THE USE OF PARABIOSIS IN THE STUDY OF FOOD INTAKE REGULATION AND BODY FAT CONTENT

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ABSTRACT

THE USE OF PARABIOSIS IN THE
STUDY OF FOOD INTAKE REGULATION
AND BODY FAT CONTENT

By

Nathan William Shier

A technique has been developed which makes it possible to determine the individual body weights of rats joined in parabiosis. In using parabiotic animals for dietary experimentation separate weight determinations of each parabiont without pair separation is necessary. Flat balance pans, attached to each of two dietetic scales, were brought to close approximation. Parabiotic rats were placed dorsally on the scales with the connection between them situated between and parallel to the balance pans. The difference between balance readings or the uncorrected weight difference was ΔA. The actual weight difference between the two animals was predicted by projecting ΔA on a standard curve. With the total weight of the parabiotic pair and the predicted weight difference of the parabionts, two equations with two unknowns were solved which gave the weights of the individual parabionts to within $\pm$ 3% of their actual weights. In developing the standard curve, giving weight differences of parabionts, forty pairs of rats with a variety of weight differences (ranging from 2 to 327 grams) and ratios were placed on the scales, as described above, and ΔA recorded. The animals in each pair were then separated and weighed individually with

the actual differences in their weights recorded as ΔB. A linear function was obtained when each ΔB was plotted as a function of its respective ΔA ($r = 0.982$).

Parabiosis, the union of two living organisms, has been used in studying the hormonal control of food and water intake. These studies are difficult to interpret because the investigator had no way of feeding separate diets to each parabiont. A feeder has now been developed which permits feeding each parabiotic animal a separate diet. This feeder and its use are described. Only a few days of training are required to get the parabiotic rats accustomed to proper use of the feeder. To check whether one rat can consume any of the other's ration, a chromium sesquioxide marker was placed in the food of one parabiont and the feces of the other assayed for chromium oxide by acid digestion and spectrophotometry. No chromium oxide was found in the excreta of the parabiont whose feed had not been marked. This feeder allows, without restraint, ad libitum food consumption in parabiotic rats with excellent weight gains and food consumption even when used continuously.

An improved scapular supportive suture has been developed giving excellent support at the pectoral girdle with virtually no scapular separation which has been a major problem in previous parabiotic experiments.

Dye dilution studies with Evans Blue indicated an improved rate of cross circulation which could have been produced by the scapular supportive suture described above. If soft tissue sutures are physically

stressed, healing will be delayed and cross circulation impaired. With increased rates of cross circulation, improved hormonal responses should be evident in parabiosis especially with hormones or metabolites quickly cleared from the circulation after crossing from one parabiont to the other.

The primary causes of death in parabiotic animals are rejection (32.88%), infection (13.25%) and pair separation (8.27%) out of a total sample size of 292 pairs. Graft success was greatly improved by using female-female pairs as opposed to males (42.80% vs. 30.40% respectively). Rejection was lowest in littermates as opposed to non-littermates. No diagnosed cases of rejection were found in Osborne-Mendel female littermates.

Parabiotic rats do not appear different, physiologically or anatomically, as compared to sham operated single animals except for a shorter nose to anus length, decreased body fat content and reduced body weight. Some male parabiotic animals did have reduced adrenal vitamin C content indicative of "stress" which may alter experimental variables making it advisable to index "stress" in all parabiotic experiments.

When both animals in a pair are fed a grain diet and compared to single sham operated controls on the same diet, increased body weight gain (though not statistically significant) and food efficiency but decreased body fat as a percentage of body weight is evident in the parabionts. All fat depots removed from parabiotic animals fed a grain ration (high carbohydrate diet) were smaller than single grain fed controls.

In parabiotic pairs where both rats were fed a high fat diet, compared to single high fat fed controls, parabionts ate fewer calories, were less efficient in converting food to body tissue, had markedly less body fat with lower fat depot weights, and had a lower rate of body weight gain. Parabionts seem more capable of "handling" increased fat in their diet without weight gain than single animals.

In pairs where one animal was fed high fat while its partner was consuming grain, animals fed high fat did not become obese, whereas, the grain fed animals lost weight. The animals fed high fat diets exhibited a similar caloric hyperphagia compared to single animals fed high fat (actually, consuming significantly more kilocalories than either single or parabiotic high fat fed controls) but, in contrast, were markedly less efficient than either control group. Compared to parabiotic high fat fed controls, high fat fed animals cross circulating with grain fed partners had lower body fat but the difference was not significant. All fat depot weights in high fat fed parabionts cross circulating with grain fed animals were statistically lower than weights recorded for single high fat fed animals. All depot weights for the high fat fed parabionts cross circulating with grain fed partners were also lower than all depot weights for control, high fat fed parabionts but no differences were significant. Body weight gain in high fat fed parabionts cross circulating with grain fed rats was depressed significantly as compared to single high fat fed animals as well as control high fat fed parabionts.

Grain fed parabionts, cross circulating with animals fed high fat,

became thinner than parabionts cross circulating with a grain fed partner. Food intake was markedly decreased in these animals as was food efficiency compared to either single or parabiotic grain controls. Body fat, body weight gain and all fat depot weights in grain fed animals cross circulating with high fat fed animals were all markedly depressed when compared to single grain fed controls. When these same values were compared to parabiotic control grain fed rats, there were no significant differences but all values for the grain fed rats cross circulating with the high fat fed were lower.

Possibly, circulatory "factors" crossing over from the high fat fed animal are depressing food intake in the grain fed animal to levels below those in parabionts where both are fed grain.

Since methoxyflurane inhalation anesthetic was used in some experiments in which heart blood was taken for insulin assay, it became necessary to determine the effects of this anesthetic on serum free fatty acids, serum glucose, adrenal weight and adrenal ascorbic acid. All of the above parameters were statistically the same between animals treated with methoxyflurane and those that were not.

Male parabionts were differentially fed as previously reported for female animals. Adrenal ascorbic acid concentration was similar for parabionts fed in the differential feeder as for single control animals; consequently, stress was not considered a major complicating factor in food intake responses.

Food intake of grain fed male parabionts cross circulating with high fat fed partners was markedly depressed as reported for female rats.

These grain fed parabionts cross circulating with high fat fed animals exhibited normal post-prandial blood levels of urea, glucose, free fatty acids and insulin even with the suppressed food intake. Possibly an anorexigenic factor (glucose, free fatty acids, or a substance liberated from the gastro-intestinal tract in response to the presence of fat) was crossing over from the high fat fed animal to the grain fed rat and producing a suppression of feed intake. A certain post-prandial blood metabolic "pattern" may serve to inhibit intake. The grain fed rat in the differentially fed pair established this pattern by receiving metabolites from its high fat fed partner rather than from its own diet; consequently, when the pattern was established, intake was suppressed.

Appetite suppressive factors may be involved, decreasing the feed intake of the grain fed rats parabiosed to high fat fed rats below the intake levels for either single or parabiotic grain fed controls, that are not calorigenic. This assumption is made on the fact that if the compound "crossing over" provided calories and reduced caloric intake on a one to one basis, body weight and body fat content should have been the same as in the grain-grain fed parabionts.

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INTRODUCTION

Parabiosis, the union of two living organisms (302), has been used extensively for over 100 years in the study of humoral factors. Parabiotic rats can be described as a whole body tissue graft with blood exchanged between the members of a pair (304). An experimental variable is imposed on one member of a pair with adaptative responses measured in its partner. If changes do occur, it can be concluded that blood factors were important in the response.

Parabiosis has not been used widely in the nutritional sciences since obtaining separate weights of members of a pair, without separation, and feeding parabionts separate diets offered problems.

With the development of the above procedures in addition to improved surgical techniques and with a greater knowledge as to how the parabiotic state, per se, affects physiology and anatomy of rats, it became possible to use parabiotic rats, effectively, in the study of the regulation of food intake and body fat content.

There is control over the ingestion of foodstuffs and the study of food intake regulation is an extremely complex and multi-faceted problem. In order to function efficiently as a physiological control mechanism, control of food intake must have many interwoven systems.

One can pose the question: Does food intake determine body energy balance or does body energy balance determine food intake? Food intake is just one system of many employed by the organism in maintaining energy homeostasis.

Actually, body weight and fatness are controlled far more precisely than food intake (1). This means that the organism makes physiological adjustments to compensate for a surfeit or deficit of calorie intake as a result of the less efficient regulation of food intake.

The whole system can be viewed in terms of coarse and fine adjustment. What is acutally regulated or the goal of regulation in the adult animal is to maintain body energy constant. Food intake brings into the body energy with a somewhat independent and coarse control. This energy is then regulated by the body with a fine adjustment in the most efficient manner considering the physiological and environmental conditions under which the animal is living. Food intake has an independent component of regulation apart from body weight regulation and vice versa but the two must be interrelated.

When one considers any control system, a natural categorization can be made: the environment and the organism. Factors in the environment can greatly affect an organism's energy balance. Availability of food, nature of the food, condition of the animal, e.g., a broken leg, air temperature (2), wind velocity (2), humidity (2) (A vapor pressure gradient is necessary for the animal to cool evaporatively

by panting or eccrine sweat gland activity.) solar radiation (2), light cycles (2), altitude (3), water availability (4,5), alterations in the food chain and levels of certain environmental elements or pollutants such as fluorides (6-8) and tannins (9) can greatly alter an animal's energy balance and food intake. The organism, whether plant or animal, compensates by a variety of mechanisms. The external morphology can be modified or the organism can move or evoke many methods of energy acquisition or dissipation.

For the present discussion it will be assumed that the organism is animal and is living in a rather stable environment to which it has acclimatized. Discussion will be focused on what actually initiates or terminates the ingestion of food as one major component of energy balance.

In terms of energy balance, one can break down the animal kingdom into poikilotherm or homeotherm. Poikilothermic food intake is affected by sex, age and season of the year as is homeothermic intake but, within limits, poikilothermic food intake varies directly with the environmental temperature unlike that seen in warm blooded animals (10). This is probably a result of the direct relationship between "deep core body temperature" of the poikilotherm and environmental temperature. Warm blooded animals can be classified, in terms of food intake regulation, as ruminant or monogastric.

Food intake regulation is considerably different between the ruminant and monogastric because of the importance of the "volatile fatty acids" and the relatively non-involvement of glucose. In lactating ewes, blood butyrate is negatively correlated and blood free fatty acid levels, positively correlated to subsequent food intake

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In terms of energy balance, one can break down the animal kingdom into poikilotherm or homeotherm. Poikilothermic food intake is affected by sex, age and season of the year as is homeothermic intake but, within limits, poikilothermic food intake varies directly with the environmental temperature unlike that seen in warm blooded animals (10). This is probably a result of the direct relationship between "deep core body temperature" of the poikilotherm and environmental temperature. Warm blooded animals can be classified, in terms of food intake regulation, as ruminant or monogastric.

Food intake regulation is considerably different between the ruminant and monogastric because of the importance of the "volatile fatty acids" and the relatively non-involvement of glucose. In lactating ewes, blood butyrate is negatively correlated and blood free fatty acid levels, positively correlated to subsequent food intake

during the first half hour of a three hour feeding period. The lower blood butyrate levels, the higher intake and the higher free fatty acid levels, the higher intake. Blood glucose was not correlated to intake (11). Intake has also been decreased by acetic acid or acetate intravenous infusion in cows (12). Glucosensitive and acetate sensitive areas have not been found in the ruminant brain (13) and hypothalamic temperatures have not been correlated to intake (14). Cortisol acetate, given intramuscularly at 25 mg/day, increased food intake in sheep and increased blood glucose, while total glucose utilization was not reduced. There was an impairment of glucose utilization relative to blood glucose (15). There should have been a greater blood glucose utilization as a result of the absolute blood glucose level.

In warm blooded, monogastric animals most work has concentrated on the human, dog, cat, monkey, rat and mouse. There obviously are enormous species variations in size, surface covering, basal metabolism, anatomy, physiology, genetics, etc., but much valuable information can be obtained on animals other than man using experimental techniques that cannot be performed on humans. Large variations in food intake also exist between male and female and the physiological state of the organism, i.e., age, pregnancy, lactation and work load. In humans, there is also the strong esthetic, psychological drive associated with food (16).

Human subjects must be used in studying the psychological component to the regulation of food intake. The counterparts of these investigations in animals are classified as behavioral or motivational. Using human subjects, it would be possible to answer such questions as

these: Does a primary disorder in hunger cause abnormalities in food intake? or Can disorders in food intake exist with normal sensations of hunger? (19) The overweight person does not recognize a point of satiety or even displeasure in eating as readily as a lean individual. "Full" and "not hungry" have different meanings to the overweight (17). Oralpharyngeal sensations in humans as well as animals seem to be potent regulators of behavior related to food ingestion (18). One can measure behavior or motivation in animals but it is hard to determine if this represents a change in hunger sensation.

In man it may be difficult to determine if basic organic illness creates psychological distress or if psychological illness is primary to an impairment in food intake regulation. In some societies purely psychological factors such as a fear of becoming fat can cause individuals to greatly curtail intake when there is actually no need to do so. Social pressures as to how a society measures beauty create strong motivational stimuli to behavior (20). Psychological misperceptions of hunger can also develop in the youngster resulting from "misperception" by the mother. The mother tries to abate many emotional needs in the child by offering food when the emotionalism is not associated with food. The food, though, does help the situation and, therefore, the conditioned child turns to food for future emotional needs (21).

A major component of the food intake regulation in any species of animal, e.g., the rat, involves the general behavior that the animal displays towards food. The total amount of food an animal consumes per 24 hours is important but equally as important are the amounts of food eaten per "meal" and the frequency of meals which establishes a

recognizable feeding behavioral pattern.

An experimental manipulation may produce a decrease in food intake but, if another variable is also altered, it is not clear if the second alteration is a result of food intake depression or a primary effect of the experimental variable. To answer this question a second group of animals are fed the reduced intake (pair feeding) without the experimental variable. If the second alteration still exists then the effect is said to be a primary result of a decrease in food intake. This type of conclusion can be misleading because even though the amount of food was pair fed, the pattern of consumption may still be quite different. Correcting for the pattern of intake, as well as the amount, gave strikingly different results when rats were fed diets containing high levels of fluoride compared to correction for amount only (7).

Techniques are available allowing accurate monitoring of the pattern of intake as well as offering food at preferred levels and frequencies (22, 23). Spillage is a problem in these devices. Animals may visit the food cup to play or spill the food. If this happens, the animal is usually omitted from the data. Even though food is offered at a particular frequency one can still not be sure that the animal will eat all of the food that is offered at a certain time in a distinct pattern.

Food is consumed with irregular intervals, but nutrients are probably more uniformly available to the organism since the gut serves as a food reservoir. Food ingestion in rats is about 50% higher at night due to larger meals with the frequency of meals being the same for day and night. Frequency seems to be highly fixed with small variations in 24 hour intakes caused by variation in meal size.

Changes in intake when the palatability of the diet is altered are once again alterations in meal size and not frequency. The number of meals per day will vary greatly between species and between individuals within a species. For rats, the average is around 8.5 meals per day. Amounts eaten in a meal vary considerably from meal to meal (24).

When the caloric density of the diet is altered, frequency is changed for about seven days and then the sustained increase or decrease of intake will be achieved in meal size with frequency constant (24).

Food intake regulation, therefore, may involve a regulation of meal size as well as meal frequency all intricately involved in the maintenance of body energy.

The size of a meal is not correlated to the time elapsed preceding the meal since the last intake but is correlated to the time subsequent to the meal until eating is initiated again. This type of eating behavior has been called "provisional appetite." The animal eats to satisfy its subsequent energy demand (24).

It can be reasoned that if one meal size affects the size of a subsequent meal, a very precarious balance would exist. Once meal size has been established and, if meal size controls subsequent meals, then to change meal size in order to regulate calorie intake would be very difficult. Meal size correlated to subsequent time lapse is a far more labile system.

Physiological and anatomical factors regulating intake have received perhaps the bulk of experimental effort. The surface area, shape and surface covering and the ability to change these parameters are all important in energy balance (2).

As would be expected, food intake is affected by a previous food deprivation. Cicala and Bare, (25) found that deprivation up to 24 hours failed to increase total consumption during a subsequent 24 hour feeding period although during the first hour of refeeding, intake increased as the length of deprivation increased. The lack of enhanced intake during the 24 hour feeding period was attributed to the dominance of the day, night feeding cycle. Le Magnen and Tallon, (26) obtained similar results except that after 48 hours of deprivation an increase in consumption occurred for approximately 3 days. The influences of deprivation on food intake are greatly modified by the absence or presence of water (27). When rats are kept under constant illumination and tested with a 3 hour feeding schedule, intake increases up to 16 hours of deprivation and then is constant even up to 96 hours (28). With human, obese patients an increase in the "satiety response" was demonstrated after fasting. These patients did not feel hungry or experience an increased appetite during fasting and were satisfied with significantly smaller amounts of food when fed after the fast (29).

Food intake can be altered by changing the caloric density of the diet (24). These investigations produce convincing evidence that amimals do regulate their food intake in response to calories. Since changes in intake are not produced immediately, one must consider changes in body energy as a component in the regulatory response. The one major labile energy source of the body is the adipose tissue. The first major study showing compensatory increase in diet intake with diet dilution was that of Adolph, (30). A major complicating factor in diluted diets is the capacity of the gastrointestinal tract.

This may ultimately determine the upper limit of intake or dilution (31). An additional review concerning the gastrointestinal tract will be forth coming in this review (page 18).

A change in weight of food consumed in the rat may take 24 hours or longer (usually longer) in response to change in calorie density (32) and may take weeks in the dog (33). When rats are fed a high fat diet, several days are required before calorie intake approximates the previous low calorie baseline. All of these factors indicate that the oral ingestion of food is not that sensitive to changes in diet caloric density but rather a change is enacted when some physiological control mechanism within the organism is activated. Irrespective of calories, rats also respond to a dilution of only the protein fraction of the diet (34). In this manner animals can be made to consume an excess of calories. Most evidence indicates that low protein diets suppress intakes. It can be concluded that oral ingestion is controlled for meal size as well as frequency and that there are long range control mechanisms in response to a surfeit or deficit of body energy stores. This regulation involves either the nervous or endocrine systems.

Several brain areas have been studied as components in the reflex arc involved in the control of food intake. The dorsal dentate-hippocampal connections and the hippocampus are implicated in appetitive conditioning. Dentate units augment to a conditioned stimulus resulting in food but responds by inhibition if the reward is electric shock (35).

Preoptic lesions do not affect food intake even when the animal's core temperature is elevated (36). Septal lesions in rats increased not only food intake but also a non-reinforced visual discrimination

task which indicates an increased or enhanced behavioral motivation towards food (37). Bilateral amygdaloid lesions decreased such a response whether reinforced or not (37, 38). In cats amygdalar inhibitory areas (the basal parvocellular nucleus) and facilitatory areas (the anteromedial area) on food intake have been identified (39). It would appear that the facilitatory action is dominant since bilateral lesions of the amygdala results in a net inhibition of intake whereas in dogs bilateral lesions of just the dorsomedial area produced aphagia and a decreased motivation (40). In monkeys lesions in the midbrain reticular formation produce hyperphagia but caudal medullary lesions produce hypophagia (41). The entire limbic-midbrain circuit, reticular formation and hypothalamus seem to be integrally involved in the control of food intake (42-45).

From the above examples one can conclude that there are several brain areas, extra-hypothalamic, when stimulated either cause an augmentation or inhibition of food intake. The brain circuitry involved in food intake regulation is very extensive reaching from the "primitive" brain stem to the cerebral cortex. The hypothalamus is only a small part of this system and the importance of the hypothalamic areas as initiators of food intake or satiety is extremely doubtful.

Probably the two brain areas receiving the most attention in food intake regulation are the lateral and ventromedial areas of the hypothalamus. These areas were the first discovered to be implicated in the control of food intake and, unfortunately, perhaps for this reason have remained in the foreground in neurological investigation.

Many nerve fiber systems run through the lateral hypothalamic areas that are associated with such diverse functions as rage, fear,

sexual manifestation, sleep, feeding and drinking (45). When rats are lesioned in this area an aphagia and adipsia results (46). Aphagic animals, though, continued to press a lever for food which they could not eat and when the food was infected intragastrically, through chronically implanted canulas, rats fed themselves by lever pressing and regulated intake well. The rats did not eat by mouth due to a "motor" failure but the sensation or drive for food was intact. Deprivation was sensed and alleviated (47, 48). Recovery from motor failure takes far longer when laterally lesioned animals are fed intragastrically (18). Impairment of conditioned reflexes have also been reported after lateral lesions with an aversion towards foods containing large amounts of water (49).

Many papers have described the voracious appetite of animals lesioned in the ventromedial nuclear area. Food intake is doubled over normal controls and gradually decreases back to normal. The syndrome is termed "dynamic" during increased food intake and weight gain and "static" when weight stabilizes and intake has returned to normal (50-52). Young, immature rats, even after starvation, do not show this syndrome after ventromedial lesions, but somatic growth may be impaired with the production of obesity (53, 54) associated with low pituitary and plasma growth hormone (55). Lactating rats also do not show hyperphagia after ventromedial lesions (53).

Adult, lesioned rats will respond to nutrient dilution even faster than normal young rats during the "dynamic" phase but this type of response to caloric dilution is lost in the static phase. Older, "normal" rats with excess fat respond similarly to nutrient dilution as static, hypothalamic rats (52). Ventromedially lesioned rats also

display a hypokinesis and a decreased basal energy production (51). The primary cause of the obesity is usually considered to be increased food intake, although, some authors believe it to be the hypokinesis (56).

Sometimes in ventromedially lesioned rats the intake is not increased or if hyperphagia does exist the more palatable diets are consumed more readily (57). Lesioned animals, pair fed to controls, still gain more weight but the increase is slight. Their basal oxygen consumption is lower with no change in fasting respiratory quotient, but display a respiratory quotient above controls post-absorptive. Creatinine excretion is 30% higher. There is a decreased insulin sensitivity and, when body weight reaches 550 grams or higher, an abnormal estrus may occur. If these animals are fasted to reduce weight or are pair fed, the estrus is normal (58). Hypothalamic rats will still gain weight even when intake is decreasing (59) and display normal fasting gastric hunger contractions (60). Ad libitum food intake patterns are the same in hypothalamic animals as controls in that the meal size is related to the interval after the meal and intragastric infusion decreases oral intake and increases the post-prandial interval (61). In hypothalamic rats body calcium, phosphorus and iron are somewhat depleted (62) and these rats are more responsive to diet palatability (52, 63). In hypothalamic monkeys there is a marked hyperglycemia and glycosuria (64, 65) and an impairment in temperature regulation to moderate cold with obesity occurring, on occasion, without hyperphagia (66).

Obesity has been produced in birds by ventromedial lesions but the fat accumulation was not as pronounced as in mammals (67) and aphagia has been produced by bilateral lesions in the lateral

hypothalamic areas (68).

The ventromedial area does seem to have an inhibitory effect on the lateral areas as shown by electrophysiological studies (69). Anatomical connections between these two brain areas were demonstrated by Arees and Mayer, (70). The ventromedial area also has functional connections with the amygdalar complex (71).

Several studies have cast a doubt on the primary importance of these hypothalamic areas in controlling food intake from a motivational or behavioral approach. Rats after recovery from ventromedial or lateral lesions still are able to regulate ingestive behavior and control body weight (72). It must be remembered, though, that the body weight may be at a different level after the lesion than before. Rats lesioned in the ventromedial hypothalamus still responded normally to intragastric loads by decreasing oral intake (73) and during the "dynamic" phase rats fed themselves intragastically by lever pressing and controlled food intake and body weight very well (18).

One may also question the fact that lesioned obese animals do not represent, functionally, the type of obesity that occurs naturally. Zucker genetically obese rats were compared to lesioned animals for similarity of various parameters. Both types of animals exhibit hyperphagia (74) but lesioned animals in the "static" phase do not respond to caloric dilution by increasing intake (75), whereas, Zucker rats do with a 20% cellulose dilution but compensate only partially for a 50% dilution (74). Lesioned animals do not decrease intake on a high fat diet (76) but the Zucker rats respond by compensating at 8% fat but not at 60% (74). Zucker rats respond differently to cold by not augmenting circulating free fatty acids, by not increasing the

release of radioactivity from the thyroid and by not regulating body temperature (77) as do lesioned rats and lean controls. However, ventromedially lesioned monkeys have difficulty in maintaining body temperature in response to cold (66). d-amphetamine produces a smaller depression in food intake in Zucker rats than in either lesioned rats or normal animals (74). Zucker rats consume more water than lean or lesioned rats (78). Estrus seems to be abnormal in the Zucker rats (74) as has been shown for lesioned animals (58). One can conclude that there are many similarities but also some differences and it seems reasonable to say that the genetic Zucker rats may have some impairment of the ventromedial area of the hypothalamus. In interpreting lesion experiments the sex (79) as well as the age (80) of the animal are very important.

Electrical recordings from the brain are also helpful in identifying or defining satiety or deprivation. Deprivation is characterized, electrically, as an EEG of low voltage and high frequency whereas the fed state is characterized by low frequency, high amplitude. Electrical activity in the brain, therefore, is correlated to appetitive drives (81).

When the brain is either stimulated or lesioned, various hormone titers are altered that could effect changes in appetite or food intake. Certain hypothalamic areas in fasted cats when stimulated cause a marked elevation or depression of plasma free fatty acids and, other areas, an elevated or depressed plasma glucose. Triglycerides and cholesterol were unaffected (82, 83). Plasma immunoreactive growth hormone levels were not affected by hypothalamic stimulation in conscious cats while cortisol levels were significantly enhanced (83).

Stimulation of the ventromedial hypothalamus of the rat produces a marked elevation of plasma glucose probably due to an increased hepatic glucose production, but plasma insulin levels stay at low levels even with the rise in glucose. The insulin inhibition of release was eliminated with adrenalectomy. Plasma glucagon levels rose with or without adrenalectomy, with antiglucagon serum inhibiting partially the hyperglycemic response (84).

In the fasted rat lateral hypothalamic stimulation will not change free fatty acid or insulin levels but will slightly increase glucose. If the animal is fed glucose the insulin levels increase with a drop in free fatty acids. In rats recovered from lateral lesions there is a marked increase in blood glucose and insulin after a meal with fasting free fatty acid levels 50% of normal. Ventromedial lesions produced an immediate elevation in blood glucose. In hyperphagic rats, serum insulin increased with eating and remained high (85).

Cholinergic and adrenergic drugs modify food intake when injected into the third ventricle of the brain (86-88). Choinergic pathways produced mainly increased water intake whereas adrenergic compounds and cholinergic drugs enhanced food intake (88). Glucochloralose stimulates food intake but galactochloralose does not (89). Other drugs producing hyperphagia are chromic acid, bipiperidyl mustard (90) and goldthioglucose (91-105).

Perhaps the most widely studied hyperphagic drug is gold thioglucose. Mice, rats and dogs all become hyperphagic and obese after injections of goldthioglucose (91, 92). The hyperphagia, as in ventromedially lesioned animals, usually subsides with time until

intake is normal, but in certain instances, female animals do not return completely back to the baseline (94). After fasting, the hyperphagia begins again (93). Earlier work concludes that the primary lesion is in the ventromedial nuclear area with the oligodendroglia cells taking up gold and being destroyed (95, 96). Exceptions are diabetic animals whose oligodendroglia are not destroyed with no subsequent hyperphagia or obesity (95, 97). Animals treated with goldthioglucose usually show no change in fasting blood glucose but glucose utilization is increased. There is an increased insulin sensitivity and increased gastric secretion in the Pavlov but not the Heidenhain pouch (91).

Constant light produces changes in ad libitum food intake independent of goldthioglucose (98). Other manifestations are centrolobular fatty liver (99), estrous malfunction (94), ulcerogenesis (101), augmentation of ACTH response to stress (101), increased tumorigenesis (102, 106), increased pancreatic insulin stores and secretory capacity in vitro in response to glucose or theophylline (107), while the diaphragm and adipose tissue respond normally to insulin (108). More C-14 carboxyl-labeled acetate is retained in goldthioglucose treated animals at weight maintenance but when fasted the C-14 retention is the same as controls (109). Daily mobilization of fat is 50 to 60% of the control level (110). Epididymal and mesenteric adipose tissue display normal in vitro glucose metabolism in the presence of insulin and growth hormone (111), and there is no increase in lipogenesis from acetate (112).

Several recent investigations have disclaimed the conclusion that the primary lesion is in the ventromedial nucleus when goldthioglucose

is injected (99, 103, 104). The lesion may be secondary to vascular damage and edema around the hypothalamus (99). Goldthioglucose is known to cause damage not only to the ventromedial hypothalamus but also to the fornix, premammillary nuclei, arcuate nuclei, anterior hypothalamus, preoptic nuclei, vagohypoglosal complex, dorsal hippocampus, hypocampal commissure and septal nuclei (105). It is still extremely interesting to note that other goldthiosugar compounds, e.g., malate and galactose do not cause brain damage, hyperphagia or obesity (97). There is also a dose response to goldthioglucose for food intake as well as obesity and one can reason from this evidence that there is some quantitation of neurons influencing food intake (99).

During food ingestion cardiac output, heart rate and systolic blood pressure are all elevated and blood flow is directed to the vascular bed of the superior mesenteric artery. It has been postulated that this general sympathomimetic state marks the end of eating (113). The carotid reflex is also elevated (114).

Another basic theory for food intake regulation is the "thermostatic" (115-125). The theory is based on the observation that rats when exposed to cooler environmental temperatures increased their food intake but at high temperatures intake was curtailed. Food intake is used as a means of temperature regulation. If the animals are able to dissipate more heat, more food is consumed (115). Skin temperatures of the thumb increase after a high protein meal in obese as well as non-obese subjects (117). In rats, brain temperature increases have been recorded in relation to feeding (118) and the magnitude of elevation was related to the length of the meal (119). In other work no correlation between brain temperature and intake was observed in

ruminants. This may be a major difference between ruminant and monogastric animals (120). Laboratory monkeys do not increase food consumption in response to cold (122). Hypothalamic hyperphagic rats have colonic temperatures higher than controls that were not caused by increased food intake, obesity, or the failure of heat dissipation mechanisms. There is also an increase of body temperature in normal animals with short and sometimes long term fasting (125) Also, thyroxin will stimulate appetite but will increase body temperature and conversely thiouracil and thyroidectomy are hypothermic but appetite depressing (125).

The gastrointestinal tract has been widely studied in relation to food intake. It can be broken down conveniently into the oralpharyngeal surface, the stomach and the small intestine.

One major variable in the buccal cavity is the teeth. When molars are extracted from rats, body weight decreases as a result of a decrease in food intake but the decrease is more than in pair fed controls (126). This indicates that the stimulation of the teeth during eating may be important to obtain greater digestibility which in turn would affect future intake. The teeth are also very important in rendering food in a size easily swallowed (136). Animals can regulate food intake without the influence of oralpharyngeal input but in the normal animal these mechanisms are utilized for control (126-135).

The oralpharyngeal surfaces are a rich source of sensory receptors (127) and combined with taste and smell are important in discriminating between food objects which can affect intake by conditioning or emotion especially in the human (128, 129). Sensory cues seem to be dispensable (130) since rats can regulate intake by feeding themselves

intragastrically on liquid diets (128). Under ad libitum conditions rats seem to eat for calories, ignoring taste and sensory perception but when the rat is in an energy deficit it becomes very discriminating and taste becomes more of a factor in regulation. This same effect is displayed by hypothalamic hyperphagic rats. Rats, after a period of deprivation, will decrease intake on cellulose diluted diets but increase intake on high fat diets (128, 131). In an intact animal, though, there is little doubt as to the importance of sensory perception.

Satiation is felt even before finishing a meal. When food touches the mouth or tongue there is a change in certain brain EEGs from fast, low voltage to slow, high voltage, indicative of satiety (132, 133). In the hungry animal many areas of the brain and hypothalamus exhibit fast, low voltage EEGs that are completely blocked by intravenous or intraarterial glucose injections. During eating, sensation from the sensory receptors inhibit the lateral areas of the hypothalamus as well as cortical and subcortical structures.

A "sensory or primary satiation" has been defined as a fast regulation coming directly from a nervous source and a "secondary or metabolic" after the food has entered the blood. Thiel et al., (134) have pointed out that in interpreting food preference one has to consider the strain, sex and prior food ixperiences of the animal. This would be especially important in the human.

Gastric receptors, stimulated by the presence of food or gastric distension, do seem to be involved in food intake regulation. When dogs are fed intragastrically with greater than 33% of their normal oral intake, food intake is partially depressed but is always greater than the oral intake alone. Inert material placed into the stomach

before a meal will decrease intake as does a water filled balloon. Also, as the intragastric load increases the duration of sham feeding decreases (137, 138). Rats react much the same way to intragastric loading and when the rate and volume of intragastric feeding is controlled to simulate the rats normal feeding cycle the oral inhibition is much greater (139). The same effects are seen for man (140, 141) and pigeons (142) and the amount of food in the mackerel's stomach determines the amount of oral intake (143). The time after intragastric loading until oral intake, increases with the volume and caloric content of the load (144). This kind of does response relationship gives strong evidence for the stomach distension theory.

Intragastric loading not only affects intake but motivation because milk injected into the stomach can serve as a reward for learning (145). After vagotomy, gastric secretion is inhibited (146) and the ingestion of a liquid diet becomes frequent and smaller in volume (147). Pressure placed around the outside of the dogs stomach also reduces intake (148). To confuse the issue, it has been shown in man (149) and in rats (150) without a stomach that normal hunger drives seem to exist. Also, in man, gastric and duodenal motility were not found to be correlated to hunger sensations (151).

Perhaps the major component of the gastrointestinal tract implicated in food intake regulation is the upper small intestine. Two components are involved: nervous and hormonal. Intraduodenal feeding experiments have produced conflicting results. Intraduodenal loading with food or inert materials does seem to temporarily inhibit intake (152). Dogs fed jejunally would not eat food by mouth but, when the vagus was severed, intake became voracious (153). In other work

distension in the jejunum of dogs produced anorexia 3 out of 5 times even after vagotomy, bilateral splanchnicectomy and excision of the lumbar chain (154). Ehman et al., (155) also found a decrease in oral intake with increased upper intestinal bulk or osmotic pressure but concluded that this mechanism would only be effective in gastrectomized animals or in animals with denervated stomachs. It would appear that some humoral factor was involved.

Support for a hormonal hypothesis was found when food placed in the upper small intestine inhibited gastric contractions even in denervated stomachs (156). Parabiotic experiments have also implicated humoral factors. Intestines were crossed in parabiotic animals so that food from one animal would pass through its stomach and into the duodenum of the partner and vice versa. One rat would eat continuously while the other would display anorexia. Gastric emptying time determined which rat was the "eating rat." The animal that received food first into the intestine would stop eating. Neural and humoral factors inhibiting eating must have originated in the upper small intestine. The other animal kept eating even with the oralpharyngeal and gastric stimulation (157).

Recently, gastrointestinal hormones have been persued for possible mechanisms in the control of food intake in addition to their direct roles in inhibiting gastric secretion (secretin) (158), gastric emptying (pentagastrin) (159), stimulating gastric acid secretion (gastrins I and II) (160), as well as showing insulin releasing activity (secretin, gastrins, cholecystokinin-pancreozymin) (161-163) and glucagon releasing activity (cholecystokinin-pancreozymin) (163). For an excellent early review see Grossman, 1950 (164).

An intestinal mucosal extract from the upper two-thirds of the small intestine when injected intraperitoneally or intraarterially completely inhibits food intake for about 90 minutes in rats. Extracts of the lower one-third of the small intestine were not active and all extracts were not active after treatment with trichloroacetic acid. When secretin or pancreozymin was injected, food intake was not affected (165). Similar results were shown with mice (166). Secretin and cholecystokinin-pancreozymin have been shown by several investigators to be ineffective on food intake (165-168). Cholecystokinin-pancreozymin does have a sedative action and it has been proposed that this influence on the sleep-awake cycle may bring about short term satiation (169). Some workers have proposed a central action for the hypothesized intestinal hormones that inhibit intake (166, 170). The interaction of the gastrointestinal hormones is very complex. There is an overlapping of effects which adds considerable confusion (171). There does seem to be, though, an effect on food intake from an intestinal "enterogastrone" unrelated to the gastrins, secretin or cholecystokinin-pancreozymin. There also seems to be an involvement, especially, from fat. Oral intake of a high fat diet does decrease beginning within a day or so. Fat also seems to release an "enterogastrone" other than the ones mentioned above, since, fat produces a potent inhibition of gastric activity when fed orally or placed directly into the duodenum. This effect is probably not mediated through secretin since fat is not a potent stimulator for this hormone (171) and in a non-gastrin stimulated stomach cholecystokinin-pancreozymin is slightly stimulatory to the stomach (169, 171). The factor, therefore, extracted from the duodenum which inhibits intake in rats and mice could be related to

the physiological effect of fat in inhibiting gastric activity, causing release of cholecystokinin-pancreozymin which may have a sedative action, and in decreasing food intake by an unidentified factor. Liver extracts, when added to the diet, were reported to have food intake stimulating properties (172).

Perhaps the most widely studied and most controversial theory involving circulatory substances on the control of food intake is the glucostatic theory (173-197). Very generally, the glucostatic theory states that food intake is related to blood glucose utilization. The greater the utilization, the greater the inhibition on food intake. According to this theory there are specific "glucoreceptors" in the hypothalamic brain areas but, perhaps also peripherally, that are sensitive to blood glucose levels. The rate of utilization of these receptors gives rise to the inhibitory stimulus (173). The oligodendrocytes may play a key chemosensitive role between the capillary and neuron (174).

D-glucose has an extremely high penetration in the brain and as blood levels increase so do the cerebral and hypothalamic levels (175). Glucose given by gavage was shown to decrease food intake even when plasma osmolarity was maintained by intravenous water infusion; hence, the suppressive effects were not due to an osmotic stress (176). Glucose, in a subcutaneous dialysis bag, caused food intake suppression (177). In general, the ventromedial hypothalamus is excited with a concomitant depression of the lateral area, as determined by brain electrodes, when glucose is administered intravenously or intraventricularly in the brain (178-180). Food intake is also significantly depressed in Houssay diabetic animals in which hyperglycemia

has been introduced. The high blood glucose levels seemingly stimulate a reduction in food intake (181, 182).

Hypothalamic hyperphagic animals have a lower than normal non-fasted blood glucose level (183). Food intake can be stimulated by administering an inhibitor of glucose metabolism, 2-deoxy-D-glucose (184, 185).

An extension to the glucostatic mechanism places the glucoreceptors in the liver and signals are then relayed to the brain. Investigators not able to reduce food intake by intravenous glucose injections were successful when the glucose was administered intraportally (186-188).

Glucose utilization may only be important in controlling intake after a certain threshold of carbohydrate load is reached (189).

An interesting effect is seen in hypothalamic hyperphagic rats in that intraperitoneally administered glucose produces a greater anorexia than in normal animals (190). If the glucose receptors were located only in the ventromedial hypothalamus, one would expect a decrease in the glucose anorexic effect if the glucose were acting only on this area of the brain. Other investigators have reported no effect of IV administered glucose in dogs (191), IP glucose in rats (192) or by oral or IV administration of glucose in chickens (193).

The greatest discrepancy in results in testing the glucostatic theory seems to involve the IV administration of glucose. Many problems arise in IV injections if the dose and rate of transfusion are not highly controlled. Different femoral A-V differences of glucose will be seen whether insulin is injected IV or IA (197). With the rapid intravascular rise of a test substance "diffusion delay" can produce large A-V differences, but actual cellular uptake could be

negligible. Large negative A-V differences could be caused by "back-diffusion" from interstitial spaces; thus, A-V differences should be determined repeatedly over a reasonable length of time and the criterion for cellular utilization is a maintained positive A-V difference simultaneously with a constant or decreasing arterial level of test substance (198). Poor technique in many IV studies probably has led to erroneous interpretation of results and has led to the confusion today concerning the glucostatic theory.

Amino acids also have been implicated in food intake regulation when very low protein diets are consumed and in extreme circumstances when circulating amino acids become excessive as in high protein, amino acid imbalance, or amino acid toxic diets (199-218). In an amino acid toxic diet (6% casein + 5% methionine or tryptophan) food intake is severely depressed. The intake is most severely depressed when the amino acid added to produce the toxicity is elevated in the plasma (200). Diets high in all essential amino acids except one growth limiting one are imbalanced and also produce a decreased growth rate and food intake. If the growth limiting amino acid is infused into the carotid artery, all depressive effects are gone but if infused into the jugular vein, the animal remains in precarious balance (201) indicating that the limiting amino acid in some way acted directly on the brain in enhancing food intake.

With histidine imbalance the ratio of the other indispensable amino acids to histidine is high and inversely correlated to food intake (202-204). These effects are not due to changes in stomach emptying (199) or in diet palatability (205), since addition of small amounts of the limiting amino acid to the diet increases intake. Diets high

in indispensable amino acids are more suppressive than diets high in dispensable amino acids (205) and ad libitum fed animals are affected to a greater extent than animals trained to meal eat (204). In chickens no change in food intake was observed in relation to dietary lysine, methionine or tryptophan levels (206, 207).

There is also a decrease in intake when low protein diets are fed in addition to an impaired glucose tolerance and an increased colonic temperature, above control levels, upon refeeding. Protamine zinc insulin decreased colonic temperature more than controls during fasting and refeeding following fasting (208). On low protein diets there is a greater gainin lean body mass and an increased ability to store more fat when energy expenditure is increased by exercise or cold which results in increased food and protein intakes (209). As a possible mechanism for the decreased intake on low protein diets, the decreased dietary intake produces a decrease in metabolic activity of proteins and protein metabolites. Metabolites associated with other foodstuffs may be in surplus causing the appetite reduction. Other metabolites will then decrease and a new steady state reached (210). Many of the effects on high protein diets, imbalance, and toxic diets will abate with intake returning to normal in a week or so after liver catabolic enzymes for the excessive amino acids are induced (211).

Protein also is related to energy requirements during stress. In adult female rats food intake is mainly regulated by caloric intake on eithera 25% or 8% isocaloric protein diet. In pregnancy the intake of rats on 8% protein will increase 50% but during lactation intake is not increased and the dams lose weight. The pups from these dams are one-half the normal weanling weight (212).

All of the above effects are also seen in hypothalamic hyperphagic rats suggesting that brain areas other than the ventromedial area are important (213-216). Lesion studies have implicated the prepyriform cortical area (217). Amino acid uptake by the brain is independent of the glucose carrier system (218).

It has been postulated by Krauss and Mayer, (216) that the food intake suppression seen in the above examples are only operative in extreme situations and act as a "safety valve mechanism" to decrease intake of a potentially dangerous diet. Animals force fed imbalanced diets will eventually develop pathological symptoms (219).

In order to determine the mechanism of the anorexia of severe exercise, Baile et al., (221) injected monkeys intravenously with 10 ml of 2-8M sodium DL-lactate and markedly reduced subsequent food intake. Sodium propionate or acetate were also effective but NaCl, glycine, alanine, lysine or glucose were not. Tannic, tartaric and acetic acids ingested with food may decrease olfactory acuity and may prolong the length of time before satiety is felt (222). Tannins at high levels, though, are known to be toxic (9).

Calcium ions may alter the "set-point" for weight or hunger control. When ionic calcium in the brain ventricles is increased, a completely satiated rat eats vigorously. This response is not blocked by alpha or beta blockers (223, 224).

The secretions of various other endocrine glands affect food intake either directly or indirectly. Removal of the thyroid or adrenal glands will suppress intake (225). Thyroxin at 3 ug/100g body weight/day had no significant effect on food intake in female Sprague-Dawley-Rolfsmeyer rats over a three day period. Intake

actually did go up, in agreement with previously reported results for throxin (125), but so did body weight; consequently, intake per unit body weight did not change (226).

Five hundred ug hydrocortisone acetate per day for 3 days significantly stimulated intake in rats (226), whereas, growth hormone at 1 mg per day for 10 days increased intake but also body weight; consequently, intake per 100 grams body weight was actually decreased (226). When thyroxin, hydrocortisone acetate and growth hormonewere given simultaneously to non-lactating adult rats, a slight decrease in intake was seen (226). Hormone interactions are very complex and many variables have to be taken into consideration: dose level, species, route of administration, length of administration, effects of administered hormones on other endocrine glands producing complex interactions, and changes in body weight occurring simultaneously with changes in food intake.

In rams, injection of thyroxin or sodium acetate either alone or together inhibited intake and thiouracil had no effect but relieved some depressive effects of acetate. The acetate was given intraruminally at 6.2 g/Kg $W^{.75}$/day for four days. Thyroxin was given subcutaneously at levels ranging between 0.3 to 0.9 mg/Kg $W^{.75}$/day and thiouracil was fed at levels ranging from 0.03 to 0.06 g/Kg $W^{.75}$/day (227).

Estrogens depress food intake in female rats and are intrically involved in body weight regulation (228-231) and saccharin preference (232).

Eating stimulates insulin release even in human subjects eating an imaginary meal under hynosis (233) and insulin, conversely, will

stimulate food intake depending on the type and amount administered (234, 235). Regular insulin in 1 to 5 units/100 grams body weight per day did not affect food intake in rats but protamine zinc insulin, being absorbed more slowly, doubled food intake with a potentiation seen after removing the adrenal medullary area (234). Insulin produced hypoglycemia may act directly on the brain to increase food intake since the same effect is seem in dogs with extrinsically denervated stomachs (236).

A decrease in food intake has been shown many times in the adult human after the administration of glucagon (237-240) but has no effect in young pre-pubertal rats (241). Ventromedial lesions are ineffective in pre-pubertal rats in augmenting intake (53). The hyperglycemia produced by glucagon with the depressed food intake has recently given increased support for the influence of blood glucose in food intake regulation.

Water, lipid, and emulsified extracts of the supraopticus and paraventricularis nuclear areas of the hypothalamus of cattle were injected into rats producing reductions in water intake, urine output and food intake and increases in blood sugar level (242).

Hypophysectomy decreases body weight and food intake (243, 244). Food intake is increased when alkaline extracts of the pituitary are injected into hypophysectomized rats (243). These effects are probably due to changes in other endocrine glands as a result of hypophysectomy (243, 245). Pituitary extract injections have the opposite effect on food intake when injected into hypophysectomized animals fed diets containing a balanced low level amino acid mixture or an imbalanced diet (245). Hypoglycemia is commonly seen after hypophysectomy but,

nevertheless, food intake is depressed (246) which seems to argue against the glucostatic mechanism.

Pregnant mice treated with cortisone ate more but weighed less than controls (247) and growth was inhibited in cortisol treated rats with no effect on food intake (248). If rats are fed amino acid imbalanced diets and injected with cortisol the usual growth and food intake depression seen in imbalanced diets is overcome (249). These rats also preferred the imbalanced diets (249). The effect of cortisol seems to be in elevating the most limiting plasma amino acid (249). Adrenalectomized rats ate less but, when force fed control amounts of food, were the same as control animals in body weight and body fat (250). Other work has shown no relationship between the adrenal hormones and other endocrine glands, e.g., feeding optimum levels of thyroid in the diet abates partially the detrimental effects of cortisone (252).

In male guinea pigs hydrocortisone acetate, 5 mg injected subcutaneously twice daily, develop a steroid diabetes. There is extreme hyperglycemia, glucosuria, reduced body weight gain, increased food intake, obesity and increased nitrogen loss. At first, pancreatic beta cells show hyperplasia and hypertrophy and degranulation with hydropic degeneration after prolonged administration. Higher doses produce a loss of appetite and no diabetes (253). Once again the hormone effect is species and dose dependent.

Fat mobilizing substance 1A has recently been found to have anorexigenic properties. Animals on high protein diets produce more of this urinary substance and rats conditioned to meal eating produce less (254).

There has been rather recent evidence (1971) that essential fatty acids may act on the brain and may affect the synthesis or release of hypothalamic releasing factors (220).

The lipostatic theory of food intake regulation is the last major, well established, concept for explaining the control of energy intake. This theory has been receiving current publicity, Lepkovsky, 1973 (255). Briefly, the lipostatic theory states that body fat stores remain constant or, in other words, lipostatic, through the action of circulating metabolites in equilibrium with adipose tissue. The limiting factor for food intake is the rate at which absorbed metabolites can be removed from circulation involving some form of synthesis or transport of fat (256). Forced feeding experiments have, in general, supported this theory but the evidence is somewhat contradictory. When White Leghorn Cockerels are force fed, body fat content increases. After force feeding there is an anorexia until body fat decreases to normal. Evidently, the sensing mechanism can sense calories from adipose tissue as well as the diet (257). Force feeding excessive calories in rats results also in an increased body fat content but the effect after force feeding depends somewhat on the environmental temperature. These animals exhibit anorexia and decrease body weight until the control levels are reached at which time control intake is resumed at either 5 degrees or 27 degrees centigrade, but at the higher temperature these rats contained more body fat (258).

Animals injected with insulin become obese and after treatment are anorexic. If the obese animals are given ventromedial hypothalamic lesions, there is little augmentation of food intake or weight gain. If obese lesioned animals are force fed to increase their obesity,

anorexia also exists after force feeding until their "normal" obese fat levels are regained (259).

Creating obesity by stimulating the lateral hypothalamus produces anorexia after stimulation until body weight approaches prestimulation levels (260).

If gonadal fat organs are removed and then goldthioglucose injected, the same amount of body fat will be stored compared to just goldthioglucose injected animals. The inguinal fat organs develop to twice their usual size in order to make up for the removed gonadal fat organs. The amount and efficiency of food utilization seems to be regulated about a set-point for body weight (261).

If the gonadal fat organs of the goldthioglucose obese mouse are removed, food intake increases until the same level of obesity is obtained. If the gonadal fat depots are removed before obesity, there is an increased food efficiency causing the same level of obesity with less food intake over a shorter period of time (261).

There also seems to be signals from the periphery. If genetically obese yellow mice are injected with goldthioglucose, they gain less weight and have a reduced hyperphagia (261). A dose response was also demonstrated between extent of lesion, food intake and body fat (261).

Similar experiments in removing adipose depots were conducted by Schemmel et al., (262). Either inguinal or testicular depots were unilaterally or bilaterally removed and the rats then fed a high fat diet. All food intakes were the same and all body fats, at autopsy, were the same between control and experimental groups. These results are similar to Liebelt's when adipose tissues are removed prior to the development of goldthioglucose obesity. The fact that the rats

gained the same level of obesity over the same length of time and with the same amount of food infers, at least, a slightly increased food efficiency to make up for the fat already removed. Increasing food efficiency accomplishes the same as increasing the total amount of food at a constant efficiency. The net result is caloric hyperphagia which is what is important in regulating energy balance.

Studies have also been conducted with certain types of stomach tumors that are highly lipolytic. When these tumors are placed into goldthioglucose treated and yellow obese rats, body fat and food intake decrease but when placed in non-obese animals, body fat decreases with food intake remaining the same until the animal becomes moribund (263).

A direct contradiction to the lipostatic theory was advanced by Mu et al., (264). These investigators were not able to correlate the extent of an electrolytic lesion of the hypothalamus to any particular degree of body fatness. Their hypothalamically lesioned rats ate the same amount of high fat diet as regular diet. One must also entertain the possibility that in nutritional obesity the brain centers controlling intake may be abnormal or, on the contrary, they may be normal but simply stressed by a dietary condition that they were not made to handle (265).

One last major area of food intake regulation concerns unidentified humoral factors that may be functional in controlling intake. These substances may actually be one or several of the factors already discussed or possibly new "hunger" or "satiety" factors not yet isolated. As one would expect, this area of research is highly controversial and very speculative. Cross circulation techniques are, though, becoming

quite sophisticated and several very interesting papers have been published. It seems quite possible that circulating factors, specifically regulating energy balance, either elaborated from the brain or the periphery will be isolated. Investigations of this type have primarily been of two types: 1. acute cross circulation experiments, and 2. parabiosis.

Several factors, affecting food intake, that are effective by perfusing into animals have been demonstrated. Islets of Langerhans were dissected from the mouse pancreas and bathed with effluent which had passed over pieces of brain either from the medial or lateral hypothalamus. Beta cells released insulin when bathed with effluent from the ventrolateral hypothalamus only (260).

In the cross-perfused cat, electrocortical activation was produced in one animal after bulbar reticular formation stimulation in the other. This effect has been attributed to the release of a humoral factor (266). Neurohumoral factors were also demonstrated as being released from the hypothalamus of cross-perfused monkeys. Perfusate was collected from hypothalamic brain areas in starved monkeys and perfused into similar brain areas of sated monkeys. After perfusion, the satiated monkeys began eating. If the donor monkey was fed, there was no effect. Perfusate from the ventromedial area of a fed monkey inhibited feeding in a deprived monkey when perfused into its ventromedial nucleus. Perfused areas that stimulated eating were from the periformical regions. Adrenergic like compounds may be involved in the response (268, 269). Similar cross perfusion experiments have implicated humoral substances involved in the control of body temperature (270).

The involvement of systemic hormones in the control of food intake was suggested in the early 1900's (271). The experimental evidence upon which the hypothesis was based is as follows: Blood taken from food deprived dogs when injected intraperitoneally increased stomach contractions in fed dogs. The converse has also been reported (272). Even as late as 1964, Jefferson et al., (267) have demonstrated a brain humoral factor that activates gastric motility. The problem is that gastric motility is a poor index of hunger and subsequent food intake.

