	2.13
3A	3.41	4.10	4.82	4.35		2.05	3.11	3.63	2.93	3.33
5A	3.71	3.42	4.72	4.05	3.49	2.98	4.60	2.99	2.35	3.74
6A	2.39	4.56	4.05	3.26	4.23	4.94	5.30	3.97	2.92	6.15
7A	5.09	5.01	5.02	6.74	4.25	5.50	3.64	4.01	3.86	3.27
8A	4.91	5.04	4.83	3.91	3.07	4.11	4.29	4.09	3.81	3.05
1B	5.03	5.06	2.68	5.76	4.34	3.42	2.24	4.24	2.83	2.57
3B	4.46	4.82	4.00	4.42	4.96	4.51	4.30	5.03	2.67	2.94
4B	5.30	6.08	6.09	4.98	5.14	5.63	6.20	6.28	5.36	4.69
5B	4.96	4.51	3.12	4.22	4.38	2.95	4.00	5.48	1.90	3.08
6B	6.25	7.14	5.32	4.52	6.29	5.91	6.50	6.65	1.35	3.69
7B	5.92	5.28	5.01	5.07	4.81	6.53	4.98	5.53	6.79	6.12
8B	5.64	6.11	5.46							

TABLE 19

FOOD INTAKE RECORDS FOR INDIVIDUAL ANIMALS AFTER d-AMPHETAMINE (15 mg/kg)
(2nd 10 DAYS)

Injection No.	11	12	13	14	15	16	17	18	19	20
Animal No.										
1A	2.80	5.38	4.05	3.89	3.82	4.34	4.01	4.71	3.62	4.52
2A	4.26	5.23	3.62	4.46	3.52	3.24	2.89	3.88	4.83	4.85
3A	4.65	5.74	3.53	4.11	4.31	3.36	4.74	4.08	4.42	4.09
5A	4.39	5.41	3.54	4.26	5.34	3.16	2.99	3.79	3.40	
6A	3.67	4.91	4.19	4.78	4.34	5.19	4.26	3.40	5.41	3.76
7A	4.06	6.17	4.54		4.50	2.93	4.89	4.72	5.76	5.00
8A	3.68	4.10	4.67	3.86	3.39	4.23	4.65	4.33	5.81	4.66
1B	4.18	3.50	4.67	5.73	5.34	5.43	4.39	2.86	4.83	4.18
3B	4.40	3.82	3.65	3.38	2.90	4.95	3.26	2.72	3.77	5.20
4B	4.74	6.35	3.50	2.93	4.69	5.77	3.21	4.55	5.25	6.28
5B	6.90	4.07	3.63	4.64	4.61	6.51	3.44	5.01	6.28	5.47
6B	4.98	4.22	4.61	5.67	5.62	5.13	4.07		5.93	6.43
7B	5.82	5.99	5.91	5.69	5.95	6.85	6.31	7.71	6.89	7.57

TABLE 20

FOOD INTAKE RECORDS FOR INDIVIDUAL ANIMALS AFTER d-AMPHETAMINE (15 mg/kg)
(3rd 10 DAYS)

Injection No.	21	22	23	24	25	26	27	28	29	30
Animal No.										
1A	4.28	2.31	3.67	3.57	4.07	2.51	3.30	3.81	2.82	5.41
2A	4.57	3.48	4.85	4.14	4.65	4.16	3.90	4.53	3.89	3.79
3A	4.68	4.12	3.29	4.12	3.81	3.12	3.63	4.06	2.30	3.91
5A	3.96	4.63	5.40	3.45	3.31	4.34	4.35	4.82	4.34	5.25
6A	5.23	4.62	4.31	3.74	2.80	4.01	4.86	4.66	3.89	5.00
7A	4.69	3.17	4.39	2.21	5.26	4.76	4.36	3.01	4.26	4.17
8A	4.60	4.18	3.66	4.12	4.25	3.53	4.02	3.95	4.24	4.82
1B	5.08	5.21	4.65	4.73	3.86	2.99	2.80	2.93	3.54	4.88
3B	3.69	3.18	4.26	3.21	3.40	2.94	3.46	2.85	3.82	3.81
4B	4.90	5.33	6.24	6.13	5.43	4.10	3.93	5.56	6.03	4.29
5B	5.44	3.03	5.28	4.77	2.96	5.55	3.51	3.61	5.21	4.78
6B	5.44	3.38	5.55	3.39	5.93	3.81	3.74	5.11	6.70	4.93
7B	7.14	6.14	6.77	5.97	6.05	5.34	6.17	5.65	6.75	3.01

TABLE 21

FOOD INTAKE RECORDS FOR INDIVIDUAL ANIMALS AFTER d-AMPHETAMINE (15 mg/kg)
(LAST 5 DAYS)

Injection No.	31	32	33	34	35
Animal No.					
1A	2.38	3.09		3.27	2.29
2A	3.32	3.39	3.86	3.23	3.63
3A	1.93	3.57		4.31	3.57
5A	2.97	4.29	4.20	3.96	3.80
6A	2.85	2.35	1.57	5.24	3.41
7A	4.18	2.98	3.58	3.82	5.53
8A	4.94	4.37	4.28	4.04	3.75
1B	2.99	3.71	3.41	3.14	2.83
3B	3.83	4.19	3.43	4.62	3.75
4B	3.39	4.05	5.22	4.84	4.29
5B		3.05	4.50	5.09	4.81
6B	3.04	4.55	3.78	3.27	5.49
7B	6.78	6.03	8.32	4.19	6.03

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