Siegel et al., in 1952 (273) tested once again the hormone hypothesis. Rats were trained to eat their food in two-hour intervals. After intraperitoneal injection of blood serum from food deprived or satiated rats, food intake was unaltered. This obviously was a better means of measuring hunger than gastric contractions. In 1954, Siegel et al., (274) administered orally to 24 hour starved rats blood serum from food deprived donors or food sated rats. Food was then offered. Intakes were not different from controls in either group. At this time the hormone hypothesis was seriously doubted. In 1965, Davis, (275) reported that humoral satiety factors are not present immediately after the termination of a meal and, therefore, are not involved in limiting meal size. Siegel did not get a response possibly because he collected blood too soon after feeding his rats.

In 1967, Davis reported the existence of a food controlling hormone when he mixed hungry rats blood with satiated blood and obtained a 50% reduced food intake in the hungry rat. Food intake was not reduced if the donor rat was also deprived or meal fed for 30 minutes immediately before transfusion. No evidence for a hormonal factor increasing

intake was found. Davis concludes that satiety factors gradually accumulate in animals fed ad libitum and disappear with fasting (276, 277). Davis was also able to obtain a dose response by fasting his fed rats for one to five hours and injecting this increasingly "deprived blood" into starved rats and observing a slow increase of food intake to normal levels (278). The electrical cortical graph of hungry rats transfused with "satiated blood" displays the high amplitude, low frequency typical of sated animals (279).

In continuously or intermittent cross circulation of monkeys, using arterial-venous shunts, blood mixing of a deprived monkey produced no change in intake of an eating monkey and the intake of a deprived monkey was not affected by blood from a feeding monkey. The fed monkeys lost only slight amounts of body weight, whereas, the starved monkeys lost significant amounts (280).

At the adipose tissue level, inhibitors of lipolysis, upon epinephrine stimulation, have been reported in the adipose tissue of hypothalamic rats (281, 282).

Experiments with parabiosis have been somewhat contradictory but also hampered by problems in methodology. Hervey, 1959 (283) studied food intake in parabiotic pairs of rats with one member of each pair being hypothalamically lesioned in the ventromedial nuclei. The rats with lesions became obese and hyperphagic while their partners exhibited signs of anorexia and became thin. It was concluded that the normal rat's hypothalamus responded to factors circulating over from the overfeeding animal and demonstrated a feedback system in food intake regulation. Wei Han et al., (284) and Fleming, (285) carried out similar experiments and found only slight decreases in intake of the

non-lesioned partners. Hervey's rats, though, were fed ad libitum, whereas, Wei Han's and Fleming's animals were placed in a very restricted feeder (284) and conditioned to eat in a limited time. When one rat in a pair was starved, the intake of its partner did not increase (284, 286). Their results agree with those of Davis in that there does not seem to be a hormone initiating eating but one that signals satiety (276, 277).

Many of the genetically obese mice and rats have been parabiosed to lean littermates. The obese hyperglycemic mouse has been parabiosed by Haessler and Crawford, (287). The lean member of the pair lost weight and the obese gained, but the lean animals of this experiment did not refuse food as in Hervey's work (283). Adipose tissue from these animals still resembled "lean" and "obese" characteristics in their respective animals. The obese hyperglycemic mouse is hyperphagic (288) without electrolytic brain lesions, but individual food intake could not be measured.

Yellow obese mice are also hyperphagic (288) and have been parabiosed to lean littermates by Weitze, (289) and later by Wolff, (290). Weitze found that the lean member of a pair kept the yellow mouse from becoming fat and concluded that the etiology of the obesity was endocrine. Wolff found opposite results with parabiosis having no effect on the weight gains of either the yellow obese or the lean mouse. Neither investigator reported food intake data.

One of the most interesting parabiosis studies was done with the genetically diabetic mouse (db/db) (291). The db/db mice are the most hyperphagic of any of the genetic obese strains eating approximately twice the intake of normal, lean mice. In this respect the hyperphagia

resembles very closely the hyperphagia of the hypothalamic obese mouse or rat. There was no effect on the diabetes when the diabetic was parabiosed to a normal littermate and the normal did not contract the disease but, as in many other studies mentioned (280, 283, 287), the lean normal partners lost weight, were hypoglycemic and died. The obese, hyperphagic gained weight and eventually died as a result of the death of its partner.

George Bray, (292) parabiosed the Zucker strain of obese rat and found no effect of parabiosis on the weights of the partners. Only three pairs were done, however, and no mention was made as to testing the efficiency of the unions. Also, parabiosis was performed at six weeks of age when the obese rats could be identified and, quite conceivably, a circulating substance could have been involved in the syndrome prior to this time. The Zucker rat is hyperphagic (288) but Bray made no mention of food intake in his study.

Hausberger (293, 294), after parabiosing obese hyperglycemic mice, also reports weight suppression in the obese mouse with the complete prevention of obesity in some. After separation, all obese mice gained weight quickly. Adipose tissue from obese animals, when successfully grafted to lean animals, acquired characteristics of lean adipose tissue and vice versa.

Fleming (285) reported, in a limited number of pairs, similar results as Hausberger. One member of a parabiotic pair was ventromedially lesioned but did not become obese or hyperphagic until after separation from the normal rat. Fleming termed these animals "inhibited hyperphagics." Fleming was also able to show inhibition of feeding in normal parabiotic pairs. One rat was fed for 2 hours. Both rats were

fed for a third hour and for the remaining 2 hours of the 5 hour period the second rat was fed alone. The animal fed last decreased its intake in about a 3 week period. The effect was also reversible (285).

Other time lapse feeding trials were conducted by Schmidt (286) and Schmidt and Andik (295). One rat of a pair was fed for 2 hours. For the next 2 hours no animal received food and for the 4th and 6th hour the second animal was fed. Contrary to Fleming's results, no significant depression of intake was seen in the animal fed last, but a slight decrease was evident from the data.

In all parabiotic experiments no means were available to measure individual weights of parabionts or to measure separate, ad libitum food consumption. The latter measurement is extremely important. The conditioned response to eat is extremely strong and may override satiety signals that may be operative in ad libitum fed animals. All time lapse feeding studies reviewed above were done with "conditioned" animals.

Also, partitioned cages used to measure separate intake in parabiotic animals were very stressful and animals could not be left in them continuously (284, 291). The present study was undertaken to 1. determine methods for measuring individual body weights in parabiotic rats, 2. develop methods to measure ad libitum separate food intakes in parabiotic rats, 3. improve surgical procedures in order to avoid many animals separating after a short post-surgical period, and 4. feed, ad libitum, one member of a parabiotic pair a high fat diet and the other a grain diet. This design simulates many of the experiments discussed above in that a caloric hyperphagic rat is cross circulating with a lean rat consuming a diet of normal caloric density except that the hyperphagic rat is not known to have any "potent" genetic anomally

precipitating obesity nor has it been deranged in any way, i.e., brain lesions, except for parabiosis.

PART 1

A METHOD FOR DETERMINING GROWTH RATES OF INDIVIDUAL RATS IN PARABIOSIS

INTRODUCTION

The inability to obtain separate weights which are essential in preparing growth curves for each member of a parabiotic pair has greatly impeded the use of parabiosis in nutrition studies. In using parabiosis in various phases of dietary research, e.g., obesity, individual weight curves are a necessity. At present, the only methods of securing individual weights of parabionts involves: 1. Estimating weights visually, or 2. Dividing the total weight of the pair by two. The system described in this report permits the determination of each parabiont's weight with an error of $\pm 3\%$ or less.

TECHNIQUE

Two dietetic scales, each with a weight capacity of 500 grams, were modified by replacing each balance pan with a six by ten inch, flat aluminum rectangle permitting an adequate surface area for animal placement. The modified balance pans were approximated to within 2.5mm, the scales zeroed and the bases of the scales secured. Parabiotic rats, just prior to weighing were lightly anesthetized with a mixture of methoxyflurane (2,2-Dichloro-1, 1-difluoroethylmethyl ether) and butylated hydroxytoluene, METOFANE, inhalation anesthetic (Pitman-Moore, Division of the Dow Chemical Company, Indianapolis). The balance pans were stabilized by blocks placed under them while each animal was positioned

on a scale with the suture line between them stretching along and between the inner edges of the pans. The animals were weighed on their backs to facilitate proper placement on the scales. The blocks under the pans were then removed (Figure 1).

After the balances came to rest, the smaller scale reading was subtracted from the larger and this difference in scale readings recorded as the uncorrected weight difference or ΔA.[1] As an example, for one parabiotic pair, the difference in scale readings or ΔA was 40. From a standard curve, to be described later, ΔA or 40 was used to predict the actual weight difference between rats in a pair. The predicted weight difference using ΔA of 40 was 47 grams. Both animals were then weighed on one balance and the pair weight recorded as 291 grams. From the combined weight and the predicted weight difference the individual weights were calculated by solving two equations for two unknowns.

$A + B = 291$; $A - B = 47$; $2B = 244$ and $B = 122$; $(A + B) - B = 169$

In developing a standard curve for predicting the actual differences in weights of parabionts, each of 40 parabiotic pairs, joined surgically by the Bunster-Meyer procedure (296), was placed on the balances, by the method described, and the scale readings recorded. The actual weight difference between two animals chosen for any parabiotic pair ranged from zero to over three hundred grams thereby covering a wide range.

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[1] Three weight differences between rats in a parabiotic union are considered in this report. To clarify them they will be referred to as: 1. The uncorrected weight difference which is the difference between the observed scale readings, 2. The predicted difference which is secured from the standard curve for parabiotic rats in Fig. 2 and the uncorrected weight difference, and 3. The actual difference which is the difference between the weights of the individual parabionts secured after their surgical separation.

If the union between the parabionts were rigid, each scale reading would reflect half of the total pair weight. If the union were so flexible that the weight of one rat would not affect that of the other, the animals would weigh independently and each scale would measure the correct animal weight. Since the union is flexible, but not to the point of independent weighing, the scale reading of the heavier parabiont will be lower than its actual weight and the scale reading of the lighter animal will be greater than its actual weight. If the animals weigh exactly the same, the difference in scale readings will be zero.

The difference in the two scale readings for each pair or the uncorrected weight difference, ΔA, should be directly and linearly related to the actual weight difference of each pair ΔB, as discussed later. To secure the actual weight differences the parabionts were surgically separated along the suture line, with a minimum loss of blood, and the weights of the individual rats recorded. The actual difference between the weights of each pair (ΔB) was plotted against the difference between the scale readings for each pair (ΔA). The plot of ΔB against ΔA was a linear regression ($P < 0.001$) with a correlation coefficient of 0.982 and a regression coefficient of 1.361 for the methods used in this report. The data for 10 pairs of the forty used in obtaining the standard curve (Table 1) indicated that the error for the calculated weights of all animals designated A was 0.96% and for all animals designated B, 1.99%.

DISCUSSION

The weights of the individual rats joined in parabiosis can be determined with a high degree of accuracy by the procedure described in this report. By this means the use of parabiotic animals can be extended to those disciplines where the growth rates of individual animals are important in evaluating experimental results.

The flexibility of the parabiotic union largely accounts for the weight interdependence of the two animals thus joined. A physical system was initially used to evaluate the effect of a flexible union on weights in an artificial parabiotic union. Both dietetic scales used had the same maximum load and spring tension. Cylindrical bronze weights were connected in pairs with rubber bands. Several weight pairs were used with the weight difference between weights in a pair differing over a range similar to that of the rats that were joined in parabiosis. The results of this study produced a straight line relationship between the differences in observed scale readings or uncorrected weight differences (ΔA) and the actual differences in the weights of the cylinders in a pair (ΔB), (Curve X, Fig. 2, $\Delta B = -1.041 + 5.216\ \Delta A$, $r = 0.998$). This preliminary observation stimulated work with the parabiotic animals.

The slope of the curve for parabiotic rats (Fig. 2) differs from that of the cylindrical weights due to a difference in the nature of the unions. The more rigid the union, the closer the slope is to the ordinate in which case ΔA approaches 0. An approach to that situation is seen in curve X, Fig. 2, where the cylindrical weights were joined

by a strong rubber band which permitted relatively little flexibility in the union. The opposite extreme, curve 0, Fig. 2, $\Delta A = \Delta B$, demonstrates independent weighing when the union between attached weights is extremely flexible so that one weight does not affect the other. The slope relating ΔA to ΔB for parabiotic animals could range anywhere between these extremes.

These observations emphasize the fact that when it is necessary to follow the growth rates of the individual rats joined in parabiosis, the same surgical procedure must be used in joining all pairs, i.e., one pair cannot be joined by supportive sutures through their pectoral girdles and another by sutures through the pelvic girdles, etc. The variability in the techniques used for joining parabiotic animals makes it essential for the investigator to check the slope of the line relating ΔA to ΔB. The curve for parabiotic animals in Fig. 2 was established with parabiotic Osborne-Mendel rats joined by supportive sutures through the pectoral girdles and supportive sutures placed around the proximal ends of the medial femurs.

One would expect any significant deviations from the regression to occur during the first two to three weeks of parabiosis. During this period, when the wound is healing and animals are first experiencing the parabiotic relationship, maximum stresses will be placed on the union; consequently, the standard curve was tested against several pairs of parabiotic rats that had been joined for several weeks. The individual weights were determined by the technique described in this paper. The actual animal weights were obtained by surgically separating the pairs. The sum of the actual individual weights equaled the pair weight before separation and each calculated weight, using the regression

equation $\Delta B = -1.023 + 1.361 \Delta A$, was within $\pm 3\%$ error of its actual weight.

As a check on the value of this procedure, the scale reading for each rat in each of the forty pairs was assumed to be the correct animal weight. These scale readings differed markedly from the actual weights ($P < 0.001$) with the difference between these readings equaling 9.99%. Weights calculated by the procedure described in this report did not differ statistically from the actual weights and under these conditions the total error was 2.50%. The latter encompasses all observations made with this procedure and is greater than the error terms for the latter part of the work after experience and familiarity with the technique had been developed. Even a difference of 2.50% is what one might expect when routinely weighing animals in non-parabiotic experiments. For this reason the weighing procedure should find ready acceptance in a number of disciplines where the body weight of each rat joined parabiotically is of importance.

Table 1

Per cent error between actual and calculated weights of individual parabionts.*

Actual Weight		Calculated Wt.		Per Cent Error	
Rat A	Rat B	A	B	A	B
72	66	72	66	0.0	0.0
143	145	143	145	0.0	0.0
291	82	294	79	1.0	3.7
409	155	402	162	1.7	4.5
410	272	416	267	1.5	1.8
590	263	585	268	0.8	1.9
352	149	352	149	0.0	0.0
558	307	549	316	1.6	2.9
277	143	280	140	1.1	2.1
311	218	305	225	1.9	3.0

* The mean per cent error for all rats in column A was 0.96 and for all in column B, 1.99. The smaller animal was always placed on balance B; consequently, the same error in grams was a greater percentage of actual body weight. The average per cent error for all animals was 1.46. The mean actual weight ($\bar{X}$) was statistically equal to the mean calculated weight ($\bar{Y}$), ($\bar{X} - \bar{Y}$) = 0. Paired-T test

Figure 1. Two parabionts being weighed on modified dietetic scales as described in text.

Figure 2. Each point in curves (X) and (●) represents the weight difference between two attached weights or parabiotic rats plotted as a function of differences in scale readings (see text). (ΔB) equals (ΔA) when two attached objects weigh independently, curve (0).

Curve (●) was obtained using 40 pairs of parabiotic rats with varying weight differences between the two animals of each pair. Curve (●) is described by the equation $\Delta B = -1.023 + 1.361 \Delta A$; the correlation coefficient (r) of 0.982; ΔB intercept + 0 ($\alpha = 0$, T-test). The relationship between ΔA and ΔB is significant (analysis of variance) $P < 0.001$; the standard error of the regression coefficient (s_b) = 0.0413; the 95% confidence limits for the true regression coefficient (β) are: $1.277 \leq \beta \leq 1.445$; the standard error of the sampled mean $\overline{\Delta B}$ (at $\overline{\Delta A}$) = 2.3549 ($s_{\overline{\Delta B}}$).

The 95% confidence belt for the means of the population regression and the 95% confidence intervals for a single observation were calculated but not plotted on figure 2. All points were within the 95% confidence interval for a single observation. The values given below will enable the reader to calculate and plot confidence intervals if the need arises. The mean for ΔA ($\overline{\Delta A}$) = 121.43; sum squares for ΔA ($SS_{\Delta A}$) = 129,969.024; the sample size (n) = 40; the mean square deviation (MS_d) = 221.843. Appropriate values for ΔA are: 49.0, 69.6, 113.3, 159.7 and 233.6. The ΔA values in table 1 may also be used.

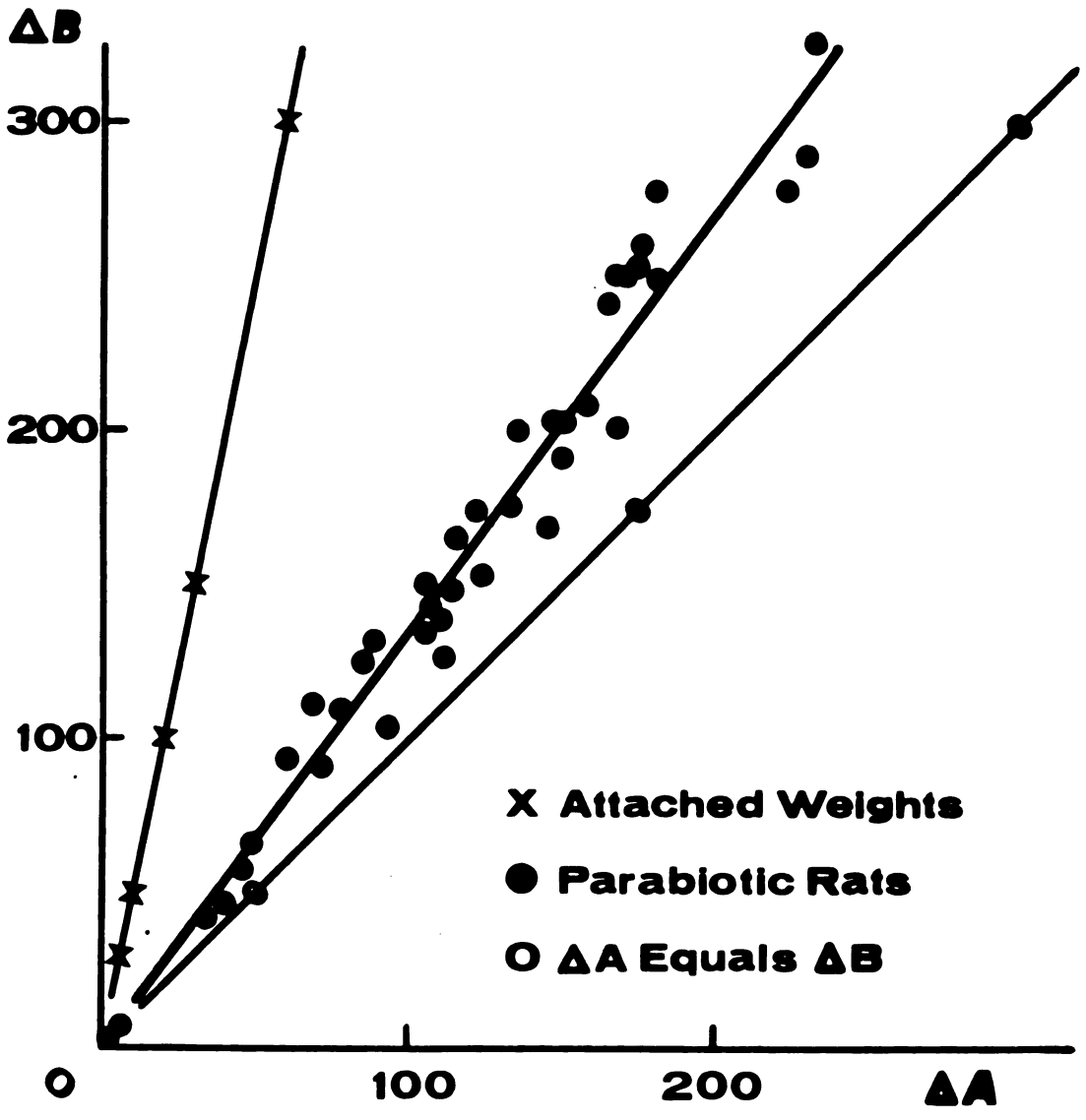

Figure 2. See opposite page.

Note: Part 1 was published as Shier, N. W. and O. Mickelsen (1972) A method for determining growth rates of individual rats in parabiosis. J. Appl. Physiol., 32:425.

PART 2

AN AD LIB. DIFFERENTIAL FEEDER FOR PARABIOTIC RATS

INTRODUCTION

Parabiotic rats have been used for studying the regulation of body fat content and food intake for many years (283, 287). These experiments have been greatly impeded by the lack of a reliable ad lib. differential feeder for parabiotic rats.

A restrictive differential feeder was designed by Wei Han et al. (284), 1963. A partition was placed lengthwise down the middle of a regular, galvanized rat cage. An adjustable opening was made in the partition through which the parabiotic union protruded. When parabionts were placed into this restrictive feeder, allowing only forward and backward motion, their food intake decreased and they lost weight. Parabionts were then placed into the feeder for approximately 15 hours, overnight, and on this schedule the rats ate enough food to just maintain body weight.

Coleman and Hummel (291), 1969, tried to place parabiotic, diabetic mice into the feeder described above and reported that the animals never did eat, struggled and even tore the skin at the site of the union.

The partitioned feeder was used with some success by Fleming (285), 1969; Schmidt and Andik (295), 1969; and Schmidt (286), 1973, but only after training their rats to meal feed within a couple of hours. The rats seemed to associate the feeder with food and did not mind the restraint. Meal fed animals may be poor subjects for the mechanistic study of the control of food intake as the urge to eat after 22 hours of

deprivation is so strong that many "normal" factors employed to suppress food intake may be overridden. It is apparent that a relatively inexpensive, easily used and reliable ad lib. differential feeder is needed before parabiotic rats can be used routinely in laboratory investigations in which parabiotic, differential feed intakes are of importance.

TECHNIQUE

Female Sprague-Dawley rats, approximately one month old, were placed in parabiosis using a modification of the procedure of Bunster and Meyer (296), 1933). Three weeks to a month were required for surgical recovery and to determine whether the "union" was viable or not. The animals were, therefore, young adults when used for experimentation. Various dimensions (length from the cephalic end of the union to the tip of the nose, the width and thickness of the head, the length of the union, the nose to anus length, thickness of the scapular area and the width at the pectoral girdle) were made on approximately 37 pairs of animals. These dimensions would determine minimal sizes for the various components of the feeder. The feeder was made universal so that as the animals grew larger the feeder could be opened for just one animal or both. Feeders were constructed out of sheet metal, one millimeter thick, and bolted into regular galvanized animal cages. Since the feeder occupies considerable space, it is recommended that a double cage be used which would give the animals sufficient room to move around. Many feeder designs were tried and when one particular design seemed to work fairly well, a cardboard model was made and, with blueprints, was given to a

metal shop for construction of five feeders for preliminary testing and, when shown to be satisfactory, an additional 35 feeders were ordered.

To test whether a feeder was functioning properly or not, a chromium sesquioxide marker was incorporated at a 1% level (w/w) in the feed on just one side of the feeder. At various times during the day the pair feeding from this feeder would be placed into a partitioned cage (284) for one-half to one hour periods, so that fecal samples could be collected separately.[1] The feces from the animal not fed chromium were analyzed for the marker. If only slight levels ($<$ .25% of dry fecal weight; See Table 6, Page 63) of chromium were found it was assumed that the animals were consuming their own diets. The feces were pre-digested with nitric acid, wet ashed and assayed for dichromate according to the method of Czarnocki et al. (297) with the following modifications. After the color changed to red during the digestion period, digestion was continued for an additional 15 minutes when 2 additional ml of 70% perchloric acid were added and digestion continued for another 10 minutes. The digestate was suspended in 110 ml of distilled water and immediately filtered through Whatmann qualitative paper instead of allowing it to stand overnight.

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[1] Later it was found not necessary to place animals in a restrictive cage to collect feces. Fecal excreta given by rats fed the labeled diet are easily discernable, being a bright green. Even if the chromium fed animal has eaten some of the non-labeled food, its feces are still easily detectable.

RESULTS

Many different feeder designs were tried with varying degrees of success. Eight designs were relatively good; each was tested to determine which one would most completely prevent cross-feeding as evidenced by the chromium marker. A parabiotic pair was placed in each of the eight feeders and food intake measured. A high fat ration (298) containing a 1% chromium sesquioxide label was placed on one side of the feeder; a grain ration (317) on the other side. Fecal samples from the animal not receiving the label were collected over several days and assayed for chromium. As seen in Figure 3, feeder 3 was extremely effective in controlling the feed consumed by the rats. Food intake and weight gain in differential feeder design 3 were improved over control values secured from parabiotic animals whose feed was in a cup sunk into the floor of the cage (Tables 2 and 3). This cup was wide enough so that both parabionts could eat at the same time.

From these preliminary studies it was clear that feeder design 3 insured that each member of a parabiotic pair consumed only its own ration resulting in excellent food intakes and body weight gains in each animal of a pair. Tables 4, 5 and 6 show feed consumption, weight gains and the chromium results, respectively, of five pairs of rats each placed in a differential feeder of design 3. Only negligible amounts of chromium were detected and, once again, food intake and body weight gains were significantly better than that of controls. Several photographs of the differential feeder can be seen in Figure 4 (A, B, C, D, E, F) and detailed drawings are shown in

Figures 5-9. In all food intake studies, using parabiotic rats in ad lib. differential feeders, a chromium tag is placed on one side of each feeder at the beginning, middle and end of the experiment as a check on proper usage of the feeder. The label was placed in the high fat diet for studies in which one rat received high fat and its partner a grain ration because of the ease of mixing and minimal spillage. Fecal boli under the cage from the animal not consuming chromium may be contaminated if the marker is placed in an easily spilled diet.

Food intake measurements were made on 12 single, sham operated control rats and 24 pairs or 48 parabiotic rats for a 19 day period (Figure 10). Although the feed intakes of the female parabiotic rats in feeder design 3 were greater than those of the controls, this difference disappeared when the intakes were expressed on the basis of body weight (Table 7). Intakes were actually a little better in the parabiotic animals as compared to single controls.

DISCUSSION

The feeder described offers a reliable and easily used method for determining ad lib. food intakes of the individual animals in parabiosis. The feeder is adjustable for different animal sizes and permits each food cup to be removed separately.

When animals are first placed in the feeder their food consumption equals that of the controls in 3-4 days. Another feature of the feeder is that feed spillage by the individual animals can be measured accurately without contamination by excreta. Spillage collection bins could be constructed around each food cup, or a partition could

be constructed between the food cups extending from the floor of the cage to the bottom of the excreta collection pan which would separate very accurately the feed spillage. These techniques were not necessary for work done in this laboratory since, in crucial intake measurements, one diet was high fat and, consequently, only the loose grain ration was spilled. Spillage is also quite localized around each cup making differential collection fairly simple if both animals are fed an easily spilled food.

Food intakes are statistically the same for parabiotic rats fed in the differential feeder compared to single rats eating out of conventional food cups placed on the floor of the cage but compared to parabiotic rats exposed to a food cup placed in the floor of the front of the cage the differentially fed parabionts have significantly higher intakes. When using the feeder, each animal is obligated to take a proper eating position. One animal cannot keep the other from eating as is the possibility when the feeder is in the floor along the front of the cage. In such a case the dominant animal can maneuver its body so that the other animal cannot reach the food cup.

Several possible errors might arise in using the differential feeder. The feeder should be placed in animal racks that allow sufficient room between the bottom of the cage and the excreta collection pan to prevent animals from reaching their feces.

A crucial factor as to whether the feeder is used properly or not is the scapular supportive suture. The scapular suture must be intact allowing a limited mobility at the pectoral girdle; otherwise, animals can twist around and will not learn proper feeder usage. With a good scapular suture the rats are committed to use the feeder in the manner designed.

In chromium determinations fecal samples under the cage could be contaminated by chromium in powdered diets when the diet is spilled, as alluded to earlier. Even though the spillage does not fall directly on the fecal material, small amounts of feed containing chromium oxide may be deposited, by air currents, on the feces of the animal fed the unmarked ration. (This is suggested by the observation that the amount of spillage was correlated with the degree of chromic oxide detection in feces collected under cages where the marker was incorporated into a loose grain ration $\overline{r} = 0.311$.) This was not a significant r value but the t-statistic was rather high (1.601) for a sample size as large as 25; consequently, some of the low levels of chromium oxide detected were probably from this source.

Table 2

FOOD CONSUMPTION IN GRAMS (AVERAGE/DAY) OF PARABIOTIC RATS IN AD LIB. DIFFERENTIAL FEEDER DESIGN 3 COMPARED TO AN AVERAGE CONTROL VALUE FOR RATS PERMITTED TO EAT FROM A CUP IN THE FLOOR OF THE CAGE

Feeder Design 3*		Control**
15.43 ± 0.77 (SE)	P > 0.05	14.58 ± 0.99 (SE)

*Average for 7 days

**Average for 8 pairs, each on diet for 7 days; 56 values

Table 3

WEIGHT GAINS IN GRAMS/DAY FOR PARABIOTIC RATS IN DIFFERENTIAL FEEDER DESIGN 3 COMPARED TO CONTROL VALUES FOR RATS PERMITTED TO EAT FROM A CUP IN THE FLOOR OF THE CAGE

Feeder Design 3*			Control**		
Combined	Right	Left	Combined	Right	Left
6.0	3.0	3.0	1.9 ± 0.4	1.3 ± 0.3#	0.6 ± 0.3

*Over a 10 day period

**Average values for 8 pairs over a 15 day period

#Since this animal is gaining weight faster than its partner, it is possible that this is the dominant member of the pair.

Table 4

AD LIB. FEED CONSUMPTION* OF PARABIOTIC RATS
IN FEEDER DESIGN 3 VS. CONTROL CAGE (TOTAL FOR 8 DAYS)

Parabiotic Pair	Food consumption (g) when parabionts were in: Feeder Design 3		Control Cage
1	129		102
2	135		130
3	142		120
4	123		116
5	122		116
Totals	691		584
$\bar{Y} \pm SE$	130 ± 3.3	$P < 0.05$	117 ± 4.0

*High fat diet

Table 5

PAIR WEIGHT GAINS IN AD LIB. DIFFERENTIAL FEEDER DESIGN 3 VS. CONTROL CAGE (TOTAL FOR 8 DAYS)

Parabiotic Pair	Weight gains (g) when parabionts were in: Feeder Design 3		Control Cage
1	25		10
2	30		32
3	30		20
4	48		20
5	40		27
Totals	173		109
$\bar{Y} \pm$ SE	35 ± 3.7	$P < 0.05$	22 ± 3.3

Table 6

Cr_2O_3 IN FECES OF RATS FED NON-LABELED* HIGH FAT DIET IN FEEDER DESIGN 3 (all values on dry weight basis)

Cage**	Fecal Weight (g)	Cr_2O_3 in Feces (mg)	(%)
1	4.62	2.6	0.07
2	7.60	4.2	0.05
3	3.36	5.6	0.17
4	4.81	1.0	0.02
5	6.22	32.7	0.53
$\bar{Y} \pm SE$			0.16 ± 0.08

*Animals fed a 2% Cr_2O_3 labeled ration produced feces containing 20-23% chromium sesquioxide. This differs from above data, $P < 0.001$.

**Rats in cages 1, 2 and 5 were fed for 8 days; those in 3 and 4 were fed for 11 days.

Table 7

PARABIOTIC M-1 FOOD INTAKES IN GRAMS IN DIFFERENTIAL FEEDER VS. CONTROLS
(average / animal / day for 19 days) - SPRAGUE-DAWLEY FEMALE RATS

				SIGNIFICANCE	
Comparison of Means	n	df	Mean ± S. D.	Variance	t	P
Control vs. Parabiont	19, 19	30#	15.365 ± 1.637, 13.113 ± .957	2.681, 1916	5.176*	.001

#Degrees of freedom calculated
*Approximate t-test

PARABIOTIC M-1 FOOD INTAKES IN DIFFERENTIAL FEEDER VS. CONTROLS
(average / 100 g body weight / day for 19 days) - SPRAGUE-DAWLEY FEMALE RATS

				SIGNIFICANCE	
Comparison of Means	n	df	Mean ± S. D.	Variance	t	P
Control vs. Parabiont	19, 19	36	6.298 ± .662, 6.591 ± .498	.439, .248	1.539	NS

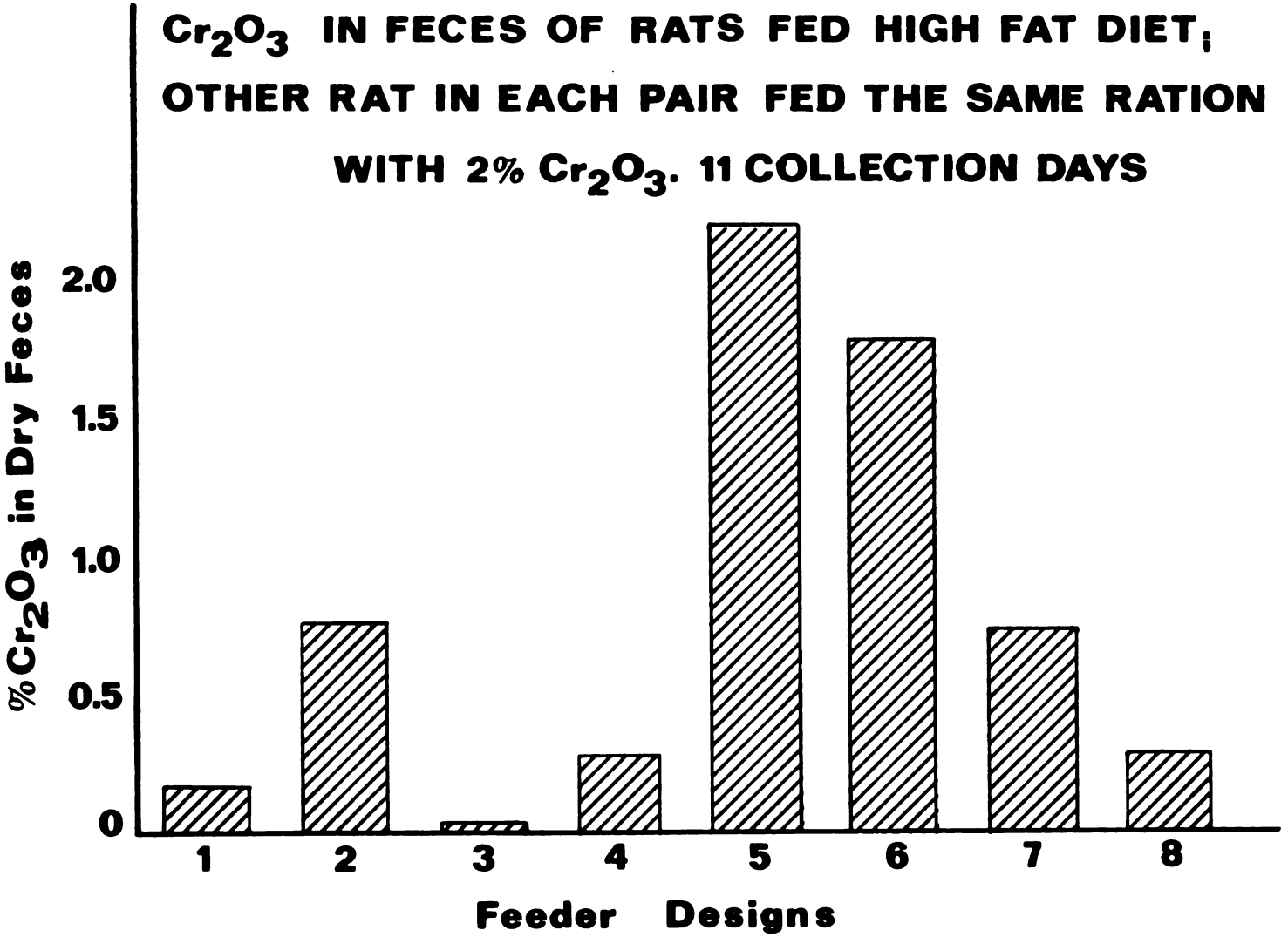


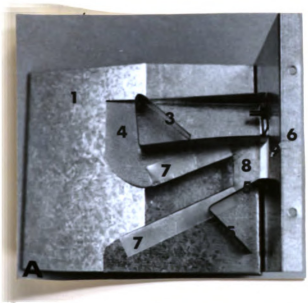
Figure 3. Cr_2O_3 in feces of rats fed high fat diet; other rat in each pair fed the same ration with 2% Cr_2O_3. Eleven collection days.

Figure 4. A. Side view of ad lib. differential feeder; B. End view; C. Top view; D and E. Parabionts feeding from differential feeder. 1) Side panels function in directing parabionts to the food cups with each animal on its proper side of the feeder; 2) Food cups. Each can be weighed separately. The end of each cup at (a, see Figure 4C) is extended slightly above the head opening (10, see Figure 4B). This acts as a head divider between the parabionts and prevents the mixing of food from one cup with that of the other; 3) Top pieces were necessary so that the animals would enter the feeder on the same plane. The top piece extends, at right angles, towards the top of the feeder to inhibit the animals from crawling over the horizontal component of part 3; 4) Body divider in which is cut a groove allowing passage of the parabiotic union (see Figure E). The width of the groove is slightly smaller than the thickness of an animal's head; so, an animal cannot stick its head through the groove to the other side. Each parabiont is therefore blocked from the other side of the feeder; 5) Bottom platform on which an animal rests its feet while eating; 6) Front piece including head holes (10) through which the animals protude their heads in order to eat; 7) Aluminum crimps for protection from sharp metal edges; 8) The solid front part of the body divider becomes part of the head divider; 9) Slip groove allowing the side panels (1) to be opened up or closed, independent of each other. Adjustments can be made for the body size of each individual animal.

Parts 3 and 5 are soldered in place on the front piece and parts 1 and 4 are bolted to the front piece with 10-24-0.5 (3/16" diameter) machine screws. The feeder is bolted into standard galvanized rat cages with machine screws placed through holes drilled in the front piece shown on Figure 4A. Free ends of the side panels are also bolted to the galvanized cage.

There is only one way in which the food can be reached. Both parabionts must enter the feeder so that each is on its proper side. There are then no impediments between the animals and the food. For proper usage of the feeder, a good scapular supportive suture is necessary to minimize flexibility at the pectoral girdle (see surgical procedures in this monograph). The feeder can be likened to a metabolism feeder for single animals only made with a partition for parabionts.

The pictures show the feeder outside of the cage.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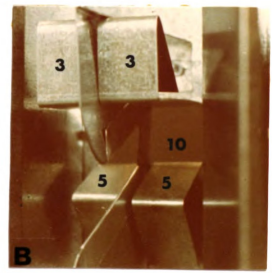
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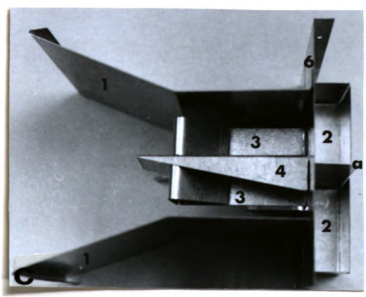
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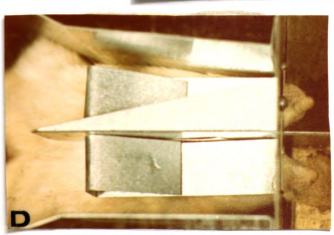
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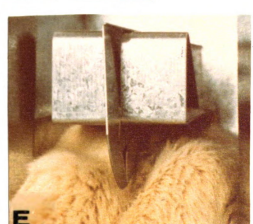
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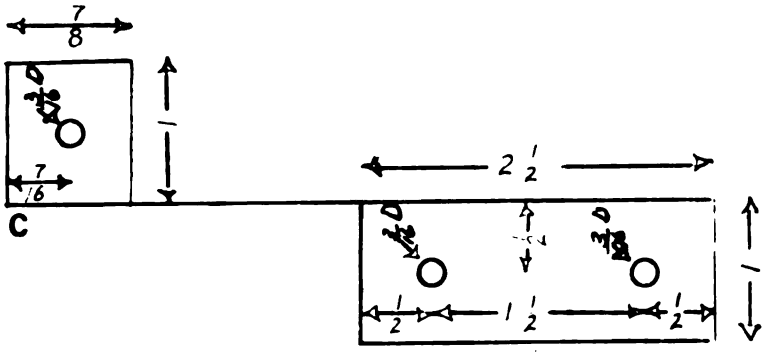


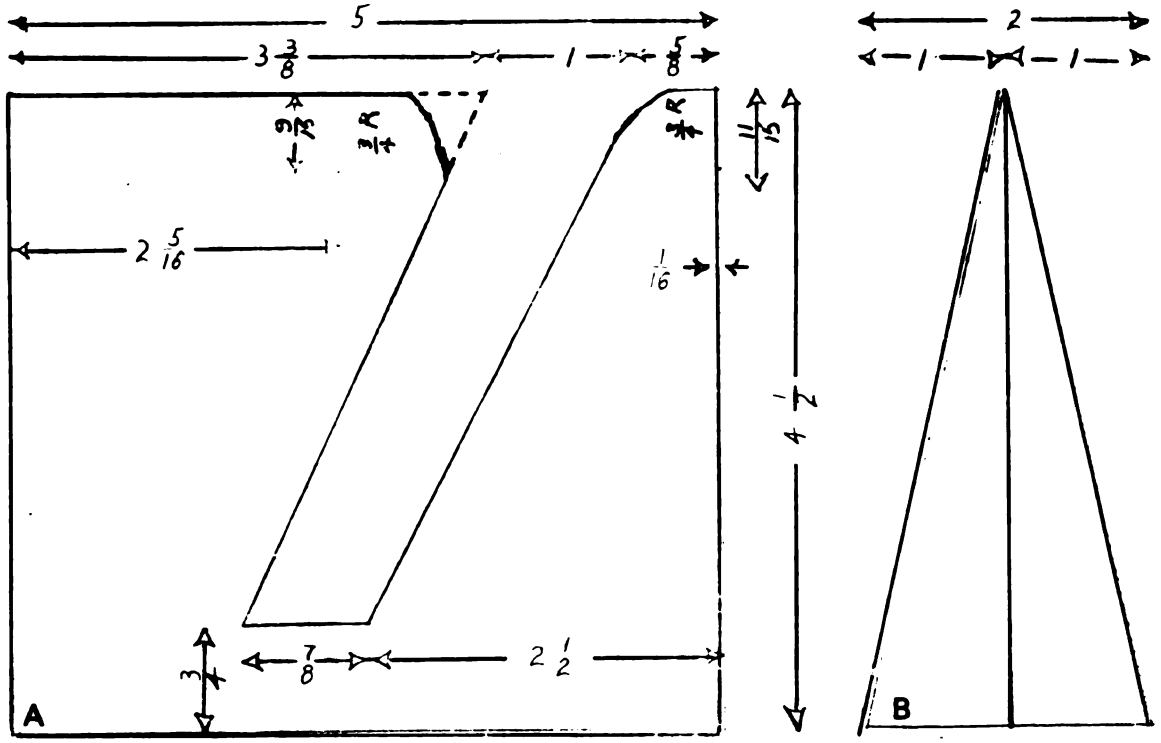


Figure 5. Drawing of the body divider (number 4 in Figure 4) of the ad lib. differential feeder. A) Side view; B) Top and C) End projections. Scale of 1:0.7. Material: Galvanized sheet metal one millimeter thick. All approximating free metal edges were secured with solder joints. (R = radius; D = diameter; All dimensions are in inches.)

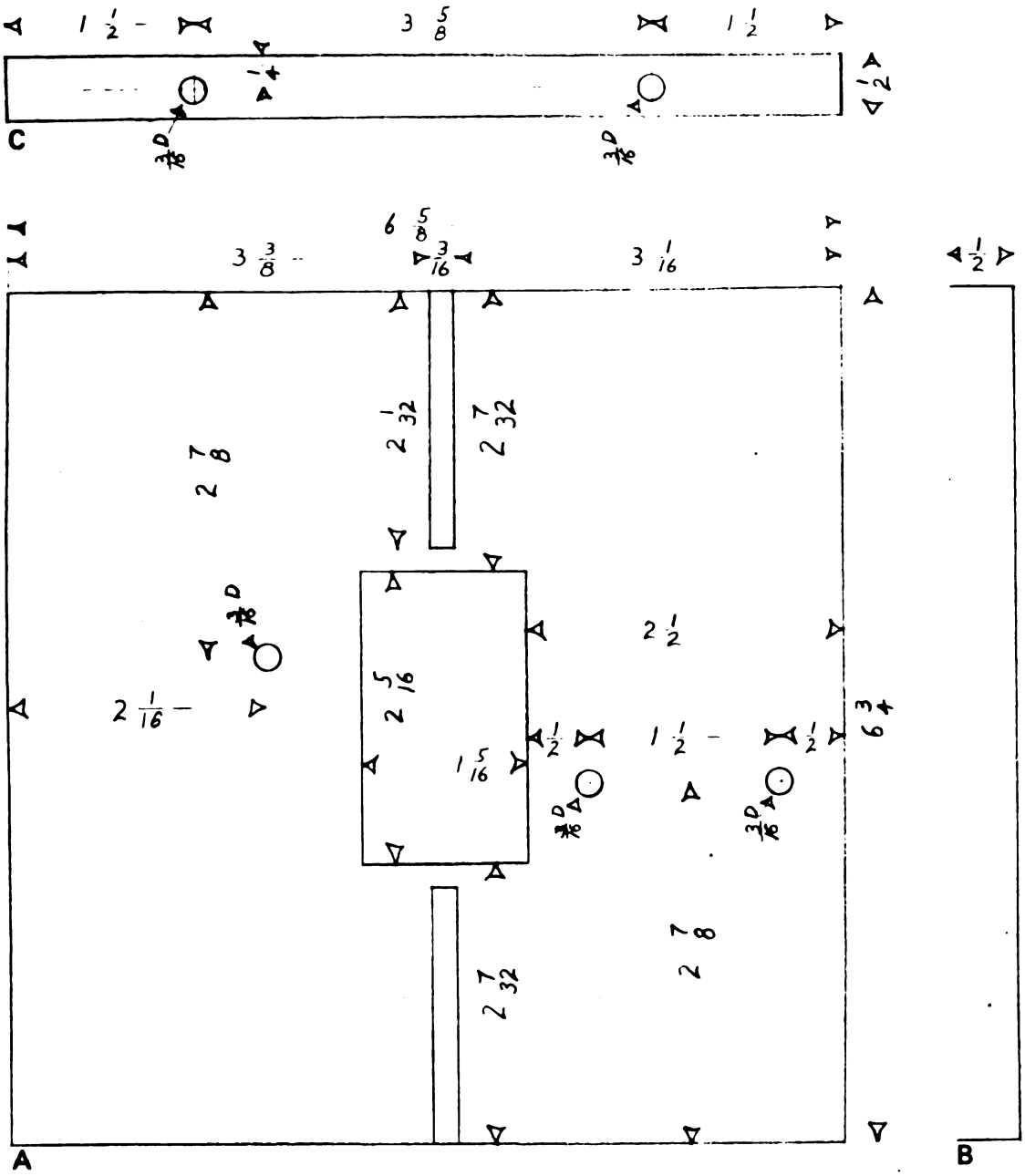


Figure 6. Drawing of the front piece (number 6 in Figure 4) of the ad lib. differential feeder. A) Front view; B) Top and C) End projections. Scale of 1:0.7. Material: Galvanized sheet metal one millimeter thick. (R = radius; D = diameter; All dimensions are in inches.)

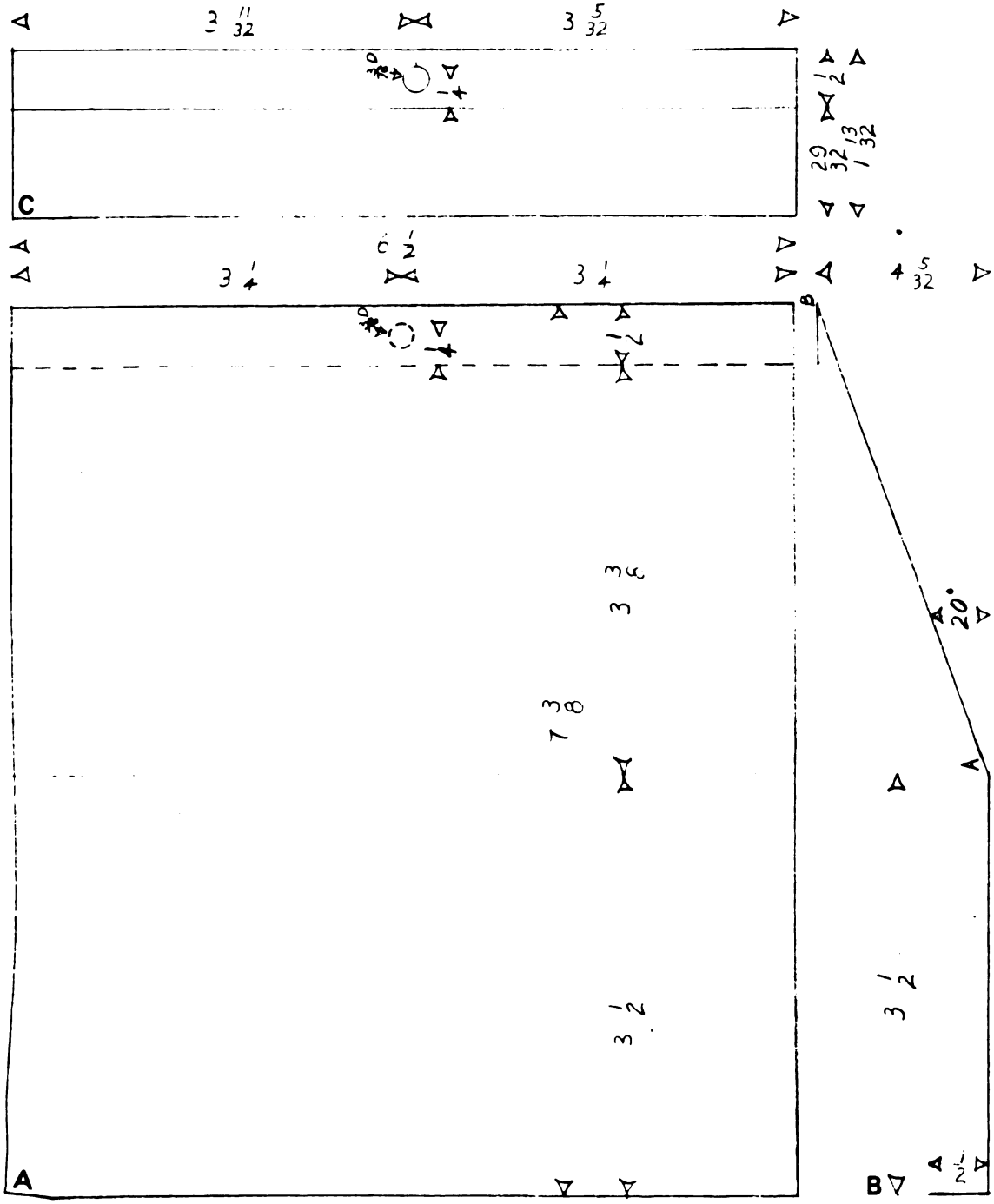


Figure 7. Drawing of a side panel (number 1 in Figure 4) of the ad lib. differential feeder. A) Side view; B) Top and C) End projections. Scale of 1:0.7. Material: Galvanized sheet metal one millimeter thick. (R = radius; D = diameter; All dimensions are in inches.)

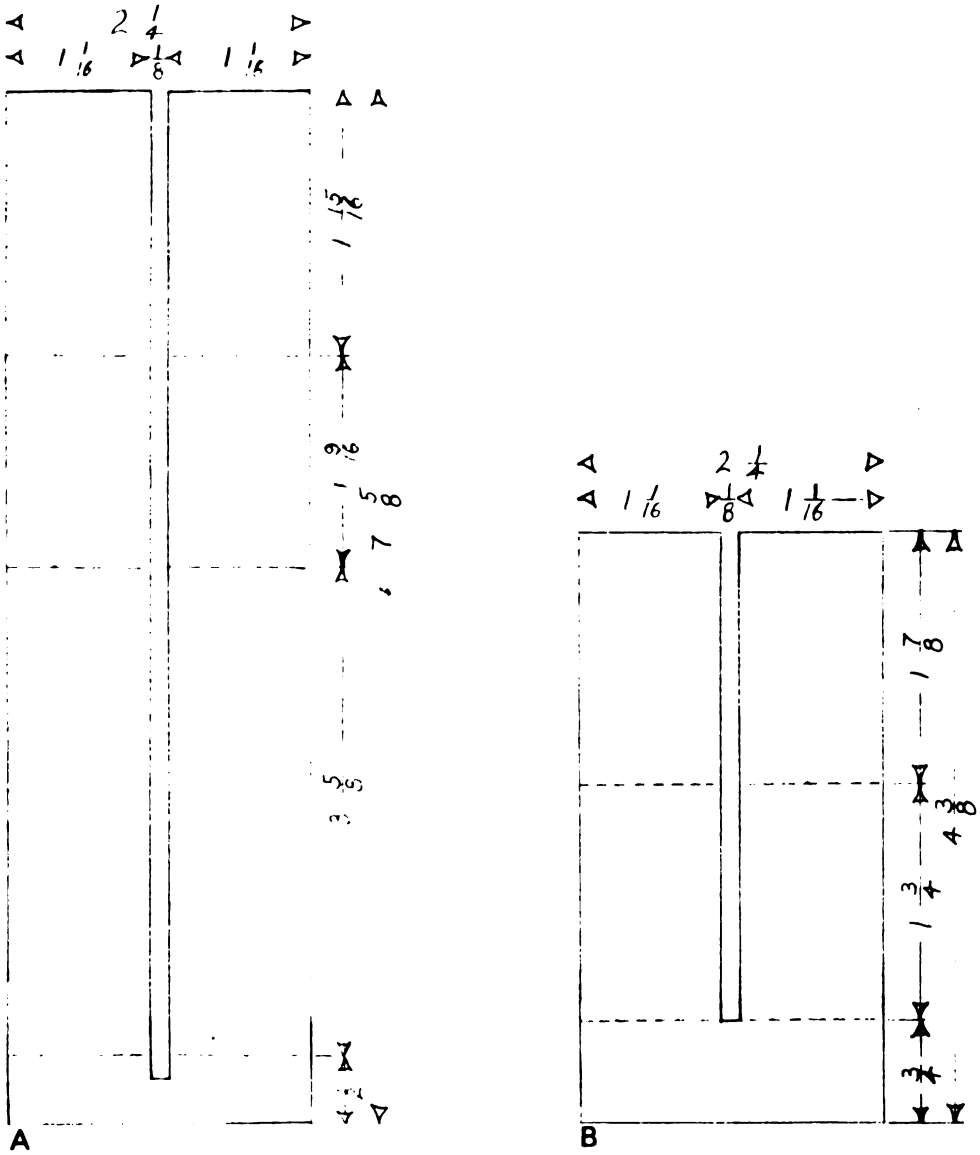
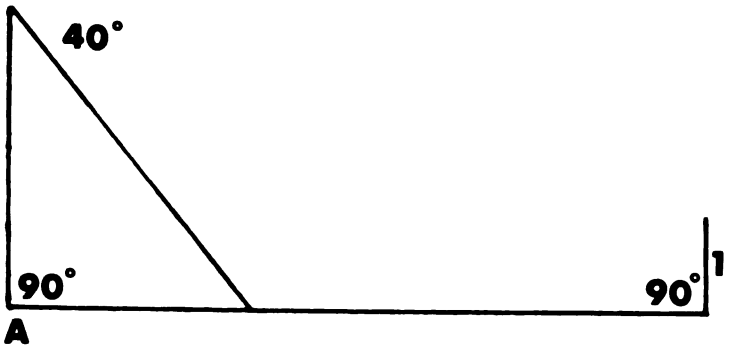


Figure 8. Flat drawings of the top piece (A) (number 3 in Figure 4) and bottom platform (B) (number 5 in Figure 4) of ad lib. differential feeder. Scale of 1:0.7. Material: Galvanized sheet metal one millimeter thick. The pieces are bent, at the dotted lines, in the forms shown on Figures 4A and 8A. (All dimensions are in inches.)



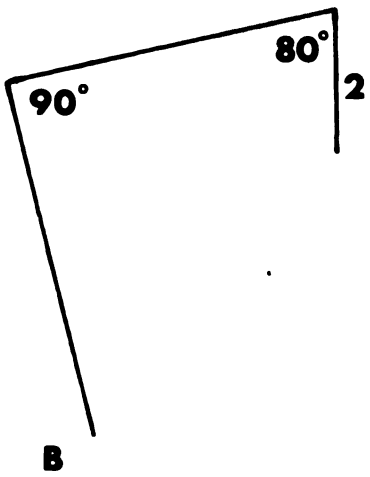


Figure 8A. The top piece (A) (number 3 in Figure 4) and bottom platform (B) (number 5 in Figure 4) bent into proper shape. Scale of 1:1. Material: Galvanized sheet metal one millimeter thick. Angles shown are approximate and the pieces may have to be adjusted slightly to the positions shown in Figures 4A and B. Flaps 1 and 2 are placed through the head holes and soldered to the back of the front piece as shown in Figure 4A.

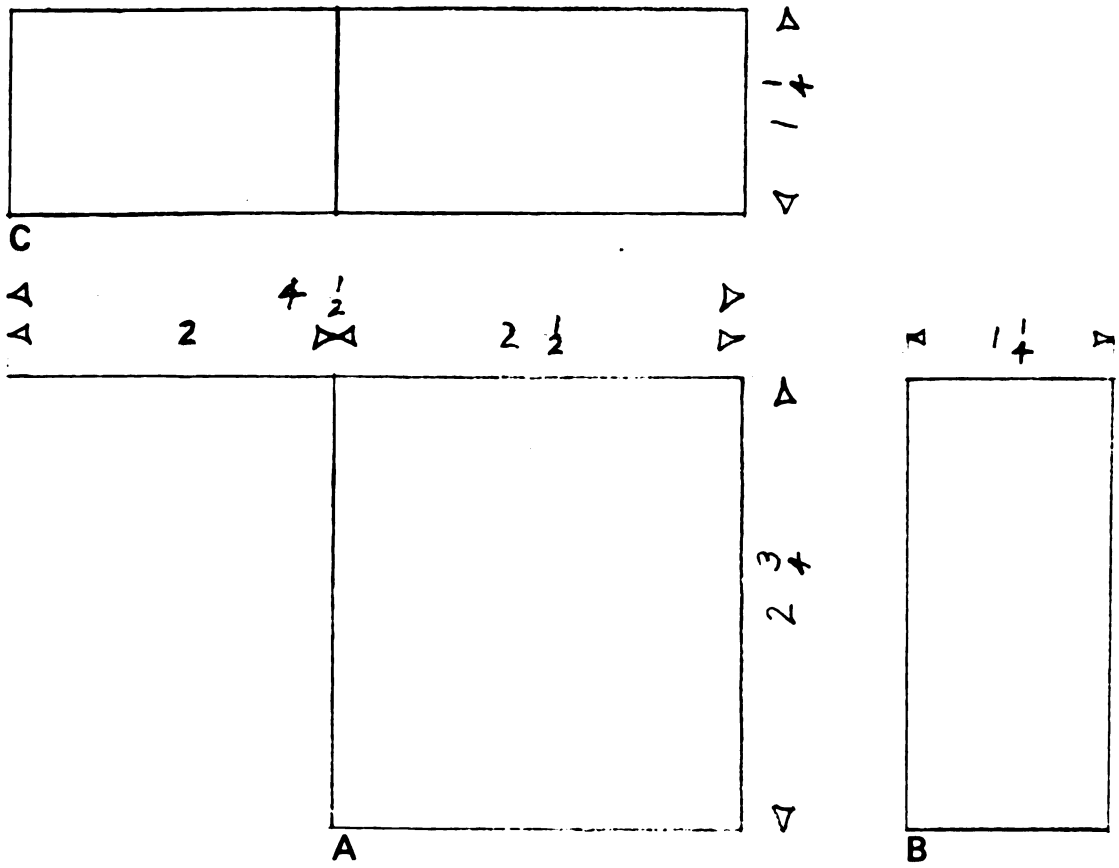


Figure 9. Drawing of food cup (number 2 in Figure 4) for ad lib. differential feeder. A) Side view; B) Top and C) Front projections. Scale of 1:0.7. Material: Galvanized sheet metal one millimeter thick. All grain food cups were soldered at the corners but high fat cups were not. (All dimensions are in inches.)

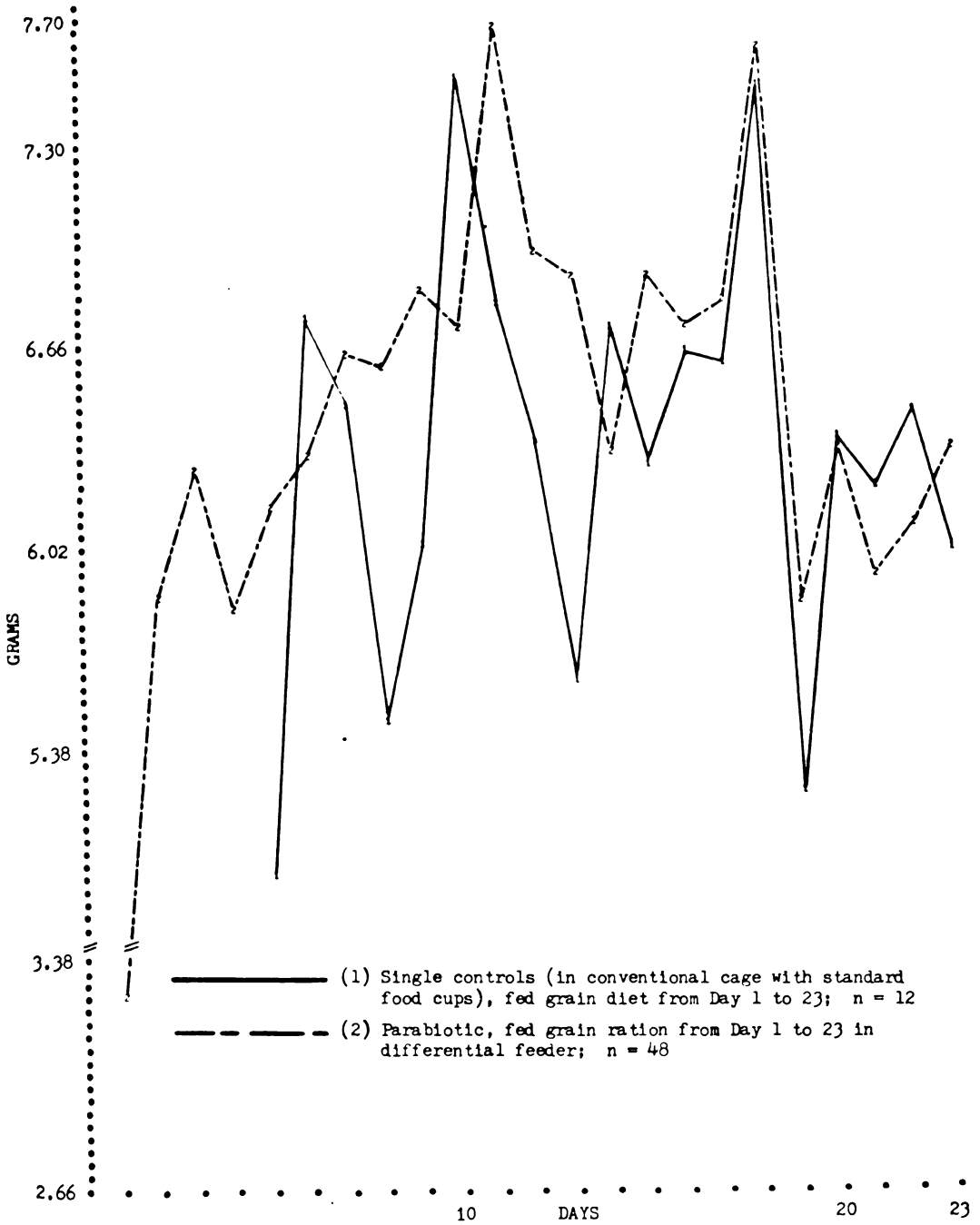


Figure 10. Food intakes per 100 grams body weight of control and parabiotic female Sprague-Dawley rats. (Av./Animal/Day)

Note: Part 2 was presented as a full length paper at the 1972 meetings of the Federation of American Societies for Experimental Biology and published in abstract form as Shier, Nathan W., Olaf Mickelsen and Dena Cederquist (1972) An ad lib. differential feeder for parabiotic rats. Federation Proc., 31:689.

PART 3

PARABIOTIC SURGICAL PROCEDURES

INTRODUCTION

Sauerbruck and Heyde (299), 1908, described a surgical procedure for parabiosis in which only skin and muscles were sutured together. After placing a small incision in the side of the abdominal musculature of each animal, the dorsal muscle flaps were sutured together followed by the ventral flaps producing an open cavity between the two animals (coelio-anastomosis). This procedure was used extensively until an improved method appeared in 1933 (296).

In the Sauerbruck and Heyde method, animals usually became highly infected and many pairs died from infection or physical trauma. The animals would pull on the soft tissue sutures tearing them apart.

Bunster and Meyer's modifications are as follows: 1) A supportive suture was placed through the scapulae and the iliac bones; 2) The cut edges of the abdominal area were sutured together as one mass closing off the open cavity between the animals and 3) A suggestion was made to place a few sutures through the thoracic muscles closing off the thoracic pocket between the animals. This technique seemed to give adequate support and protection for the soft-tissue and muscle sutures until healing. This technique is preferred at present.

One major problem still existed with the Bunster-Meyer technique. In many pairs the scapulae would become separated after a week or 10 days. Bunster and Meyer offered no reason for this but stated that the skin sutures were healed by this time and that the scapular separation

was of little significance. Williams (300), 1968, also noticed considerable scapular separation. In many of this author's early pairs, scapular separation was noticed before as well as after skin healing. If separation occurs after skin healing, the skin is still stretched considerably which possibly produces a "stress." Schmidt and Andik (295), 1969, reported that half of their pairs (24 in all) separated completely after 2 to 4 weeks. The authors concluded that the separations were due to immune reactions. It is possible that some of their separations could have been due to defective scapular sutures. A better supportive scapular suture was needed not only to secure better parabionts but also to keep pairs more rigid at the pectoral girdle for proper use of the parabiotic differential feeder as described by Shier et al. (301), 1972.

TECHNIQUE

The surgical technique was that of Bunster and Meyer (296), 1933, except for the following modifications. The above reference contains surgical details not described below.

Thoracic sutures were not used. These sutures do not seem to be necessary and may add further trauma. The major difference in the technique reported here and that of Bunster and Meyer concerns the supportive sutures. The new suturing system for the scapular area is shown in Figure 11. The caudal supportive suture is placed around the respective medial femora of each animal and tied from the dorsal aspect. The tie should be fairly loose so as not to tie off circulation in the femoral artery and vein. This type of caudal suture has been used by Hervey (283), 1959.

The scapular suture is made as follows: A stainless steel half-curved surgical needle (three-eighths circle, cutting edge, number 12)[1] was threaded with Suprylon, gauge 3, suture.[2] The left scapula of the right animal is located and elevated and the needle is pierced through the supraspinous fossa beginning from the medial side of the scapula. The right scapula of the left animal is then located and elevated and the suture is continued across to the lateral right scapular surface of the left animal and the needle pushed through the supraspinous fossa and out the medial side. The suture is then continued posteriorly and placed through the medial side of the infraspinous fossa of the right scapula of the left animal. The suture then passes through the lateral side of the infraspinous fossa of the left scapula of the right animal emerging on the medial side (Figure 11A). The scapulae are then drawn together, as in Figure 11B, and A and B are tied snugly. The end of the suture at B is left long enough so that the suture can be passed through the supraspinous fossa of the left scapula of the right animal and, diagonally, through the infraspinous fossa of the right scapula of the left animal emerging on the medial side (Figure 11). The ends of A and B are once again tied across the top of the scapulae. The excess suture is removed and the dorsal skin suture completed over the scapular area.

Other surgical techniques and materials were as follows. The animals were anesthetized with a mixture of methoxyflurane[3] and

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[1] Miltex Instrument Company, Division of E. Miltenberg, Inc. New York, New York. 10010.

[2] J. Pfrimmer and Company. Erlangen, West Germany.

[3] Chemically, methoxyflurane is 2,2-dichloro-1,1-difluroethylmethyl ether, supplied by Pitman-Moore, Division of Dow Chemical Company. Fort Washington, Pa. 19034.

butylated hydroxytoluene, Metofane inhalation anesthetic. The hair was removed from the appropriate sides of each animal with electric clippers. The skin was prepared with Zephiran Chloride (benzalkonium chloride)[4] in an aqueous solution of approximately 0.13% concentration. All incisions were made with surgical scissors. The abdominal sutures were interrupted using a (00 gauge) braided surgical silk.[5] The ventral and dorsal skin closure was made using 9 mm stainless steel wound clips.[6]

After surgery, each animal was given 0.2 cc Longicil S[7] intramuscularly. Sterile procedure was used as much as possible. All equipment and sutures were sterilized "cold" with Zephiran Chloride. If an infection developed post-surgically in the wound or if the animal experienced a general infection due to surgical stress, additional Longicil was given at a dose of 0.2 cc upwards to 0.7 cc. Treatment was usually a single injection intramuscularly but, once on occasion, treatment was continuous for two to three days. In surgery done most recently, chloromycetin[8] or Mychel-S[9] was given in lieu of Longicil at a dose of 0.05 cc intraperitoneally of a 10% solution as a

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[4] Winthrop Laboratories, Division of Sterling Drug Company. New York, New York. 10016.

[5] American Cyanamid Company, Surgical Products Division. New York, New York. 10965.

[6] Autoclips supplied by Clay-Adams, Inc. New York, New York. 10010. (Made by Totco, Glendale, California)

[7] Fort Dodge Laboratories, Inc. Fort Dodge, Iowa. 50501. Note: One cc of Longicil-S contains 150,000 units benzathine penicillin G, 100,000 units of procaine penicillin G, and 250 mg of dihydrostreptomycin in an aqueous suspension.

[8] Brand of chloramphenicol sodium succinate. Park, Davis and Company. Detroit, Michigan. 48232.

[9] Brand of chloramphenicol sodium succinate. Rachelle Laboratories, Inc. Subsidiary of International Rectifier Corporation, 700 Henry Ford Avenue, Long Beach, California. 90801.

prophylactic and a dose of 0.05 to 0.1 cc later if infection developed. In seriously infected wounds, treatment at the above dose levels was given directly into the infected area.

RESULTS

The new scapular suture held extremely well. Virtually no scapular sutures weakened. The animals were held together firmly for the differential feeder and scapular infection was not a serious problem. Even if the animals were exhibiting immuno-incompatibility the supportive sutures still held.

DISCUSSION

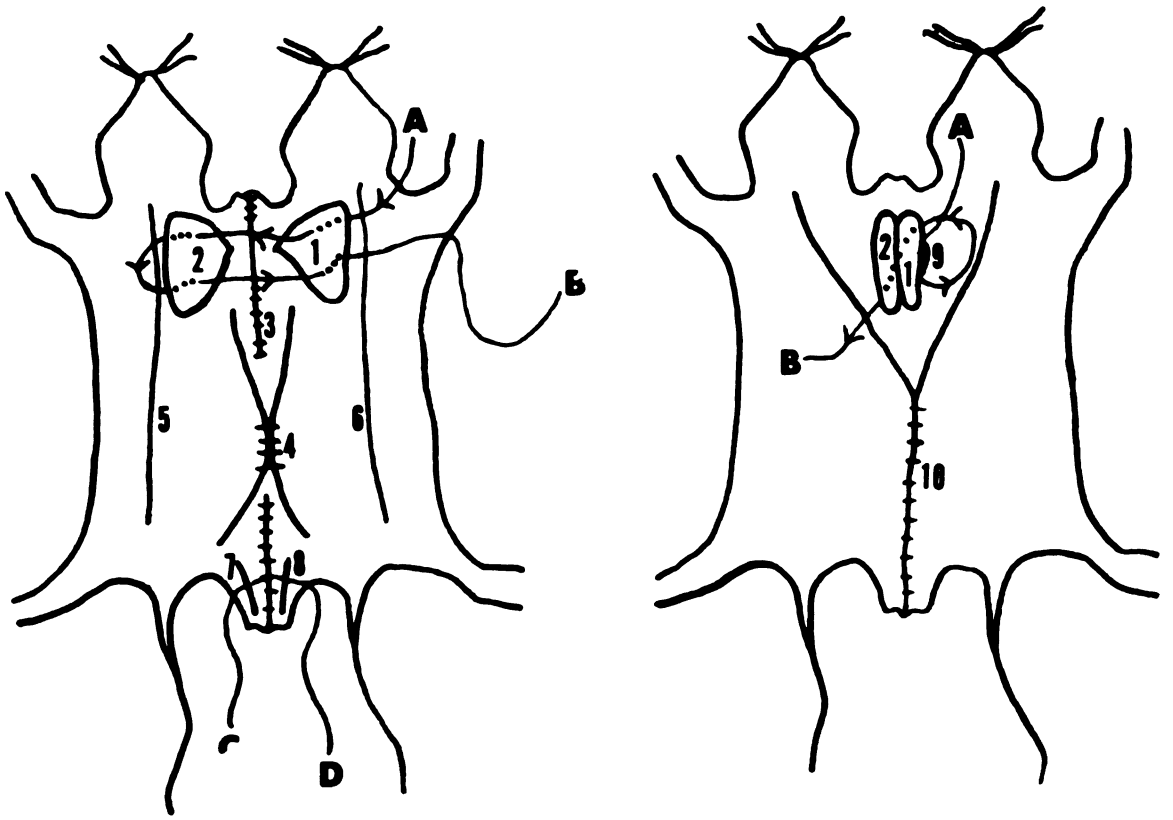
Surgery is more easily performed on weanling animals. The central areas of the scapula are cartilaginous at this age and the needle can be easily inserted without cracking the bone. Parabiosis seems to be better tolerated, behaviorly, when performed at an early age and younger, smaller animals will physically stress the union immediately after surgery far less than adults. There is no need to remove the scapular suture after healing. The suture becomes encased in connective tissue and produces no subsequent problem. Non-absorbable sutures are used both for abdominal sutures and supportive sutures to avoid the suture from loosening before a firm union has been established.

One other major problem developed that has not been previously reported in the literature. Many pairs of animals, after surgery, would bite at the abdominal sutures or auto clips probably because of

a slight irritation. The wound in many cases would be ripped open and become infected. In some instances re-suturing with a braided silk, gauge 1 suture prevented a recurrence but in many cases did not. A rather noxiously tasting substance, when placed on the area, was of little benefit. Small aluminum braces were then constructed. These braces were about 13 mm wide and extended from the tail of the animal to the pectoral girdle. The brace was sutured on the dorsal surface of the animal with four sutures (one at the caudal area, one at the mid-line and two over the pectoral region). These braces prevented the animals from "jack-knifing" and worked very well in keeping them from the wound. Within seven to ten days the brace would be sloughed off at which time the parabiotic wound would be healed. No infection ever resulted from using the braces. Sedation was never used post-surgically since, with certain experiments, it is unwise to treat with drugs that could alter experimental results unless absolutely necessary to preserve enough animals for the experiment. In all cases control animals received the same antibiotic treatments as experimental animals, but control animals were not given braces.

Wound clips were removed several weeks post-surgically and any small skin areas not healed were closed with a few silk sutures. When using wound clips one must check the animal's incisors every 2 or 3 days. Several animals have had clips caught in their teeth and could not eat.

The rats were fed, while convalescing, with low food cups, approximately $\frac{1}{2}$" high, wired to the bottom of the cage. All animals were fed with a standard, powdered grain ration. No liquid diets were used.



Animal outlines taken from Wei Han, 1963 (285)

Figure 11. Modified surgical procedure for parabiosis. (A) and (B) in the left figure are tied together after drawing the scapulae (1) and (2) together producing the knot at (9) in the right drawing; (A) and (B) in the right figure are then tied across the top of the two scapulae after passing the suture back through the scapular area as described in the text; (C) and (D) are tied loosely around the respective femurs (7) and (8); (3) the ventral skin suture; (4) abdominal sutures; (5) and (6) the dorsal skin flaps that are shown partially sutured at (10). The arrows indicate the direction of the suture and the dots indicate that the suture is under the scapula at that particular point.

PART 4

DYE DILUTION STUDIES

INTRODUCTION

After one has perfected a surgical skill or technique, it is wise to test several pairs of parabiotic animals for the rate of cross-circulation. Many authors do not report data on rates of blood exchange but it is essential to know if parabiosis between members of a certain species of animals or by a particular technique will produce viable cross-circulation.

There are several methods available to determine rates of cross-circulation. Early workers used the compound belladonna. After injecting this compound into one parabiont, the pupils of the non-injected animal were observed for dilation in 20 to 30 minutes (302). Protein bound fluorescein was used by Scheff and Plagge (303), 1955. Radioiodinated serum albumin and iron^{59} labeled erythrocytes may also be used (304) as well as chromium labeled red cells (305).

Perhaps the most widely used method is that of T-1824 or Evans Blue dye[1] injection. Evans Blue molecules adsorb primarily to the albumin fraction of the blood and, consequently, are mixed uniformily as it is cleared by the injected animal very slowly (306-309). Evans Blue is eventually cleared through the bile (310).

Hervey (283), 1959, presented a detailed description of the technique and included a mathematical formula by which one can calculate

[1] Allied Chemical, National Biological Stains and Reagents Dept. (Formerly National Aniline Division), P. O. Box 431, Morristown, New Jersey. 07960.

rate constants. Studies were not undertaken here to determine the time that cross-circulation begins in this preparation but it has been established that considerable exchange occurs at 3 days (304, 305). As the wound heals, capillaries interdigitate and grow together producing a direct blood exchange.

TECHNIQUE

Adult, female Osborne-Mendel rats were made parabiotic at weaning. Controls and parabionts were fed grain laboratory ration (317) until time of experiment.

All optical density readings for Evans Blue were done (283) using benzalkonium chloride as solvent (311). A 17% concentration was employed instead of the original 12.8% used by Caster since the commercially available concentration is now 17% and dilution would produce considerable frothing. The 17% solution proved to be as optically homogeneous and acceptable as the 12.8%.

The absorption maximum for Evans Blue in a 17% benzalkonium chloride solution was 626 mu. All readings were, therefore, done at 626.

Calculation of dye dosage: The standard curve for Evans Blue in serum was determined to be perfectly linear at least to 8 ug per ml. It is linear somewhat beyond this point but further determination was unnecessary. As suggested by Hervey (283), a dosage of 0.2 cc of a 0.25% solution per 100 grams body weight was used.

To determine whether that level of Evans Blue would be adequate, the dilution of the dye in a rat was determined mathematically. Assume a 300 gram rat is injected with 0.6 cc of a 0.25% dye solution.

$$\frac{0.25 \text{ g dye}}{100 \text{ ml solution}} = \frac{X}{0.6 \text{ ml dye solution injected}}$$

$X = 0.0015$ g injected

A 300 gram rat will have a blood volume approximating 8% of body weight or 24 ml blood or 12 ml plasma.

$$\frac{0.0015 \text{ g dye injected}}{12 \text{ ml plasma dilution}} = \frac{X}{0.1 \text{ ml plasma taken for assay}}$$

$X = 0.0000125$ g of dye found in 0.1 ml plasma or 12.5 ug.

0.05 ml plasma is then added to 6 ml zephiran chloride giving a final dilution of 1.03 ug dye per ml solution. This final concentration is on the linear standard curve and, therefore, the dye dosage is acceptable.

Zephiran chloride was used because Evans Blue does adhere to glass surfaces and can produce an error in optical density readings. Zephiran chloride detergent frees Evans Blue from the glass and also elutes the dye from its adhesion to the albumin fraction in the blood allowing a far more accurate optical density determination (311-313).

Hemoglobin was not considered to be a primary interference since its absorption maximum in zephiran chloride is absent at 540 mu and the sample reading was made at 626 mu. Hemolysis was usually slight and fairly uniform; furthermore, any major error should cancel out.

A 0.2504% solution of Evans Blue was prepared in distilled water. The solvent fraction would be 99.7496 grams converted to volume correcting for room temperature. This solution was standardized and the specific gravity determined. All blood samples were taken by cardiac puncture under sodium pentobarbital anesthesia.[1] 0.35 cc blood was taken for control serum and determination of the hematocrit. The appropriate dye injection was prepared and the syringe weighed.

[1] Dosage of 0.05 cc per 100 g body weight of a 6% solution given intraperitoneally.

The approximate position of the femoral vein is located by palpating the femoral artery in the groin. An incision of 1 to 1.5 cm is made with surgical scissors after picking up the skin with tissue forceps about 4-5 mm, lateral to the point of palpation. A "mosquito" hemostat is then used to clear any skin adhesions around the femoral vein. A hemostat is clamped on each side of the wound and allowed to hang below the leg. This keeps the wound opened and exposes the vein. The entire preparation procedure takes less than a minute and there is no blood loss. The needle (27 gauge, $\frac{1}{2}$") is inserted, proximally, into the vein. After dye injection and needle removal, a dampened gauze is pressed lightly on the vein for a few seconds. No blood is lost from the vein during or after injection. The wound is closed with one or two small silk sutures. The syringe is then re-weighed and the exact dosage calculated by difference. This procedure is fast, extremely accurate (one is confident of a complete injection) and does not appear to be debilitating to the animals. Post dye injection blood samples of 0.3 ml were taken by cardiac puncture from each animal of a pair at 10 minutes, $\frac{1}{2}$, 1 and 2 hours with the ammonium salt of heparin as anticoagulant. The total amount of blood taken was always less than 10% of the blood volume based on 8% of body weight.

The blood was centrifuged at 3000 rpm for 30 minutes and 0.1 ml plasma added to 6 ml zephiran chloride. The optical density was determined against zephiran chloride with 0.1 cc distilled water added.

Five single controls and one member of each of 5 pairs of parabiotic animals were injected and plasma optical densities determined over time by the above procedures. The 2 hour sample in one of the pairs was lost. The total amount of blood removed from the control

animals was statistically the same as that removed from the parabionts ($P > 0.05$). Any error in disproportionate blood removal was therefore eliminated making the dilution curves comparable.

The exchange rate was calculated using the $\frac{1}{2}$ hour blood samples and expressed as the percentage of plasma volume exchanged per minute. The exchange rate best fits the curve for the hyperbolic cotangent of rate times time as described by Hervey (283), 1959.

$$\text{Coth } rt = \frac{C_1}{C_2}$$

r = the exchange rate expressed as a fraction of one animal's plasma volume per minute

t = time in minutes after dye injection

C_1 and C_2 = optical densities of the injected and non-injected animal, respectively

It became necessary to express this equation in a more usable form which should be of benefit to future investigators. Hervey did not publish the mathematical "breakdown" of the above expression and a mathematical discussion of this particular equation was not found in the literature, although, Huff et al. (304), 1956, presented a mathematical tretis of parabiosis with a physical model.

In order to make calculations easier, $\frac{C_1}{C_2}$ will be equal to 2.

$$\text{Coth } rt = 2$$

$$\text{let } rt = X$$

$$\text{Coth } X = 2$$

$$\text{Coth } X = \frac{\text{Cosh } u}{\text{Sinh } u} = \frac{e^{x} + e^{-x}}{e^{x} - e^{-x}}$$

$$\frac{e^{x} + e^{-x}}{e^{x} - e^{-x}} = 2$$

$$\frac{e^x + \frac{1}{e^x}}{e^x - \frac{1}{e^x}} = 2$$

$$e^x + \frac{1}{e^x} = \frac{e^{2x} + 1}{e^x}$$

each side of the first expression was multiplied by e^x to give the second expression

$$e^x - \frac{1}{e^x} = \frac{e^{2x} - 1}{e^x}$$

each side of the first expression was multiplied by e^x to give the second expression

$$\frac{e^{2x} + 1}{e^x} \times \frac{e^x}{e^{2x} - 1} = \frac{e^{3x} + e^x}{e^{3x} - e^x}$$

$$\frac{e^{3x} + e^x}{e^{3x} - e^x} = 2$$

$$e^{3x} + e^x = 2e^{3x} - 2e^x$$

$$e^x + 2e^x = 2e^{3x} - e^{3x}$$

$$3e^x = e^{3x}$$

$$3 = \frac{e^{3x}}{e^x}$$

$$3 = e^{2x}$$

$$\ln 3 = 2x$$

$$x = \frac{\ln 3}{2}$$

since $x = rt$

$r = \frac{\ln 3}{2t}$ = a certain fraction of plasma volume exchanged/minute

let plasma volume = PV; so,

$(PV \times \frac{\ln 3}{2t})$ = the cc of plasma volume exchanged per minute;

since, 2.3026 times the common log = $\log_e$, it follows that

$$\frac{PV \ \frac{(2.3026 \times \log_{10} 3)}{2t}}{PV} \times (100) = \% \text{ plasma volume exchanged/minute}$$

The "real" numbers in the above expression will change as the original value for $\frac{C_1}{C_2}$ changes.

Thirty-minute samples are adequate in calculating exchange rates. By 30 minutes the dye concentration in a non-parabiotic injected animal is rather stable and is not being cleared from the blood significantly per unit time; consequently, any decrease in optical density reflects a true exchange in parabionts. If one waits too long to take samples, the dye will be equilibrated between the two animals and $\frac{C_1}{C_2}$ will become unity and obviously flux will be 0.

RESULTS

The dye dilution curves for single injected controls, non-injected and injected parabionts are shown in Figure 12. Dye injection was made at time 0. The parabiotic rats displayed equal optical densities at 117 minutes. In Table 8 the percent of plasma volume exchanged per minute is presented.

By definition the % exchange rate will be inversely proportional to the time for each parabiont to display equal optical densities; consequently, the percentage volume exchanged should be highly but inversely correlated to time at equal optical density. This correlation was determined excluding the parabiotic sample that had to be calculated at 1 hour instead of 30 minutes. The correlation had an r value of -0.786. This is a very good correlation but the t-statistic was 1.800 which was non-significant. The non-significance was simply a matter of a large within group variance and small sample size. The large within

group variance is actually an unfair hinderance in statistical interpretation, since, the large difference in exchange rates is a result of the effectiveness of wound healing and is totally in the realm of chance and cannot be corrected for in experimental design. Exchange rates were still highly correlated, in the correct sign, with time. As an added check on the technique the total dye removed from the animals during the experiment added to the residual dye left in circulation statistically equaled the dose of dye injected. Since samples were collected shortly after injection, it was expected that these values should be very close. Theoretically, the only difference would be the dye cleared by the animal from the time of injection to the first 10 minute sample. This difference is a small percentage of actual dose.

DISCUSSION

The control curve in Figure 12 agrees very well with that reported by Gibson and Evans (309) in that the slope is fairly constant for 8-10 minutes after injection of the dye. Optical densities seemed to be slightly more stable after 25 minutes.

At 120 minutes there is a slight discrepancy between the curves for parabionts and controls. Exactly the same procedure was used in all animals including dye dosage; consequently, theoretically all curves should terminate at approximately the same optical density. The dye dosage was given on a body weight basis and since blood volume is correlated to body weight, one would expect similar optical densities assuming that the dynamics of clearing a small percentage of the initial dose was the same in all animals. Sometimes, in parabiotic animals,

a small unhealed area or irritation exists within the union and that some dye is probably lost from the systemic circulation when crossing from one animal to the other. This would decrease the optical densities. The difference between all curves at 120 minutes, although, are not statistically different ($P > 0.05$).

Hervey (283) reported a range for percent exchange of one animal's plasma volume to its partner per minute as 0.3 to 2.1% (1.0 ± 0.2 [std. error]) for eight pairs. Similar results were reviewed by Finerty (302), 1952. In the present study the average percentage exchange was 2.338 ± 1.802 (std. deviation) for five pairs with a range of 0.51 to 5.03. Four out of five of the present pairs had exchanges higher than Hervey's mean. The presence or absence of a coelio-anastomosis makes no difference on exchange rates (283). Hervey also states that his lowest rates were in pairs done by the unmodified Bunster and Meyer procedure and that the scapulae were separated. The improved exchange rates in the present study possibly are a result of the improved scapular supportive sutures previously described. With improved cross-circulation rates it will become easier to demonstrate the exchange of various test substances from one animal to its partner.

It is very important to reiterate the variability in cross-circulation data and if possible increased sample sizes should be used.

Plasma volumes were calculated in parabiotic rats at the time when optical densities were equal in both animals. This same time was used for control animals. These volumes were used in calculating final percentages of plasma volume exchanged.

Table 8

PARABIOTIC PLASMA EXCHANGE RATE AS DETERMINED BY DYE T-1824 INJECTED INTO OSBORNE-MENDEL FEMALE RATS

Percent plasma volume exchanged / minute *
5.03
1.57
1.34
3.24
.51
average 2.34

*Optical density of plasma was determined one-half hour after dye injection, except for the last sample which was taken at one hour.

PART 5

CAUSES OF DEATH IN PARABIOTIC RATS

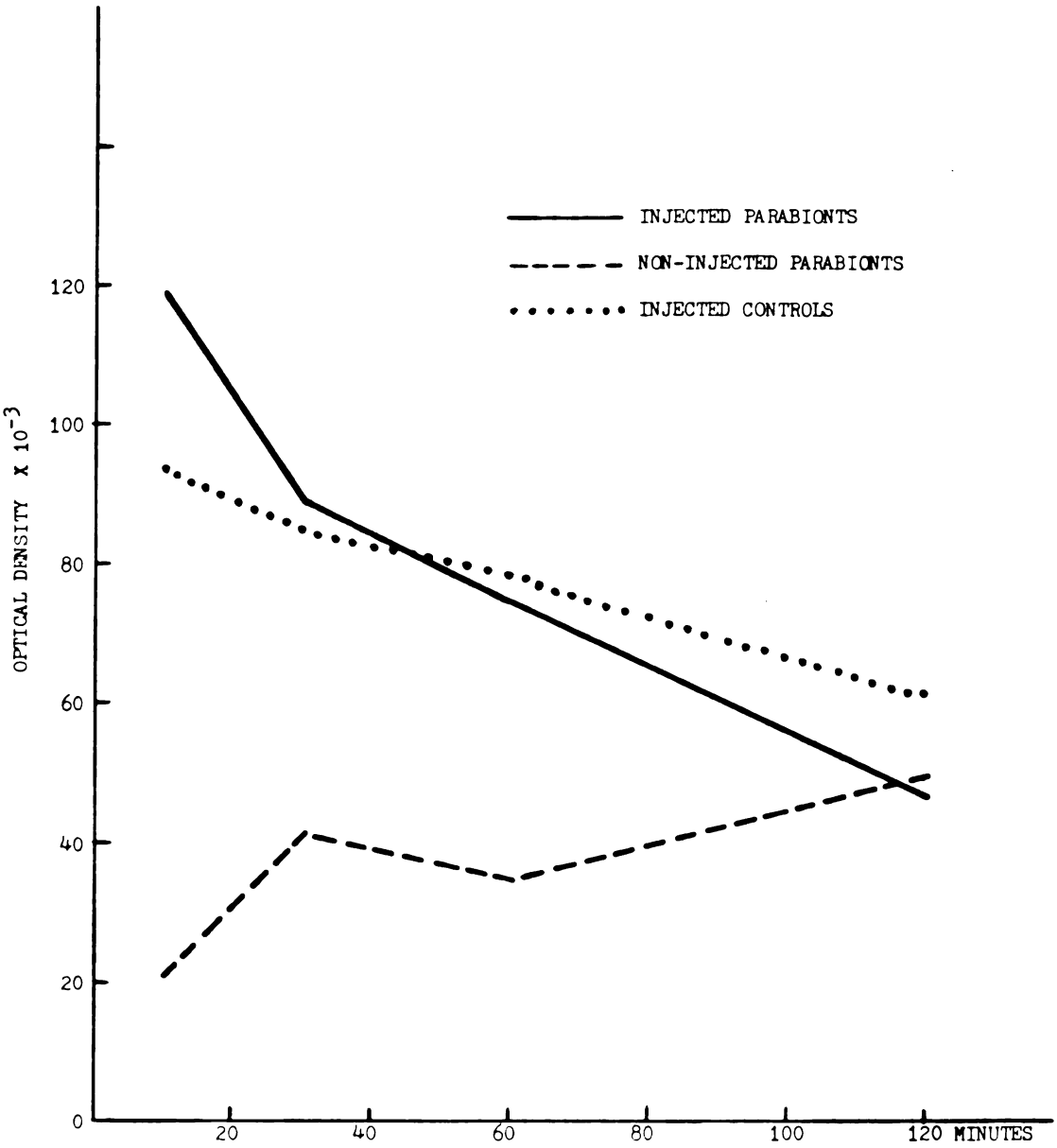


Figure 12. Evans Blue dye dilution curves for control and parabiotic female Sprague-Dawley rats.

INTRODUCTION

The major cause of death in parabiotic rats is tissue rejection referred to as "Parabiosis Intoxication" (314-316, 302). Other causes of death have been reported as excessive anesthesia, fighting, peritonitis and shock (315).

TECHNIQUES

Sprague-Dawley, Osborne-Mendel and S5B/Pl rats (both male and female as well as littermates and non-litttermates) were joined parabiotically by procedures described previously (see surgical techniques, this thesis). As a tissue incompatibility control, 10 pairs of Sprague-Dawley and S5B/Pl rats were also made.

Careful surgical and post-surgical records were kept on all pairs.

RESULTS

Table 9 summarizes the surgical and parabiotic success for all groups tried as well as the itemization of the causes of death.

Two hundred and thirty-two pairs were lost out of 292 tried or 79.45% excluding the known tissue incompatable pairs of Sprague-Dawley and S5B/Pl rats. It is not uncommon for death rates to be this high in parabiosis which produces a tremendous disadvantage for the investigator.

It also must be recognized that before an experiment has ended, especially obesity experiments which may last for months, some of the pairs surviving the first months of parabiosis will have died resulting from some of the reasons discussed later. It is wise to start with 4 or 5 times as many pairs as needed in the experimental design to insure against the items listed in Table 9.

The number of deaths by rejection was 96 or 32.88%. Many animals diagnosed as "uncertain" may have rejected and, if these numbers are added to the known rejection figures, the rejection total increases to 136 or 46.58%. Both percentage figures are within a reasonable range of what one would expect for parabiosis. Forty pairs were lost from wound infection or 13.25% and 25 pairs or 8.28% separated. Out of all male pairs 42.86% rejected compared to 30.40% for females. For female littermates, 26.83% rejected compared to 45.00% for male littermates. For female non-littermates, 33.33% rejected versus 40.91% for male non-littermates.

In the Sprague-Dawley group the female non-littermates had a slightly lower rejection rate than female littermates but the male littermates rejected less than male non-littermates.

Osborne-Mendel female littermates had no diagnosed cases of rejection compared to 30.19% for Osborne-Mendel female non-littermates. There were no male Osborne-Mendel littermate pairs to compare to non-littermates.

Female S5B/Pl littermate pairs had an extremely low rejection rate surpassed only by one Osborne-Mendel non-littermate group (Table 9). The highest rejection was, as expected, in the differential pairs, equalling 80.00% and if the one "uncertain" is included, 90.00%.

In some groups all pairs died. These include: 1) male non-littermate Sprague-Dawley, 2) male non-littermate Osborne-Mendel, and 3) female S5B/Pl littermate rats.

DISCUSSION

Male rats, in general, have a significantly lower success rate in parabiosis than females with respect to rejection as well as total pairs surviving a several month test period (42.80% vs. 30.40%). Similar problems were found with male animals by other investigators (302). Male parabionts fight a great percentage of the time. In Table 9 only two pairs were shown to die from fighting but the incidence of fighting at various intervals of time during experiments was quite high, especially in male animals. S5B/Pl females also fight quite a bit but these rats are known to be quite temperamental. Only one or two incidences of fighting were seen in other female pairs. These pairs were removed from the experiment.

Since serious problems do arise with male parabionts and because the investigator is at a great disadvantage because of rejection, all precautions must be taken to increase success ratios; consequently, female animals are usually used if at all possible. Food intakes were taken over long enough periods of time to minimize the "estrous" interferences (228-231).

Osborne-Mendel female littermates displayed the lowest rate of rejection followed by S5B/Pl and finally Sprague-Dawley rats with 0.00, 18.2, and 45.0% rejection rates, respectively. Osborne-Mendel female non-littermates rejected fewer times than Sprague-Dawley female

non-littermates with 30.19% rejection compared to 38.70%, respectively. Male non-littermate Osborne-Mendel rats rejected fewer times (30.00%) as compared to Sprague-Dawley (50.00%). Over-all rejection for S5B/Pl rats was 18.2%, for Osborne-Mendel, 25.74%, and for Sprague-Dawley, 42.54%.

All Sprague-Dawley rats were ordered and, regardless if ordered as littermates or not, one cannot be certain that littermates were always joined. Littermates may also be present in groups ordered as non-littermates and, by chance alone, some littermate pairs would be joined. This explains the more erratic rejection results for Sprague-Dawley rats as opposed to Osborne-Mendel when comparing littermates with non-littermates.

Osborne-Mendel rats were from the breeding colony of Dr. Rachel Schemmel at Michigan State University, allowing excellent control over littermate and non-littermate pairs.

If all littermates, whether male or female or whether Osborne-Mendel, S5B/Pl or Sprague-Dawley, are compared to all non-littermates, the littermates do have a lower rejection rate of 30.39% versus 34.21%. One must remember that these numbers are heavily biased by the abnormally high littermate rejection for Sprague-Dawleys, the rejection rate being very low for all other female littermate pairs.

The total percentage of Osborne-Mendel rats dying was 88.97%, 66.42% for Sprague-Dawleys and 100.00% for S5B/Pl pairs. The reason for this is due to the percent deaths other than rejection. Sprague-Dawley rats had the lowest non-rejection deaths at 38.88%, followed by Osborne-Mendel with 63.23% and S5B/Pl pairs with 81.81%. The major cause of death in Osborne-Mendel and S5B/Pl pairs other than rejection

was infection. Probably, the best animal used for parabiosis was the female Osborne-Mendel littermate, although, the rate of infection was high. However, infection can be treated whereas rejection cannot. One should be cautioned in using littermate animals that are too highly inbred. The litters may become small and the size of the animal also decreases (53). The above animals could not be secured in sufficient numbers at any one time in order to do the amount of parabiosis necessary. The non-littermate Osborne-Mendel and littermate S5B/Pl females, male Sprague-Dawleys and all other male pairs were not suitable for parabiosis at all. The animals offering the most viable pairs, coupled with availability, were littermate and non-littermate Sprague-Dawley rats; consequently, these rats became the primary experimental subjects for food intake studies.

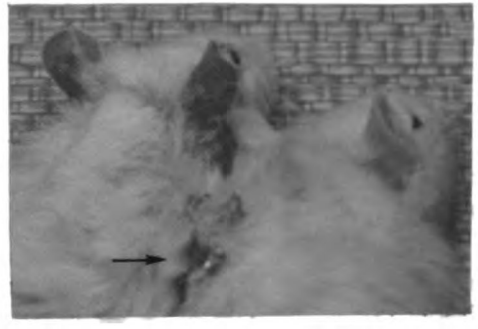
Table 9

CAUSES OF DEATH FOR VARIOUS GROUPS OF PARABIOTIC RATS

CAUSE OF DEATH	SPRAGUE-DAWLEY								OSBORNE-MENDEL								S5B/P1		SD + S5B/P1*	
	FEMALE NLM** n = 62		FEMALE LM*** n = 40		MALE NLM n = 12		MALE LM n = 20		FEMALE LM n = 20		FEMALE NLM n = 23		FEMALE NLM n = 83		MALE NLM n = 10		FEMALE LM n = 22		MALE n = 10	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
REJECTION+	24	38.7	18	45.0	6	50.0	9	45.0			2	8.7	30	36.1	3	30.0	4	18.2	8	80.0
SEPARATION	3	4.8							2	10.0	6	26.1	12	14.5	1	10.0	1	4.6		
INFECTION	2	3.2	1	2.5			1	5.0	7	35.0	2	8.7	17	20.5	4	40.0	6	27.3		
RESPIRATORY DISEASE	2	3.2	1	2.5									2	2.4			1	4.6		
SURGERY			1	2.5	2	16.7					1	4.4								
GUT HERNIATION									1	5.0			1	1.2						
GUT OBSTRUCTION			1	2.5																
WOUND CLIP IN TEETH	1	1.6			1	8.3														
FIGHTING					2	16.7							1	1.2			1	4.6	1	10.0
BITING WOUND									2	10.0	2	8.7								
TAPE TOO TIGHT+									1	5.0							2	9.1		
GANGRENE											1	4.4								
TUMOR													1	1.2						
METOFANE OVERDOSE															1	10.0				
COLD ENVIRONMENT++																	2	9.1		
UNCERTAIN	4	6.5	5	12.5	1	8.3	4	20.0	3	15.0	2	8.7	15	18.1	1	10.0	5	22.7	1	10.0
TOTALS	36	58.1	27	67.5	12	100.0	14	70.0	16	80.0	16	69.6	79	95.2	10	100.0	22	100.0	10	100.0

*Parabiosis between a Sprague-Dawley and an S5B/P1
**Non-Littermate
***Littermate
+Tape was sometimes wrapped around the tails of the animals to help take stress off the surgical union.
++One morning, in the Winter, the heat went off in the animal room.

1



Rejection in parabiotic animals is termed "Parabiosis Intoxication" (314). Clinical symptoms are easily recognizable as shown. One animal develops severe anemia and the other marked hyperemia. Symptoms are extremely distinct in the ears but may also be apparent in the tail. The union between the animals is not physiological and begins to break down as shown at the arrow.

PART 6

VARIOUS PHYSIOLOGICAL AND ANATOMICAL VALUES FOR "NORMAL" PARABIOTIC RATS

INTRODUCTION

Parabiosis has been used over a century in the study of many physiological problems but few investigators have considered how the parabiotic state modifies the animal's anatomy and physiology. Perhaps the only investigator that dealt with this subject in any detail was Hervey (283). Hervey's parabionts grew more slowly and at any age weighed less than single controls. At 5 months the average weight of his parabiosed animals was approximately 71-85% that of control animals. Weight gained per unit of food intake, i.e., food efficiency, was the same for the above animals whether single or parabiotic.

Hervey's "normal" parabionts contained one-half the body fat of the controls, the vicera appeared to be normal but smaller, and the parabionts were shorter than "normal" animals. Hervey concluded that energy metabolism was the same for single controls and parabionts since feed efficiencies were identical.

Individual organ weights, as affected by parabiosis, for the most part, have not been reported except for a brief comment by Hervey. In his "hooded" rats the heart and kidney weights for all female and male parabiotic animals were slightly lower compared to controls. The liver also weighed less in parabiotic males when compared to single controls but was almost identical in weight for females. The variability was great and no statistics were reported.

TECHNIQUES

Osborne-Mendel and Sprague-Dawley, male and female rats were used. Parabiosis was performed during the first one to two months of life. All single controls were subjected to comparable sham surgery. Surgery was performed as indicated in the surgical section of this monograph. The animals were fed either a standard grain (317) or high fat diet (298). At the beginning of experiments all animals were of the same age. Each experimental group received rats staggered over the range of body weights so that each group would have close to the same average body weight and standard deviation. Several litters were used. Therefore, each experimental group received an equal number of animals from each litter as far as possible.

Nose to anus length was determined with a metric rule with the animals on their backs. Fat pads were removed following the methods of Schemmel (318), 1967.

Blood volume was determined by the Evans Blue method. (See dye dilution methods in this monograph or von Porat (306), 1951). Packed cell volume was determined using heparinized, fire polished, micro-hematocrit capillary tubes purchased under the brand name capilets.[1]

All animals were placed on experiment immediately after surgery in order to follow growth curves continuously from weanling on; consequently, many animals were lost during the rejection phase of parabiosis as well as other complicating factors (see causes of death in parabiosis, this monograph).

[1] Dade, Div. American Hospital Supply Corp. Miami, Florida. 33152.

RESULTS

Figure 13 shows growth curves for parabiotic female Osborne-Mendel rats fed either a 60% high fat ration (298) or a ground grain diet (317) compared to controls. All rats except the high fat single controls showed a decrease in body weight for about 2 days as a consequence of surgery.

The single high fat control curve deviated from the other curves almost immediately, whereas, the other 3 curves remained together until about day 70 when the parabiotic high fat animals began to gain more body weight compared to controls and parabionts fed the grain ration.

The grain fed parabionts did have an initial slower rate of weight gain compared to grain fed sham operated single rats, but this slight difference was made up rapidly and at the end of 181 days, the weights of the grain fed single and parabiotic rats were statistically the same. The final weights for the single high fat fed animals were statistically greater than those for the high fat fed parabiotic animals ($P < 0.10$). The significant level is actually much better than 0.1. The approximate-t test was used, because of high variance, with the "t" value equalling 2.783. Since the F-statistic for within group variance was also high, critical values for the t-statistic had to be calculated which makes the test quite rigorous. The calculated critical value for the probability of 0.05 was $\pm$ 2.820. The t-value was only 0.037 away from 0.05 and the statistic tables do not list a probability factor between 0.05 and 0.10. It is evident that the probability of the differences between the weights of the single and parabiotic high fat fed animals having

occurred by chance is far less than 0.10. The single high fat controls were significantly heavier than the grain fed controls ($P<0.01$) or grain fed parabionts ($P<0.01$). The high fat fed parabiotic animals weighed more than the single grain fed rats ($P<0.01$) or grain fed parabionts ($P<0.01$).

Under the particular conditions of this experiment the grain fed controls and parabionts did weigh the same with the controls measuring slightly longer than the parabionts. The fat depot weights (Table 13) were lower in the parabionts but body fat was not determined. In subsequent studies the grain parabionts did weigh considerably less than grain controls which is usually the case. The parabiotic Osborne-Mendel rats did do exceptionally well.

The data in Tables 10 and 12-15 are a compilation of all animals in parabiosis in two experiments such as the one for which body weights are given in Figure 13. Combination was necessary to improve sample sizes since so many animals died in each experiment. Care was taken to include as many animals as possible from each of the two experiments in each of the basic experimental groups, viz., single and parabiotic grain fed and single and parabiotic high fat fed animals. Since the experiments ran different lengths of time, this pairing was necessary to hold constant the within-group variance and to make statistical comparisons valid.

Table 10 shows the difference in body lengths between single sham operated controls and parabionts, fed either the high fat or grain diet. The animals were fed for a minimum of 165 days and a maximum of 220 days. The data are a compilation of 2 experiments as discussed previously. Animals were in parabiosis for at least $5\frac{1}{2}$ months when

measurements were taken. All parabionts were shorter than controls except the comparison between single grain fed animals and high fat parabionts (groups B, C).

In subsequent experiments body length was also recorded as shown in Table 11. As can be seen, regardless of sex or species, parabionts were always shorter than controls ($P < 0.001$, except for the fourth group, $P < 0.01$). These animals had all been fed grain ration. The length of time the rats were parabiosed for the various groups was $8\frac{1}{2}$ months to $5\frac{1}{2}$ weeks. The failure to grow in length seemingly occurs within the first several weeks of parabiosis; this deficiency persists for the life of the animal.

The only fat depot weight (Tables 12 and 13) that was markedly larger in the controls as compared to the parabionts, regardless of how expressed, was the perirenal ($P < 0.05$). All of the parabiotic depot weights are smaller, absolutely or on a body weight basis, except the intermuscular which is slightly larger. On a sign test or frequency test, this would undoubtedly be significant even though most individual fat depot weights were not statistically different between the control and parabiont.

The growth curves in Figure 13 were terminated at 181 days when significant separations were evident between high fat and grain single animal curves. The animals, however, remained on the diets for an additional 284 days. At sacrifice, they were $16\frac{1}{2}$ months old when the control animal weighed 337 grams while the parabionts averaged 319. The parabiotic rat weighed approximately 18 grams less than controls.

Body fat was calculated using the prediction equations of Grewal et al. (319), 1973, by multiplying the various fat depot weights

(inguinal, interscapular, genital, renal-retroperitoneal, mesenteric and omental) and the body weight by appropriate constants and then summating. To increase sample size, animals were added from another identical experiment giving six animals in the single grain as well as the parabiotic grain categories; consequently, neither group was biased. The range in age was $6\frac{1}{2}$ to $16\frac{1}{2}$ months with four animals in each experimental group in the older category. The average age was about one year. The average animal weights at the beginning of the experiment for the single grain was 54.3 grams and 58.5 for the parabionts. These weights were statistically the same. At sacrifice the single grain fed animals weighed 315 grams with the parabiotic weighing 287. This is a difference of about 28 grams per animal. Body fat calculated by prediction equations for single grain fed rats was 79 grams or 25% of body weight and for parabionts, 56 grams or 19% of body weight. This is a decrease of 23%. The decrease in grams fat was 24 grams which is about 86% of the total weight difference (28 grams) between the single and parabiotic groups. It seems clear that either directly or via food intake alterations, body fat was regulated at a lower level in parabiotic rats.

Tables 14 and 15 compare internal organ weights between control and parabionts, all fed a grain ration. On an absolute basis only the lungs were heavier in the controls ($P < 0.05$) with the empty stomach being markedly heavier in the parabionts on a body weight basis.

Since adrenal weights may be used as a "stress" indicator, an additional study was made of adrenal weights between controls and parabionts with a greater sample size. Thyroid weights were also determined. When sacrificed, animals had been in parabiosis for 9

to 12 months. Adrenal weights were not different, expressed absolutely or corrected for body weight, between control and parabiont (Table 16). When corrected for body weight, the parabionts actually had lighter adrenals. The thyroid weights (Table 17) were different on an absolute basis ($P < 0.05$) but the significance was lost when expressed on a body weight basis.

Blood volume using T-1824 dilution was determined in four parabiotic pairs (Table 18). The blood volume of the parabionts was slightly higher than the controls but the difference was non-significant. A possible explanation for this difference is that dye seems to be lost from the systemic circulation in the parabiotic union.

DISCUSSION

The main reason for the preliminary growth curves plotted in Figure 13 was to determine how parabiosis affected body weight gain when rats were fed the high fat diet. Would parabiotic rats become nutritionally obese on a high fat diet? The average body weight for the parabionts fed the high fat ration was approximately half way between the single grain fed and single high fat fed rats. Body weight gains of the parabionts fed the high fat ration were even less than the grain controls for the first 40 days post surgery but then they reached and surpassed the single grain controls at day 65. The parabiotic grain animals did not catch the single grain controls until 130 days after surgery when the two curves became congruent.

The decreased growth rates after surgery were probably a result of surgical stress combined with parabiotic stress. The single grain

controls had an initial faster growth rate but then leveled off at about 60 or 70 days, whereas, the parabionts had a slower rate of growth but maintained it for a longer period of time until they caught up with the single controls.

The high fat fed parabionts did not gain as much weight as the single high fat fed controls. The controls were sham operated and housed 2 in a cage to simulate parabiosis. Since the grain parabionts were similar in weight to single grain controls, the parabiotic state, per se, was not causing the decreased gain of the parabionts fed the high fat ration.

All subcutaneous and abdominal fat organs weighed less in the parabionts compared to controls. There seemed to be a lower body fat content in parabiotic animals fed a low fat diet and a decreased ability to gain weight on a high fat diet. Hervey (283) reported that his "normal" parabiotic rats had about one-half the body fat as "normal" single animals both fed a standard laboratory rat ration. Hervey also reported significantly lower body weights for his parabionts as compared to controls. His parabiotic rats at 150 days weighed about 71-85% that of control rats. Parabiosis seems to affect normal body weight gain independently of surgical stress.

Parabiotic animals are shorter. Supportive data once again comes from Hervey (283) whose parabionts were markedly shorter than controls. There was a slight effect of ration on nose to anus length (Table 10). All high fat fed animals were slightly longer than grain fed animals whether single or parabiotic and all parabiotic rats (except the high fat fed parabionts versus single grain fed rats) were shorter in body length. In the exception noted above the single controls were longer

and the comparison had a rather high t-statistic but significance was not quite reached. Similar results were reported for non-parabiotic rats by Schemmel et al. (320), Schemmel (318), and Mickelsen et al. (321).

All fat depot weights per 100 grams body weight for young adult Osborne-Mendel rats were in general agreement with those reported by Schemmel (318). Interscapular, genital and perirenal values agree extremely well with Schemmel's data while the mesenteric, omental and xiphoid are just slightly different. It is quite evident from these data that parabiotic animals do have lighter depot weights. The subcutaneous and abdominal depots seemed to be affected equally.

The inguinal fat depot is reported only from the non-operated side since it was difficult to remove the depot effectively from the operated side. In performing parabiotic surgery the inguinal depot on the side of the surgery was incised across the belly but left in the animal. This cleaned the fat away from the abdominal area so the abdominal sutures could be placed more easily.

The only organ weight that was affected by parabiosis was the stomach. Parabionts had a heavier stomach per 100 grams body weight as compared to controls ($P < 0.01$), but the absolute stomach weight was the same between the two groups. More than likely, the significance on a body weight basis was caused by some parabionts being physically smaller with normal visceral formation, which would give a greater stomach weight per unit body weight. Slightly lower food intake in parabiotic animals may have been a contributing factor.

Hervey (283) has reported the same liver weights for single and parabiotic female "hooded" rats which is in agreement with the data

presented here for female Osborne-Mendel rats. All absolute liver weights agree with those reported by Schemmel (318), 1967, for adult female (6 to 7 months old) Osborne-Mendel rats fed a grain ration. Liver weights per 100 grams body weight reported here also agree with those reported in the Handbook of Biological Data (322) and by Marshall et al. (323). Humoral factors implicated in liver regeneration have been reported to cross the parabiotic union from an animal with liver injury to an intact animal (302, 324).

Hervey (283) reported heart weights to be slightly lower for parabiotic rats but gave no statistics. The present results show no difference in heart weight between control and parabiont. Animals sharing a common circulation evidently are not experiencing circulatory stress manifested by cardiac hypertrophy. Weights reported here agree with those of Schemmel (318), but are slightly smaller on a body weight basis than those reported in the Handbook of Biological Data (322). The comparison with Schemmel's animals is, of course, more meaningful since her animals were of the same sex, species and age as the ones reported here.

Lung weights are normal and are the same on a body weight basis for control and parabionts. The data are in agreement with the Handbook of Biological Data (322). Lungs were weighed mainly to detect serious respiratory lesions. Infected lungs are heavier due to congestion. All animals with either external respiratory symptoms, heavy lungs or other noticeable lung diseases at autopsy were elimated from the comparison.

The pancreas in the rat is diffuse but, since its color is quite distinct from all surrounding tissues, most of the organ can be extirpated.

Pancreatic weights agree with those reported by Malaisse (325) and are statistically identical for control and parabionts.

Parabiosis had no effect on kidney weights even with a reasonable rate of cross-circulation. Hervey (283) reported slightly lower kidney weights for parabionts but once again no statistics were given. Combined kidney weights reported here agree with those of Schemmel (318). Kidney weights reported in the Handbook of Biological Data are slightly higher than the results reported above, but Marshall's (323) weights are practically identical with those reported here. Humoral substances involved in renal hypertrophy have been reported for parabiotic mice (326).

A "malignant hypertensive vascular disease" has been reported in parabiotic rats (327). It is said to arise "spontaneously" and responds well to cortisone treatment. This disease appears to be quite distinct from parabiosis intoxication. Heart and kidney weights are greatly enlarged in this syndrome; consequently, one reason for taking these weights at autopsy was to determine to what extent the disease occurred in this particular colony of parabiotic rats. Particular types of animals as well as certain surgical procedures may be predisposing to this anomoly. Since all heart and kidney weights in the current study were normal, it is likely that our parabiotic rats did not have "malignant hypertensive vascular disease."

Spleen weights can be used as a diagnostic aid to tissue rejection. Degenerative changes have been reported in the spleen of both parabionts in a pair undergoing visible rejection (302). In the present study, spleen weights in parabionts diagnosed as having a normal union were the same as control weights on a body weight basis. These spleen weights agree with those in the literature (318, 322).

Gastro-intestinal tract weights agree well with those of Spector (322), on a body weight basis and are also statistically the same for control and parabiotic animals.

If one accepts the suggestion that the size of the adrenal gland is related to the stress undergone by the animals, then it appears that the parabiotic rats used in this study were not stressed. This is based on the observation that the adrenal weights of the parabiotic and control rats were almost identical. Adrenal weights reported here agree, on a body weight basis, with those reported by Marshall et al. (323) for female adult rats.

Thyroid weights for parabiotic rats were almost identical on a body weight basis between control and parabiotic animals. These weights agree well with those reported by Braham et al. (328). Thyroid weights reported in the Handbook of Biological Data are 0.001 mg per 100 grams body weight and, according to this author, are in error.

A normal blood volume range for the rat with a body weight between 250-300 grams is 16 to 22 ml (329). Blood volumes reported here are within the normal range. Parabiotic values are not statistically different from controls but are a little high and probably a result of a loss of small amounts of dye in the parabiotic union. Blood volumes for parabionts are averages per pair, because meaningful optical densities can only be taken when optical densities are equal between the members of a pair. One animal could have a greater absolute amount of dye with a larger blood volume but still resulting in an identical optical density as its partner with less dye and a lower blood volume. Nephrectomized rats in parabiosis with intact animals have been reported to exhibit hypervolemia (330).

Packed cell volume is normal for parabiotic rats as compared to controls and standard values (329). Immune reactions could cause hemolysis giving parabiotic animals low hemotocrits (302). Anemia due to translocation of red blood cells has been reported in parabiotic mice (331).

Hervey (283) concluded that his "normal" parabiotic rats had one-half the body fat content as controls because each animal, either at the brain or peripheral level or both, was regulating body fat in which not only is its own body fat a component but also, added in parallel, the body fat of its partner. Evidence has been presented here supporting Hervey's data showing a lower fat content in parabionts.

Food intake alterations in parabiotic animals may be a result of an experimental variable but also may be secondary to another alteration produced by the parabiotic state, per se. For the parameters reported here, parabiotic animals were in agreement with control data and parabiotic rats appear to be "normal" except for a decreased nose to anus length, a decreased body weight, and lower weights of the fat depots which may reflect alower body fat content as suggested by Hervey (283). This lower body fat level may be related to a greater energy expenditure by the parabiotic rats. This possibility is suggested by the fact that the feed intake of the parabiotic rats when expressed on the basis of body weight was almost the same as that of the sham operated controls. To evaluate that suggestion will require energy balance studies.

Table 10

NOSE TO ANUS LENGTH (cm) - 200 DAY OLD OSBORNE-MENDEL FEMALE PARABIOTIC RATS FED EITHER GRAIN OR HIGH FAT RATION

A Single High Fat	B Single M-1	C Parabiotic High Fat	D Parabiotic M-1
25.0	24.0	22.5	23.0
24.5	23.0	21.0	23.0
24.0	23.0	22.0	22.0
22.5	22.5	22.0	22.0
22.0	21.5		19.0
	22.5		20.5
			20.0
			21.0

SIGNIFICANCE

Comparison of Means	n	df	Mean ± S. D.	Variance	t	P
AB	5, 6	9	23.60 ± 1.29, 22.75 ± .75	1.68, .56	1.327	NS
AC	5, 4	7	23.60 ± 1.29, 21.88 ± .63	1.68, .40	2.422	.050
AD	5, 8	11	23.60 ± 1.29, 21.31 ± 1.44	1.68, 2.07	2.892	.020
BC	6, 4	8	22.75 ± .75, 21.88 ± .63	.56, .40	1.795	NS
BD	6, 8	12	22.75 ± .75, 21.31 ± 1.44	.56, 2.07	2.183	.050
CD	4, 8	10	21.88 ± .63, 21.31 ± 1.44	.63, 2.07	.734	NS

Table 11

NOSE TO ANUS LENGTH (cm)

Comparison of Means	n	df	Mean + S. D.	Variance	t	P
Sprague-Dawley Female Littermate Rats AB*	32, 18	49#	18.14 ± .79, 20.24 ± .39	.63, .16	12.426**	.001
Sprague-Dawley Male Littermate Rats AB	16, 8	22	20.18 ± .74, 22.47 ± .58	.55, .34	7.580	.001
Sprague-Dawley Female Non-littermate Rats AB	40, 9	47	19.43 ± .82, 21.13 ± .53	.68, .28	5.927	.001
Sprague-Dawley Male Non-littermate Rats AB	12, 5	15	21.88 ± .81, 23.43 ± .94	.65, .88	3.204	.010
Osborne-Mendel Female Non-littermate and Littermate Rats Combined AB	32, 33	63	19.94 ± .91, 21.56 ± .85	.82, .72	7.210	.001

*Parabionts, Controls

**Approximate t-test

#Degrees of freedom calculated

Table 12

COMPARISON OF FAT DEPOT WEIGHTS (GRAMS) BETWEEN SINGLE AND PARABIOTIC OSBORNE-MENDEL FEMALE RATS FED A GRAIN RATION

Fat Depot	Comparison of Means: Single M-1	Parabiotic M-1	n	df	Variance	t	P
Mesenteric and omental	5.787 ± 2.015	4.013 ± 2.140	5, 5	8	4.061, 4.580	1.350	NS
Perirenal	4.712 ± 1.542	2.853 ± 1.060	6, 6	10	2.376, 1.124	2.433	.050
Xiphoid	.367 ± .167	.283 ± .127	6, 6	10	.028, .016	.847	NS
Genital	11.042 ± 1.957	8.142 ± 1.168	4, 4	6	3.829, 1.364	2.545	.050
Interscapular	2.435 ± 1.317	1.526 ± .791	6, 6	10	1.734, .625	1.450	NS
Intermuscular	.281 ± .032	.289 ± .106	5, 5	-+	.001, .011	.163*	NS
Inguinal**	4.149 ± 1.253	2.794 ± .870	6, 6	10	1.570, .756	2.177	.100

+Weighted critical values calculated
*Approximate t-test
**Weights for unoperated side only

Table 13

COMPARISON OF FAT DEPOT WEIGHTS (grams / 100 grams body weight) BETWEEN SINGLE AND PARABIOTIC OSBORNE-MENDEL FEMALE RATS ON GRAIN (M-1) RATION

Fat Depot	Comparison of Means: Single M-1	Parabiotic M-1	n	df	Variance	t	P
Mesenteric and omental	1.852 ± .590	1.384 ± .476	5, 5	8	.348, .227	1.382	NS
Perirenals	1.504 ± .486	.968 ± .217	6, 6	10	.236, .047	2.466	.050
Xiphoid	.114 ± .044	.096 ± .031	6, 6	10	.002, .001	.835	NS
Genitals	3.310 ± .712	2.549 ± .086	4, 4	-+	.565, .007	2.012*	NS
Interscapular	.779 ± .416	.526 ± .250	6, 6	10	.173, .063	1.276	NS
Intermuscular	.087 ± .014	.095 ± .019	5, 5	8	.0002, .0003	.779	NS
Inguinal**	1.287 ± .376	.967 ± .237	6, 6	10	.141, .056	1.760	NS

+Weighted critical values calculated
*Approximate t-test
**Weights for unoperated side only

Table 14

COMPARISON OF ORGAN WEIGHTS (grams) BETWEEN SINGLE AND PARABIOTIC OSBORNE-MENDEL FEMALE RATS ON GRAIN DIET

Organ	Comparison of Means: Single M-1	Parabiotic M-1	n	df	Variance	t	P
Liver	11.306 ± 1.227	10.214 ± 2.957	6, 6	-+	1.505, 8.744	.836*	NS
Heart	1.021 ± .124	.983 ± .236	6, 6	10	.015, .056	.354	NS
Lungs	2.440 ± .476	1.728 ± .287	5, 5	8	.227, .083	2.860	.050
Pancreas	1.250 ± .208	1.017 ± .225	6, 6	10	.043, .051	1.858	.100
Kidneys	2.499 ± .502	2.395 ± .539	6, 6	10	.252, .290	.344	NS
Spleen	1.065 ± .368	.874 ± .244	6, 6	10	.135, .059	1.061	NS
Stomach (empty)	1.390 ± .195	1.428 ± .312	6, 6	10	.038, .097	.256	NS
Gastro-intestinal tract and stomach (empty)	7.330 ± 1.263	6.855 ± 1.091	6, 6	10	1.594, 1.190	.697	NS
Adrenals	.068 ± .015	.062 ± .011	6, 6	10	.0002, .0001	.767	NS

+Weighted critical values calculated
*Approximate t-test

Table 15

COMPARISON OF ORGAN WEIGHTS g/100 g BODY WEIGHT
BETWEEN SINGLE AND PARABIOTIC OSBORNE-MENDEL FEMALE RATS

Organ	Comparison of Means: Single M-1	Comparison of Means: Parabiotic M-1	n	df	Variance	t	P
Liver	3.610 ± .285	3.504 ± .419	6, 6	10	.081, .176	.509	NS
Heart	.326 ± .026	.343 ± .040	6, 6	10	.001, .002	.883	NS
Lungs	.774 ± .066	.616 ± .143	5, 5	8	.004, .021	2.242	.100
Pancreas	.404 ± .085	.362 ± .076	6, 6	10	.007, .006	.891	NS
Kidneys	.790 ± .072	.834 ± .038	6, 6	10	.005, .001	1.307	NS
Spleen	.334 ± .080	.307 ± .066	6, 6	10	.006, .004	.626	NS
Stomach (empty)	.441 ± .023	.499 ± .036	6, 6	10	.001, .001	3.291	.010
Gastro-intestinal tract and stomach (empty)	2.326 ± .211	2.425 ± .305	6, 6	10	.044, .093	.656	NS
Adrenals	.022 ± .003	.023 ± .008	6, 6	-+	.00001, .00007	.273*	NS

+Weighted critical values calculated
*Approximate t-test

Table 16

ADRENAL WEIGHTS (mg) - OSBORNE-MENDEL FEMALE RATS FED A GRAIN (M-1) RATION

A and B are adjusted per 100 g body weight; C and D are unadjusted.

Parabionts		Controls	
A	C	B	D
20.144	41.9	19.883	50.9
21.282	41.5	24.349	76.7
15.000	36.0	14.894	49.3
27.137	67.3	19.419	63.5
29.964	82.1	14.528	46.2
24.933	74.3	20.707	84.9
24.867	74.6	27.122	75.4
19.653	62.3	18.729	54.5
15.542	50.2	20.517	47.6
23.864	52.5	21.262	64.0
27.213	49.8	24.710	76.6
		21.595	70.4

SIGNIFICANCE

Comparison of Means	n	df	Mean ± S. D.	Variance	t	P
AB	11, 12	21	22.69 ± 4.82, 20.64 ± 3.69	23.22, 13.61	1.150	NS
CD	11, 12	21	57.50 ± 15.49, 63.33 ± 13.44	239.81, 180.59	.967	NS

Table 17

THYROID WEIGHTS (mg) - OSBORNE-MENDEL FEMALE RATS FED A GRAIN (M-1) RATION

A and B are adjusted per 100 g body weight; C and D are unadjusted.

Parabionts		Controls	
A	C	B	D
9.135	19.0	5.898	15.1
7.744	15.1	6.127	19.3
6.583	15.8	8.248	27.3
10.000	24.8	11.346	37.1
9.197	25.2	7.610	24.2
7.248	21.6	5.951	24.4
8.167	24.5	6.367	17.7
6.246	19.8	9.210	26.8
6.409	20.7	11.078	25.7
8.864	19.5	8.704	26.2
6.721	12.3	8.645	26.8
		6.902	22.5

SIGNIFICANCE

Comparison of Means	n	df	Mean ± S. D.	Variance	t	P
AB	11, 12	21	7.85 ± 1.31, 8.01 ± 1.89	1.71, 3.57	.235	NS
CD	11, 12	21	19.85 ± 4.19, 24.43 ± 5.62	17.53, 31.56	2.199	.050

Table 18

BLOOD VOLUME (ml) IN SINGLE AND PARABIOTIC OSBORNE-MENDEL FEMALE RATS FED GRAIN RATION *

A Single	B Parabiotic
21.06	30.46
21.83	23.51
19.81	26.87
20.89	18.35

*Optical density of plasma was determined two hours after dye T-1824 injection.

SIGNIFICANCE

Comparison of Means	n	df	Mean ± S. D.	Variance	t	P
AB	4, 4	-+	20.90 ± .83, 24.80 ± 5.15	.69, 26.53	1.495**	NS

+Weighted critical values calculated

**Approximate t-test

Table 19

PACKED CELL VOLUME IN SINGLE AND PARABIOTIC OSBORNE-MENDEL FEMALE RATS FED GRAIN RATION

A Single	B Parabiotic
44.75	38.50
43.75	38.00
44.63	43.25
45.50	43.00
44.50	43.50
	40.00
	46.75
	44.38
	46.10
	45.90

SIGNIFICANCE

Comparison of Means	n	df	Mean ± S. D.	Variance	t	P
AB	5, 10	-+	44.63 ± .63, 42.94 ± 3.14	.39, 9.83	1.641*	NS

+Weighted critical values calculated

*Approximate t-test

PART 7

ENERGY METABOLISM

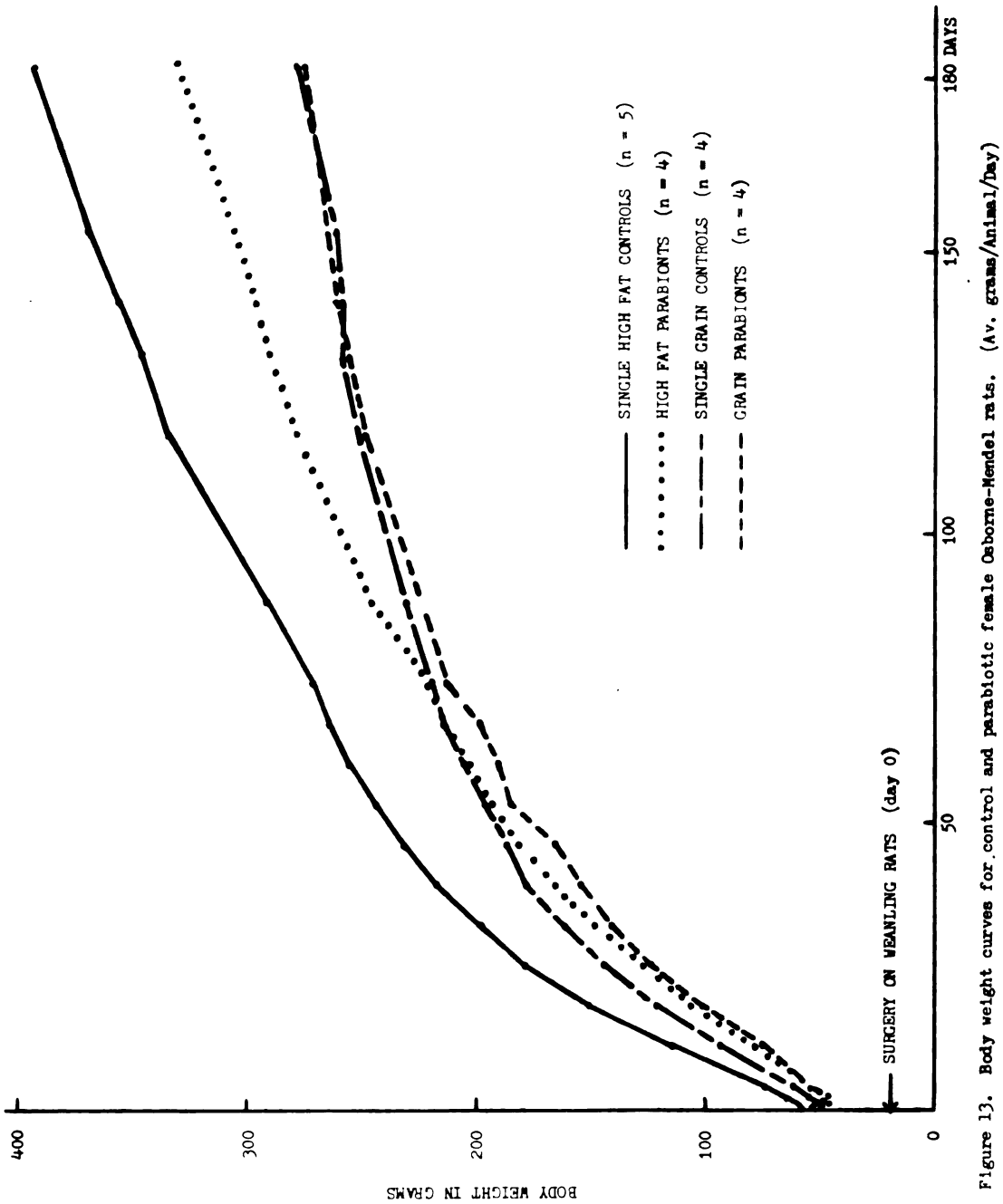


Figure 13. Body weight curves for control and parabiotic female Osborne-Mendel rats. (Av. grams/Animal/Day)

INTRODUCTION

Food intake may be altered in parabiotic animals by a change in energy metabolism caused by the parabiotic state, per se, and not necessarily a consequence of the experimental variable. It is essential that oxygen consumption in parabiotic animals be measured to determine if the parabiotic state does influence energy metabolism. Oxygen consumption of parabiotic animals could not be found in the literature.

TECHNIQUES

Oxygen consumption determinations were made by a "closed circuit" technique (332) modified as follows. Room air was used in lieu of pure oxygen and was injected into a desiccator with a calibrated syringe and needle at a rate necessary to maintain constant intra-chamber pressure. Chamber pressure was monitored by a U-tube extending from the top of the desiccator and partially filled with Brodies solution. The desiccator was made air tight by coating all cracks with stopcock grease. Intra-chamber temperature was monitored by a thermistor probe.[1] Soda lime was used for carbon dioxide absorption.

Oxygen consumption can be read directly from the calibrated syringe. With this procedure the exact volume of the exposure chamber, corrected

[1] Yellow Springs Instrument Company, Inc. Yellow Springs, Ohio. 45387. Model 43TA; Serial 9888; Probe No. 401.

for animal size, is of no importance and saves many calibrations. All animals were tested in the same exposure chamber.

Male Sprague-Dawley rats both single and parabiotic were tested. The rats were fasted for approximately 17 hours, overnight, and all measurements were made in the mid-afternoon (1:00 to 4:00). This time was chosen as the afternoon would approximate the best basal conditions for measurements. In the morning, animals are just settling down from an active night and their body temperatures would still be rather high relative to basal. All feed prior to fasting was a standard grain ration (317).

All gas volumes were converted to Kelvin before any further calculation or analyses.

Control oxygen consumptions were done with two single animals in the desiccator to simulate the parabiotic dualty. No measurements were made until the chamber reached constant temperature and each pair of animals were tested over three or four individual periods of approximately 15-20 minutes each. After each "run" the chamber was opened and flushed out with room air to avoid long exposure periods to high relative humidities as no dessicant was used. Activity was visually monitored and all determinations thrown out where activity was excessive since oxygen consumption would then be above basal levels. On each experimental day one pair of controls and one parabiotic pair were measured alternating the initial determination on each day insuring a completely "staggered" design.

After oxygen consumption determination the animals were sacrificed by first lightly anesthetizing with methoxyflurane inhalation anesthetic.[2]

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[1] Pitman-Moore, Division of Dow Chemical Co. Fort Washington, Pa. 19034.

This was done in order to obtain heparinized heart blood for insulin assay and to separate the parabionts. All animals, single controls and separated parabionts, were weighed and then decapitated. Immediately after decapitation as much blood as possible was collected into centrifuge tubes (without anticoagulent) and placed in beakers of ice water. Proper aliquots of serum were frozen at -40°F for future assay of combined thyroxin and triiodothyronine, glucose, urea nitrogen, and free fatty acids.

After blood collection, the abdomen was opened and the adrenals removed, weighed and frozen at -40°F in 5 ml of 6% trichloroacetic acid to await assay of adrenal ascorbic acid. Approximately 5 minutes were required for the entire operation. Thyroid and testicular weights were also determined. Organs were placed between layers of slightly dampened towels, after removal and before processing, to impede evaporation.

Serum thyroxin and triiodothyronine were assayed by the "Standard 11, T_4 by column test."[3] For that assay, thyroxin and triiodothyronine (in 0.5 ml serum) were freed from proteins by adding 5 ml of 0.15N NaOH. After making the ion exchange resin[3] alkaline, the sample was placed on the column. The resin adsorbed thyroxin as well as other iodinated organic compounds, inorganic iodine, proteins and amino acids. The proteins and iodotyrosines were eluted (alcohol-acetate buffer and 15% acetic acid) from the column while thyroxin, thyronine and inorganic iodide remained adsorbed. The thyroxin and triiodothyronine were then moved down the column with glacialacetic acid and eluted with 50% acetic acid into test tubes. The hormones were then determined colorimetrically.

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[3] Bio·Rad Laboratories, 220 Maple Avenue, Rockville Centre, New York. 11570.

The colorimetric reaction is based on the fact that bromine replaces iodine on the thyroid hormone molecules and the free iodine catalyzes the reduction of cerium by arsenic as follows:

$$2\,Ce^{+4} + As^{+3} \xrightarrow[\text{Iodine}]{\text{Yellow}} 2\,Ce^{+3} + As^{+5}$$

Colorless

As free iodine increases, the greater the catalysis of cerium reduction. As cerium is reduced, the solution turns from yellow to colorless. Higher hormone concentrations will therefore have a higher percent transmittance or lower absorbance. All samples must be read in exactly 20 minutes from the initiation of the reaction. The rate of the reaction and color disappearance is linearly related to the initial hormone concentration.

Different volumes of serum were used as a standard curve, giving varying levels of thyroid hormone eluted from the column into a constant volume. In using the column, therefore, one can start with different volumes of equal concentration and end with equal volumes of varying concentration both lower and higher than the original concentration. The standard curve was used to check the linear functioning of the system and to make sure all samples were read on the linear phase of the curve. Since actual concentration values for the standard were not known, the data cannot be expressed in exact units but the relative values can be compared between experimental and control groups giving statistically valid conclusions. A value of .04 ug I^- per ml was arbitrarily ascribed to the serum used for the standard curve thus giving numerical values to the data.

Before the ascorbic acid assay, the adrenals were removed from the freezer and allowed to stand at room temperature for a few minutes.

While a few ice crystals were still present, the adrenals were cut into small pieces with operating scissors and then homogenized on a "polytron" homogenizer.[4] The homogenate was transferred to disposable centrifuge tubes and centrifuged for about 10 minutes at 3000 rpm. 0.5 ml of the supernate was taken for total ascorbic acid assay by the osazone method of Schwartz and Williams (333). The three hour incubation period recommended by Schwartz was not long enough to adequately react the more concentrated samples; consequently, the incubation period was increased to 6 hours at 37°C. Five hundred microliters of the supernate were used, since both adrenals were assayed together, doubling the ascorbic acid concentration. Two drops of 2,6-dichlorophenolindophenol were used instead of one.

Serum glucose was measured by the glucose oxidase method of Keston (334), as modified by Teller (335), after the proteins were precipitated by the Somogyi procedure. Chromogen and enzymes were purchased under the trade-mark "Glucostat."[5] The semi-micro method was used and the reaction was allowed to go to completion.

Serum urea nitrogen was analyzed by the modified Berthelot reaction (336-339). Reagents were purchased under the trade-mark "UN-TEST."[6]

Blood for the insulin immunoassay was taken by heart puncture with heparin (ammonium salt) as anticoagulant. Heart blood was used since cerebral spinal fluid and tissue fluid proteins may bind insulin if serum is taken when the animal is decapitated. The immunoassay

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[4] Patent-Lizeng Prof. P. Willems, Luzern Kinematisches Hochbrequenz-Gerat; Kinematica Gmbh. Luzern-Schweiz.

[5] Worthington Biochemical Corporation, Freehold, New Jersey. 07728.

[6] Hyland Division, Travenol Laboratories, Inc. Costa Mesa, California. 92626.

method and calculations are according to Hales and Randle (340). Iodinated insulin^{125}, insulin binding reagent, standard and millipore filters were purchased from Amersham/Searle.[7] Bovine albumin was used in place of horse serum, No. 2. Samples were counted by liquid scintillation.

Serum free fatty acids were measured by the extraction and titrametric method of Ko and Royer (341). 0.4 ml of serum was used with palmitate as standard. One normal NaOH was used to absorb CO_2 rather than ascarite.

RESULTS

All oxygen consumption values when translated into energy units were the same for parabiotic and control animals for each day. The values expressed on the basis of body weights were greater but not statistically so for the parabiotic rats.

Although adrenal weights on an absolute as well as body weight basis for parabiotic animals, compared to controls, were slightly larger, there was no significance to these differences (Tables 21 and 22). Within group variance was high for parabionts with the elevated mean and within group variance heavily biased by one reading (96.6 mg). Without this reading the means are far closer (60.0 mg compared to 57.0 mg) for parabiotic and controls respectively and would still be non-significant.

Thyroid weights approached significance for the absolute weights. When these weights were expressed on a body weight basis, they were

[7] Amersham/Searle Corporation, 2636 S. Clearbrook Drive, Arlington Heights, Illinois. 60005.

different ($P < 0.01$); the parabiotic rats had the heavier thyroids (Table 23).

Testicular weights were identical regardless of how expressed (Table 24).

The serum thyroxin and triiodothyronine iodine level for parabionts was low but still within the normal range of 2.9 to 6.5 ug%.[8] The levels for control animals were in the mid-range for normal but were significantly higher than the parabionts ($P < 0.05$).

No difference was seen for serum glucose or plasma insulin between controls and parabionts as shown in Tables 26 and 27.

Serum free fatty acid levels are not elevated to the same extent in parabiotic rats as in controls in response to fasting (Table 28).

In this particular group of parabionts the level of adrenal ascorbic acid was lower than controls (Table 29) even with no statistical difference in adrenal weights; nevertheless, the adrenal weights were absolutely and per unit body weight larger for parabionts.

Fasting urea levels were different between parabionts and controls only at the 0.10 level (Table 30). The variance was high for parabionts with a couple of animals having high values.

DISCUSSION

An excellent measurement of metabolic rate is "specific metabolism."

$$\text{Specific metabolism} = \frac{\text{Cal/24 hours}}{\text{Body weight in Kg}^{0.73}}$$

Specific metabolism for control and parabiont were practically

[8] Standard II, T-4 by column test, Instruction Manual, Bio·Rad Laboratories, 220 Maple Avenue, Rockville Centre, New York. 11570.

the same. The normal Kcal produced per kilogram body weight per day is 130 for the rat (229) with the values reported here agreeing quite well considering normal variability for different species of rat. All values are close to the ones normally reported for rats. There should be no effect on parabiotic food intake caused by changes in energy metabolism produced by the parabiotic state, per se.

The male adrenal weights are a little heavier than those reported by Marshall et al. (323), but are still within normal species variability. Weights of the adrenal per 100 grams body weight reported by Tullner and Edgcomb (342) are identical to those of the control animals reported here. The value of 0.05 grams per 100 grams body weight reported by Spector (322) seems to be incorrect. Parabiotic adrenal weights seem to be slightly heavier than controls but, as stated earlier, biased by one extremely high value.

Thyroid weights for male control and parabiotic Sprague-Dawley rats lie within the general ranges reported by Braham (328) with the controls having significantly lighter thyroids than parabionts ($P < 0.01$). Absolute thyroid weights for both groups were not different with all weights in the normal range. The significance on a body weight basis was caused by the smaller physical size of the parabionts. Body weight for the controls averaged 389 grams and for parabionts 334 grams. The animals were all of the same age and in parabiosis for approximately $5\frac{1}{2}$ months at the time of sacrifice. Body size usually is smaller in parabionts but most internal organ weights are, on an absolute basis, the same as single control animals. The amount of organ tissue per unit body weight is, therefore, increased.

No difference in testicular weights were found between single

controls and parabionts and all values were in agreement with weights reported by Tullner and Edgcomb (342), 1962, for Sprague-Dawley rats.

Serum thyroid hormone iodide levels for parabiotic animals were significantly below control values. Normal ranges for serum hormonal iodine range from 3.0 to 6.0 ug% (343) and specifically for the technique used here[9] 2.9 to 6.5 ug%. As seen from Table 25, all values fall into the normal range but the parabiotic levels are at the lower range.

As shown by adrenal ascorbic acid data (to be discussed later), parabionts in this study probably were slightly stressed. A stress syndrome independent of the adrenals has been described whereby thyroid stimulating hormone secretion is inhibited. The effect is either on the pituitary or hypothalamus. This inhibition could cause a lowered serum thyroxin level (344).

ACTH-corticoid activation has been shown to inhibit thyroid stimulating hormone secretion (345). Since these parabionts may be slightly stressed, this corticoid activation could inhibit TSH synthesis or release at the pituitary level causing a decreased thyroid activity and serum thyroid hormone.

Hypervolemia in parabiotic animals (302) may slightly dilute thyroid hormone levels but other blood constituents (glucose, urea and insulin) were normal; consequently, this does not seem to be a factor. Blood volume in parabiotic animals reported here was slightly elevated but not significantly over control values (see blood volume discussion in this thesis).

Specific metabolism was the same for parabionts and controls.

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[9] Bio-Rad Laboratories, 220 Maple Avenue, Rockville Centre, New York. 11570.

This is of major concern in food intake studies and since thyroid weights and thyroid hormone levels were normal it appears that parabiotic rats can be used in food intake experimentation without alterations in energy metabolism caused by the parabiotic state, per se.

As stated earlier, the difference on a body weight basis between thyroid weights of parabionts and controls is probably a result of the decreased physical size of parabionts disproportionate to internal organ weights since parabionts do have normal absolute thyroid weights but a decreased body weight.

Lower circulating thyroid hormone levels are not inconsistent with a statistical agreement in energy metabolism between parabionts and controls. PBI, e.g., can range from 4-8 ug% with basal metabolic rates remaining normal (346). Even hypothyroids can have normal basal metabolic rates (347). It is quite conceivable that free serum thyroxin is the same for the two groups. Only total thyroxin and T_3 were measured. Free hormone is only 0.10% of the total and is in equilibrium with the total bound hormone. It is the free thyroxin that enters cells and acts as a feedback regulator for TSH secretions. Usually, the concentration of free thyroxin changes proportionately with changes in bound hormone; consequently, measurement of the total hormone pool is a good indication of thyroid function. The total thyroid hormone binding capacity can vary directly to the total bound hormone but inversely to turn-over rate; consequently, free thyroxin (resulting from furn-over) can be the same in groups of animals with different total bound iodine. These animals would also have similar metabolic rates (348).

Parabiosis may affect the total circulating thyroid hormone level

by altering the thyroglobulin level or through some "stress" mechanism. Over a reasonable range of these kinds of effects the free serum thyroid hormone can be regulated within normal ranges. This is done by alterations in the "turn-over" rate if thyroglobulin levels and total bound hormone are low.

Fasting serum glucose and plasma insulin levels (Tables 26 and 27) were the same for parabionts and controls. Fasting serum glucose levels agree well with those reported by Blazquez and Quijada (349, 350). Immunoreactive insulin levels, during fasting, are reported to be around 25 micro units per ml. As shown in Table 27, fasting insulin levels for parabiotic and control animals are identical and agree with other published values (351). Chlouverakis (352) reported a small but significant decrease in blood glucose for parabiotic mice with no changes in serum insulin levels. Data reported here (Table 26) shows a slight but non-significant decrease in fasting serum glucose in parabiotic rats. There was no parabiotic effect in rats on plasma insulin levels (Table 27). This agrees with the report of Chlouverakis that parabiosis had no effect on serum insulin levels in parabiotic mice.

The effect of fasting on serum free fatty acid levels is shown in Table 28. The difference between parabionts and controls was unexpected. Fasting free fatty acid levels averaged 0.60 ueq./ml for parabionts and 1.07 ueq./ml for controls. These values (especially parabiotic values) are within normal ranges as shown by Regouw et al. (353), Takagi et al. (354), Harper (351), Gordon (355), Dole (356) and Grossman et al. (357). Post prandial levels approximate 0.5 ueq./ml with fasting levels ranging from 0.6 to 0.8 ueq./ml.

Fasting serum free fatty acid levels for parabiotic animals were in perfect agreement with values reported in the literature for single rats. The difference between parabionts and controls was caused by an elevation, slightly over the "normal" ranges, in the control animals. Free fatty acid data determined on other single animals fed different diets were then compared to these values: (A) Animals fed a high fat diet (60% fat) measured .367 ueq./ml serum; (B) Grain fed animals 0.252 ueq./ml; (C) Animals fasted for 17 hours, anesthetized with metofane but did not go through oxygen consumption measurement had levels of 0.685 ueq./ml; (D) The parabionts that were fasted for 17 hours, anesthetized with metofane to obtain heart blood and exposed to oxygen consumption determination displayed free fatty acid levels of 0.600 ueq./ml; and finally, (E) The controls for the latter group, 1.070 ueq./ml. It can be seen in comparing groups (C) and (D) that the oxygen consumption procedure did not effect free fatty acid levels and that parabionts agree almost exactly with controls. The second group of controls, (C) above, were housed one in a cage. The elevated free fatty acid levels in the original control group, group (E) above, could have been caused by a particular type of emotional excitement. The second control group, group (C), was killed immediately after the 17 hour fast. The first control group, (E), was also housed separately, but were measured in pairs for oxygen consumption which was done to simulate the paired parabiotic state. By pairing animals, immediately before oxygen consumption determination, that had not previously been caged together established a particular state of "emotional" excitement. This type of excitement is known to primarily elaborate norepinephrine which is a potent fat mobilizer but

a very weak ACTH stimulator; consequently, serum free fatty acids rose but the ACTH-corticoid stress system was not stimulated as shown by adrenal ascorbic acid data (to be discussed later). This system would not be activated in parabionts as they have been living together for many weeks. The demarcation of adrenal response to different types of "stress" or emotionalism is discussed by Russell (358).

As a sensitive index of "stress", adrenal ascorbic acid levels were determined. Parabiotic rats did have significantly lower adrenal ascorbic acid than controls ($P < 0.02$) (Table 29). Adrenal weights of these parabionts were slightly but not significantly larger.

The parabiotic rats, evidently, still had not completely adapted to parabiosis. The parabiotic data are biased by two vary low values but the average vitamin C levels without these values are still somewhat lower than that of the controls.

Adrenal ascorbic acid for control animals, fasted for 17 hours and subjected to metofane after oxygen consumption determination, was 1.96 mg/g tissue. Concentration for male, Sprague-Dawley grain fed animals was 3.05 mg/g tissue. These data demonstrate the decreased adrenal ascorbic acid seen after fasting or hypoglycemia. Hypoglycemia is a potent stimulator of epinephrine and ACTH secretion. ACTH stimulates the adrenal cortex to secrete glucocorticoids and in the process of stimulation ascorbic acid is decreased. Epinephrine stimulates liver phosphorylase activity and, therefore, to a lower degree, fat depot fatty acid mobilization. Animals, not exposed to oxygen consumption procedures but still fasted and subjected to metofane, had an adrenal ascorbic acid level of 1.9 mg/g tissue. This value is almost identical to the first group of controls reported above showing no

additional ACTH-corticoid or epinephrine activation, which gives further support for the argument that the elevated free fatty acid release in the first group of controls resulted from norepinephrine stimulation. These data also indicate that the oxygen consumption assay system did not cause an increased stress as evidenced by similar free fatty acid and ascorbic acid values as data from animals not having gone through this procedure. The lower adrenal ascorbic acid in parabionts, therefore, was due to the parabiotic state.

Parabiotic serum urea nitrogen was higher but not statistically different from controls. This possibly was due to a higher fasting rate of gluconeogenesis stimulated by a lower blood glucose. Normal serum urea nitrogen values are around 8-20 mg%.[10] The values reported here fall within the normal range.

In conclusion, no changes were observed in energy metabolism measurements between control and parabionts. Adrenal and testicular weights, expressed absolutely or per unit body weight, were not different between experimental groups. Thyroid weights were not different, absolutely, but were on a body weight basis because of the smaller physical size of parabionts relative to certain internal organ weights. The serum thyroid hormones were lower in parabionts but since energy metabolism was not affected it was concluded that the total bound hormone was decreased with free thyroxin unchanged, possibly due to an increased turnover rate from the total bound hormone. Total serum thyroid hormone could have been lower because of the "stress factor" with the ACTH-corticoid system inhibiting TSH at the pituitary level. Stress was indicated by lower adrenal ascorbic acid in the parabionts.

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[10] Hyland, Div. Travenol Laboratories, Inc. Costa Mesa, California. 92626.

No change was noted in serum glucose and urea or plasma insulin. Fasting free fatty acids were normal in parabionts but elevated in controls for reasons discussed.

The only major factor that could affect food intake would be the slight degree of stress. No parabiotic adrenal weights thus far reported have been different from controls but ascorbic acid was lower for parabionts presented here. This difference existed even after $5\frac{1}{2}$ months. Usually adaptation occurs before this length of time (359). Parabiotic and control animals were fasted for the same length of time and handled in the same manner. Everything was controlled with the state of parabiosis as experimental variable. It is possible that parabiotic rats are "stressed" differently by various experimental procedures than single control rats. It is suggested that when possible some measurement of stress be made in parabiotic experiments to obviate the possibility that experimental results were actually a manifestation of a general "stress syndrome."

Table 20

ENERGY METABOLISM: PARABIOTIC VS. CONTROL - ADULT SPRAGUE-DAWLEY MALE RATS

SIGNIFICANCE

	n	df	Mean ± S. D.		Variance		t	P
ml O_2 consumed / hour	6, 6	10	460.86 ± 32.06,	465.89 ± 30.46	1027.93,	927.59	.278	NS
Kcalories heat / 24 hours	6, 6	10	53.37 ± 3.71,	53.95 ± 3.53	13.78,	12.45	.276	NS
Specific metabolism	6, 6	10	119.45 ± 12.84,	108.98 ± 7.35	164.84,	54.08	1.733	NS
Kcal. / Kg BW / 24 hours	6, 6	10	160.77 ± 19.52,	141.38 ± 9.87	381.00,	97.32	2.172	.100

Table 21

ADRENAL WEIGHT (mg) - SPRAGUE-DAWLEY MALE RATS *

A Parabiotic	B Control
61.7	57.7
52.9	55.8
49.4	57.6
70.0	55.2
63.7	53.8
96.6	59.7

*Samples were collected after oxygen consumption determinations preceded by a 17 hour fast. Animals were maintained on a grain (M-1) ration.

SIGNIFICANCE

Comparison of Means	n	df	Mean ± S. D.	Variance	t	P
AB	6, 6	-+	65.7 ± 16.9, 58.0 ± 4.2	284.6, 17.3	1.086**	NS

**Approximate t-test

+Weighted critical values calculated

Table 22

ADRENAL WEIGHT, mg/100 g BODY WEIGHT - SPRAGUE-DAWLEY MALE RATS*

A Parabiotic	B Control
19.10	14.95
16.03	13.88
13.28	14.96
20.06	15.51
22.20	13.55
27.60	14.74

*Samples were collected after oxygen consumption determinations preceded by a 17 hour fast. Animals were maintained on a grain (M-1) ration.

SIGNIFICANCE

Comparison of Means	n	df	Mean ± S. D.	Variance	t	P
AB	6, 6	-+	19.80 ± 5.04, 14.60 ± .74	25.36, .54	2.503**	.100

**Approximate t-test

+Weighted critical values calculated

Table 23

THYROID WEIGHTS (mg) - SPRAGUE-DAWLEY MALE RATS *

A and B are unadjusted; C and D are adjusted per 100 g body weight.

Parabionts		Controls	
A	C	B	D
22.3	6.90	22.3	5.79
22.2	6.73	14.3	4.02
22.3	5.99	21.5	5.42
21.8	6.25	15.7	3.88
17.8	5.09	16.4	4.35
		21.1	5.47
		15.0	3.73

*Samples were collected after oxygen consumption determinations preceded by a 17 hour fast. Animals were maintained on a grain (M-1) ration.

SIGNIFICANCE

Comparison of Means	n	df	Mean ± S. D.	Variance	t	P
AB	5, 7	10	21.3 ± 2.0, 18.0 ± 3.4	3.8, 11.8	1.883	.100
CD	5, 7	10	6.19 ± .72, 4.67 ± .87	.51, .75	3.224	.010

Table 24

TESTICULAR WEIGHTS (g) - SPRAGUE-DAWLEY MALE RATS *

A and B are unadjusted; C and D are adjusted per 100 g body weight.

Parabionts		Controls	
A	C	B	D
3.4545	1.07	3.8007	.99
3.6895	1.12	3.5963	1.01
4.0049	1.08	3.8449	.97
4.0628	1.16	3.8272	.94
3.4996	1.22	3.3647	.89
2.4063	.69	4.0165	1.04
		3.9140	.97

*Samples were collected after oxygen consumption determinations preceded by a 17 hour fast. Animals were maintained on a grain (M-1) ration.

SIGNIFICANCE

Comparison of Means	n	df	Mean ± S. D.	Variance	t	P
AB	6, 7	-+	3.5196 ± .6006, 3.7663 ± .2182	.3607, .0476	.954*	NS
CD	6, 7	-+	1.06 ± .19, .97 ± .05	.34, .002	.377*	NS

+*Weighted critical values estimated
*Approximate t-test

Table 25

THYROID HORMONE IODIDE (conc. ug %) - SPRAGUE-DAWLEY MALE RATS*

A Parabionts	B Controls
3.34	3.34
4.14	8.04
3.34	9.58
2.56	5.06
3.12	3.52
1.84	4.46
	4.32

*Samples were collected after oxygen consumption determinations preceded by a 17 hour fast. Animals were maintained on a grain (M-1) ration.

SIGNIFICANCE

Comparison of Means	n	df	Mean ± S. D.	Variance	t	P
AB	6, 7	-+	3.06 ± .78, 5.47 ± 2.39	.61, 5.72	2.527**	.050

** Approximate t-test

+Weighted critical values calculated

Table 26

GLUCOSE IN ASSAY MEDIUM (mg %) SPRAGUE-DAWLEY MALE RATS*

A Parabionts	B Controls
9.20	11.50
10.40	11.70
10.00	12.70
8.70	10.15
9.60	8.40
6.55	9.55
	11.55

*Samples were collected after oxygen consumption determinations preceded by a 17 hour fast. Animals were maintained on a grain (M-1) ration.

SIGNIFICANCE

Comparison of Means	n	df	Mean ± S. D.		Variance		t	P
AB	6, 7	11	9.08 ± 1.37,	10.79 ± 1.48	1.88,	2.20	2.152	.100

Table 27

INSULIN IN PLASMA (micro-units / ml) - SPRAGUE-DAWLEY MALE RATS *

A Parabionts	B Controls
37.04	27.49
22.52	33.25
27.65	26.70
39.80	46.51
24.25	34.83
29.86	14.39
	27.33

*Samples were collected after oxygen consumption determinations preceded by a 17 hour fast. Animals were maintained on a grain (M-1) ration.

SIGNIFICANCE

Comparison of Means	n	df	Mean ± S. D.	Variance	t	P
AB	6, 7	11	30.19 ± 6.93, 30.07 ± 9.78	47.99, 95.74	.024	NS

Table 28

SERUM FREE FATTY ACIDS (ueq. / ml) - SPRAGUE-DAWLEY MALE RATS*

A Parabionts	B Controls
.448	1.240
.750	.990
.438	.558
.425	1.253
1.025	1.010
.515	1.290
	1.143

*Samples were collected after oxygen consumption determinations preceded by a 17 hour fast. Animals were maintained on a grain (M-1) ration.

SIGNIFICANCE

Comparison of Means	n	df	Mean ± S. D.	Variance	t	P
AB	6, 7	11	.600 ± .241, 1.069 ± .254	.058, .065	3.392	.010

Table 29

VITAMIN C (ug) / mg ADRENAL WEIGHT - SPRAGUE-DAWLEY MALE RATS *

A Parabiotic	B Control
.7731	2.5685
.8015	2.2258
1.4089	1.6111
1.4557	1.0186
1.0989	2.3987
1.5124	2.2259

*Samples were collected after oxygen consumption determinations preceded by a 17 hour fast. Animals were maintained on a grain (M-1) ration.

SIGNIFICANCE

Comparison of Means	n	df	Mean ± S. D.	Variance	t	P
AB	6, 6	10	1.1751 ± .3329, 1.9653 ± .5439	.1108, .2959	3.086	.020

Table 30

UREA NITROGEN IN SERUM (conc. mg %) - SPRAGUE-DAWLEY MALE RATS *

A Parabionts	B Controls
14.09	14.79
27.94	16.53
22.21	17.34
18.86	10.95
18.95	16.94
16.37	18.78
	11.88

*Samples were collected after oxygen consumption determinations preceded by a 17 hour fast. Animals were maintained on a grain (M-1) ration.

SIGNIFICANCE

Comparison of Means	n	df	Mean ± S. D.	Variance	t	P
AB	6, 7	11	19.74 ± 4.86, 15.32 ± 2.93	23.60, 8.56	2.025	.100

PART 8

PARABIOTIC RATS DIFFERENTIALLY FED GRAIN AND HIGH FAT RATIONS

INTRODUCTION

The development of a procedure for determining separate growth rates of individual rats in parabiosis and a technique for ad lib. differentially feeding parabiotic rats, permitted the evaluation of the influence of parabiosis on the development of dietary induced obesity. To that end, one rat in each parabiotic pair was fed a high fat ration (298) while the other was fed a low fat grain ration (317). When conventional Sprague-Dawley rats are fed the high fat ration, obesity becomes prominent in a few months (318).

TECHNIQUES

All weighing, feeding and surgical procedures are those described in technique sections of this monograph.

Animals were adult, female Spargue-Dawley rats placed in parabiosis at approximately 1-2 months of age. All pairs were convalesced for about 4-8 weeks. Only pairs diagnosed as excellent "unions," totally recovered from surgery and showing no overt signs of "emotional stress" or physical illness were used. Animals were allowed to adapt to parabiosis for 4-8 weeks after surgery while consuming a standard grain (M-1) ration (317).

While animals were healing from surgery, the differential feeders were bolted into "triple" rat cages (one feeder at each end). A

partition, made of galvanized sheet metal, was then placed across the center of the cage dividing it into two equal areas. The compartments were equal in surface area to $1\frac{1}{2}$ regular galvanized rat cages. The added room was necessary for animal comfort as the feeder takes up considerable cage room. It is important to use a rat cage rack that has a reasonable degree of space (approximately 3 inches) between the bottom of the rat cage and the waste collection pan to minimize coprophagy. A distance greater than that recommended is not advisable as diet spillage will become too diffuse and difficult to collect.

In preliminary studies it was noticed that members in a parabiotic pair compete for water, one rat frequently preventing the other from drinking. For this study, therefore, two water bottles were offered with the spouts about $1\frac{1}{2}$ inches apart. Parabionts would drink each from its own bottle. Water bottles were emptied, flushed and refilled with fresh water every other day. Bottles and spouts were changed when necessary (approximately every third week).

The animal room was equipped with a humidifier and, if night work was absolutely necessary, low intensity red lights were used. A 12 hour light-dark cycle was used and was automatically controlled. Room temperature was controlled at 73 ± 2 degrees Fahrenheit and was checked every day. Air conditioning was set to allow for adequate ventilation. Any "upsets" in the room environment were recorded to help in data interpretation at the termination of the experiment.

Respiratory disease was a problem and any animal exhibiting symptoms was omitted from the study. Litter papers were changed twice a week.

Fresh high fat diet was made every 5-6 days, removing any possibility

of rancidity. Food cups were filled every day of the 62 day period to eliminate any changes in intake as a result of the level of food in the cup and also to supply fresh food daily.

After all physical preparations were completed, wound clips were removed from the animals and the rats examined one last time for any open wounds and body weights recorded (360).

Sham operated control rats were treated and handled exactly the same as parabionts. They were housed two in a cage to mimic the parabiotic duality.

Since surgery extended over a 2-3 month period, several groups of weanling animals had to be ordered. All experimental groups were given, as far as possible, the same number of pairs from each of the different ordered groups.

Estrus was not considered a major variable because food intakes were made over long periods and fluctuations in intake caused by the cycle should average out.

The controls needed were as follows: 1) Sham operated single controls, high fat fed; 2) Sham operated single controls, grain fed; 3) Parabiotic controls with both members of a pair feeding on high fat diet; and 4) Parabiotic controls with both parabionts eating grain ration. Single controls ate out of conventional glass food cups with parabiotic controls using differential feeders. For ease of discussion, experimental members of a parabiotic pair will be listed in two separate groups according to the ration consumed during the study. The two experimental groups were: 5) A high fat fed parabiont cross-circulating with a grain fed partner; and 6) Grain fed parabionts cross-circulating with group (5).

The entire feeding experiment lasted for 62 days. All animals were fed grain ration from day 1 to 23, inclusive, at which time groups 1, 3 and 5 were placed on the high fat ration with groups 2, 4 and 6 remaining on the grain ration. Animals were fed after day 23 for an additional 39 days.

Food intakes and body weights were determined throughout the experiment, body weights on four occasions and food intakes every day. A chromium sesquioxide check was made at the end of the 23 day control period and just before the end of the experimental period to make sure the feeders were used properly (see development of an ad lib. differential feeder for parabiotic rats in this monograph for the procedure).

Animals were sacrificed on day 62 when it was clear from body weight data that single high fat fed controls had gained a significantly greater amount of weight than the single grain fed rats. Since the animals were young adults when the experiment began, most of the body weight gain of the high fat fed group over the grain fed group was assumed to be fat accretion. This was later verified.

All animals were sacrificed on the same afternoon so that the experimental end-point would be well defined. Final body weights were taken after separating the pairs. The wound on each animal was then sutured shut to impede moisture loss. Fat organs were later dissected and weighed according to the methods of Schemmel (318).

The gastrointestinal tract, having been removed to dissect out the mesenteric and omental fat depots, was then slit from end to end with scissors and gently flushed out with water. The stomach was cut free from the duodenum and weighed separately. The intestinal tract

was then weighed. The cecum was weighed full and, after cleaning, was weighed with the intestinal tract minus the stomach. All weighings were done on a Mettler balance[1] using tared aluminum dishes. Dissections were alternated with one animal in each experimental group completed before beginning the second animal in any group. After one animal in each group had been dissected (series one), the next series would be started with a group other than the group that started the first series. In this way groups were staggered, as well as animals within a group. Any slight error occurring from the time of death to dissection would be completely randomized over all groups.

After dissection, the fat organs and empty gastro-intestinal tract were placed back into the carcass, the skin flaps folded over the opened abdominal area, the animal placed in a plastic freezer bag and frozen to -20 C.

At a convenient time the carcasses were analyzed for total body fat according to the method of Mickelsen and Anderson (361), with the following modifications. Goldfisch extraction was used in lieu of the Soxhlet. A colloid mill was not used with homogenate from the Waring Blendor extracted directly. Omitting the colloid mill does not decrease assay sensitivity.[2] Room temperature was taken so that volume of water used to facilitate homogenization could be accurately converted to weight. Room temperature was used simply for expediency and it was assumed that the water had reached room temperature.

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[1] Type H15, 160 g capacity. Mettler Instrument Corporation, Highstown, New Jersey. 08520.

[2] Personal communication from Dr. Rachel Schemmel, Department of Food Science and Human Nutrition, Michigan State University, East Lansing, Michigan. 48824.

RESULTS

Figures 14 and 15 show food intake data on an absolute basis and as a percentage of body weight, respectively, from days 1 through 62. Parabiotic animals were placed in the differential feeders for the first time on day 1 and adapted extremely fast and were consuming normal food intakes by day 4. It is important to look at data trends with all groups plotted on the same set of coordinates (Figures 14 and 15), but this produces a very complicated figure. To clarify group responses, 15 figures were plotted including all possible combinations of the six experimental groups taken 2 at a time (Figures 16-30). Day 23 is marked on each figure indicating the day on which groups 1, 3 and 5 were started on high fat diet. The first high fat intakes are plotted on day 24.

A chromium sesquioxide assay in which a 1% chromium oxide labeled diet was fed to just one animal of a pair in each differential feeder at the termination of the control period (day 23) and at the end of the experimental period (day 62, inclusive) was undertaken to determine if each animal in a pair was eating its proper diet. These tests were conducted during food intake measurement. Since chromium sesquioxide has no caloric value that would decrease slightly the energy content of the ration containing it. To keep the level of non-nutritive components of the different diets as uniform as possible, the 2% non-nutritive fiber[3] in the high fat ration was reduced to 1% whenever

[3] Cellulose type. General Biochemicals, Mogul Corporation, Laboratory Park, Chagrin Falls, Ohio. 44022.

chromium sesquioxide was added. Since 1% of the chromium compound was used, the non-nutritive level was maintained at 2%. When chromium sesquioxide was added to the grain diet, a similar amount of non-nutritive fiber was added to any unlabeled grain so that differences in grain intakes would not be altered among the grain fed groups.

Throughout the study, the control animals received the same diet as their parabiotic counterparts. This was true for the regular diets as well as those containing the added chromium compound. In all groups and at all times the differential feeder insured that each animal had access only to its assigned ration (Table 31). Levels of chromium detected were within the range of contamination or experimental error. All t-values were extremely low. This means that all differentially fed rats ate their proper diets. Because of the new, permanent scapular suture as well as feeder design, it wound be highly improbable for parabionts to use the feeder improperly, but, nevertheless, "chromium checks" were done as a precaution. Food intakes and body weight gains were excellent for the animals receiving their rations in the differential feeders during the control period (to be discussed later).

Food intake data, statistically analyzed, is shown in Tables 32-37. The list of numbers under the heading of "comparison of means" represent the same experimental groups listed on the food intake graphs, i.e., 1) Single high fat controls; 2) Single grain controls; 3) Parabiotic high fat controls; 4) Parabiotic grain controls; 5) Parabionts fed high fat from days 23 to 62 and cross-circulating with a grain fed animal; and 6) Grain fed animals cross-circulating with the high fat fed animals in group 5 above. Tables 32-34 are not adjusted for body weight whereas Tables 35-37 are. Tables 32 and 35 show average

mean intake comparisons for the control period during the last 19 days. The last 19 days were used since the parabiotic animals required the first 4 days to adjust to the differential feeder. Tables 33 and 36 list average intake comparisons for the last 25 days of the experimental period. The superscript "prime" indicates that the data were collected while the animals were fed the experimental rations. Records for the last 25 days were used because when the high fat diet replaced the grain ration on day 23, considerable instability in intake was observed (to be discussed later) for about 2 weeks. The intakes were fairly stable for the last $3\frac{1}{2}$ weeks of the study; consequently, these data were used for statistical comparison. Tables 34 and 37 compare grams of food intake, absolutely and on a body weight basis, between controls and experimental values of each group omitting groups 1, 3 and 5, as these are obviously different (Table 34). For example, in Table 34, 2 and 2' indicates a comparison of means between the food intake, in grams, for group 2 during the last 19 days of the control period versus the grams food intake for group 2 during the last 19 days of the experimental period. An equal number of days were used to compare control and experimental periods within one experimental group to hold sample size "bias" at a minimum.

Differences in intakes between groups during the control period were non-significant for 13 out of the 15 mean comparisons (Table 35). The only two that were slightly different were the comparisons of 2 vs. 4 and 4 vs. 6. The means for group 2 are the same as for group 6. The two comparisons were different, therefore, because group 4 (grain fed parabionts) was slightly high. The t-values are close to non-significance and during the subsequent days of intake (days 24-62)

the two grain control groups of 2 and 4 exhibited identical intakes. All intakes, therefore, during the control period were satisfactory and conditions were excellent for initiation of differential feeding.

On day 23, the high fat diet was fed to all animals in groups 1, 3 and 5. Figures 14-30 show the very dramatic changes that occurred in intake between days 23 and 38. Group 5 displayed the greatest drop in grams intake on day 24 followed by group 3 and then 1. Single high fat controls required longer periods for food intake adjustment in response to an increased caloric density than either groups 3 or 5. Group 5 increased intake on day 25, as shown on Figure 26, and then became congruent with the curve for group 3. Groups 3 and 5 decreased intake from day 26 to 31 at the same rate. At day 31, group 3 regulated intake lower than group 5 and usually remained lower for the remainder of the experiment. When high fat is fed to both parabionts of a pair (group 3), food intake is regulated in response to caloric density far more efficiently than in single control animals.

Groups 2 and 4 continued approximately the same intake as during the control period. The slopes of intake curves, as shown in Figure 15, are slightly downward. This usually happens when intake is expressed on a body weight basis because increases in intake lag slightly behind in proportion to gains in body weight causing less intake per unit body weight.

Rats in group 6 showed the most erratic changes in food intake between days 23 and 38 (Figures 14, 15, 20, 24, 27, 29 and 30). Intake fell precipitously as soon as their partners were fed the high fat diet. Intake, for the first week or so, was depressed so markedly that one member of group 6 died. At day 31, group 6 began to adjust

intake upward and around day 39 stabilized and remained fairly constant to the termination of the study. The final stabilized intake (2.8 g per 100 g body weight) was markedly lower than control intakes (6.5 g per 100 g body weight) for group 6 (see Figure 24 or Table 37) ($P < .001$).

There was a significant difference in the feed consumed by the different groups of rats except for the single and parabiotic rats, all of which were fed the grain ration (Table 36). It was anticipated that there would be differences in feed intakes when comparisons where made between those fed the grain and high fat rations. This difference was expected since the weight of ration consumed decreases as the caloric density increases (There are 3.24 kcal/g in the grain and 6.62 in the high fat ration.). The feed intakes were significantly different when the two rations and the two types of animals (parabiotic and conventional) were compared (Table 36).

The feed intake per unit of body weight of the high fat fed rats parabiotically joined to rats receiving the grain ration was greater than the average of a parabiotic pair where both were fed the high fat ration (3' vs. 5', Table 36). More surprisingly, the intake of the high fat fed parabiotic rats joined to a rat receiving the grain ration was higher than the intake of similar weight conventional rats fed the high fat ration (1' vs. 5', Table 36). When the parabionts in a pair were both fed the high fat ration, their intake was less than that of the conventional animal fed the same ration (1' vs. 3', Table 36). The comparison statistics of (2', 6') and (4', 6') were also expected and is clearly shown on Figure 15. Group 6, parabionts cross-circulating with high fat fed animals, regulated intake far below other grain controls which indicates a physiological suppression of intake.

Comparisons of the experimental period versus the control period are shown in Table 37. Once again, the comparisons of (1, 1'; 3, 3'; and 5, 5') were expected to be highly different since the comparisons were between grain and high fat diets. (2, 2' and 4, 4') are different because of the decreasing slope due to disproportionality between intake and body weight when intake is expressed as a percentage of body weight. The actual decrease for group 2 is similar to that of group 4.

Group 6 is not only significantly lower than either groups 2 or 4 during the experimental period but also the experimental period for group 6 compared to control values for 6 is also markedly lower with an extremely large t-value demonstrating, once again, suppression of intake of group 6 when cross-circulating with a high fat fed animal. On an absolute basis groups 2 and 4 ended the experimental period consuming an average of approximately 1 gram of food less than the amounts consumed during their control periods. These two groups have almost identical consumption curves. Group 6, however, on an average consumed about 3 grams less food per day at the end of the experimental period as compared to control values (Table 34).

Kcal intakes for all six groups during the control and experimental periods are shown in Figures 31-40. To expedite calculations food intake was converted to Kcal every third day. Figures 31-35 are absolute Kcal intakes while 36-40 are adjusted on a body weight basis.

As shown on Table 41, all control Kcal intakes are non-significant for the 15 comparisons which gave an excellent baseline on which to start high fat feeding.

The results of high fat feeding, adjusted for body weight, are shown in Figures 36-40 with the statistical analyses in Table 42. As

expected, the grain fed controls, both single and parabiotic (comparison 2', 4'), are statistically the same for caloric intakes. After changing all intakes to calories an interesting comparison was observed that was not readily apparent by just comparing intakes in grams. After such conversion, the comparison of (2', 3') is now non-significant. In other words, parabiotic high fat fed animals, regulated their caloric intakes so well that they adjusted to the level of the grain fed single controls. The values for the high fat fed parabionts are actually slightly lower than for grain fed single controls. The comparison of (3', 4') is equally as interesting in that the parabiotic high fat controls (both animals fed high fat) regulated their energy intakes significantly lower ($P < 0.05$) than the grain fed parabiotic controls (both animals eating grain) (Figure 34).

The ability of parabiotic animals to regulate their caloric intake when both are fed the high fat diet is in contrast to single animals fed the same diet. Single high fat controls consumed significantly more calories ($P < 0.01$) compared to their grain fed counterparts (comparison 1', 2'). The single high fat fed animals also consumed more energy than either parabiotic high fat fed or the parabiotic grain fed rats (groups 3 and 4).

Caloric intakes of group 5 (high fat fed parabionts cross-circulating with grain fed animals) were definitely augmented to the point where their intakes were all significantly higher (usually at $P < 0.001$) than any of the other groups (1, 2, 3, 4 and 6; Table 42).

The above relationships are true not only with between group comparisons but also comparing control and experimental periods within each group (Table 40). Groups 2 and 4 show a drop in intake due, once

again, to any weight gain disproportionate to changes in food intake. This is a normal occurrence. Even with this kind of mathematical relationship between energy intake and body weight, the caloric intakes for single high fat fed animals (group 1) were significantly higher during the experimental period. With the kind of relationship shown in groups 2 and 4, the difference in group 1 is even made more pronounced as group 1 gained the most weight during the experimental period.

The parabiotic rats where both members of the pair were fed the high fat ration, consumed the same number of calories on a body weight basis after the high fat ration was started as was consumed while being fed the grain ration (Table 45; Figure 34). There was a marked increase in energy intake for about 10 days after the high fat ration was started but from then on the caloric intake was the same as that of the parabionts when fed the grain ration.

Energy intakes for group 5 are augmented over the control values for group 5 whereas intakes for group 6 are suppressed compared to their grain control values (Table 45; Figures 36-40).

Group 3, parabiotic rats both fed the high fat ration, not only regulated their caloric intakes at a lower level than the other high fat fed groups, but this regulation also occurred at a faster rate (Figure 36). The initial caloric hyperphagia due to high fat feeding was not as pronounced in high fat fed parabionts as in single high fat fed animals. This hyperphagia for group 5, when initially placed on high fat, was absent as compared to groups 1 and 3 (Figure 36).

A point of concern is brought to the attention of the reader concerning intake data for day 60. Something evidently happened in

the animal room on this particular day augmenting intakes over "normal" levels in every group. There is a greater effect in some groups than others making data comparison impossible. All intakes for the few days following day 60 were returning to values existing previous to that day, especially group 6 which is of major concern. The data for day 60 were left in for completeness of the record and since intakes lasted almost a month, the effect would be diluted out. Nothing "environmentally adverse" was recorded for that particular day so, whatever happened, probably occurred over night which is the prime time that food intake may be affected.

Food efficiency data is presented on Tables 43-45. Eleven out of the 15 mean comparisons were statistically the same during the control period (Table 43), offering a fairly good baseline. It is not known why the efficiencies for group 1 were a little high (or less efficient). Group 1 was composed of the same animal stock as all other groups.

As expected, group 1, when offered the high fat ration on day 23, exhibited a marked increase in food efficiency compared to all other groups (Table 44) and also compared to the control values for group 1 (Table 45). Group 3 (parabionts both fed high fat) showed statistically no change in efficiency when placed on high fat and actually became slightly less efficient. The opposite to the animals in group 1 is seen in group 5. These animals (cross-circulating with grain fed animals) became markedly less efficient when fed the high fat ration. Three totally different responses were seen in groups 1, 3 and 5 when offered the high fat diet. Group 1 became more efficient which is the normal response. There was no change in efficiency in group 3 and group 5 became markedly less efficient. The efficiency of group 5 was

the same as that of the parabiotic grain fed animals (comparison 4', 5'; Table 44). Group 6 was less efficient than any of the other groups (Table 44). Group 2, during the experimental period, did become less efficient as body weight gain began to plateau. Body weight gain plateau was somewhat delayed in parabiotic grain fed animals (group 4); consequently, these animals were a little more efficient (compared to group 2) during this period of time. This may be a result of the parabiotic state, per se, and is extremely hard to control. Even though values are different for groups 2 and 4, their ranges are quite distinct from the other basic responses in groups 1, 3 and 6. Group 5 has a similar value as groups 2 and 4, but comparisons to control values for group 5 or to other high fat fed groups are more important. The last comparison is (3', 4') and demonstrates that, when high fat is fed to both members of a pair, the loss of efficiency with time, as seen in control groups 2 and 4 on grain diet, is decreased. In other words, since efficiency for group 3 is the same for control and experimental periods, there was no decrease with time as is normal and shown by control groups 2 and 4. It is important, therefore, to compare efficiency not only between groups but also the rate of change of efficiency for any particular group as a function of time. All efficiency data are manifested in body weight and body fat data as subsequently discussed. It is important to note, although, that changes in efficiency may not be produced by body weight changes. Food efficiency varies with absolute food intake with body weight increasing, decreasing or remaining constant.

Body weight curves are plotted in Figures 41 and 42 and analyzed statistically in Table 46. Absolute weight is plotted against time in Figure 41 while Figure 42 is a plot of body weight gain versus time.

It can readily be seen from Figure 41 that all single animals are markedly heavier than parabiotic animals at the beginning of the experiment as well as throughout the entire experiment.

All slopes in Figure 41 are similar in the control period except for group 1. This slope was slightly flatter and correlated well with the lower efficiency for this group (Table 43). Group 1, according to design, was to be fed high fat. One can observe the marked effect that high fat diet produced on the weight of these animals (Figure 41) and on food efficiency (Table 44). Such a large effect was still seen in this group of rats even with the initial low efficiency.

All groups of animals gained weight during the experimental period (Figure 41). The change in slope at day 23 indicates how much the experimental conditions altered the rate of weight gain. The group affected the greatest was group 6.

In Figure 42 the rate of weight gain for all groups except 1 is practically identical during the control period. The decreased efficiency in group 1 is, once again, reflected here. The gain in weight for all groups was still practically the same in the control period and gave a very good baseline for experimental group comparisons. The rate of weight gain for group 1 was greater and highly statistically different ($P < .001$) from any of the other groups (Figure 42 and Table 46).

The gain in weight for group 3 (both parabionts fed high fat) was almost identical to that reported in Figure 13, being less than half that of the high fat fed single controls. The gain in weight for group 3 was statistically different from groups 2, 4 and 6 and only weakly different from group 5 ($P < .100$). In the high fat fed animals, therefore, group 1 gained the most weight followed by group 3 and then

group 5. These data agree perfectly with the ability of the rats to regulate calorie intakes and with food efficiency for these 3 groups.

The weight gain of the animals in group 2 was not different from either groups 4, 5 or 6. Group 4's gain was not different from 5 or 6 and group 5 was not different from 6. Group 6, although not statistically different from groups 2, 4 and 5, does have the lowest rate of body weight gain.

The gain in weight for group 5 (cross-circulating with a grain fed group) was practically identical to both grain fed control groups (Figure 42). The feed efficiencies were also grouped in this identical order.

Group 3 gained more weight than either groups 2, 4, 5 or 6 even with the same food efficiency during the grain control period as during the high fat experimental period. Efficiency was actually slightly less with the slope of the body weight gain curve slightly lower. These animals gained weight because the efficiency did not decrease with time as in groups 2, 4, 5 or 6.

Body fat, as a percentage of body weight, is shown in Table 47. As expected, body fat for the single high fat controls was significantly greater than that for any other group ($P < .001$). Group 3 (both animals fed high fat diet) had markedly more fat than the respective controls in group 4 (both animals fed grain ration). Percent body fat for high fat fed and grain fed female Sprague-Dawley rats as reported here agrees almost exactly to values reported by Schemmel (318). Schemmel's values are 30.38% and 14.83% for the high fat fed and grain fed female Sprague-Dawley rats, respectively. Values shown on Table 47 are 27.24% for high fat and 13.79% for grain. These animals were at approximately the same age as Schemmel's.

Group 3 had almost exactly the same body fat content as group 2 with an extremely low t-value. The same is true for the comparison between group 2 and group 5; so, two of the three high fat fed groups had statistically the same body fat as single grain fed controls.

There was a large decrease in body fat in group 4 (both parabionts fed grain) compared to single grain controls. The P-value was 0.10, but the t-statistic was extremely high and just missed 0.05; consequently, the probability was a lot less than 0.10 that the difference was caused by chance. These data show once again, as all previous data, that grain fed parabionts have a lower body fat content compared to single grain fed animals and that high fat fed parabionts are resistant to the development of obesity when fed a high fat diet.

The lowest body fat was seen in group 6, approximating one-half that of normal single grain fed animals and was markedly lower than groups 2, 3 or 5. Group 6 was not statistically different from 4, but the body fat for group 6 was lower by almost 17%. Among grain fed animals the lowest body fat was reached in grain fed parabionts cross-circulating with high fat fed animals. The second highest levels were in parabionts both fed grain and the highest in single grain fed controls. Cross-circulation with a high fat fed animal further decreased body fat when compared to parabionts both fed grain.

The comparison between group 3 and 5 was non-significant and the body fat contents of these two groups were almost identical to group 2 as stated earlier.

The last comparison of means in Table 47 is that of group 4 versus group 5. As expected, group 5 does have a higher fat content which is bordering on significance at $P < 0.10$.

Adipose depot weights were obtained from these animals to determine if any depot was affected more than others or if the decreased body fat content of parabiotic rats was a general loss of fat affecting all fat depots equally. Data are presented for inguinal fat organs (Tables 48-50), the renal-retroperitoneal depots (Table 51), perimetrial fat organs (Table 52) and the total fat removed in each group (Table 53).

It was very difficult to remove the inguinal depots in parabiotic rats on the side of the union. When separating parabionts at autopsy, the inguinal depot closest to the union was distrubed. The inguinal data are therefore reported inclusive (Table 49) and exclusive (Table 48) of the inguinals closest to the union.

When the rats were joined in parabiosis, the inguinal depot on the side of the surgery, for parabionts and controls, was incised across the "belly" and freed of its adhesions to the abdominal musculature. The fat organ was left inserted and no part of it was removed. This procedure was done as an aid in making the abdominal incisions and sutures. As an adjunct to the present study, it was determined if incising the inguinal depot produced any effect on the subsequent weight or size of the depot with animals fed grain or high fat diet. In single control animals both incised and non-incised inguinal depots could be removed with controlled uniformity (Table 51) without interference as with the parabiotic union described above. If the incised and non-incised inguinal organs were combined or just the non-incised included there was no difference as to whether a test was significant or not (comparing Table 48 with 49). The data will therefore be analyzed from Table 49. About the same trend in significance is seen in Table 49 as for body fat as a percentage of body weight (Table 47).

The inguinal depot seems to be markedly affected by parabiosis as well as differential high fat and grain feeding.

Parabiotic grain fed controls had inguinal weights about one-half the size of single grain fed controls (comparison 2, 4; Table 49). Group 3 (high fat fed parabionts) had the same size inguinal depots as single grain controls (group 2) and, as seen for body fat data, group 5's data agreed with groups 2 and 3. There was a marked depression of the inguinal depots for animals in group 6 compared to all groups except group 4, in which the absolute weight was lower but not statistically so. Rats in group 6, attached to high fat fed animals, did not display any marked change in inguinal depot weights other than that already produced by parabiosis. Single high fat controls displayed excessively enlarged inguinals (group 1).

Table 50 shows the effect of incising the inguinal depot across the "belly" on subsequent depot development. By using a general pooled-t test no effect was seen on depot development in animals fed a grain or high fat ration. When the data were paired by comparing pairs of incised and non-incised depots from the same animal, a significant difference was seen in the grain fed but not the high fat fed animals This difference was strong ($P < .01$) even with a loss of half of the degrees of freedom by pairing. It is concluded that incised depots are smaller in grain fed animals not "stressed" by excessive fat storage but when "stressed" by feeding high fat diet the tissue had the same potential for fat storage.

The significance for the combined renal-retroperitoneal fat depots (Table 51) were almost identical to the inguinal (Table 49). The major comparison that changed from significant to non-significant, or

borderline levels, was the comparison betwen group 5 and group 6. The absolute weight of the depot is lower in group 6 than group 5 but statistically the weights appear to be similar.

The combined perimetrial depots (Table 52) show two major changes in significance over the inguinal data. Comparing group 2 with 4, the perimetrial depots appear to be statistically similar, although, the grain parabionts still had lower values. There is, as with the renal-retroperitoneal data but in contrast to the inguinal weights, no difference between groups 5 and 6.

The following conclusions in all depot weight data seem to be evident. In all depots removed, the single high fat controls exhibited significantly increased weights over all other groups ($P < .001$). Group 3 (both parabionts fed high fat) produced depot weights statistically the same, for all fat organs, as the single grain controls. These observations are in agreement with total body fat determinations. The parabiotic state, per se, does not seem to markedly affect the perimetrial depots but does the inguinal and renal-retroperitoneal (comparisons 2 and 4; Tables 48 and 51). Group 5 (high fat fed parabionts cross-circulating with grain fed animals) had depot weights in all cases comparable to groups 2 and 3. Group 6 (grain fed; cross-circulating with high fat fed animals) had lower depot weights in all cases compared to groups 2 and 3 but did not differ statistically in any case from group 4 although all absolute weights were further depressed below group 4. The major depot affected in a grain fed animal cross-circulating with a high fat fed animal was the inguinal. This effect is a further reduction of depot size from the parabiotic effect, per se. In all cases high fat fed parabionts had larger depots compared

to grain parabionts (comparisons 3, 4) and high fat parabionts, in all cases, had the same weights as high fat parabionts attached to grain fed animals (comparisons 3, 5).

All depot sizes are statistically the same between groups 4 and 5, although the weights for group 5 are absolutely larger. In all cases the absolute weights for group 5 are lower than absolute weights for group 3. Attachment to a grain fed animal decreased further the depot weights of animals in group 5, compared to group 3. Within high fat fed groups, the single controls exhibited the largest fat organs followed by group 3 and finally group 5.

The total fat excised as depots in all groups (Table 53) closely reflects body fat as a percentage of body weight (Table 47).

All depot weight data show the same trends as shown earlier in this thesis in that depot weights in parabionts are usually lower (see various physiological and anatomical parameters for "normal" parabiotic rats, Part 6).

At autopsy, the empty stomach, empty intestinal and full cecal weights were determined. After the full cecum was weighed, it was cleaned and the empty organ added to the intestinal tract. These animals offered an opportunity to study the effects of cross-circulation in parabiosis on the characteristic weight changes seen in the gut after feeding grain and high fat diets to single rats.

In all comparisons involving a grain fed and high fat fed animal, whether control or parabiotic, the grain fed animals had larger stomachs expressed as a percentage of body weight (Table 55). These results were expected for single animals and, as seen, parabiosis did not seem to alter the response. The parabiotic state, per se, did not produce

an effect as shown by comparison 2, 4 (Table 55) when grain ration was fed. On high fat diet the parabionts had heavier stomachs compared to single high fat controls (comparison 1, 3). This may be of importance in implicating gastro-intestinal tract hormones in food intake regulation. Differential feeding produced little effect. Grain fed parabionts (group 6), cross-circulating with a high fat fed animal, still displayed a heavier stomach and this value was statistically the same with the major control for group 6, group 4 (comparison 4, 6). The same was true for the high fat fed animal cross-circulating with a grain fed animal. The stomach weights for group 5 were similar to the grain fed parabionts, group 3.

Virtually no effects were seen in the empty intestinal weights plus cecum. The only 3 differences were interactions 1, 4; 1, 5; and 1, 6 (Table 57). These differences did not seem important since the intestinal weights of rats in group 4 were the same as those of 3 and 5. Group 5 is the same as 3 and group 6 is the same as the high fat fed groups 3 and 5. No differential effects were seen.

For all comparisons of full cecal weight, grain fed animals had higher weights than high fat fed whether control or parabiotic. The differential feeding of high fat diet and grain made no difference as group 6 still had heavier full cecums compared to group 5 and group 6 also had similar weights as the parabiotic control, group 4. Group 5 had statistically the same full cecal weights as group 3. All grain controls were statistically the same (comparison 2, 4) but, exactly as with the empty stomach data, the parabionts both fed high fat had heavier cecums compared to the single high fat controls.

The only data in the preceding gastro-intestinal tract experiments

that were not expected were that the high fat fed parabionts had heavier stomachs and cecums, on a body weight basis, than the single high fat controls. These differences do not hold on an absolute basis. For stomach weights, the absolute weights observed in group 1 were markedly heavier than in group 3 animals (Table 54). Cecal absolute weights for group 1 and group 3 are the same statistically. The cecal weights for grain controls (comparison 2, 4) were non-significant no matter how the data were expressed, and stomach weights for group 2 were heavier than group 4. It does appear that differences in cecal weights between single and parabiotic high fat fed animals may be an artifact due to the smaller physical size of the parabionts disproportionate to organ weights.

DISCUSSION

Differential feeding introduced after parabiosis quickly produced an extreme and variable effect in body weight gain and food intake for group 6 from day 23 to 39. During this time span, food consumption among the grain fed parabionts was suppressed so markedly that one member of group 6 died. At approximately day 31 the animals in group 6 began to consume more food and reached a fairly steady plateau which was maintained from day 39 to 62. It is possible that some post prandial circulating substance in group 5, caused by high fat feeding, could have produced such a rapid response in group 6. Since group 6 did regulate upwards, it also seems probable that animals in group 6 simply had their meal frequency upset by being attached to an animal consuming a high fat diet. It has been reported (24)

that altering the caloric density of the diet will change meal frequency in the rat but that after 6 or 7 days frequency returns to normal and any alterations in grams of food intake to compensate for caloric density of the diet is accomplished by changing meal size. The major disruption of normal intake in this experiment lasted for approximately 7 days at which time it is postulated, the high fat fed animals (group 5) reverted back to a normal frequency and at this time the grain fed partner began to regulate intake upwards. Frequency of eating in the ad lib. differential feeder is extremely important since animals must both be willing to go into the feeder at the same time. The animals would now be on the same frequency but would vary in meal size and also the ease and time of actual consumption of the different diets. The grain fed animal would have to increase consumption per unit time in order to compete with the high fat fed animal's shorter meal both in grams and probably also in time.

Grain fed parabionts, cross-circulating with high fat fed partners, after regulating their intakes upwards, leveled off at an average consumption of 76.9% of their previous control values (days 1-23). It seems reasonable to conclude that the depressed intakes from day 39 to 62 (almost $3\frac{1}{2}$ weeks) in group 6 were caused by some factor that was cross-circulating from the high fat fed animal and would be indicative of a systemic component in the regulation of food intake. Perhaps studies using iso-caloric diets varying in different types of food components but having the same caloric density and particulate consistency would be very valuable in implicating a particular dietary factor as being appetite depressive. Caloric density problems as the one described above would thus be avoided.

A few summarizing comments would perhaps be appropriate at this time. Three basic interactions of experimental comparisons of group means are extremely important. These interactions are group 2 vs. 4 (single grain fed controls vs. parabiotic grain fed controls), group 1 vs. 3 (single high fat fed controls vs. parabiotic high fat controls), and group 5 vs. 6 (high fat fed animals cross-circulating with a grain fed rat vs. grain fed animals cross-circulating with high fat fed animals). Groups 5 and 6 constitute the parabiotic pairs that were fed different rations. The high fat fed member of a pair is in group 5 and its partner, grain fed, is in group 6. It has been shown that parabiotic animals on a "normal" diet do have less body fat as a percentage of body weight as compared to single rats fed the same diet (comparison 2, 4; Table 47). Absolute percentages are much lower for parabionts. Statistical significance is only 0.10 but the t-value is extremely high and just misses 0.05; consequently, the probability is far less than 0.10 that the difference was due to chance. Hervey (283) reported that his parabiotic rats regulated to one-half the body fat content of the controls. Hervey's rats were in parabiosis for 8 months and the pairs reported here had been sustained for 5 months. The general trend in lowered body fat reported here does corroborate Hervey's observation with the difference in magnitude being one of length of time in parabiosis.

Energy intakes were statistically the same for groups 2 and 4 with actual values slightly higher for the parabionts (group 4). Parabionts in group 4 show a higher food efficiency for the entire experiment. During the control period, this was not significant, but during the experimental period it became so. Rates of body weight gain agree

well with efficiency data in that parabionts (group 4) did gain more body weight but the increased gain was not significant compared to grain controls (group 2). Even with an increased body weight gain and food efficiency, body fat was regulated lower in parabiotic grain fed animals. Possibly, there are also circulating factors directly responsible for regulating body fattiness. The argument proposed by Hervey (283) is that each animal in parabiosis is actually responding to approximately twice as much body fat within its own regulatory system. Responses to a surfeit of fat would tend to decrease the excess. Further support for a suppression of body fat is seen in fat organ analyses. Virtually all fat organs removed from parabiotic animals were markedly lower in actual weight (Tables 48-49; 51-52). In previous experiments practically all fat organs absolutely and per unit body weight were lower in weight compared to controls (see Part 6, this thesis).

Interaction 1, 3 (single high fat controls vs. parabiotic high fat controls) provides further evidence for increased suppression of body fat in parabionts. High fat fed parabionts (group 3) compared to high fat fed controls (group 1) ate fewer calories, were less efficient, had markedly less body fat with all fat depot weights being much smaller, and had a lower rate of body weight gain. Parabionts seem to be able to handle a high fat diet far better than single animals. The decreased food intake may be a result of a greater release of gastrointestinal tract hormones in high fat fed parabiotic rats (group 3) (see literature review, GIT hormones).

In the differentially fed pairs (group 5 vs. group 6), the high fat fed animal did not become obese and the grain fed animal became leaner than the single or parabiotic control. Group 5 displayed a

caloric hyperphagia when fed the high fat diet compared to the high fat fed parabiotic controls (high fat - high fat) but was markedly less efficient. Body fat in group 5 was lower than in the parabiotic high fat fed controls but not statistically so. All fat depots for group 5 were statistically lower in weight compared to group 3 and body weight gain was depressed.

Rats in group 6 became leaner when attached to animals fed the high fat ration compared to grain fed parabiotic controls. Food intake decreased, food efficiency was the lowest for any grain fed group, body fat was extremely low, all fat depots were lighter and the total amount of body weight gain was decreased. Group 5 at this time was depositing fat and did increase body fat higher than normally seen in grain fed parabiotic animals.

The inhibition of food intake with a decreased body fat in grain fed animals attached to animals that were calorically hyperphagic and increasing body fat stores (in other words, nutritionally hyperphagic caused by high fat feeding) has not been previously reported. The reduction in weight of a lean parabiont joined to one that is overeating or is obese is a phenomenon that has been observed by a number of investigators. This was true of Hervey's (283) work when he joined hypothalamically lesioned hyperphagic and obese animals with normal rats. Haessler and Crawford (287) noted a similar change when they joined obese, hyperglycemic mice with normal mice. The hyperphagic diabetic mouse was used by Coleman and Hummel (291); and most recently Chlouverakis (352) reported that lean mice, when joined to obese diabetic mice, lost weight.

The data reported here, using a nutritional model, are in

agreement with all of the above papers. Possibly, overly fed or obese animals, in this case group 5, respond to excessive caloric intake by trying to correct the energy imbalance. This response, if humorally mediated, could cross-over to the lean animal causing a reduction of its feed intake and consequently a loss in body weight.

Meal fed animals have an almost identical suppression of food intake as that seen in group 6. Even with a reduction of food consumption equal to 25%, meal feeders have increased food efficiencies, stomach weights and body fat contents with body weight gains remaining constant and equal to that of the ad lib. fed controls.[1] It is doubtful whether meal eating can explain the changes that occurred in the parabiotic rats. Although the parabiotic rats consumed only 76% of their "normal" feed intake, there was a reduction in their feed iffiiciency (Table 44), body fat content (Table 47), and rates of body weight gain (Table 46; Figure 42). Even stomach weights were lower than in controls confirming the suggestion that "meal eating" was likely not involved in the development of the observed condition in the rats used in the present study. Animals in group 6 were probably exposed to their food with the same frequency as when their partners (animals in group 5) were also consuming grain and had, therefore, ample opportunity to increase their consumption if desired.

At the initiation of differential feeding the average body weights for animals in group 5 and 6 were 222 and 211 grams respectively; consequently, initial intake suppression in group 6 was not a result of larger, satiated animals in group 5, physically keeping the rats in group 6 out of the feeder. If any animals were dominant, it should

[1] Personal communication, Dr. Gilbert A. Leveille, Chairman, Dept. of Food Science and Human Nutrition, Michigan State University.

be those in group 6; they should have been able to lead the pair into the feeder if they wished to eat.

Grain fed animals attached to high fat fed partners do have the ability to consume their normal daily intakes if they want to. Relatively normal intakes have been seen in differentially fed pairs where cross-circulation may have been extremely low which meant that the union was only a physical one. This virtually eliminates any effect of differential feeding, per se, as causing the intake depression. In other words if these animals were not cross-circulating but still attached and feeding differentially, they would have had the ability to consume their normal intakes.

Evidence seems to support the contention that an anorexigenic circulating substance coming from the high fat fed animal was responsible for inhibiting the intake in the grain fed animal. These findings also indicate that the decreased food intake seen when single animals are placed on a high fat diet may partially be humorally mediated in addition to the long standing argument that the fat has a longer resident time in the gastro-intestinal tract increasing its satiety value.

Suggestive evidence is presented for the existence of this humoral factor in these studies. The effect would not be as marked in the grain fed animals (group 6) as only small amounts per unit time of the postulated "factor" could crossover from the high fat fed rat. Crossover rates must also exceed clearance for a physiological response to be manifested. As seen from the data (Tables 32-33) suppression of intake is about 3 grams per day in the grain fed animals (group 6) but may be 6-8 grams when both parabionts are fed the high fat ration.

If some factor is crossing from the high fat fed animal then the intake suppression in group 5 should not be as marked as in either groups 1 or 3 as some of the factor is being lost. Group 5 has the highest intake of any of the high fat fed groups or, i.e., intake was not as depressed as would be expected, indicating that an "appetite depressive factor" was partially lost.

Table 31

USE OF DIFFERENTIAL FEEDER - SPRAGUE-DAWLEY FEMALE RATS

Food with 1% chromium sesquioxide was placed on one side of the feeder; a ration containing none of the chromium compound was on the other side. Food intake of the rat fed the non-labeled diet was measured and the total amount of chromium sesquioxide recovered in the feces of that animal over a set period of time (usually two days) was determined. The label detected in the feces was used in calculating the weight of the labeled diet consumed by the rat assigned to the unlabeled diet. This amount was added to the food consumed determined by food cup weight difference. These un-corrected and corrected food consumptions are compared below for statistical difference.

Experimental Group	Comparison of Means: Uncorrected Intakes	Corrected Intakes	n	df	Variance	t	P
All pairs at end of first 23 days (control period); M-1 diet (g)	14.12 ± 2.37	14.56 ± 2.44	26, 26	50	5.60, 5.93	.660	NS
High fat fed control parabionts at end of experiment (g)	6.90 ± 1.29	7.01 ± 1.24	5, 5	8	1.68, 1.54	.135	NS
M-1 fed control parabionts at end of experiment (g)	13.28 ± 1.67	13.73 ± 1.67	5, 5	8	2.78, 2.80	.430	NS
M-1 fed parabionts cross-circulating with high fat fed animals (end of experiment) (g)	9.55 ± 3.04	9.98 ± 3.12	8, 8	14	4.26, 9.74	.281	NS
(kcal)	32.48 ±10.34	35.38 ±10.95	8, 8	14	107.00, 119.80	.545	NS

Table 32

FOOD INTAKES IN GRAMS (average / animal / day) OF SPRAGUE-DAWLEY FEMALE RATS KEPT IN A CAGE CONTAINING A DIFFERENTIAL FEEDER;[1] CONTROL PERIOD: M-1 DIET

Comparison of Means[2]	n	df	Mean ± S. D.	Variance	t	P
1,2	19, 19	36	15.249 ± 1.677, 15.539 ± 1.776	2.813, 3.155	.516	NS
1,3	19, 19	36	15.249 ± 1.677, 13.051 ± 1.574	2.813, 2.479	4.166	.001
1,4	19, 19	31#	15.249 ± 1.677, 13.105 ± 1.002	2.813, 1.004	4.783*	.001
1,5	19, 19	36	15.249 ± 1.677, 13.063 ± 1.159	2.813, 1.342	4.676	.001
1,6	19, 19	30#	15.249 ± 1.677, 13.345 ± .965	2.813, .931	4.289*	.001
2,3	19, 19	36	15.539 ± 1.776, 13.051 ± 1.574	3.155, 2.479	4.572	.001
2,4	19, 19	30#	15.539 ± 1.776, 13.105 ± 1.002	3.155, 1.004	5.202*	.001
2,5	19, 19	32#	15.539 ± 1.776, 13.063 ± 1.159	3.155, 1.342	5.089*	.001
2,6	19, 19	29#	15.539 ± 1.776, 13.345 ± .965	3.155, .931	4.731*	.001
3,4	19, 19	32#	13.051 ± 1.574, 13.105 ± 1.002	2.479, 1.004	.126*	NS
3,5	19, 19	36	13.051 ± 1.574, 13.063 ± 1.159	2.479, 1.342	.034	NS
3,6	19, 19	31#	13.051 ± 1.574, 13.345 ± .965	2.479, .931	.694*	NS
4,5	19, 19	36	13.105 ± 1.002, 13.063 ± 1.159	1.004, 1.342	.122	NS
4,6	19, 19	36	13.105 ± 1.002, 13.345 ± .965	1.004, .931	.705	NS
5,6	19, 19	36	13.063 ± 1.159, 13.345 ± .965	1.342, .931	.816	NS

*Approximate t-test

#Degrees of freedom calculated

1Food intakes were measured during the last 19 days of the 23 day control period. The data for the first 4 days were discarded since it takes that long for the animals to become adjusted to the differential feeder. The grain ration was fed to all animals. This pertains to subsequent control data.

2For all tables, these numbers refer to the grouping of rats; see technique discussion.

Table 33

FOOD INTAKES IN GRAMS (average / animal / day) - SPRAGUE-DAWLEY FEMALE RATS

EXPERIMENTAL PERIOD

1' Single High Fat
2' Single M-1
3' Parabiotic High Fat
4' Parabiotic M-1
5' Parabiotic High Fat attached to Parabiotic M-1
6' Parabiotic M-1 attached to Parabiotic High Fat

Comparison of Means	n	df	Mean ± S. D.	Variance	t	P
1',2'	25, 25	48	8.765 ± .763, 14.558 ± 1.010	.582, 1.020	22.883	.001
1',3'	25, 25	41#	8.765 ± .763, 6.298 ± .459	.582, .211	13.852*	.001
1',4'	25, 25	48	8.765 ± .763, 12.364 ± .748	.582, .559	16.846	.001
1',5'	25, 25	48	8.765 ± .763, 7.190 ± .691	.582, .478	7.649	.001
1',6'	25, 25	48	8.765 ± .763, 10.189 ± .919	.582, .844	5.962	.001
2',3'	25, 25	34#	14.558 ± 1.010, 6.298 ± .459	1.020, .211	37.224*	.001
2',4'	25, 25	48	14.558 ± 1.010, 12.364 ± .748	1.020, .559	8.727	.001
2',5'	25, 25	44#	14.558 ± 1.010, 7.190 ± .691	1.020, .478	30.431*	.001
2',6'	25, 25	48	14.558 ± 1.010, 10.189 ± .919	1.020, .844	16.000	.001
3',4'	25, 25	41#	6.298 ± .459, 12.364 ± .748	.211, .559	34.564*	.001
3',5'	25, 25	43#	6.298 ± .459, 7.190 ± .691	.211, .478	5.373*	.001
3',6'	25, 25	36#	6.298 ± .459, 10.189 ± .919	.211, .844	18.941*	.001
4',5'	25, 25	48	12.364 ± .748, 7.190 ± .691	.559, .478	25.407	.001
4',6'	25, 25	48	12.364 ± .748, 10.189 ± .919	.559, .844	9.183	.001
5',6'	25, 25	48	7.190 ± .691, 10.189 ± .919	.478, .844	13.043	.001

*Approximate t-test
#Degrees of freedom calculated

Table 34

FOOD INTAKES IN GRAMS (average / animal / day) - SPRAGUE-DAWLEY FEMALE RATS

CONTROL PERIOD vs. EXPERIMENTAL PERIOD *

Comparison of Means	n	df	Mean + S. D.	Variance	t	P
Single M-1, Single M-1 2, 2'	19, 19	32#	15.539 ± 1.776, 14.498 ± 1.138	3.155, 1.296	2.151**	.050
Parabiotic, Parabiotic M-1 M-1 4, 4'	19, 19	36	13.105 ± 1.002, 12.251 ± .813	1.004, .660	2.886	.010
Parabiotic, Parabiotic M-1 M-1 attached to Parabiotic High Fat 6, 6'	19, 19	36	13.345 ± .965, 10.262 ± .962	.931, .925	9.863	.001

*Intakes compared for the last 19 days of each period.

**Approximate t-test

#Degrees of freedom calculated

Table 35

FOOD INTAKES IN GRAMS / 100 GRAMS BODY WEIGHT (average / animal / day) - SPRAGUE-DAWLEY FEMALE RATS

CONTROL PERIOD: M-1 DIET

Comparison of Means	n	df	Mean ± S. D.	Variance	t	P
1,2	19, 19	36	6.320 ± .699, 6.241 ± .680	.489, .462	.354	NS
1,3	19, 19	36	6.320 ± .699, 6.533 ± .742	.489, .551	.913	NS
1,4	19, 19	36	6.320 ± .699, 6.708 ± .580	.489, .337	1.863	.100
1,5	19, 19	36	6.320 ± .699, 6.509 ± .580	.489, .336	.907	NS
1,6	19, 19	36	6.320 ± .699, 6.290 ± .494	.489, .244	.152	NS
2,3	19, 19	36	6.241 ± .680, 6.533 ± .742	.462, .551	1.268	NS
2,4	19, 19	36	6.241 ± .680, 6.708 ± .580	.462, .337	2.280	.050
2,5	19, 19	36	6.241 ± .680, 6.509 ± .580	.462, .336	1.308	NS
2,6	19, 19	36	6.241 ± .680, 6.290 ± .494	.462, .244	.253	NS
3,4	19, 19	36	6.533 ± .742, 6.708 ± .580	.551, .337	.808	NS
3,5	19, 19	36	6.533 ± .742, 6.509 ± .580	.551, .336	.115	NS
3,6	19, 19	33#	6.533 ± .742, 6.290 ± .494	.551, .244	1.188*	NS
4,5	19, 19	36	6.708 ± .580, 6.509 ± .580	.337, .336	1.060	NS
4,6	19, 19	36	6.708 ± .580, 6.290 ± .494	.337, .244	2.391	.050
5,6	19, 19	36	6.509 ± .580, 6.290 ± .494	.336, .244	1.251	NS

*Approximate t-test

#Degrees of freedom calculated

Table 36

FOOD INTAKES IN GRAMS / 100 GRAMS BODY WEIGHT (average / animal / day) - SPRAGUE-DAWLEY FEMALE RATS

EXPERIMENTAL PERIOD

1' Single High Fat
2' Single M-1
3' Parabiotic High Fat
4' Parabiotic M-1
5' Parabiotic High Fat attached to Parabiotic M-1
6' Parabiotic M-1 attached to Parabiotic High Fat

Comparison of Means	n	df	Mean ± S. D.	Variance	t	P
1',2'	25, 25	48	2.845 ± .266, 5.391 ± .438	.071, .191	24.030	.001
1',3'	25, 25	42#	2.845 ± .266, 2.681 ± .224	.071, .050	3.294*	.010
1',4'	25, 25	48	2.845 ± .266, 5.493 ± .374	.071, .140	26.596	.001
1',5'	25, 25	48	2.845 ± .266, 3.121 ± .324	.071, .105	2.609	.020
1',6'	25, 25	48	2.845 ± .266, 4.529 ± .423	.071, .179	14.802	.001
2',3'	25, 25	37#	5.391 ± .438, 2.681 ± .224	.191, .050	30.597*	.001
2',4'	25, 25	48	5.391 ± .438, 5.493 ± .374	.191, .140	1.345	NS
2',5'	25, 25	48	5.391 ± .438, 3.121 ± .324	.191, .105	21.568	.001
2',6'	25, 25	48	5.391 ± .438, 4.529 ± .423	.191, .179	8.243	.001
3',4'	25, 25	40#	2.681 ± .224, 5.493 ± .374	.050, .140	34.182*	.001
3',5'	25, 25	44#	2.681 ± .224, 3.121 ± .324	.050, .105	6.391*	.001
3',6'	25, 25	33#	2.681 ± .224, 4.529 ± .423	.050, .179	19.822*	.001
4',5'	25, 25	48	5.493 ± .374, 3.121 ± .324	.140, .105	24.008	.001
4',6'	25, 25	48	5.493 ± .374, 4.529 ± .423	.140, .179	9.882	.001
5',6'	25, 25	48	3.121 ± .324, 4.529 ± .423	.105, .179	12.407	.001

*Approximate t-test
#Degrees of freedom calculated

Table 37

FOOD INTAKES IN GRAMS / 100 GRAMS BODY WEIGHT (average / animal / day) - SPRAGUE-DAWLEY FEMALE RATS

CONTROL PERIOD vs. EXPERIMENTAL PERIOD *

Comparison of Means	n	df	Mean + S. D.	Variance	t	P
Single M-1, Single High Fat 1, 1'	19, 19	-+	6.320 ± .699, 2.845 ± .266	.489, .071	20.241**	.001
Single M-1, Single M-1 2, 2'	19, 19	32#	6.241 ± .680, 5.391 ± .438	.462, .191	4.585**	.001
Parabiotic, Parabiotic M-1 High Fat 3, 3'	19, 19	-+	6.533 ± .742, 2.681 ± .224	.551, .050	21.658**	.001
Parabiotic, Parabiotic M-1 M-1 4, 4'	19, 19	31#	6.708 ± .580, 5.493 ± .374	.337, .140	7.668**	.001
Parabiotic, Parabiotic M-1 High Fat attached to Parabiotic M-1 5, 5'	19, 19	28#	6.509 ± .580, 3.121 ± .324	.336, .105	22.239**	.001
Parabiotic, Parabiotic M-1 M-1 attached to Parabiotic High Fat 6, 6'	19, 19	36	6.290 ± .494, 4.529 ± .423	.244, .179	11.800	.001

*Intakes compared for the last 19 days of each period.
+Weighted critical values calculated
**Approximate t-test
#Degrees of freedom calculated

Table 38

KCAL. INTAKES (average / animal / day) - SPRAGUE-DAWLEY FEMALE RATS

CONTROL PERIOD: M-1 DIET

Comparison of Means	n	df	Mean ± S. D.	Variance	t	P
1,2	6, 6	10	52.892 ± 3.842, 55.374 ± 6.345	14.763, 40.258	.820	NS
1,3	6, 6	-+	52.892 ± 3.842, 45.369 ± 8.873	14.763, 78.736	1.906*	NS
1,4	6, 6	10	52.892 ± 3.842, 45.353 ± 2.520	14.763, 6.352	4.019	.010
1,5	6, 6	10	52.892 ± 3.842, 46.535 ± 4.577	14.763, 20.949	2.605	.050
1,6	6, 6	10	52.892 ± 3.842, 46.513 ± 3.454	14.763, 11.929	3.024	.020
2,3	6, 6	10	55.892 ± 6.345, 45.369 ± 8.873	40.258, 78.736	2.247	.050
2,4	6, 6	-+	55.892 ± 6.345, 45.353 ± 2.520	40.258, 6.352	3.595*	.020
2,5	6, 6	10	55.892 ± 6.345, 46.535 ± 4.577	40.258, 20.949	2.767	.020
2,6	6, 6	10	55.892 ± 6.345, 46.513 ± 3.454	40.258, 11.929	3.004	.020
3,4	6, 6	-+	45.369 ± 8.873, 45.353 ± 2.520	78.736, 6.352	.031*	NS
3,5	6, 6	10	45.369 ± 8.873, 46.535 ± 4.577	78.736, 20.949	.286	NS
3,6	6, 6	-+	45.369 ± 8.873, 46.513 ± 3.454	78.736, 11.929	.294*	NS
4,5	6, 6	10	45.353 ± 2.520, 46.535 ± 4.577	6.352, 20.949	.554	NS
4,6	6, 6	10	45.353 ± 2.520, 46.513 ± 3.454	6.352, 11.929	.665	NS
5,6	6, 6	10	46.535 ± 4.577, 46.513 ± 3.454	20.949, 11.929	.010	NS

*Approximate t-test

+Weighted critical values calculated

Table 39

KCAL. INTAKES (average / animal / day) - SPRAGUE-DAWLEY FEMALE RATS

EXPERIMENTAL PERIOD

1' Single High Fat	2' Single M-1	3' Parabiotic High Fat	4' Parabiotic M-1	5' Parabiotic High Fat attached to Parabiotic M-1	6' Parabiotic M-1 attached to Parabiotic High Fat

Comparison of Means	n	df	Mean ± S. D.	Variance	t	P
1',2'	9, 9	-+	59.151 ± 5.101, 49.070 ± 1.996	26.018, 3.986	5.521*	.001
1',3'	9, 9	16	59.151 ± 5.101, 42.277 ± 3.908	26.018, 15.269	7.878	.001
1',4'	9, 9	13#	59.151 ± 5.101, 41.142 ± 2.677	26.018, 7.168	9.379*	.001
1',5'	9, 9	16	59.151 ± 5.101, 48.947 ± 5.305	26.018, 28.139	4.160	.001
1',6'	9, 9	16	59.151 ± 5.101, 35.078 ± 3.831	26.018, 14.675	11.321	.001
2',3'	9, 9	13#	49.070 ± 1.996, 42.277 ± 3.908	3.986, 15.269	4.644*	.001
2',4'	9, 9	16	49.070 ± 1.996, 41.142 ± 2.677	3.986, 7.168	7.112	.001
2',5'	9, 9	-+	49.070 ± 1.996, 48.947 ± 5.305	3.986, 28.139	.065*	NS
2',6'	9, 9	13#	49.070 ± 1.996, 35.078 ± 3.831	3.986, 14.675	9.717*	.001
3',4'	9, 9	16	42.277 ± 3.908, 41.142 ± 2.677	15.269, 7.168	.716	NS
3',5'	9, 9	16	42.277 ± 3.908, 48.947 ± 5.305	15.269, 28.139	3.037	.010
3',6'	9, 9	16	42.277 ± 3.908, 35.078 ± 3.831	15.269, 14.675	3.946	.010
4',5'	9, 9	13#	41.142 ± 2.677, 48.947 ± 5.305	7.168, 28.139	3.941*	.010
4',6'	9, 9	16	41.142 ± 2.677, 35.078 ± 3.831	7.168, 14.675	3.892	.010
5',6'	9, 9	16	48.947 ± 5.305, 35.078 ± 3.831	28.139, 14.675	6.359	.001

*Approximate t-test
+Weighted critical values calculated
#Degrees of freedom calculated

Table 40

KCAL. INTAKES (average / animal / day) - SPRAGUE-DAWLEY FEMALE RATS

CONTROL PERIOD vs. EXPERIMENTAL PERIOD

Comparison of Means	n	df	Mean + S. D.	Variance	t	P
Single M-1, Single High Fat 1, 1'	6, 9	13	52.892 ± 3.842, 59.151 ± 5.101	14.763, 26.018	2.550	.050
Single M-1, Single M-1 2, 2'	6, 9	-+	55.374 ± 6.345, 49.070 ± 1.996	40.258, 3.986	2.357*	.100
Parabiotic, Parabiotic M-1 High Fat 3, 3'	6, 9	-+	45.369 ± 8.873, 42.277 ± 3.908	78.736, 15.269	.803*	NS
Parabiotic, Parabiotic M-1 M-1 4, 4'	6, 9	13	45.353 ± 2.520, 41.142 ± 2.677	6.352, 7.168	3.046	.010
Parabiotic, Parabiotic M-1 High Fat attached to Parabiotic M-1 5, 5'	19, 25	42	44.412 ± 3.939, 48.186 ± 4.624	15.518, 21.385	2.854	.010
Parabiotic, Parabiotic M-1 M-1 attached to Parabiotic High Fat 6, 6'	6, 9	13	46.513 ± 3.454, 35.078 ± 3.831	11.929, 14.675	5.879	.001

*Approximate t-test
+Weighted critical values calculated

Table 41

KCAL. INTAKES / 100 GRAMS BODY WEIGHT (average / animal / day) - SPRAGUE-DAWLEY FEMALE RATS

CONTROL PERIOD: M-1 DIET

Comparison of Means	n	df	Mean ± S. D.	Variance	t	P
1,2	6, 6	10	21.953 ± 1.536, 22.287 ± 2.324	2.360, 5.399	.294	NS
1,3	6, 6	-+	21.953 ± 1.536, 22.756 ± 4.198	2.360, 17.627	.440*	NS
1,4	6, 6	10	21.953 ± 1.536, 23.286 ± 1.132	2.360, 1.281	1.712	NS
1,5	6, 6	10	21.953 ± 1.536, 23.251 ± 2.234	2.360, 4.990	1.173	NS
1,6	6, 6	10	21.953 ± 1.536, 21.981 ± 1.773	2.360, 3.143	.030	NS
2,3	6, 6	10	22.287 ± 2.324, 22.756 ± 4.198	5.399, 17.627	.239	NS
2,4	6, 6	10	22.287 ± 2.324, 23.286 ± 1.132	5.399, 1.281	.947	NS
2,5	6, 6	10	22.287 ± 2.324, 23.251 ± 2.234	5.399, 4.990	.732	NS
2,6	6, 6	10	22.287 ± 2.324, 21.981 ± 1.773	5.399, 1.773	.256	NS
3,4	6, 6	-+	22.756 ± 4.198, 23.286 ± 1.132	17.627, 1.281	.299*	NS
3,5	6, 6	10	22.756 ± 4.198, 23.251 ± 2.234	17.627, 4.990	.255	NS
3,6	6, 6	-+	22.756 ± 4.198, 21.981 ± 1.773	17.627, 3.143	.417*	NS
4,5	6, 6	10	23.286 ± 1.132, 23.251 ± 2.234	1.281, 4.990	.035	NS
4,6	6, 6	10	23.286 ± 1.132, 21.981 ± 1.773	1.281, 3.143	1.520	NS
5,6	6, 6	10	23.251 ± 2.234, 21.981 ± 1.773	4.990, 3.143	1.091	NS

*Approximate t-test

+Weighted critical values calculated

Table 42

ENERGY INTAKES KCAL / 100 GRAMS BODY WEIGHT (average / animal / day) - SPRAGUE-DAWLEY FEMALE RATS

EXPERIMENTAL PERIOD

1' Single High Fat	2' Single M-1	3' Parabiotic High Fat	4' Parabiotic M-1	5' Parabiotic High Fat attached to Parabiotic M-1	6' Parabiotic M-1 attached to Parabiotic High Fat

Comparison of Means	n	df	Mean ± S. D.	Variance	t	P
1',2'	25, 25	41#	19.874 ± 2.205, 18.473 ± 1.340	4.862, 1.794	2.715*	.010
1',3'	25, 25	42#	19.874 ± 2.205, 18.156 ± 1.395	4.862, 1.947	3.292*	.010
1',4'	25, 25	39#	19.874 ± 2.205, 18.964 ± 1.239	4.862, 1.534	1.799*	.100
1',5'	25, 25	48	19.874 ± 2.205, 21.509 ± 2.226	4.862, 4.956	2.609	.020
1',6'	25, 25	41#	19.874 ± 2.205, 15.317 ± 1.368	4.862, 1.871	8.781*	.001
2',3'	25, 25	48	18.473 ± 1.340, 18.156 ± 1.395	1.794, 1.947	.819	NS
2',4'	25, 25	48	18.473 ± 1.340, 18.964 ± 1.239	1.794, 1.534	1.345	NS
2',5'	25, 25	41#	18.473 ± 1.340, 21.509 ± 2.226	1.794, 4.956	5.843*	.001
2',6'	25, 25	48	18.473 ± 1.340, 15.317 ± 1.368	1.794, 1.871	8.243	.001
3',4'	25, 25	48	18.156 ± 1.395, 18.964 ± 1.239	1.947, 1.534	2.164	.050
3',5'	25, 25	42#	18.156 ± 1.395, 21.509 ± 2.226	1.947, 4.956	6.381*	.001
3',6'	25, 25	48	18.156 ± 1.395, 15.317 ± 1.368	1.947, 1.871	7.265	.001
4',5'	25, 25	39#	18.964 ± 1.239, 21.509 ± 2.226	1.534, 4.956	4.995*	.001
4',6'	25, 25	48	18.964 ± 1.239, 15.317 ± 1.368	1.534, 1.871	9.881	.001
5',6'	25, 25	41#	21.509 ± 2.226, 15.317 ± 1.368	4.956, 1.871	11.849*	.001

*Approximate t-test
#Degrees of freedom calculated

Table 43

FOOD EFFICIENCY (Kcal. / g body weight gain) - SPRAGUE-DAWLEY FEMALE RATS

CONTROL PERIOD: M-1 DIET

Comparison of Means	n	df	Mean ± S. D.	Variance	t	P
1,2	3, 3	4	66.891 ± 5.272, 45.143 ± 2.252	27.792, 5.071	6.571	.010
1,3	3, 4	5	66.891 ± 5.272, 41.394 ± 7.198	27.792, 51.814	5.139	.010
1,4	3, 4	5	66.891 ± 5.272, 31.680 ± 10.178	27.792, 103.584	5.386	.010
1,5	3, 4	5	66.891 ± 5.272, 40.229 ± 3.667	27.792, 13.442	7.970	.001
1,6	3, 4	5	66.801 ± 5.272, 49.836 ± 19.729	27.792, 389.245	1.428	NS
2,3	3, 4	5	45.143 ± 2.252, 41.394 ± 7.198	5.071, 51.814	.853	NS
2,4	3, 4	-+	45.143 ± 2.252, 31.680 ± 10.178	5.071, 103.584	2.563*	.100
2,5	3, 4	5	45.143 ± 2.252, 40.229 ± 3.667	5.071, 13.442	2.025	.100
2,6	3, 4	-+	45.143 ± 2.252, 49.836 ± 19.729	5.071, 389.245	.472*	NS
3,4	4, 4	6	41.394 ± 7.198, 31.680 ± 10.178	51.814, 103.584	1.558	NS
3,5	4, 4	6	41.394 ± 7.198, 40.229 ± 3.667	51.814, 13.442	.288	NS
3,6	4, 4	6	41.394 ± 7.198, 49.836 ± 19.729	51.814, 389.245	.804	NS
4,5	4, 4	6	31.680 ± 10.178, 40.229 ± 3.667	103.584, 13.442	1.581	NS
4,6	4, 4	6	31.680 ± 10.178, 49.836 ± 19.729	103.584, 389.245	1.636	NS
5,6	4, 4	-+	40.229 ± 3.667, 49.836 ± 19.729	13.442, 389.245	.957*	NS

+Weighted critical values calculated

*Approximate t-test

Table 44

FOOD EFFICIENCY (Kcal. / g body weight gain) - SPRAGUE-DAWLEY FEMALE RATS

EXPERIMENTAL PERIOD

1' Single High Fat
2' Single M-1
3' Parabiotic High Fat
4' Parabiotic M-1
5' Parabiotic High Fat attached to Parabiotic M-1
6' Parabiotic M-1 attached to Parabiotic High Fat

Comparison of Means	n	df	Mean ± S. D.	Variance	t	P
1',2'	5, 5	-+	32.724 ± 1.455, 148.555 ± 4.419	2.118, 19.531	55.666*	.001
1',3'	5, 5	8	32.724 ± 1.455, 48.438 ± .725	2.118, .526	21.608	.001
1',4'	5, 5	8	32.724 ± 1.445, 96.554 ± 1.829	2.118, 3.347	61.054	.001
1',5'	5, 5	-+	32.724 ± 1.455, 98.913 ± 5.054	2.118, 25.539	28.143*	.001
1',6'	5, 5	-+	32.724 ± 1.455, 226.116 ± 5.840	2.118, 34.101	71.855*	.001
2',3'	5, 5	-+	148.555 ± 4.419, 48.438 ± .725	19.531, .526	49.987*	.001
2',4'	5, 5	8	148.555 ± 4.419, 96.554 ± 1.829	19.531, 3.347	24.311	.001
2',5'	5, 5	8	148.555 ± 4.419, 98.913 ± 5.054	19.531, 25.539	16.535	.001
2',6'	5, 5	8	148.555 ± 4.419, 226.116 ± 5.840	19.531, 34.101	23.682	.001
3',4'	5, 5	8	48.438 ± .725, 96.554 ± 1.829	.526, 3.347	54.672	.001
3',5'	5, 5	-+	48.438 ± .725, 98.913 ± 5.054	.526, 25.539	22.107*	.001
3',6'	5, 5	-+	48.438 ± .725, 226.116 ± 5.840	.526, 34.101	67.517*	.001
4',5'	5, 5	-+	96.554 ± 1.829, 98.913 ± 5.054	3.347, 25.539	.981*	NS
4',6'	5, 5	-+	96.554 ± 1.829, 226.116 ± 5.840	3.347, 34.101	47.342*	.001
5',6'	5, 5	8	98.913 ± 5.054, 226.116 ± 5.840	25.539, 34.101	36.831	.001

+Weighted critical values calculated
*Approximate t-test

Table 45

FOOD EFFICIENCY (Kcal. / g body weight gain) - SPRAGUE-DAWLEY FEMALE RATS

CONTROL PERIOD vs. EXPERIMENTAL PERIOD

Comparison of Means	n	df	Mean + S. D.	Variance	t	P
Single M-1, Single High Fat 1, 1'	3, 5	-+	66.891 ± 5.272, 32.724 ± 1.455	27.792, 2.118	10.977*	.010
Single M-1, Single M-1 2, 2'	3, 5	6	45.143 ± 2.252, 148.555 ± 4.419	5.071, 19.531	36.919	.001
Parabiotic, Parabiotic M-1 High Fat 3, 3'	4, 5	-+	41.394 ± 7.198, 48.438 ± .725	51.814, .526	1.949*	NS
Parabiotic, Parabiotic M-1 M-1 4, 4'	4, 5	-+	31.680 ± 10.178, 96.554 ± 1.829	103.584, 3.347	12.587*	.010
Parabiotic, Parabiotic M-1 High Fat attached to Parabiotic M-1 5, 5'	4, 5	7	40.229 ± 3.667, 98.913 ± 5.054	13.442, 25.539	19.390	.001
Parabiotic, Parabiotic M-1 M-1 attached to Parabiotic High Fat 6, 6'	4, 5	-+	49.836 ± 19.729, 226.116 ± 5.840	389.245, 34.101	17.275*	.001

+Weighted critical values calculated
*Approximate t-test

Table 46

TOTAL BODY WEIGHT CHANGES PER ANIMAL (g) DURING EXPERIMENTAL PERIOD - SPRAGUE-DAWLEY FEMALE RATS

1 Single High Fat	2 Single M-1	3 Parabiotic High Fat	4 Parabiotic M-1	5 Parabiotic High Fat attached to Parabiotic M-1	6 Parabiotic M-1 attached to Parabiotic High Fat

Comparison of Means	n	df	Mean ± S. D.	Variance	t	P
1,2	12, 8	-+	70.167 ± 19.803, 13.250 ± 7.592	392.152, 57.643	9.012*	.001
1,3	12, 10	20	70.167 ± 19.803, 34.100 ± 13.195	392.152, 174.100	4.912	.001
1,4	12, 8	18	70.167 ± 19.803, 17.000 ± 18.501	392.152, 342.286	6.033	.001
1,5	12, 6	16	70.167 ± 19.803, 18.667 ± 17.212	392.152, 296.267	5.412	.001
1,6	12, 6	16	70.167 ± 19.803, 6.167 ± 12.983	392.152, 168.567	7.130	.001
2,3	8, 10	16	13.250 ± 7.592, 34.100 ± 13.195	57.643, 174.100	3.961	.010
2,4	8, 8	-+	13.250 ± 7.592, 17.000 ± 18.501	57.643, 342.286	.530*	NS
2,5	8, 6	-+	13.250 ± 7.592, 18.667 ± 17.212	57.643, 296.267	.720*	NS
2,6	8, 6	12	13.250 ± 7.592, 6.167 ± 12.983	57.643, 168.567	1.287	NS
3,4	10, 8	16	34.100 ± 13.195, 17.000 ± 18.501	174.100, 342.286	2.291	.050
3,5	10, 6	14	34.100 ± 13.195, 18.667 ± 17.212	174.100, 296.267	2.025	.100
3,6	10, 6	14	34.100 ± 13.195, 6.167 ± 12.983	174.100, 168.567	4.123	.010
4,5	8, 6	12	17.000 ± 18.501, 18.667 ± 17.212	342.286, 296.267	.172	NS
4,6	8, 6	12	17.000 ± 18.501, 6.167 ± 12.983	342.286, 168.567	1.221	NS
5,6	6, 6	10	18.667 ± 17.212, 6.167 ± 12.983	296.267, 168.567	1.420	NS

+Weighted critical values calculated
*Approximate t-test

Table 47

BODY FAT AS PERCENTAGE OF BODY WEIGHT - SPRAGUE-DAWLEY FEMALE RATS

1 Single High Fat	2 Single M-1	3 Parabiotic High Fat	4 Parabiotic M-1	5 Parabiotic High Fat attached to Parabiotic M-1	6 Parabiotic M-1 attached to Parabiotic High Fat
24.77	8.00	10.32	6.53	12.63	12.32
19.40	10.81	19.00	12.38	16.71	10.34
22.93	18.08	14.55	9.12	13.72	7.13
23.05	14.56	10.23	12.73	6.77	4.29
19.51	16.80	18.93	5.28	14.83	8.42
33.33	16.15	15.09	14.23	18.77	9.46
26.30	15.39	12.02	10.67		
29.18	10.50	17.64	7.20		
26.46		13.75	14.93		
31.27		16.98	10.67		
34.69					
35.94					

BODY FAT AS PERCENTAGE OF BODY WEIGHT (SIGNIFICANCE)

Comparison of Means	n	df	Mean ± S. D.	Variance	t	P
1,2	12, 8	18	27.24 ± 5.67, 13.79 ± 3.57	32.11, 12.77	5.942	.001
1,3	12, 10	20	27.24 ± 5.67, 14.85 ± 3.29	32.11, 10.82	6.093	.001
1,4	12, 10	20	27.24 ± 5.67, 10.37 ± 3.30	32.11, 10.86	8.047	.001
1,5	12, 6	16	27.24 ± 5.67, 13.91 ± 4.12	32.11, 16.99	5.095	.001
1,6	12, 6	16	27.24 ± 5.67, 8.66 ± 2.77	32.11, 7.67	7.510	.001
2,3	8, 10	16	13.79 ± 3.57, 14.85 ± 3.29	12.77, 10.82	.657	NS
2,4	8, 10	16	13.79 ± 3.57, 10.37 ± 3.30	12.77, 10.86	2.103	.100
2,5	8, 6	12	13.79 ± 3.57, 13.91 ± 4.12	12.77, 16.99	.058	NS
2,6	8, 6	12	13.79 ± 3.57, 8.66 ± 2.77	12.77, 7.67	2.909	.020
3,4	10, 10	18	14.85 ± 3.29, 10.37 ± 3.30	10.82, 10.86	2.978	.010
3,5	10, 6	14	14.85 ± 3.29, 13.91 ± 4.12	10.82, 16.99	.508	NS
3,6	10, 6	14	14.85 ± 3.29, 8.66 ± 2.77	10.82, 7.67	3.850	.010
4,5	10, 6	14	10.37 ± 3.30, 13.91 ± 4.12	10.86, 16.99	1.893	.100
4,6	10, 6	14	10.37 ± 3.30, 8.66 ± 2.77	10.86, 7.67	1.065	NS
5,6	6, 6	10	13.91 ± 4.12, 8.66 ± 2.77	16.99, 7.67	2.587	.050

Table 48

INGUINAL FAT DEPOT WEIGHT (g), NON-OPERATED SIDE ONLY
SPRAGUE-DAWLEY FEMALE RATS

1 Single High Fat	2 Single M-1	3 Parabiotic High Fat	4 Parabiotic M-1	5 Parabiotic High Fat attached to Parabiotic M-1	6 Parabiotic M-1 attached to Parabiotic High Fat
4.8030	1.8057	2.3509	.8702	2.1466	1.2428
8.4109	3.4458	3.5627	.5081	.9051	.7568
4.6897	2.2709	2.3864	.9643	1.2466	1.1212
3.1104	1.9540	2.4206	1.8411	2.4236	1.2075
7.1400		2.1461	2.4894	2.3798	.9466
3.7407		2.0521	1.6470	2.4521	1.8959
6.5328			1.6977		
			1.0851		

SIGNIFICANCE

Comparison of Means	n	df	Mean ± S. D.	Variance	t	P
1,2	7, 4	9	5.4896 ± 1.9225, 2.3691 ± .7436	3.6961, .5529	3.059	.020
1,3	7, 6	-+	5.4896 ± 1.9225, 2.4865 ± .5469	3.6961, .2991	3.951*	.010
1,4	7, 8	-+	5.4896 ± 1.9225, 1.3879 ± .6431	3.6961, .4136	5.387*	.010
1,5	7, 6	-+	5.4896 ± 1.9225, 1.9256 ± .6757	3.6961, .4566	4.585*	.010
1,6	7, 6	-+	5.4896 ± 1.9225, 1.1951 ± .3880	3.6961, .1506	5.774*	.010
2,3	4, 6	8	2.3691 ± .7436, 2.4865 ± .5469	.5529, .2991	.290	NS
2,4	4, 8	10	2.3691 ± .7436, 1.3879 ± .6431	.5529, .4136	2.375	.050
2,5	4, 6	8	2.3691 ± .7436, 1.9256 ± .6757	.5529, .4566	.979	NS
2,6	4, 6	8	2.3691 ± .7436, 1.1951 ± .3880	.5529, .1506	3.313	.020
3,4	6, 8	12	2.4865 ± .5469, 1.3879 ± .6431	.2991, .4136	3.363	.010
3,5	6, 6	10	2.4865 ± .5469, 1.9256 ± .6757	.2991, .4566	1.580	NS
3,6	6, 6	10	2.4865 ± .5469, 1.1951 ± .3880	.2991, .1506	4.717	.001
4,5	8, 6	12	1.3879 ± .6431, 1.9256 ± .6757	.4136, .4566	1.516	NS
4,6	8, 6	12	1.3879 ± .6431, 1.1951 + .3880	.4136, .1506	.647	NS
5,6	6, 6	10	1.9256 ± .6757, 1.1951 ± .3880	.4566, .1506	2.296	.050

*Approximate t-test
+Weighted critical values calculated

Table 49

RIGHT AND LEFT COMBINED INGUINAL FAT DEPOT WEIGHT (g)
SPRAGUE-DAWLEY FEMALE RATS

1 Single High Fat	2 Single M-1	3 Parabiotic High Fat	4 Parabiotic M-1	5 Parabiotic High Fat attached to Parabiotic M-1	6 Parabiotic M-1 attached to Parabiotic High Fat
8.7974	3.7164	3.5193	1.6622	3.3325	1.7670
17.5194	6.8282	6.4746	1.1368	2.5012	1.2045
9.7568	3.4861	5.2678	1.4849	2.7201	1.8806
7.0609	3.7213	4.3500	3.4273	3.7612	2.1338
8.4480	3.8883	3.4189	3.6357	3.2561	2.3294
13.4524	4.5844	3.3674	3.2773	5.5206	3.3768
7.6060	5.9528	8.0612	2.8323		
9.6243	5.0704	4.0012	2.1812		
11.9704		5.9616	3.1863		
7.7468		3.0406	5.1029		
10.9018					
7.8497					

SIGNIFICANCE

Comparison of Means	n	df	Mean ± S. D.	Variance	t	P
1,2	12, 8	-+	10.0612 ± 3.0337, 4.6560 ± 1.2125	9.2033, 1.4702	5.544*	.001
1,3	12, 10	19#	10.0612 ± 3.0337, 4.8453 ± 1.6303	9.2033, 2.6580	5.133*	.001
1,4	12, 10	-+	10.0612 ± 3.0337, 2.7927 ± 1.1994	9.2033, 1.4384	7.616*	.001
1,5	12, 6	-+	10.0612 ± 3.0337, 3.5153 ± 1.0810	9.2033, 1.1686	6.675*	.001
1,6	12, 6	-+	10.0612 ± 3.0337, 2.1154 ± .7271	9.2033, .5287	8.600*	.001
2,3	8, 10	16	4.6560 ± 1.2125, 4.8453 ± 1.6303	1.4702, 2.6580	.273	NS
2,4	8, 10	16	4.6560 ± 1.2125, 2.7927 ± 1.1994	1.4702, 1.4384	3.260	.010
2,5	8, 6	12	4.6560 ± 1.2125, 3.5153 ± 1.0810	1.4702, 1.1686	1.822	.100
2,6	8, 6	12	4.6560 ± 1.2125, 2.1154 ± .7271	1.4702, .5287	4.531	.001
3,4	10, 10	18	4.8453 ± 1.6303, 2.7927 ± 1.1994	2.6580, 1.4384	3.207	.010
3,5	10, 6	14	4.8453 ± 1.6303, 3.5153 ± 1.0810	2.6580, 1.1686	1.766	.100
3,6	10, 6	-+	4.8453 ± 1.6303, 2.1154 ± .7271	2.6580, .5287	4.589*	.010
4,5	10, 6	14	2.7927 ± 1.1994, 3.5153 ± 1.0810	1.4384, 1.1686	1.208	NS
4,6	10, 6	14	2.7927 ± 1.1994, 2.1154 ± .7271	1.4384, .5287	1.243	NS
5,6	6, 6	10	3.5153 ± 1.0810, 2.1154 ± .7271	1.1686, .5287	2.632	.050

*Approximate t-test
+Weighted critical values calculated
#Degrees of freedom calculated

Table 50

INGUINAL FAT DEPOT WEIGHT (g) - SPRAGUE-DAWLEY FEMALE RATS

A Single M-1 Non-operated	B Single M-1 Operated	C Single High Fat Non-operated	D Single High Fat Operated
1.806	1.911	4.803	3.994
3.446	3.382	8.411	9.109
2.271	1.215	4.690	5.067
1.954	2.630	3.110	3.950
9.744	9.161	7.140	6.312
7.509	5.395	3.741	3.865
6.797	5.981	6.533	5.438
3.023	2.556	19.499	21.148
3.537	3.652	17.894	18.138
7.410	5.668	17.423	23.687
10.156	9.386	9.545	6.997
5.166	4.741	8.693	6.485
3.433	2.775		
4.117	3.156		
5.911	5.447		
3.643	2.819		
2.132	2.297		

SIGNIFICANCE

Comparison of Means	n	df	Mean ± S. D.	Variance	t	P
AB	17, 17	32	4.9444 ± 2.6466, 4.2455 ± 2.3586	7.6045, 5.5563	.674	NS
CD	12, 12	22	9.2901 ± 5.7847, 9.5159 ± 7.1747	33.4632, 51.4764	.085	NS
AB	17, 17	16	4.9444 ± 2.6466, 4.2455 ± 2.3586	7.6045, 5.5563	3.469*	.010
CD	12, 12	11	9.2901 ± 5.7847, 9.5159 ± 7.1747	33.4632, 51.4764	.345*	NS

*Paired t-test

Table 51

RIGHT AND LEFT COMBINED RENAL-RETROPERITONEAL FAT DEPOT WEIGHT (g)
SPRAGUE-DAWLEY FEMALE RATS

1 Single High Fat	2 Single M-1	3 Parabiotic High Fat	4 Parabiotic M-1	5 Parabiotic High Fat attached to Parabiotic M-1	6 Parabiotic M-1 attached to Parabiotic High Fat
10.2807	2.6617	3.4948	2.0827	5.4603	2.3910
13.2615	5.2006	7.0497	1.0845	.8278	.3936
7.0669	2.9731	3.8359	.5145	2.7840	1.9284
7.7873	1.4982	2.1793	3.1264	3.3616	2.1633
11.2597	3.9022	1.8812	2.8421	4.3118	.8036
11.7681	4.1748	1.6024	3.2265	2.3633	2.0682
8.6041	5.1450	4.5425	1.9796		
9.7558	4.6622	3.9339	.5801		
14.7327		4.7352	2.3086		
6.0672		1.9709	2.8778		
14.1097					
10.9569					

SIGNIFICANCE

Comparison of Means	n	df	Mean ± S. D.	Variance	t	P
1,2	12, 8	18#	10.4709 ± 2.7625, 3.7772 ± 1.3060	7.6315, 1.7055	7.264*	.001
1,3	12, 10	20	10.4709 ± 2.7625, 3.5226 ± 1.6935	7.6315, 2.8681	6.927	.001
1,4	12, 10	-+	10.4709 ± 2.7625, 2.0623 ± 1.0208	7.6315, 1.0419	9.774*	.001
1,5	12, 6	16	10.4709 ± 2.7625, 3.1848 ± 1.6044	7.6315, 2.5740	5.924	.001
1,6	12, 6	-+	10.4709 ± 2.7625, 1.6247 ± .8193	7.6315, .6712	10.230*	.001
2,3	8, 10	16	3.7772 ± 1.3060, 3.5226 ± 1.6935	1.7055, 2.8681	.349	NS
2,4	8, 10	16	3.7772 ± 1.3060, 2.0623 ± 1.0208	1.7055, 1.0419	3.132	.010
2,5	8, 6	12	3.7772 ± 1.3060, 3.1848 ± 1.6044	1.7055, 2.5740	.763	NS
2,6	8, 6	12	3.7772 ± 1.3060, 1.6247 ± .8193	1.7055, .6712	3.530	.010
3,4	10, 10	18	3.5226 ± 1.6935, 2.0623 ± 1.0208	2.8681, 1.0419	2.335	.050
3,5	10, 6	14	3.5226 ± 1.6935, 3.1848 ± 1.6044	2.8681, 2.5740	.394	NS
3,6	10, 6	14	3.5226 ± 1.6935, 1.6247 ± .8193	2.8681, .6712	2.546	.050
4,5	10, 6	14	2.0623 ± 1.0208, 3.1848 ± 1.6044	1.0419, 2.5740	1.724	NS
4,6	10, 6	14	2.0623 ± 1.0208, 1.6247 ± .8193	1.0419, .6712	.889	NS
5,6	6, 6	10	3.1848 ± 1.6044, 1.6247 ± .8193	2.5740, .6712	2.121	.100

*Approximate t-test
#Degrees of freedom calculated
+Weighted critical values calculated

Table 52

RIGHT AND LEFT COMBINED PERIMETRIAL FAT DEPOT WEIGHT (g)
SPRAGUE-DAWLEY FEMALE RATS

1 Single High Fat	2 Single M-1	3 Parabiotic High Fat	4 Parabiotic M-1	5 Parabiotic High Fat attached to Parabiotic M-1	6 Parabiotic M-1 attached to Parabiotic High Fat
16.0923	4.7077	7.8207	5.0717	8.3967	5.5332
20.9786	9.3701	10.7602	2.7575	1.8428	.8670
8.7671	4.8195	5.6057	1.4107	2.7201	3.3676
12.0704	2.6656	5.1417	6.2500	6.2819	5.2111
14.9854	8.2430	3.6181	4.9865	7.1727	1.3165
18.5114	6.1069	3.2232	7.2201	5.2891	3.7244
11.5082	7.0699	8.7315	3.8212		
13.1985	7.5768	7.0060	1.4103		
18.7923		10.5293	4.3072		
8.8676		3.9361	5.5368		
20.5573					
17.4617					

SIGNIFICANCE

Comparison of Means	n	df	Mean ± S. D.	Variance	t	P
1,2	12, 8	19#	15.1492 ± 4.2725, 6.3199 ± 2.1842	18.2546, 4.7706	6.068*	.001
1,3	12, 10	20	15.1492 ± 4.2725, 6.6373 ± 2.7776	18.2546, 7.7152	5.408	.001
1,4	12, 10	17#	15.1492 ± 4.2725, 4.2772 ± 1.9502	18.2546, 3.8031	7.884*	.001
1,5	12, 6	16	15.1492 ± 4.2725, 5.2839 ± 2.5561	18.2546, 6.5335	5.165	.001
1,6	12, 6	18#	15.1492 ± 4.2725, 3.3366 ± 1.9323	18.2546, 3.7340	8.068*	.001
2,3	8, 10	16	6.3199 ± 2.1842, 6.6373 ± 2.7776	4.7706, 7.7152	.264	NS
2,4	8, 10	16	6.3199 ± 2.1842, 4.2772 ± 1.9502	4.7706, 3.8031	2.095	.100
2,5	8, 6	12	6.3199 ± 2.1842, 5.2839 ± 2.5561	4.7706, 6.5335	.818	NS
2,6	8, 6	12	6.3199 ± 2.1842, 3.3366 ± 1.9323	4.7706, 3.7340	2.652	.050
3,4	10, 10	18	6.6373 ± 2.7776, 4.2772 ± 1.9502	7.7152, 3.8031	2.199	.050
3,5	10, 6	14	6.6373 ± 2.7776, 5.2839 ± 2.5561	7.7152, 6.5335	.970	NS
3,6	10, 6	14	6.6373 ± 2.7776, 3.3366 ± 1.9323	7.7152, 3.7340	2.548	.050
4,5	10, 6	14	4.2772 ± 1.9502, 5.2839 ± 2.5561	3.8031, 6.5335	.892	NS
4,6	10, 6	14	4.2772 ± 1.9502, 3.3366 ± 1.9323	3.8031, 3.7340	.937	NS
5,6	6, 6	10	5.2839 ± 2.5561, 3.3366 ± 1.9323	6.5335, 3.7340	1.489	NS

*Approximate t-test
#Degrees of freedom calculated

Table 53

TOTAL WEIGHT OF FAT DEPOTS REMOVED (g) - SPRAGUE-DAWLEY FEMALE RATS

	1 Single High Fat	2 Single M-1	3 Parabiotic High Fat	4 Parabiotic M-1	5 Parabiotic High Fat attached to Parabiotic M-1	6 Parabiotic M-1 attached to Parabiotic High Fat
Inguinals	120.73	37.25	48.45	27.93	21.09	12.69
Perimetrials	181.79	50.56	66.37	42.77	31.70	20.02
Renal-Retroperitoneals	125.65	30.22	35.23	20.62	19.11	9.75
Total	428.17	118.03	150.05	91.32	71.90	42.46
Average per rat	35.68	14.75	15.01	9.13	11.98	7.08
No. of rats	12	8	10	10	6	6

Table 54

EMPTY STOMACH WEIGHT (g) - SPRAGUE-DAWLEY FEMALE RATS

1 Single High Fat	2 Single M-1	3 Parabiotic High Fat	4 Parabiotic M-1	5 Parabiotic High Fat attached to Parabiotic M-1	6 Parabiotic M-1 attached to Parabiotic High Fat
1.0029	1.0748	.8750	1.0477	.9589	1.1061
1.0865	1.1387	.8778	.9732	.7799	.9804
.9978	1.1740	1.0357	1.0654	.9168	1.1238
.9922	1.0429	.9777	1.1658	.9842	1.0800
1.0255	1.0961	.8854	.9094	.9405	.9457
1.0075	1.2359	.8772	.9304	.8300	1.0534
1.0908	1.1833	1.0164	.9923		
1.0313	1.2041	.9397	.9223		
1.0377	1.1669	.9166	1.0579		
.9444	1.1611	.9748	1.2982		
1.0869		.9347			
.9846		1.0011			

SIGNIFICANCE

Comparison of Means	n	df	Mean ± S. D.	Variance	t	P
1,2	12, 10	20	1.0240 ± .0456, 1.1478 ± .0601	.0021, .0036	5.493	.001
1,3	12, 12	22	1.0240 ± .0456, .9427 ± .0579	.0021, .0034	3.823	.001
1,4	12, 10	-†	1.0240 ± .0456, 1.0363 ± .1216	.0021, .0148	.302*	NS
1,5	12, 6	16	1.0240 ± .0456, .9017 ± .0797	.0021, .0064	4.185	.001
1,6	12, 6	16	1.0240 ± .0456, 1.0482 ± .0710	.0021, .0050	.884	NS
2,3	10, 12	20	1.1478 ± .0601, .9427 ± .0579	.0036, .0034	8.133	.001
2,4	10, 10	14#	1.1478 ± .0601, 1.0363 ± .1216	.0036, .0148	2.599*	.050
2,5	10, 6	14	1.1478 ± .0601, .9017 ± .0797	.0036, .0064	7.031	.001
2,6	10, 6	14	1.1478 ± .0601, 1.0482 ± .0710	.0036, .0050	3.002	.010
3,4	12, 10	13#	.9427 ± .0579, 1.0363 ± .1216	.0034, .0148	2.229*	.050
3,5	12, 6	16	.9427 ± .0579, .9017 ± .0797	.0034, .0064	1.251	NS
3,6	12, 6	16	.9427 ± .0579, 1.0482 ± .0710	.0034, .0050	3.389	.010
4,5	10, 6	14	1.0363 ± .1216, .9017 ± .0797	.0148, .0064	2.401	.050
4,6	10, 6	14	1.0363 ± .1216, 1.0482 ± .0710	.0148, .0050	.218	NS
5,6	6, 6	10	.9017 ± .0797, 1.0482 ± .0710	.0064, .0050	3.361	.010

*Approximate t-test
†Weighted critical values calculated
#Degrees of freedom calculated

Table 55

EMPTY STOMACH WEIGHT IN GRAMS PER 100 g BODY WEIGHT - SPRAGUE-DAWLEY FEMALE RATS

1 Single High Fat	2 Single M-1	3 Parabiotic High Fat	4 Parabiotic M-1	5 Parabiotic High Fat attached to Parabiotic M-1	6 Parabiotic M-1 attached to Parabiotic High Fat
.3030	.3951	.3494	.4393	.3783	.4198
.2859	.3886	.3102	.4063	.4062	.4927
.3465	.4223	.4014	.5261	.3781	.4834
.3253	.4066	.4365	.4536	.3937	.4463
.3185	.4121	.4298	.4330	.4152	.4801
.3198	.4753	.3899	.3767	.3906	.4541
.3841	.4474	.3669	.4725		
.3154	.4210	.4233	.5316		
.3034	.4558	.3819	.4464		
.3472	.4245	.3947	.5111		
.3314		.4064			
.3020		.4278			

SIGNIFICANCE

Comparison of Means	n	df	Mean ± S. D.	Variance	t	P
1,2	12, 10	20	.3235 ± .0263, .4249 ± .0273	.0007, .0007	8.831	.001
1,3	12, 12	22	.3235 ± .0263, .3932 ± .0372	.0007, .0014	5.294	.001
1,4	12, 10	14#	.3235 ± .0263, .4597 ± .0511	.0007, .0026	7.634*	.001
1,5	12, 6	16	.3235 ± .0263, .3937 ± .0149	.0007, .0002	6.002	.001
1,6	12, 6	16	.3235 ± .0263, .4627 ± .0276	.0007, .0008	10.407	.001
2,3	10, 12	20	.4249 ± .0273, .3932 ± .0372	.0007, .0014	2.235	.050
2,4	10, 10	15#	.4249 ± .0273, .4597 ± .0511	.0007, .0026	1.916*	.100
2,5	10, 6	14	.4249 ± .0273, .3937 ± .0149	.0007, .0002	2.553	.050
2,6	10, 6	14	.4249 ± .0273, .4627 ± .0276	.0007, .0008	2.672	.020
3,4	12 10	20	.3932 ± .0372, .4597 ± .0511	.0014, .0026	3.531	.010
3,5	12, 6	-+	.3932 ± .0372, .3937 ± .0149	.0014, .0002	.041*	NS
3,6	12, 6	16	.3932 ± .0372, .4627 ± .0276	.0014, .0008	4.035	.001
4,5	10, 6	-+	.4597 ± .0511, .3937 ± .0149	.0026, .0002	3.854*	.010
4,6	10, 6	14	.4597 ± .0511, .4627 ± .0276	.0026, .0008	.135	NS
5,6	6, 6	10	.3937 ± .0149, .4627 ± .0276	.0002, .0008	5.390	.001

*Approximate t-test
#Degrees of freedom calculated
+Weighted critical values calculated

PART 9

EFFECTS OF METHOXYFLURANE ANESTHETIC ON VARIOUS BIOCHEMICAL PARAMETERS

Table 56

EMPTY GASTRO-INTESTINAL TRACT WEIGHT (g) - SPRAGUE-DAWLEY FEMALE RATS

1 Single High Fat	2 Single M-1	3 Parabiotic High Fat	4 Parabiotic M-1	5 Parabiotic High Fat attached to Parabiotic M-1	6 Parabiotic M-1 attached to Parabiotic High Fat
2.9670	3.6146	3.5287	4.1751	3.4840	3.2480
3.7677	3.0263	3.2638	3.4227	2.5411	2.4252
2.6881	2.9585	2.1615	2.8710	3.0111	3.0708
3.8034	3.1689	1.9887	2.5557	3.2074	3.2573
2.7754	3.9020	2.7181	2.4135	2.6756	2.7235
2.8456	3.6589	3.1002	3.3323	2.4598	2.4385
2.8611	3.6028	2.7331	2.2003		
2.7831	2.2494	2.7071	2.3561		
2.7041	2.3470	2.0676	2.9204		
2.4690	2.3210	2.4462	3.4718		
4.0112		3.5426			
3.9343		2.9864			

SIGNIFICANCE

Comparison of Means	n	df	Mean ± S. D.	Variance	t	P
1,2	12, 10	20	3.1342 ± .5659, 3.0849 ± .6145	.3203, .3776	.200	NS
1,3	12, 12	22	3.1342 ± .5659, 2.7703 ± .5359	.3203, .2872	1.617	NS
1,4	12, 10	20	3.1342 ± .5659, 2.9719 ± .6239	.3203, .3892	.639	NS
1,5	12, 6	16	3.1342 ± .5659, 2.8965 ± .4052	.3203, .1642	.912	NS
1,6	12, 6	16	3.1342 ± .5659, 1.2586 ± .1202	.3203, .1476	1.060	NS
2,3	10, 12	20	3.0849 ± .6145, 2.7703 ± .5359	.3776, .2872	1.283	NS
2,4	10, 10	18	3.0849 ± .6145, 2.9719 ± .6239	.3776, .3892	.408	NS
2,5	10, 6	14	3.0849 ± .6145, 2.8965 ± .4052	.3776, .1642	.665	NS
2,6	10, 6	14	3.0849 ± .6145, 2.8606 ± .3842	.3776, .1476	.799	NS
3,4	12, 10	20	2.7703 ± .5359, 2.9719 ± .6239	.2872, .3892	.816	NS
3,5	12, 6	16	2.7703 ± .5359, 2.8965 ± .4052	.2872, .1642	.506	NS
3,6	12, 6	16	2.7703 ± .5359, 2.8606 ± .3842	.2872, .1476	.366	NS
4,5	10, 6	14	2.9719 ± .6239, 2.8965 ± .4052	.3892, .1642	.263	NS
4,6	10, 6	14	2.9719 ± .6239, 2.8606 ± .3842	.3892, .1476	.392	NS
5,6	6, 6	10	2.8965 ± .4052, 2.8606 ± .3842	.1642, .1476	.158	NS

Table 57

EMPTY GASTRO-INTESTINAL TRACT WEIGHT IN GRAMS PER 100 g BODY WEIGHT
SPRAGUE-DAWLEY FEMALE RATS

1 Single High Fat	2 Single M-1	3 Parabiotic High Fat	4 Parabiotic M-1	5 Parabiotic High Fat attached to Parabiotic M-1	6 Parabiotic M-1 attached to Parabiotic High Fat
.8964	1.3289	1.4087	1.7506	1.3744	1.2326
.9915	1.0329	1.1533	1.4291	1.3235	1.2187
.9334	1.0642	.8378	1.4178	1.2417	1.3208
1.2470	1.2354	.8878	.9944	1.2830	1.3460
.8619	1.4267	1.3195	1.1493	1.1813	1.3825
.9034	1.3755	1.3779	1.3491	1.1576	1.0511
1.0074	1.3857	.9867	1.0478		
.8511	.8504	1.2194	1.3580		
.7907	.8206	.8615	1.2322		
.9077	.9066	.9904	1.3669		
1.2229		1.5403			
1.2068		1.2762			

SIGNIFICANCE

Comparison of Means	n	df	Mean ± S. D.	Variance	t	P
1,2	12, 10	20	.9850 ± .1564, 1.1427 ± .2358	.0245, .0556	1.878	.100
1,3	12, 12	22	.9850 ± .1564, 1.1550 ± .2378	.0245, .0565	2.069	.100
1,4	12, 10	20	.9850 ± .1564, 1.3095 ± .2179	.0245, .0475	4.062	.001
1,5	12, 6	16	.9850 ± .1564, 1.2603 ± .0833	.0245, .0069	3.996	.010
1,6	12, 6	16	.9850 ± .1564, 1.2586 ± .1202	.0245, .0144	3.747	.010
2,3	10, 12	20	1.1427 ± .2358, 1.1550 ± .2378	.0556, .0565	.121	NS
2,4	10, 10	18	1.1427 ± .2358, 1.3095 ± .2179	.0556, .0475	1.643	NS
2,5	10, 6	-+	1.1427 ± .2358, 1.2603 ± .0833	.0556, .0069	1.436*	NS
2,6	10, 6	14	1.1427 ± .2358, 1.2586 ± .1202	.0556, .0144	1.110	NS
3,4	12, 10	20	1.1550 ± .2378, 1.3095 ± .2179	.0565, .0475	1.576	NS
3,5	12, 6	-+	1.1550 ± .2378, 1.2603 ± .0833	.0565, .0069	1.376*	NS
3,6	12, 6	16	1.1550 ± .2378, 1.2586 ± .1202	.0565, .0144	.995	NS
4,5	10, 6	-+	1.3095 ± .2179, 1.2603 ± .0833	.0475, .0069	.641*	NS
4,6	10, 6	14	1.3095 ± .2179, 1.2586 ± .1202	.0475, .0144	.522	NS
5,6	6, 6	10	1.2603 ± .0833, 1.2586 ± .1202	.0069, .0144	.027	NS

*Approximate t-test
+Weighted critical values calculated

Table 58

FULL CECUM WEIGHT (g) - SPRAGUE-DAWLEY FEMALE RATS

1 Single High Fat	2 Single M-1	3 Parabiotic High Fat	4 Parabiotic M-1	5 Parabiotic High Fat attached to Parabiotic M-1	6 Parabiotic M-1 attached to Parabiotic High Fat
1.6796	4.3396	1.5818	4.3928	1.4829	3.0357
2.5413	4.4322	1.8332	4.5599	1.7019	2.8541
1.5981	4.9075	2.2846	3.2778	2.2374	3.8488
2.3143	5.1382	2.4286	4.3388	1.7436	3.7151
1.5711	4.2482	2.2888	2.9322	1.7716	2.7642
1.6498	5.0096	1.6197	2.7397	2.3420	3.2489
1.9857	3.5290	1.5862	4.3640		
1.6538	3.1272	1.4400	3.0782		
1.4615	3.0076	.8208	3.4226		
1.7844	4.1945	1.4269	3.4716		
1.2907		2.1513			
2.1937		2.1018			

SIGNIFICANCE

Comparison of Means	n	df	Mean ± S. D.	Variance	t	P
1,2	12, 10	13#	1.8103 ± .3727, 4.1934 ± .7553	.1389, .5705	9.097*	.001
1,3	12, 12	22	1.8103 ± .3727, 1.7970 ± .4708	.1389, .2216	.077	NS
1,4	12, 10	14#	1.8103 ± .3727, 3.6578 ± .6877	.1389, .4729	7.615*	.001
1,5	12, 6	16	1.8103 ± .3727, 1.8799 ± .3349	.1389, .1122	.385	NS
1,6	12, 6	16	1.8103 ± .3727, 3.2445 ± .4502	.1389, .2027	7.197	.001
2,3	10, 12	20	4.1934 ± .7553, 1.7970 ± .4708	.5705, .2216	9.096	.001
2,4	10, 10	18	4.1934 ± .7553, 3.6578 ± .6877	.5705, .4729	1.658	NS
2,5	10, 6	-+	4.1934 ± .7553, 1.8799 ± .3349	.5705, .1122	8.406*	.001
2,6	10, 6	14	4.1934 ± .7553, 3.2445 ± .4502	.5705, .2027	2.522	.050
3,4	12, 10	20	1.7970 ± .4708, 3.6578 ± .6877	.2216, .4729	7.512	.001
3,5	12, 6	16	1.7970 ± .4708, 1.8799 ± .3349	.2216, .1122	.383	NS
3,6	12, 6	16	1.7970 ± .4708, 3.2445 ± .4502	.2216, .2027	6.233	.001
4,5	10, 6	14	3.6578 ± .6877, 1.8799 ± .3349	.4729, .1122	5.869	.001
4,6	10, 6	14	3.6578 ± .6877, 3.2445 ± .4502	.4729, .2027	1.305	NS
5,6	6, 6	10	1.8799 ± .3349, 3.2445 ± .4502	.1122, .2027	5.957	.001

*Approximate t-test
#Degrees of freedom calculated
+Weighted critical values calculated

Table 59

FULL CECUM WEIGHT IN GRAMS PER 100 g BODY WEIGHT - SPRAGUE-DAWLEY FEMALE RATS

1 Single High Fat	2 Single M-1	3 Parabiotic High Fat	4 Parabiotic M-1	5 Parabiotic High Fat attached to Parabiotic M-1	6 Parabiotic M-1 attached to Parabiotic High Fat
.5074	1.5954	.6315	1.8418	.5850	1.1521
.6688	1.5127	.6478	1.9039	.8864	1.4342
.5549	1.7653	.8855	1.6187	.9226	1.6554
.7588	2.0032	1.0842	1.6882	.6974	1.5352
.4879	1.5533	1.1111	1.3963	.7822	1.4031
.5237	1.8833	.7199	1.1092	1.1021	1.4004
.6992	1.3573	.5726	2.0781		
.5059	1.1823	.6486	1.7742		
.4273	1.0516	.3420	1.4441		
.6560	1.6385	.5777	1.3668		
.3935		.9353			
.6729		.8982			

SIGNIFICANCE

Comparison of Means	n	df	Mean ± S. D.	Variance	t	P
1,2	12, 10	-+	.5713 ± .1163, 1.5543 ± .2972	.0135, .0883	9.852*	.001
1,3	12, 12	17#	.5713 ± .1163, .7545 ± .2296	.0135, .0527	2.467*	.050
1,4	12, 10	-+	.5713 ± .1163, 1.6221 ± .2933	.0135, .0860	10.656*	.001
1,5	12, 6	16	.5713 ± .1163, .8293 ± .1820	.0135, .0331	3.680	.010
1,6	12, 6	16	.5713 ± .1163, 1.4301 ± .1678	.0135, .0281	12.769	.001
2,3	10, 12	20	1.5543 ± .2972, .7545 ± .2296	.0883, .0527	7.125	.001
2,4	10, 10	18	1.5543 ± .2972, 1.6221 ± .2933	.0883, .0860	.514	NS
2,5	10, 6	14	1.5543 ± .2972, .8293 ± .1820	.0883, .0331	5.360	.001
2,6	10, 6	14	1.5543 ± .2972, 1.4301 ± .1678	.0883, .0281	.931	NS
3,4	12, 10	20	.7545 ± .2296, 1.6221 ± .2933	.0527, .0860	7.788	.001
3,5	12, 6	16	.7545 ± .2296, .8293 ± .1820	.0527, .0331	.693	NS
3,6	12, 6	16	.7545 ± .2296, 1.4301 ± .1678	.0527, .0281	6.367	.001
4,5	10, 6	14	1.6221 ± .2933, .8293 ± .1820	.0860, .0331	5.926	.001
4,6	10, 6	14	1.6221 ± .2933, 1.4301 ± .1678	.0860, .0281	1.455	NS
5,6	6, 6	10	.8293 ± .1820, 1.4301 ± .1678	.0331, .0281	5.945	.001

*Approximate t-test
+Weighted critical values calculated
#Degrees of freedom calculated

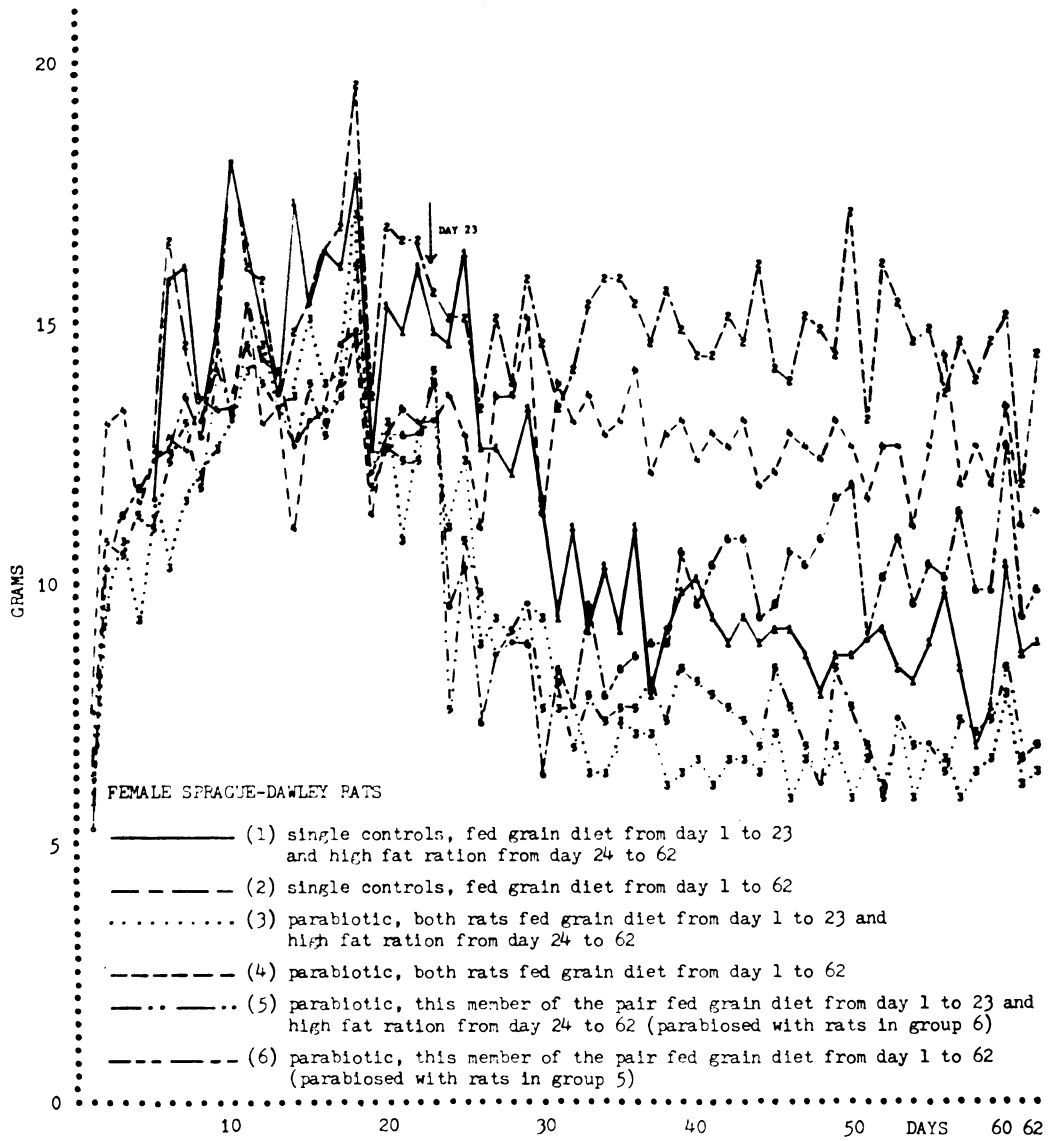

Figure 14 . Food intake of control and parabiotic female Sprague-Dawley rats. (Av./Animal/Day)

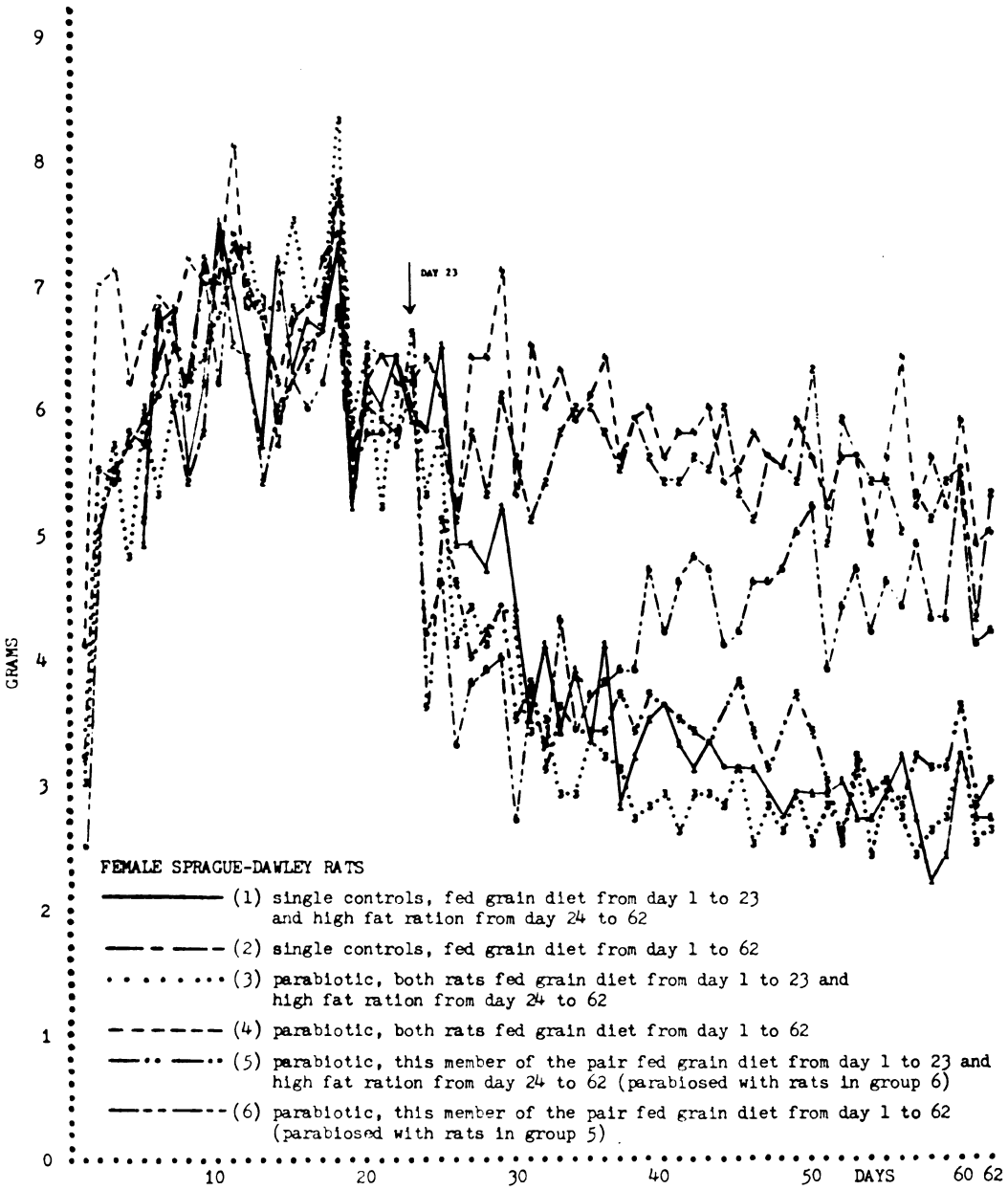

Figure 15. Food intake per 100 grams body weight of control and parabiotic female Sprague-Dawley rats. (Av./Animal/Day)

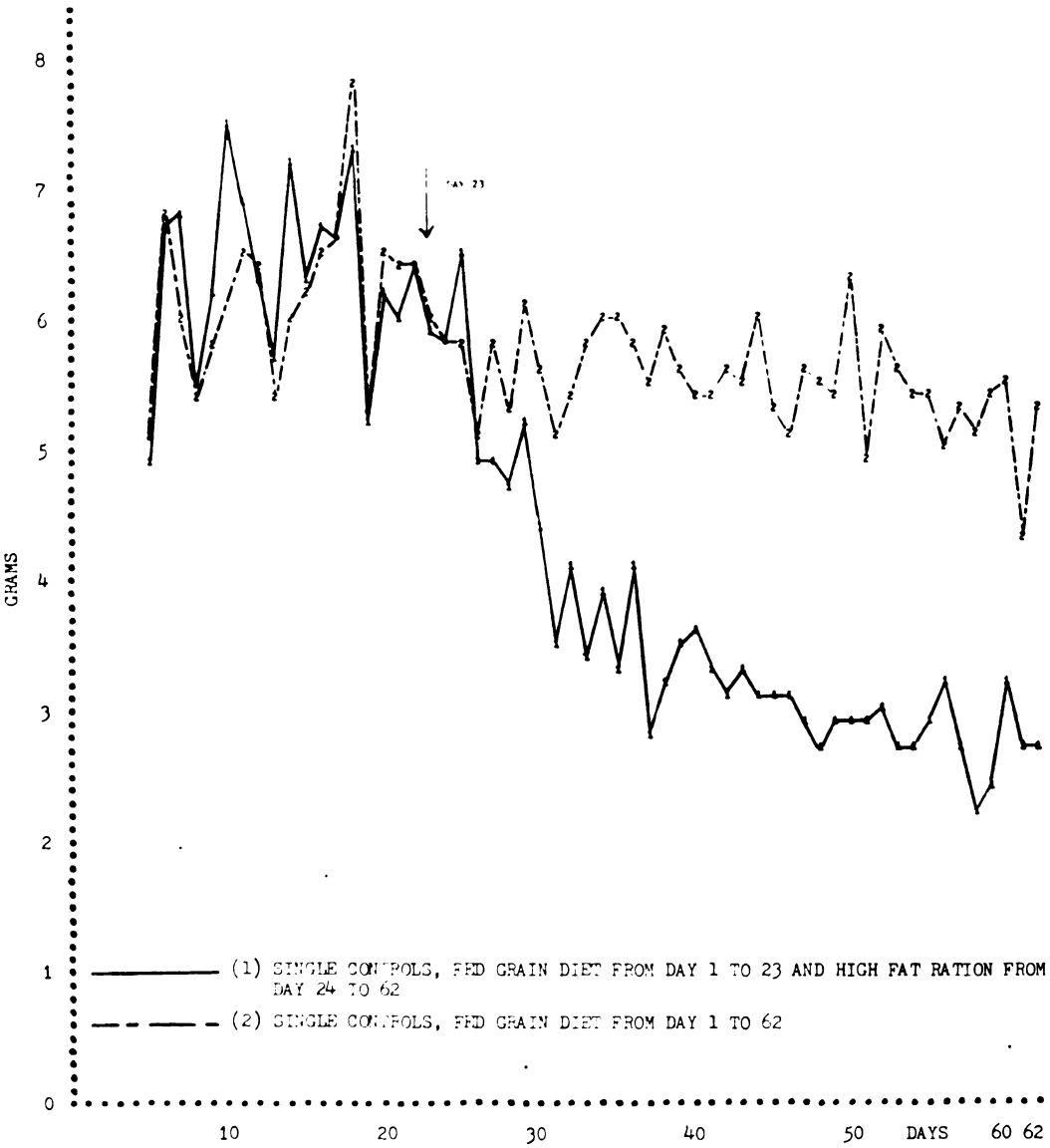

Figure 16. Food intake per 100 grams body weight of control female Sprague-Dawley rats. (Av./Animal/Day)

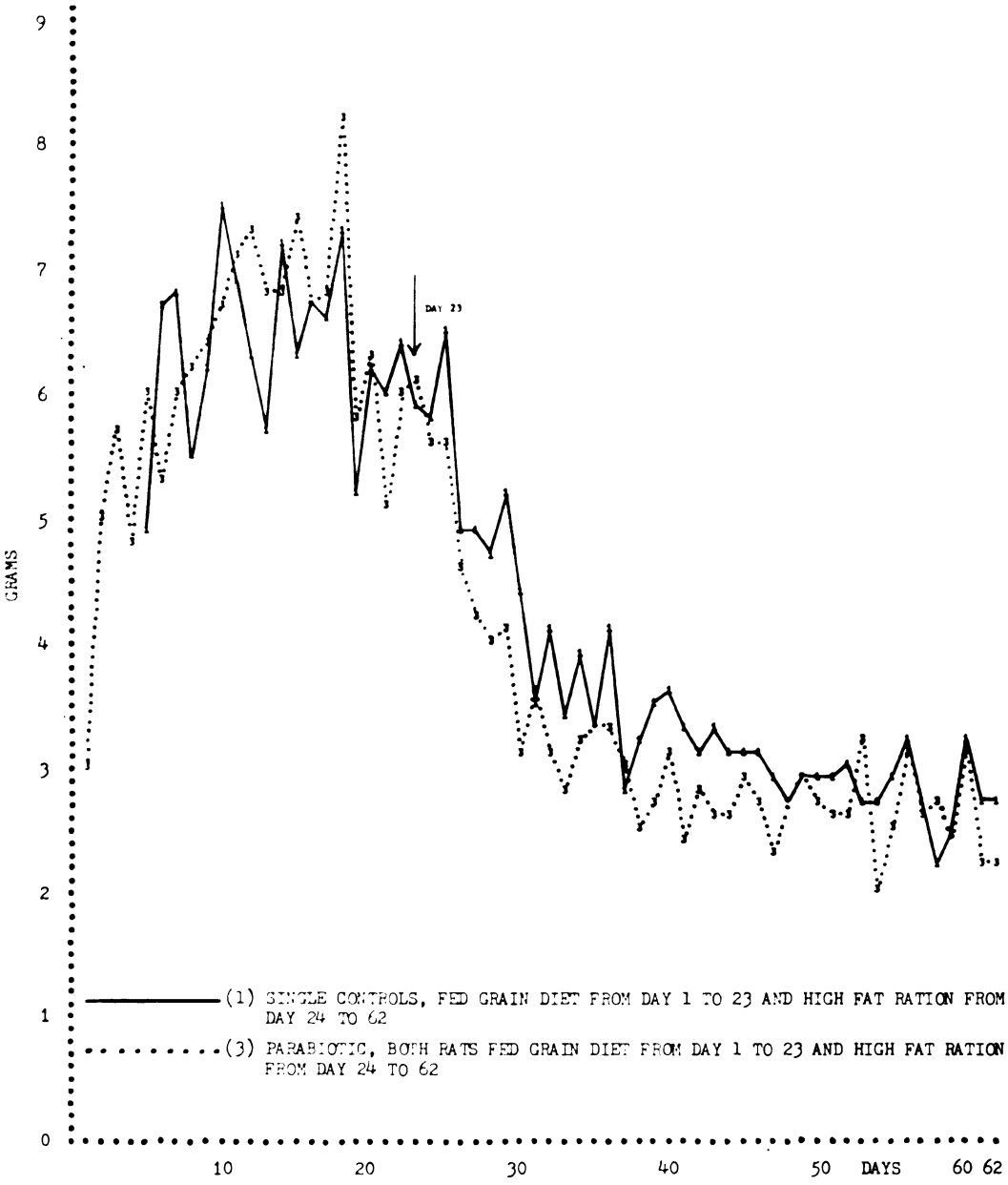

Figure 17. Food intakes per 100 grams body weight of control and parabiotic female Sprague-Dawley rats. (Av./Animal/Day)

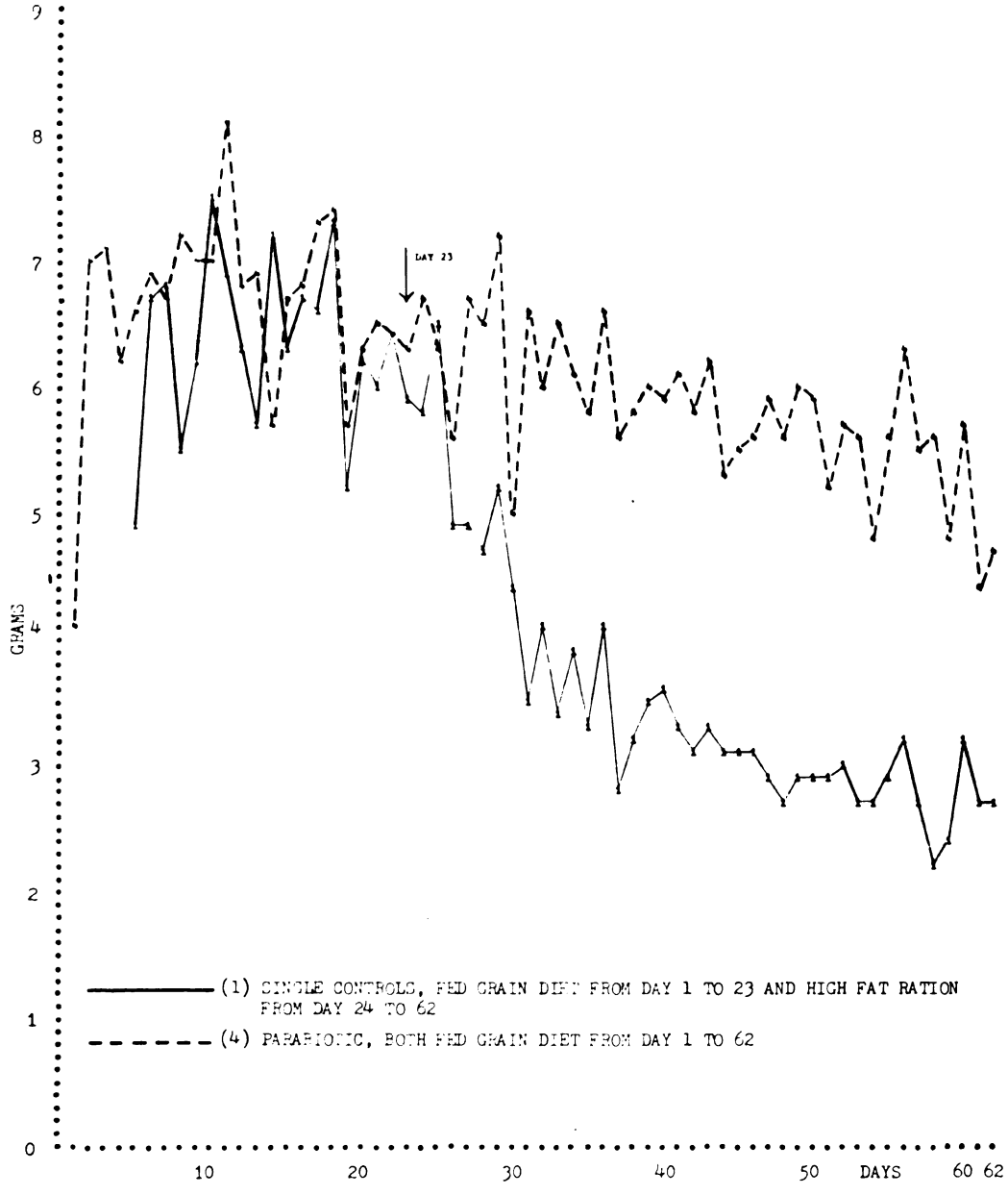

Figure 13. Food intakes per 100 grans body weight of control and parabiotic female Sprague-Dawley rats. (Av./Animal/Day)

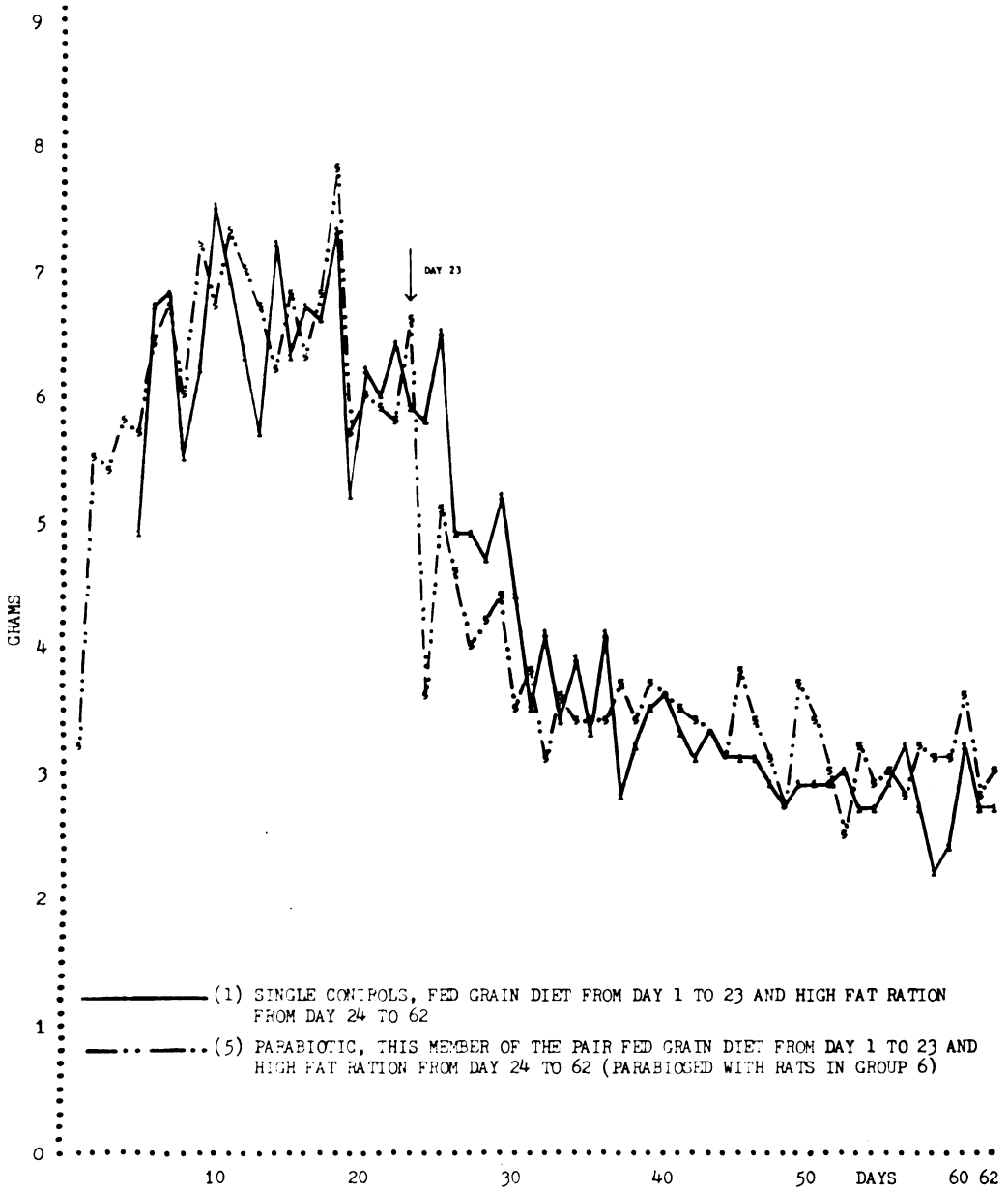

Figure 19. Food intakes per 100 grams body weight of single and parabiotic female Sprague-Dawley rats. (Av./Animal/Day)

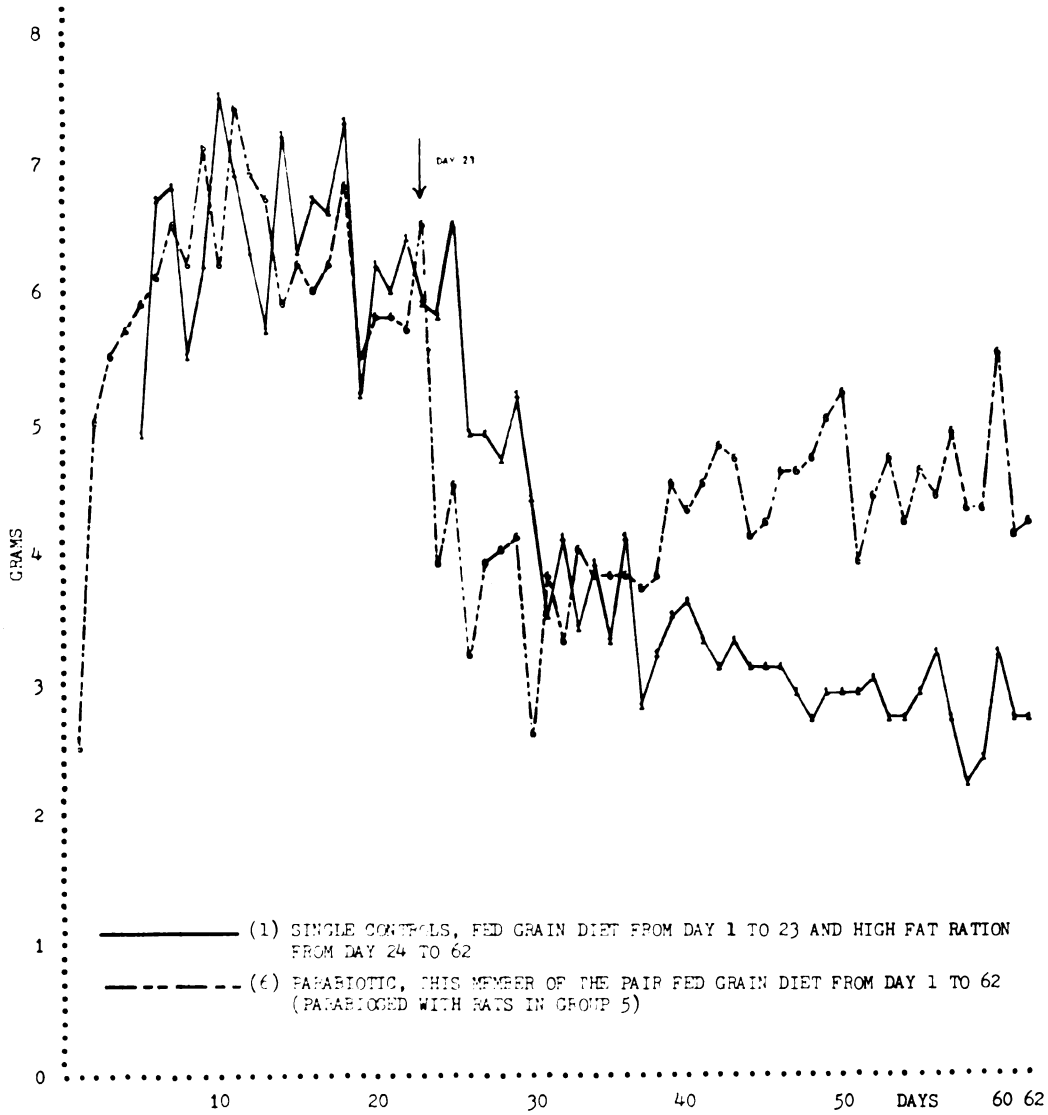

Figure 20. Food intakes per 100 grams body weight of control and parabiotic female Sprague-Dawley rats. (Av./Animal/Day)

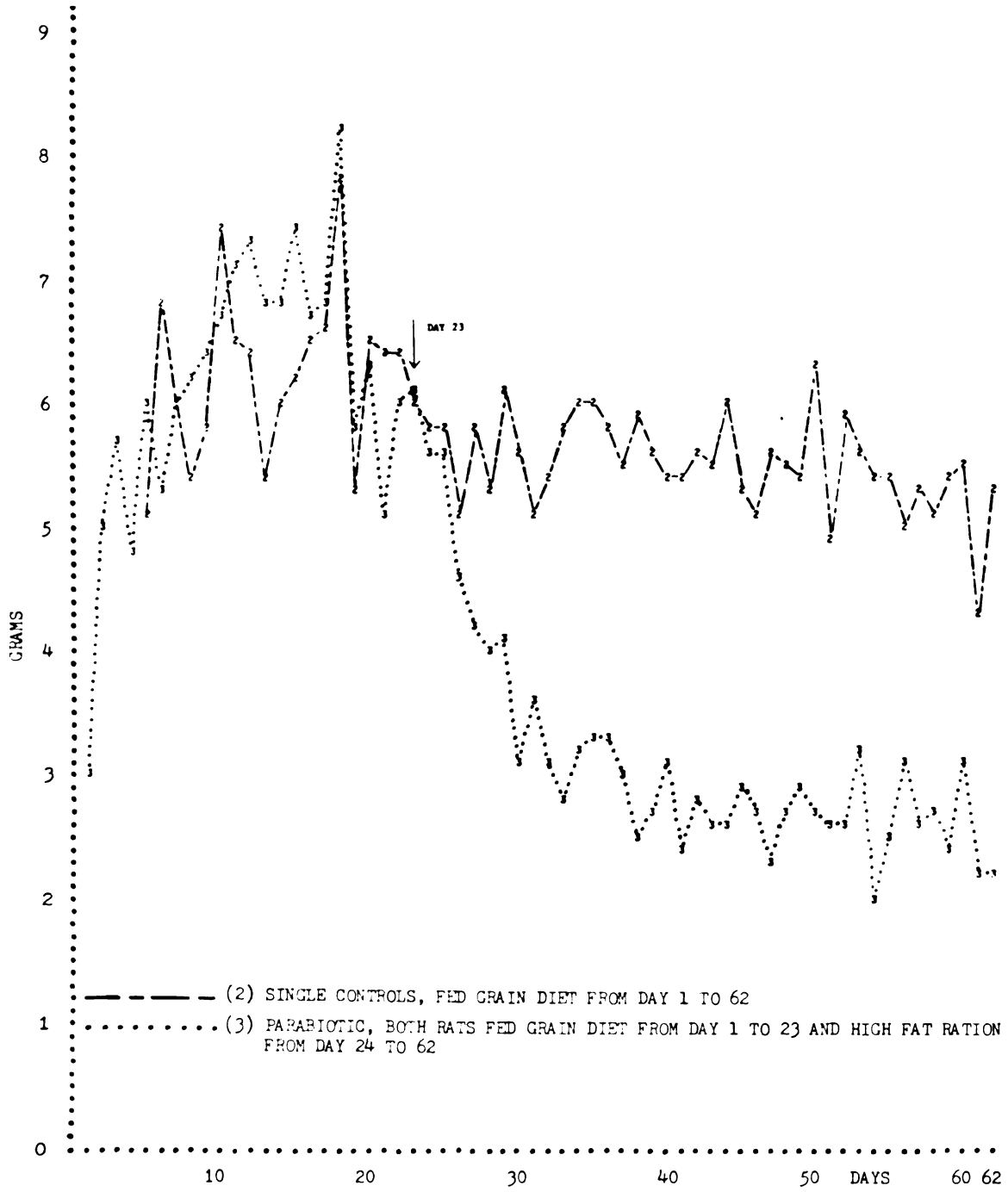

Figure 21. Food intake per 100 grams body weight of control and parabiotic female Sprague-Dawley rats. (Av./Animal/Day)

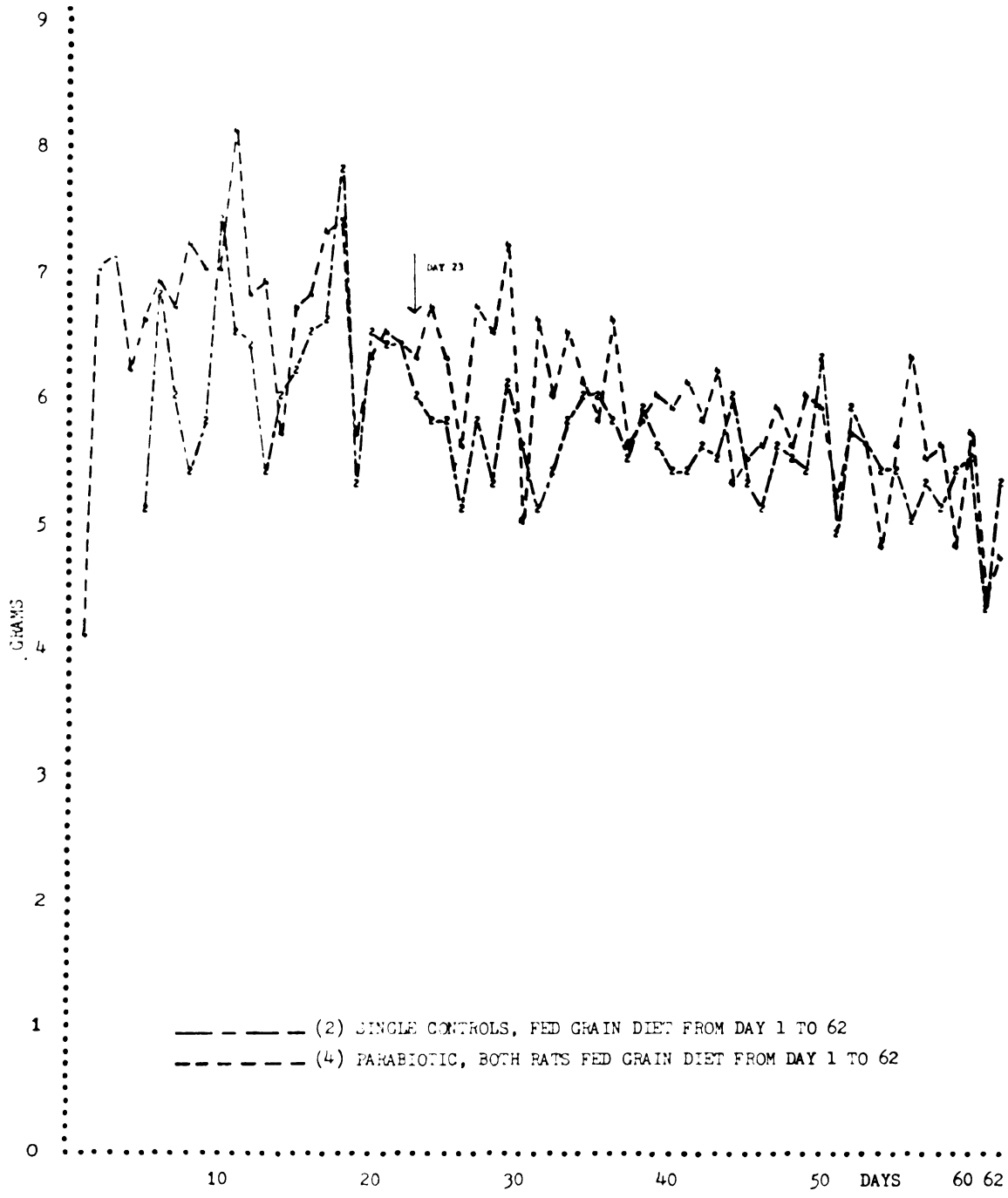

Figure 22. Food intakes per 100 grams body weight of control and parabiotic female Sprague-Dawley rats. (Av./Animal/Day)

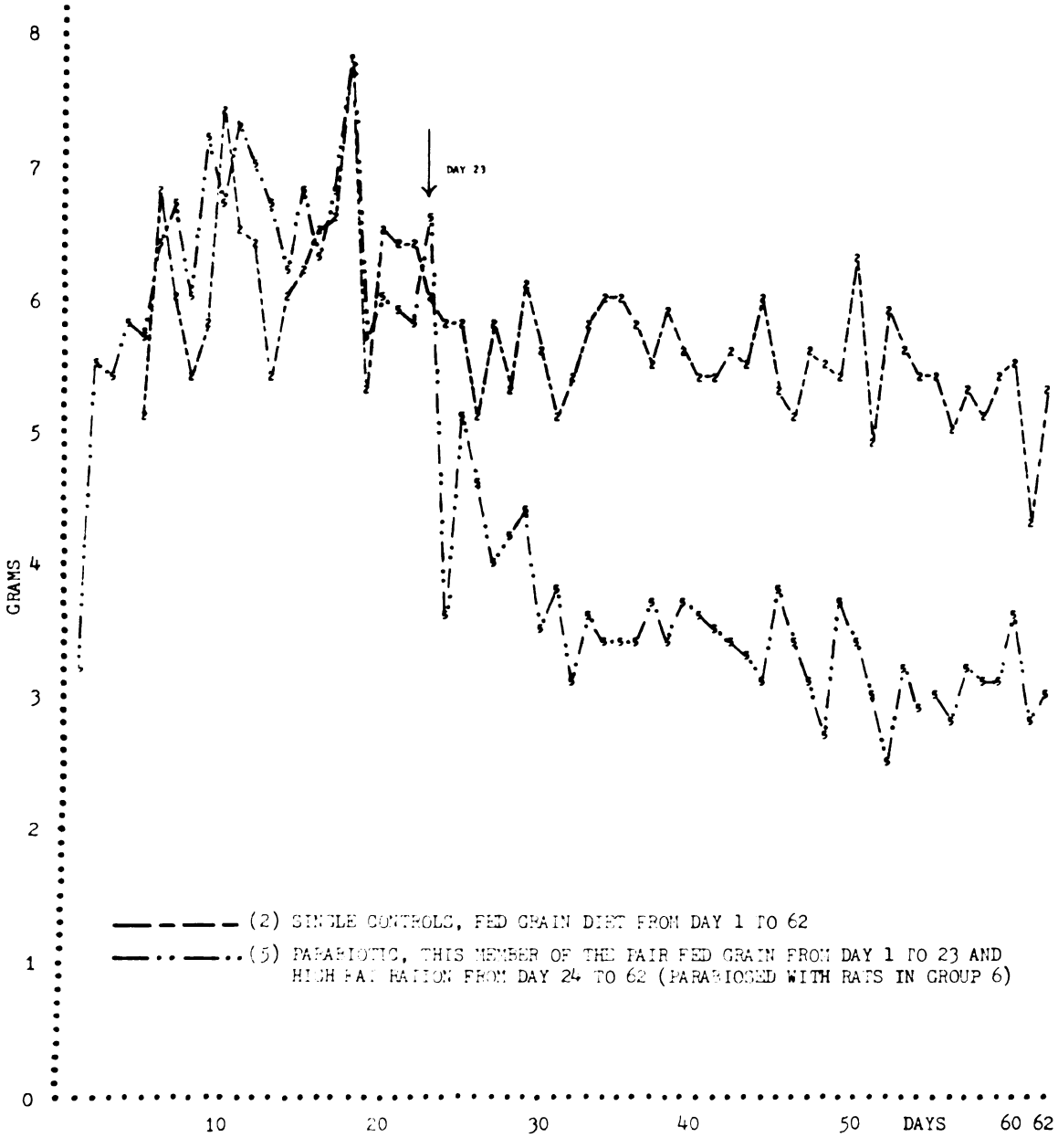

Figure 23. Food intakes per 100 grams body weight of control and parabiotic female Sprague-Dawley rats. (Av./Animal/Day)

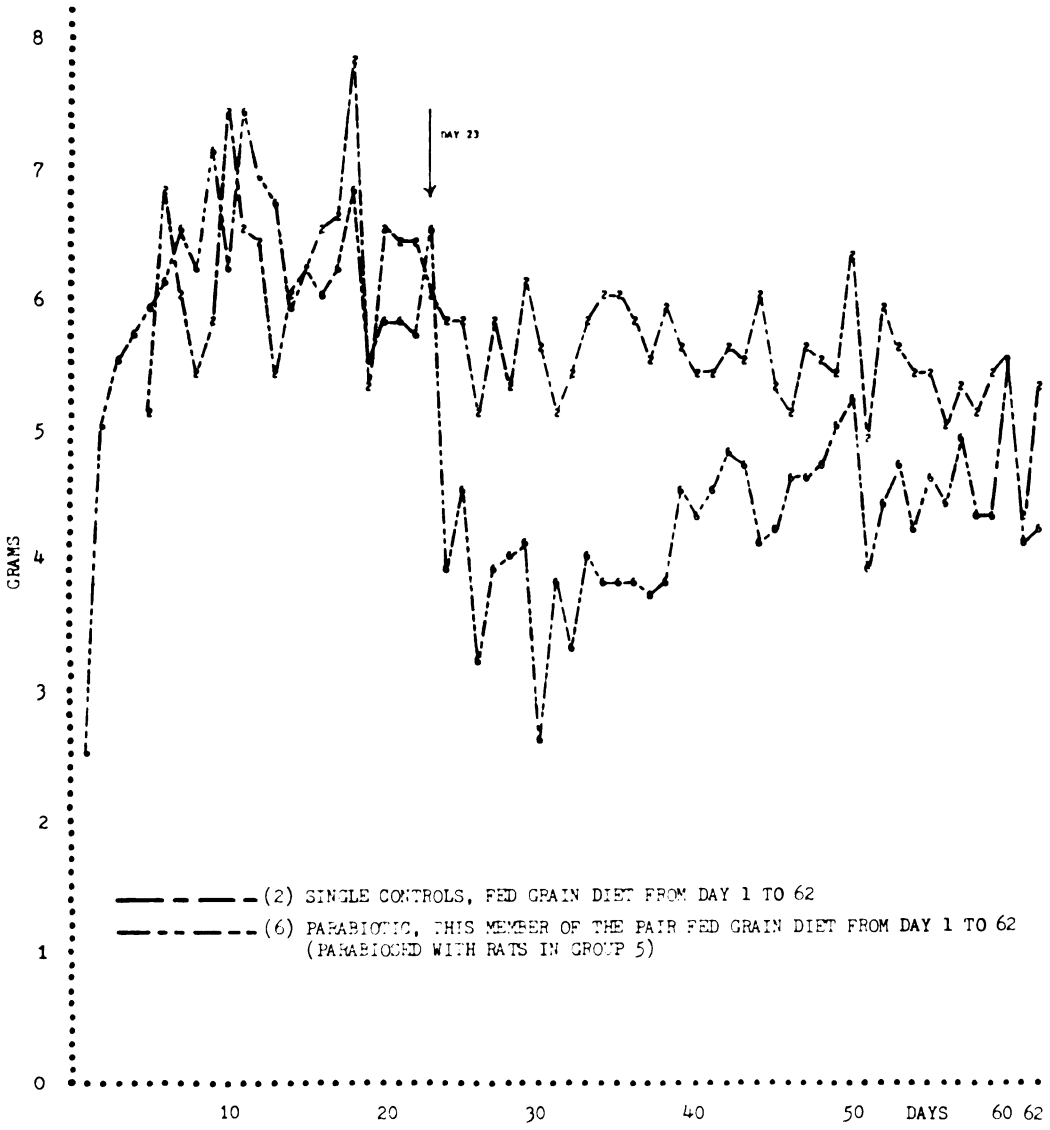

Figure 24. Food intakes per 100 grams body weight of control and parabiotic female Sprague-Dawley rats. (Av./Animal/Day)

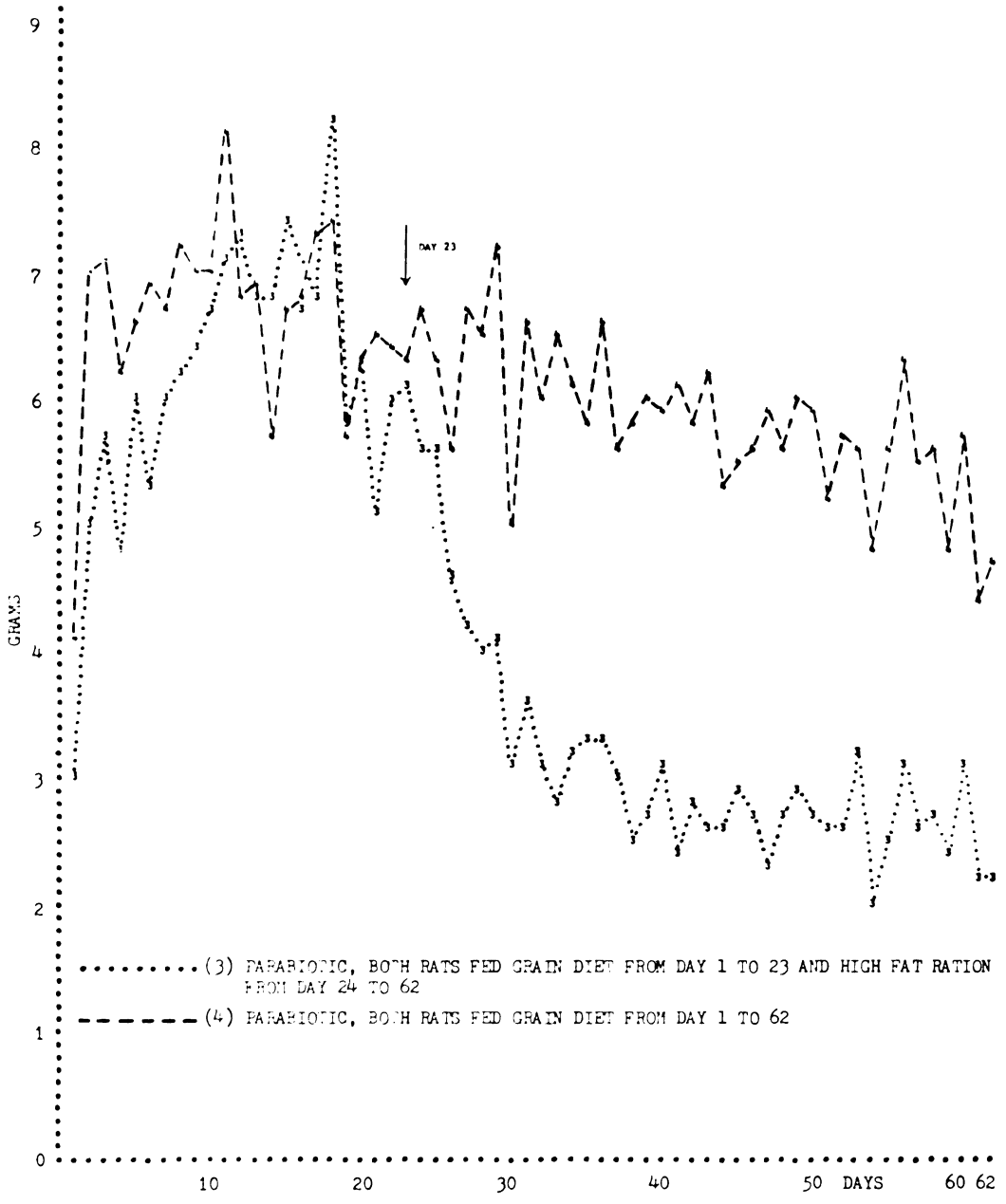

Figure 25. Food intakes per 100 grams body weight of parabiotic female Sprague-Dawley rats. (Av./Animal/Day)

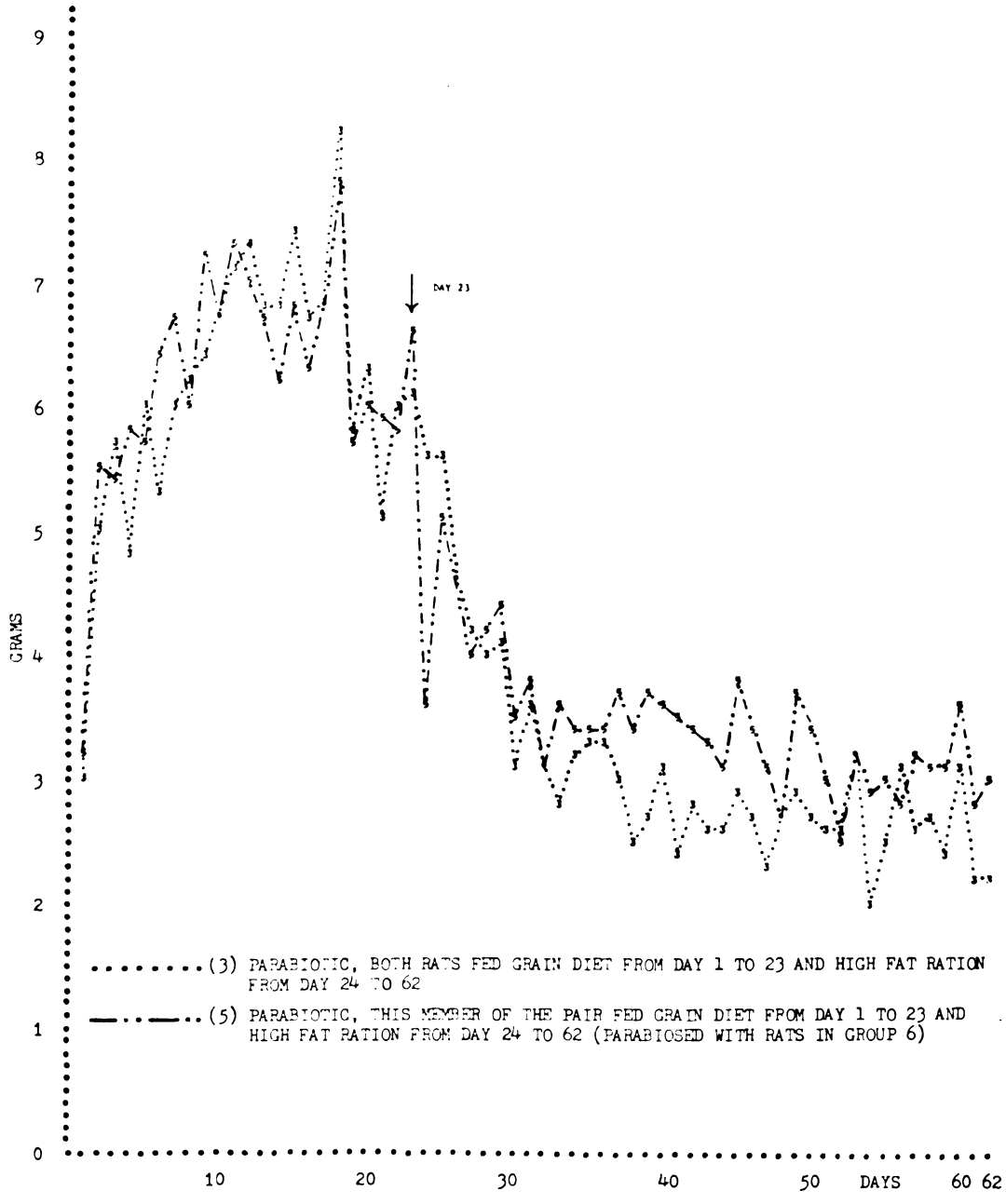

Figure 26. Food intakes per 100 grams body weight of parabiotic female Sprague-Dawley rats. (Av./Animal/Day)

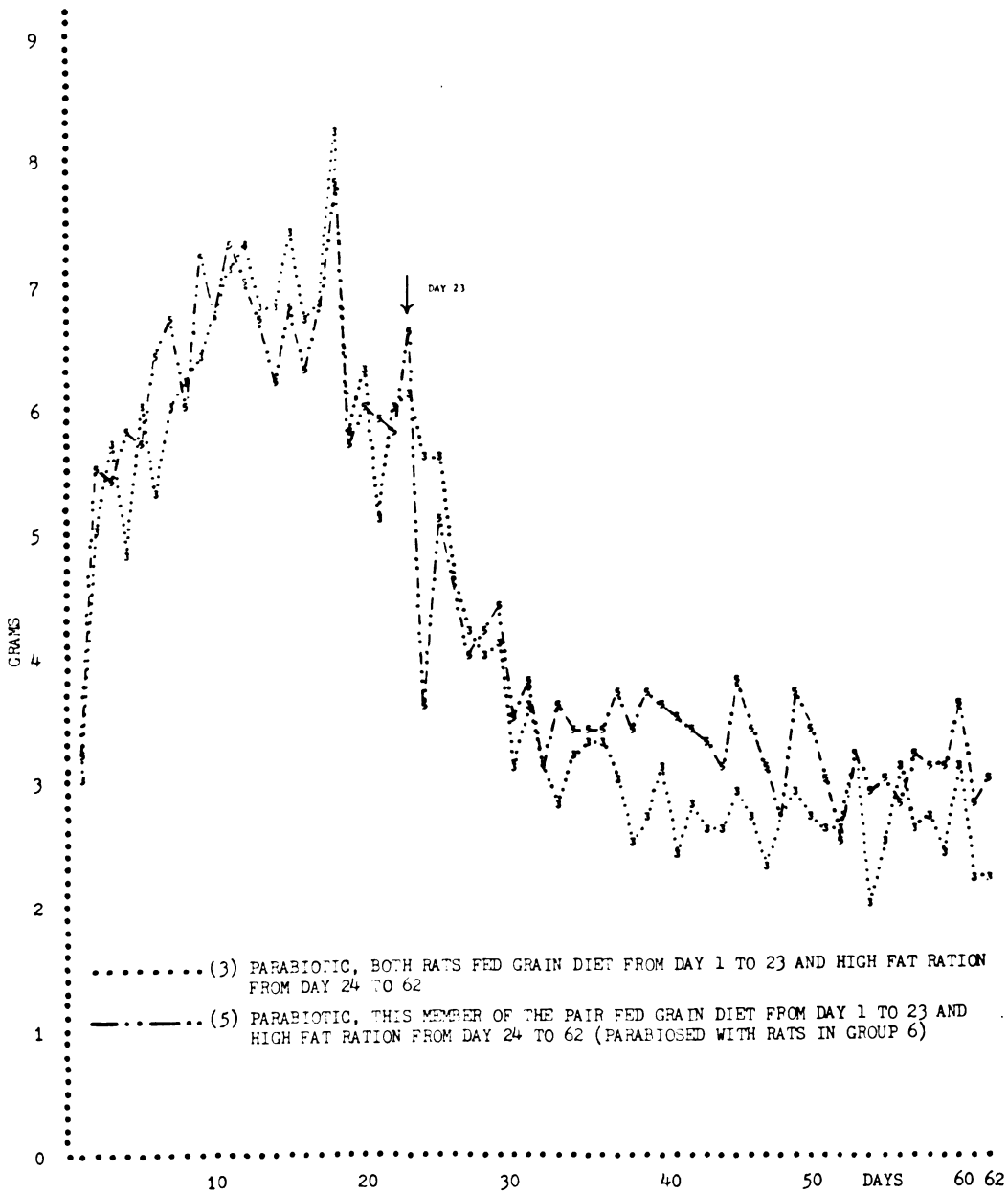

Figure 26. Food intakes per 100 grams body weight of parabiotic female Sprague-Dawley rats. (Av./Animal/Day)

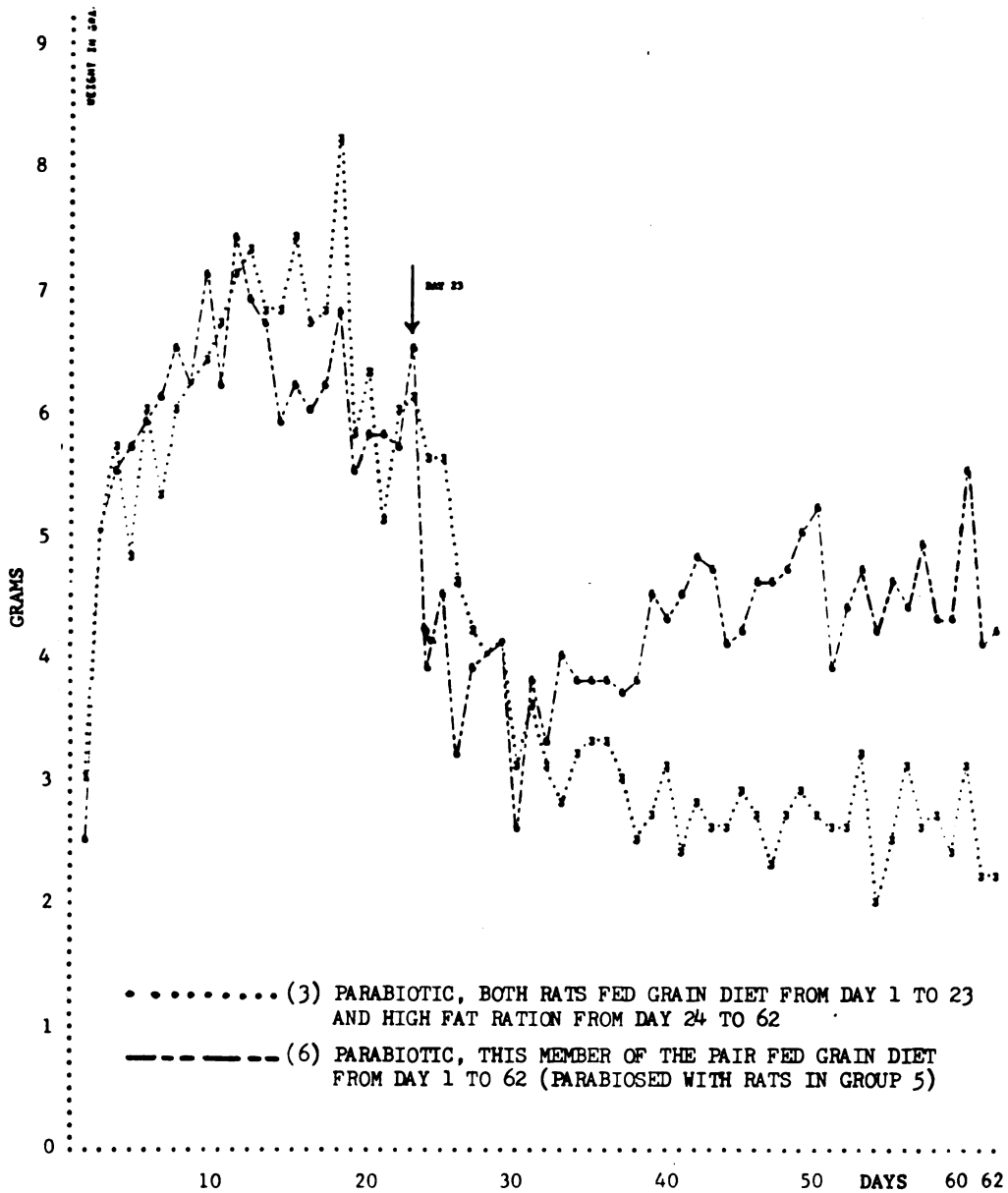

Figure 27. Food intakes per 100 grams body weight of parabiotic female Sprague-Dawley rats. (Av./Animal/Day)

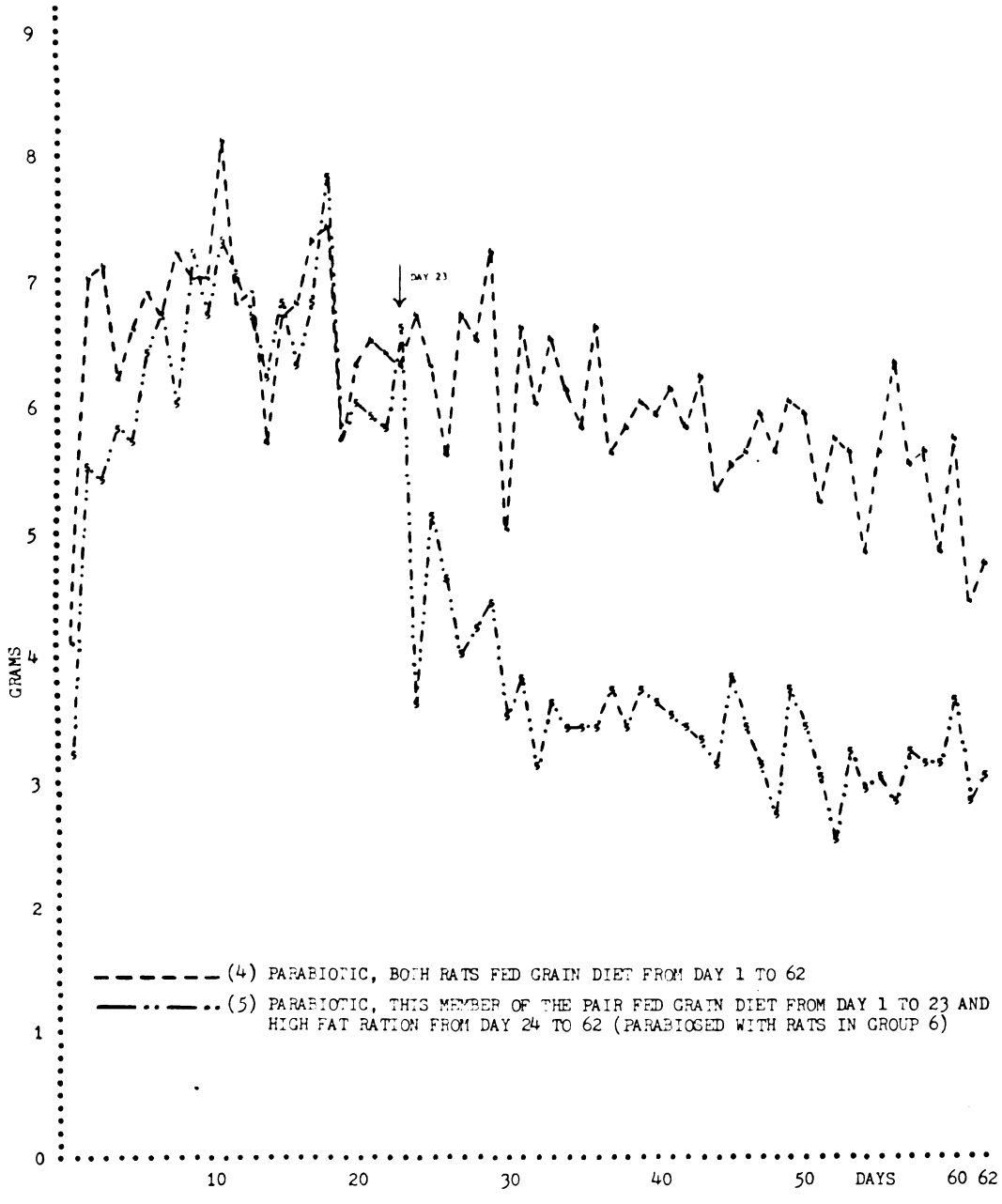

Figure 28. Food intakes per 100 grams body weight of parabiotic female Sprague-Dawley rats. (Av./Animal/Day)

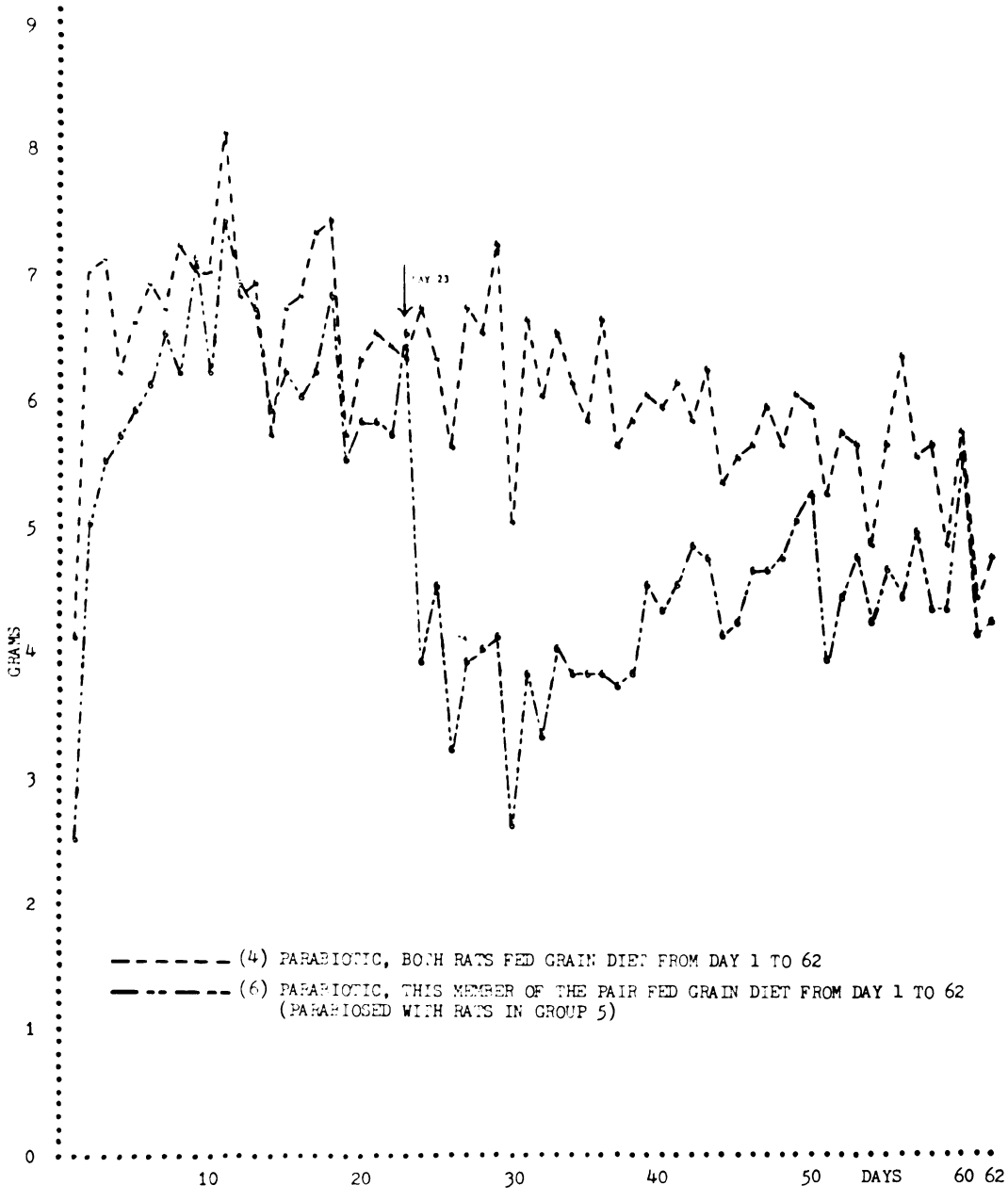

Figure 29. Food intakes per 100 grams body weight of parabiotic female Sprague-Dawley rats. (Av./Animal/Day)

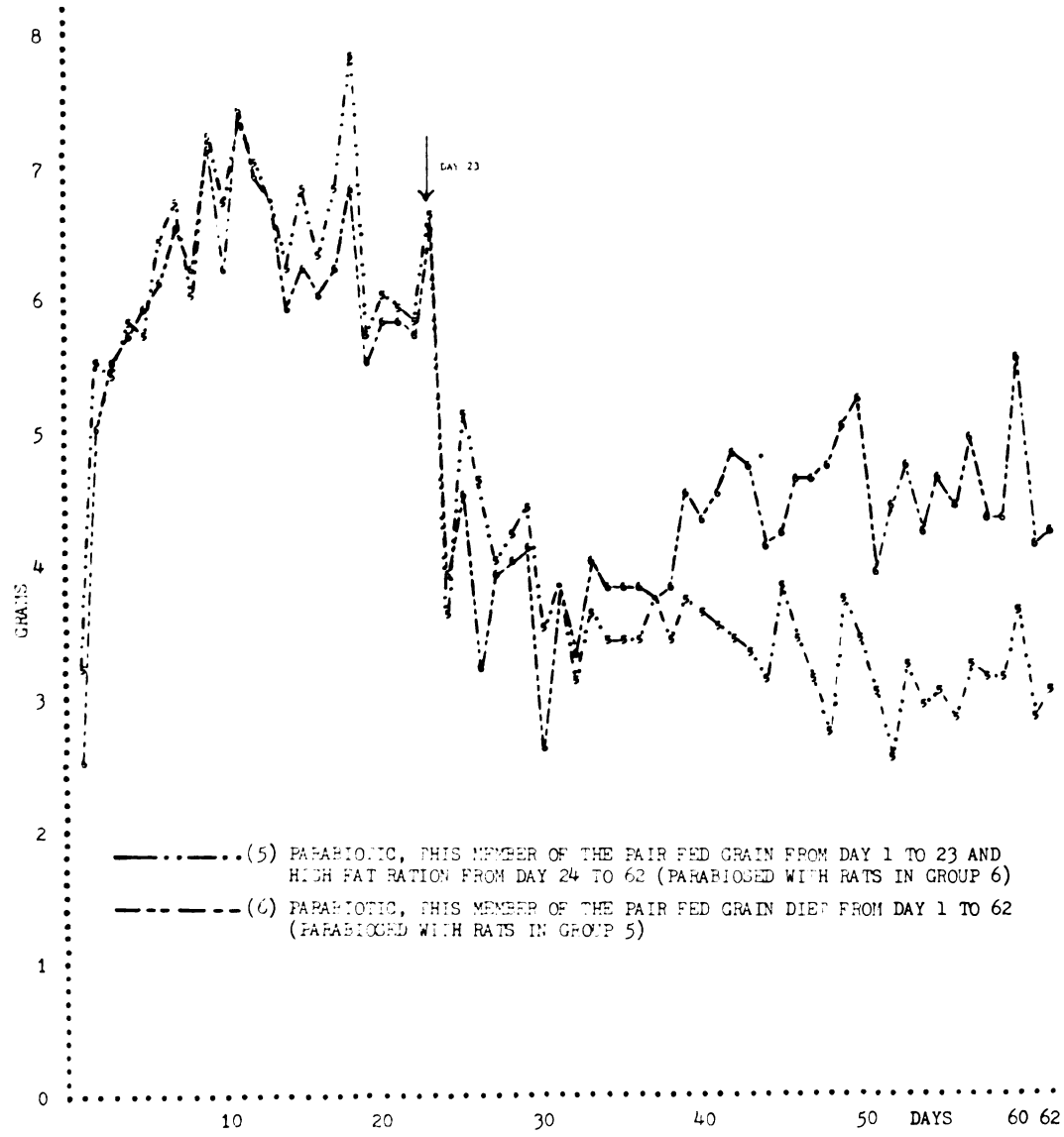

Figure 30. Food intakes per 100 grams body weight of parabiotic female Sprague-Dawley rats. (Av./Animal/Day)

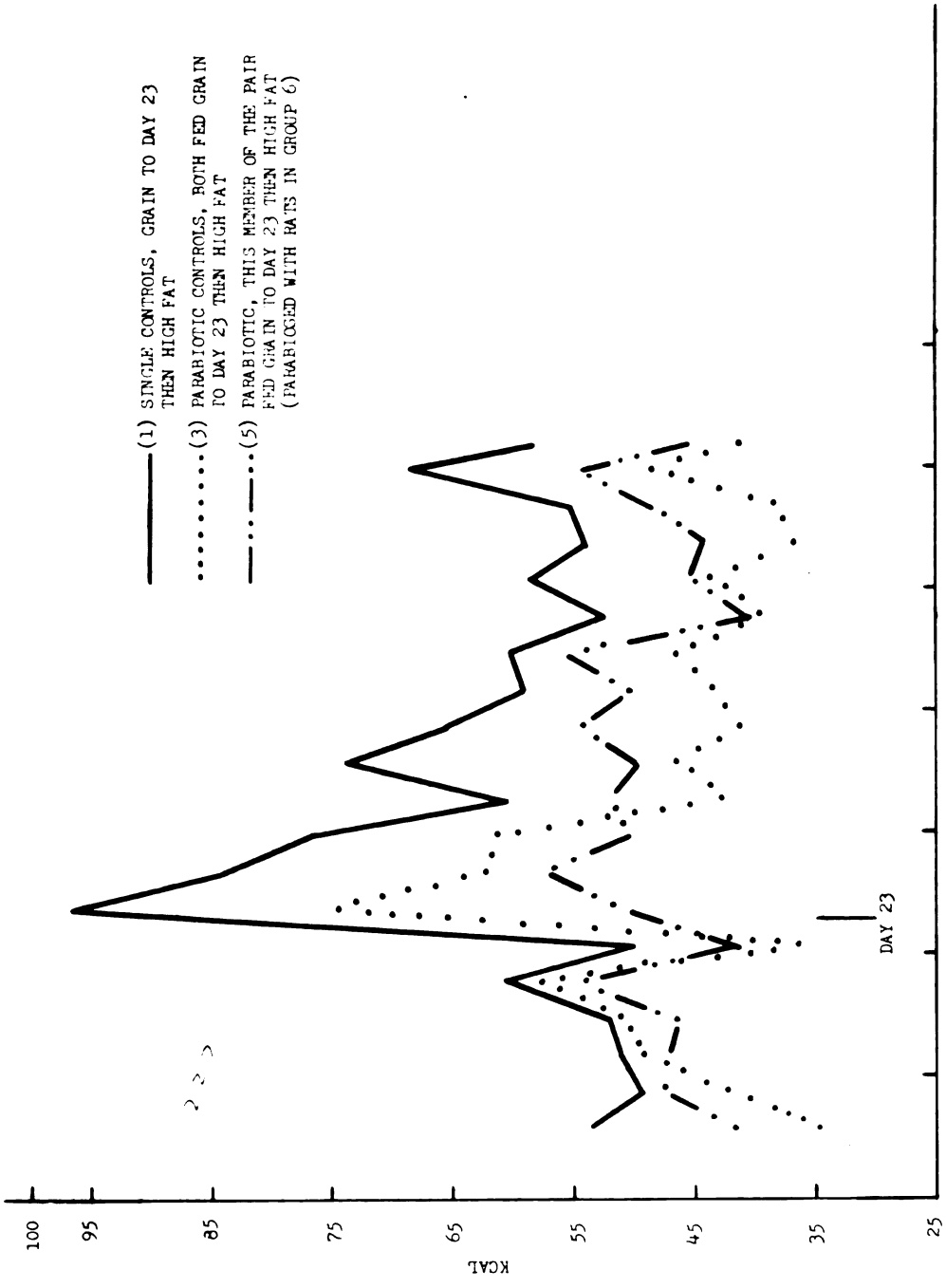

Figure 31. Kcal intakes of control and parabiotic female Sprague-Dawley rats. (Av./Animal/Day)

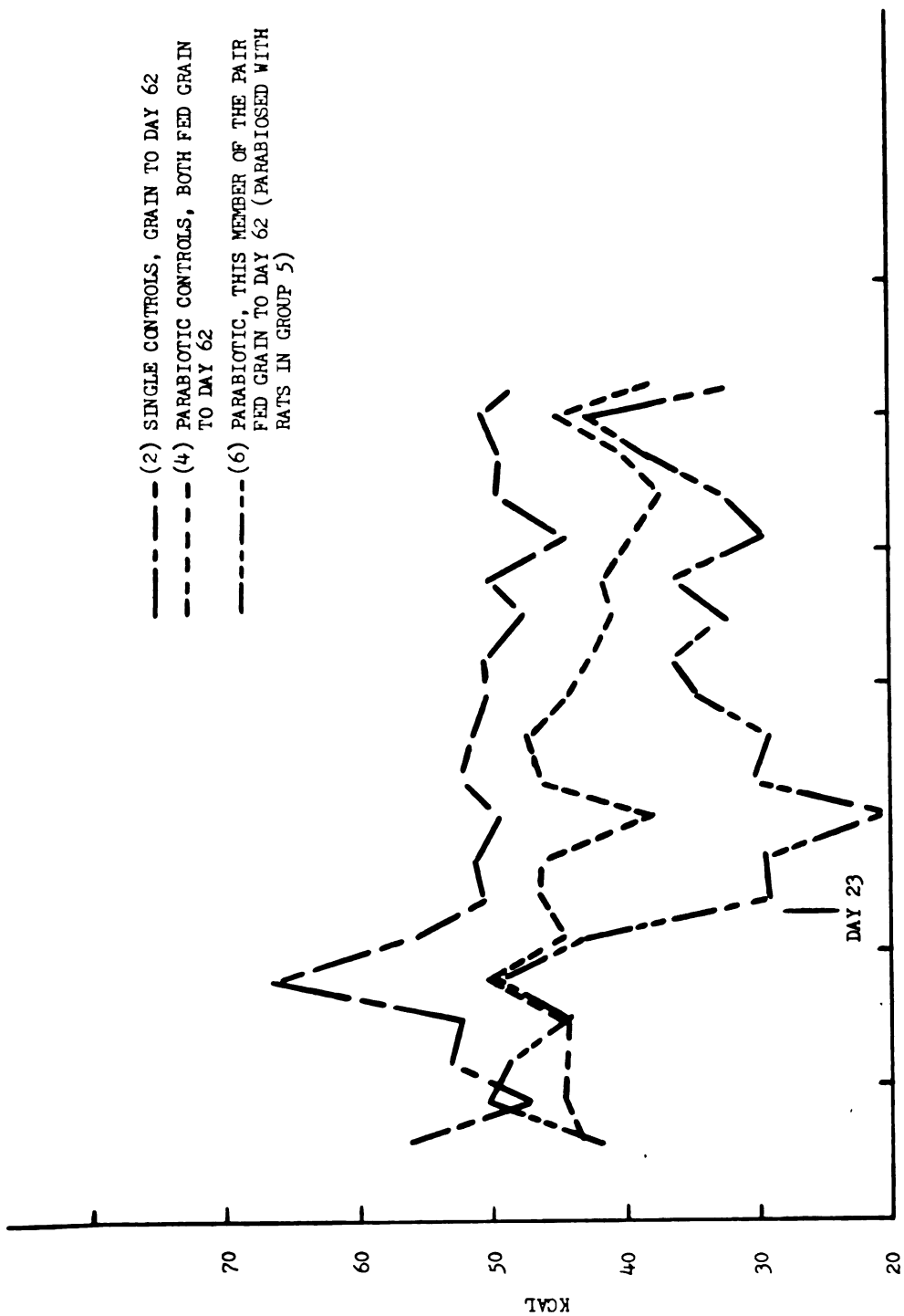

Figure 32. Kcal intakes of control and parabiotic female Sprague-Dawley rats. (Av./Animal/Day)

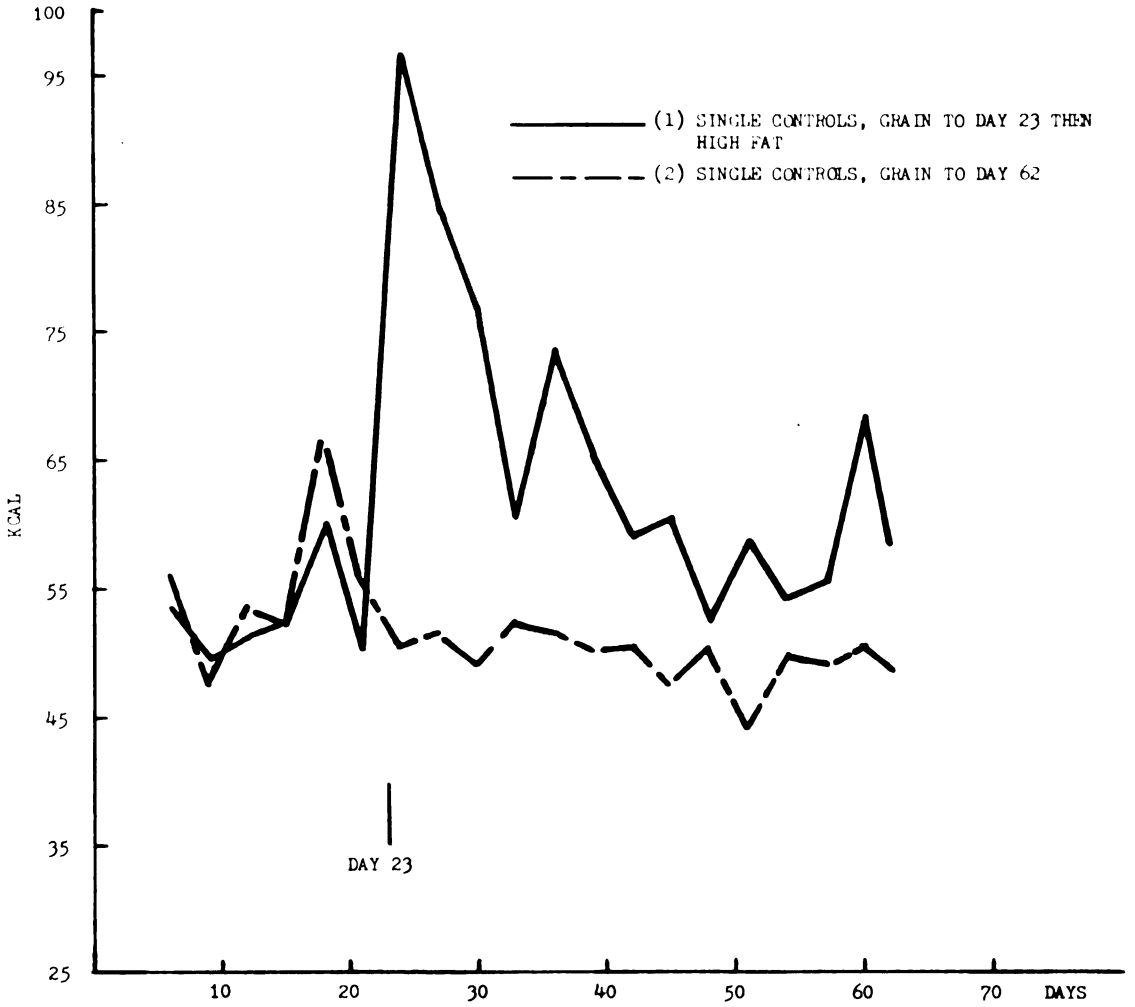

Figure 33. Kcal intakes of control female Sprague-Dawley rats. (Av./Animal/Day)

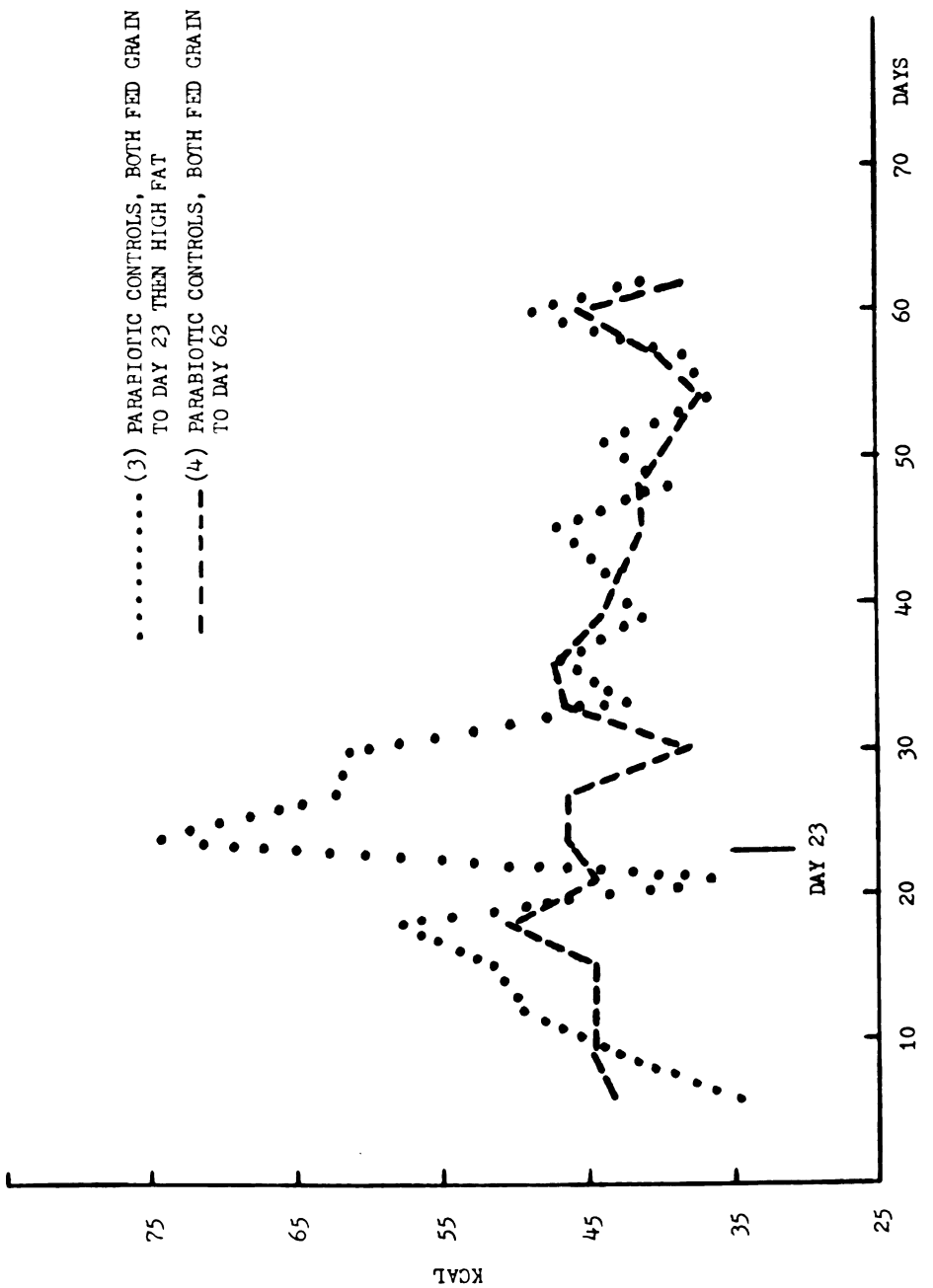

Figure 34. Kcal intakes of parabiotic female Sprague-Dawley rats. (Av./Animal/Day)

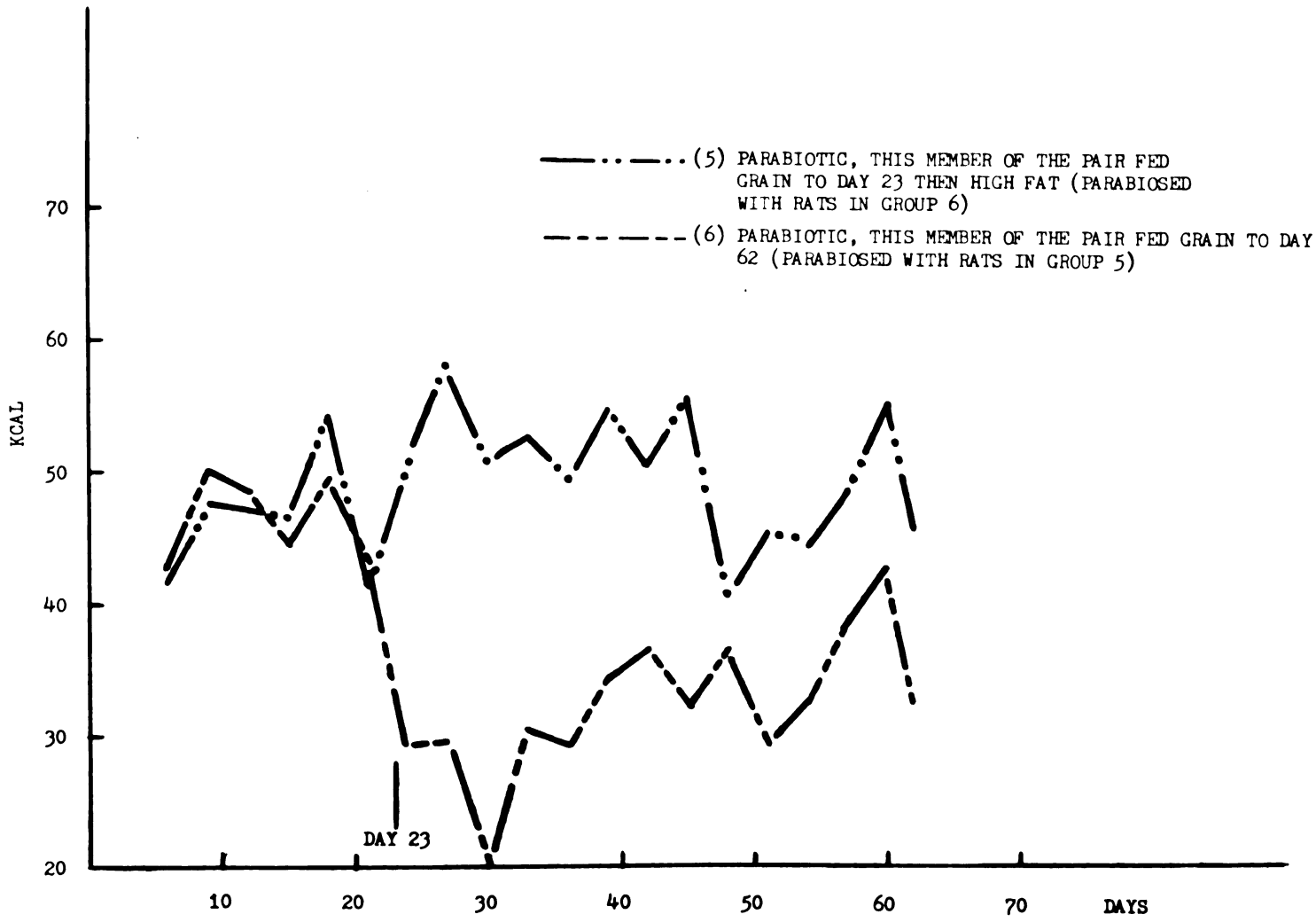

Figure 35. Kcal intakes of parabiotic female Sprague-Dawley rats. (Av/Animal/Day)

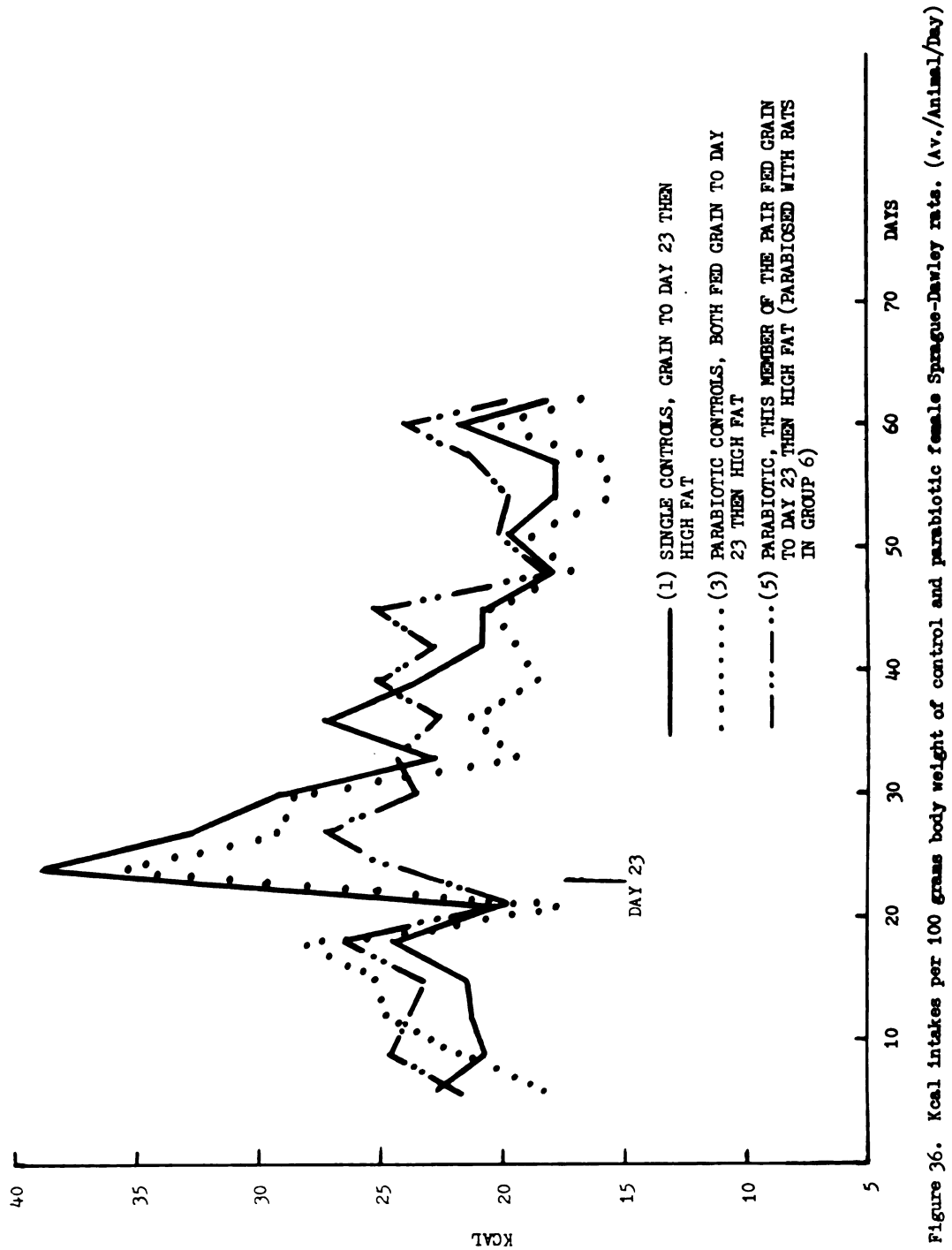

Figure 36. Kcal intakes per 100 grams body weight of control and parabiotic female Sprague-Dawley rats. (Av./Animal/Day)

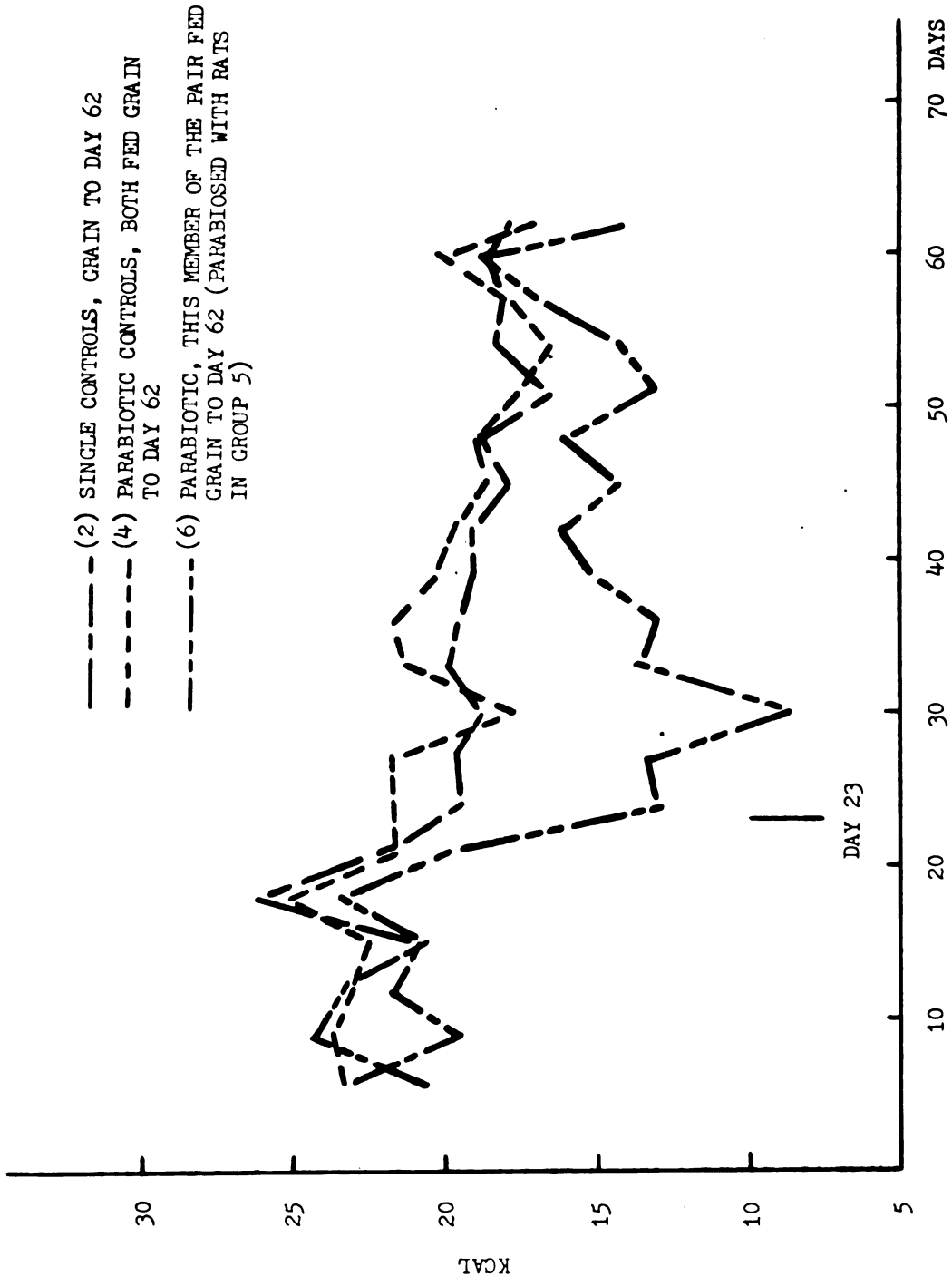

Figure 37. Kcal intakes per 100 grams body weight of control and parabiotic female Sprague-Dawley rats. (Av./Animal/Day)

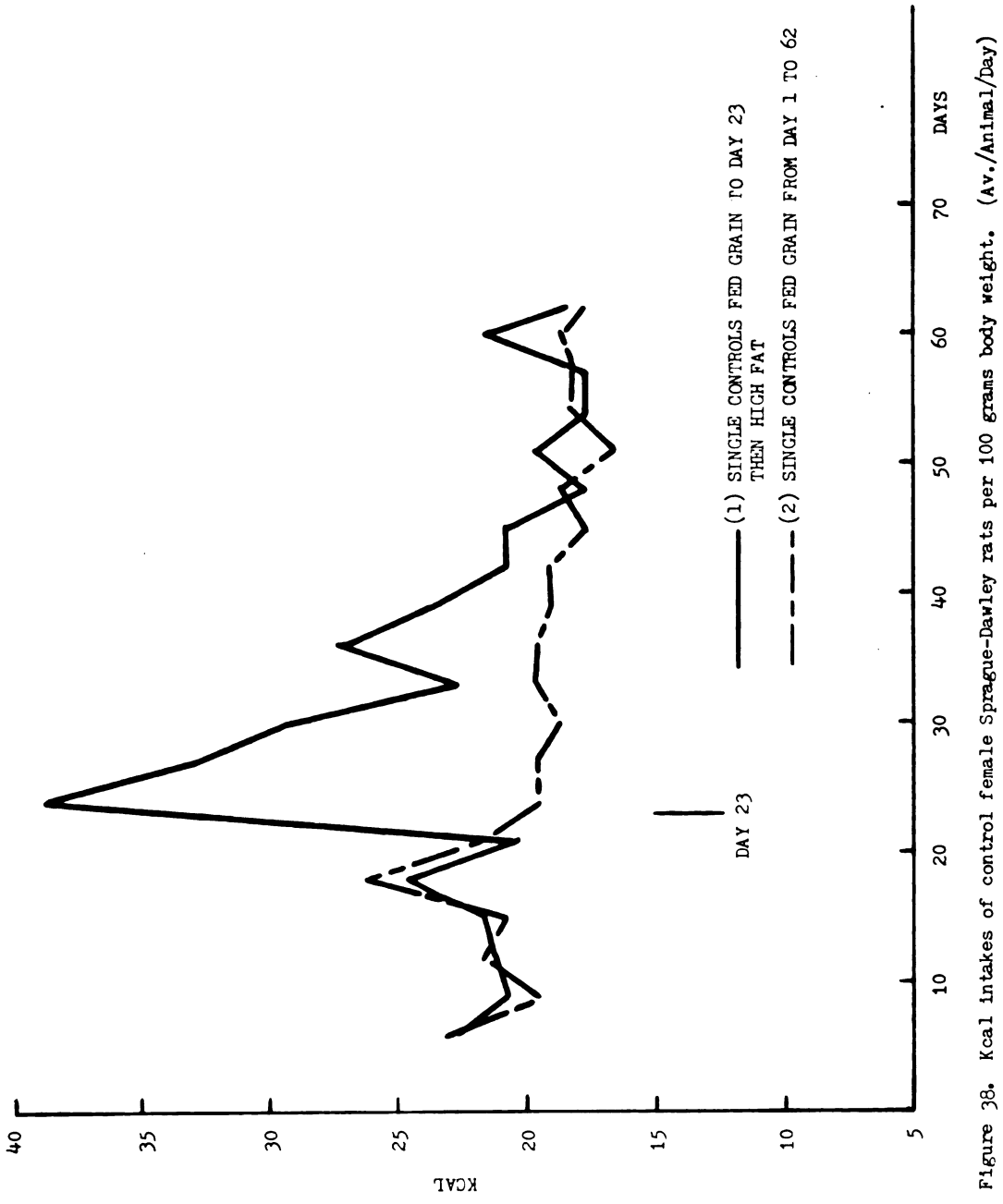

Figure 38. Kcal intakes of control female Sprague-Dawley rats per 100 grams body weight. (Av./Animal/Day)

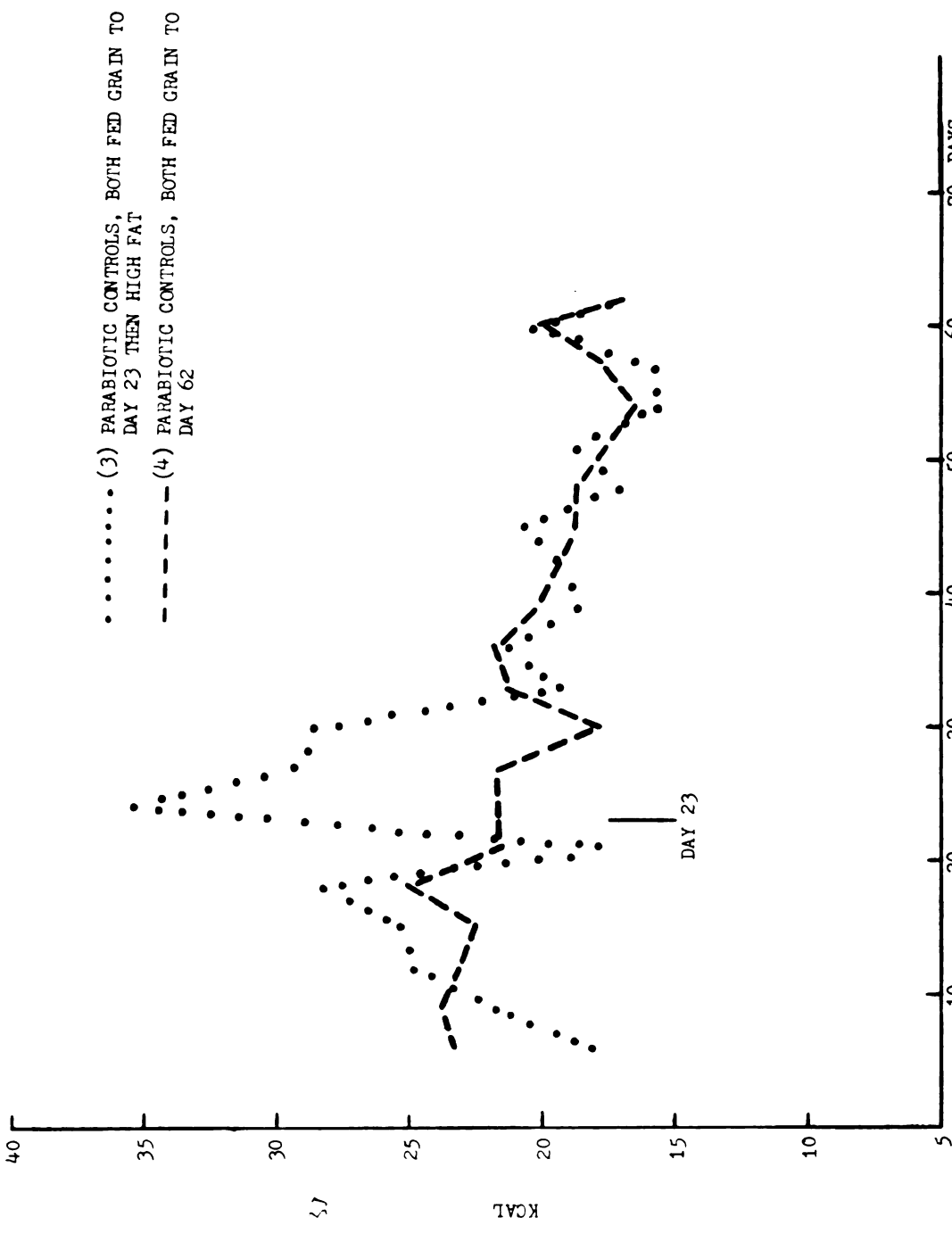

Figure 39. Kcal intakes per 100 grams body weight of parabiotic female Sprague-Dawley rats. (Av./Animal/Day)

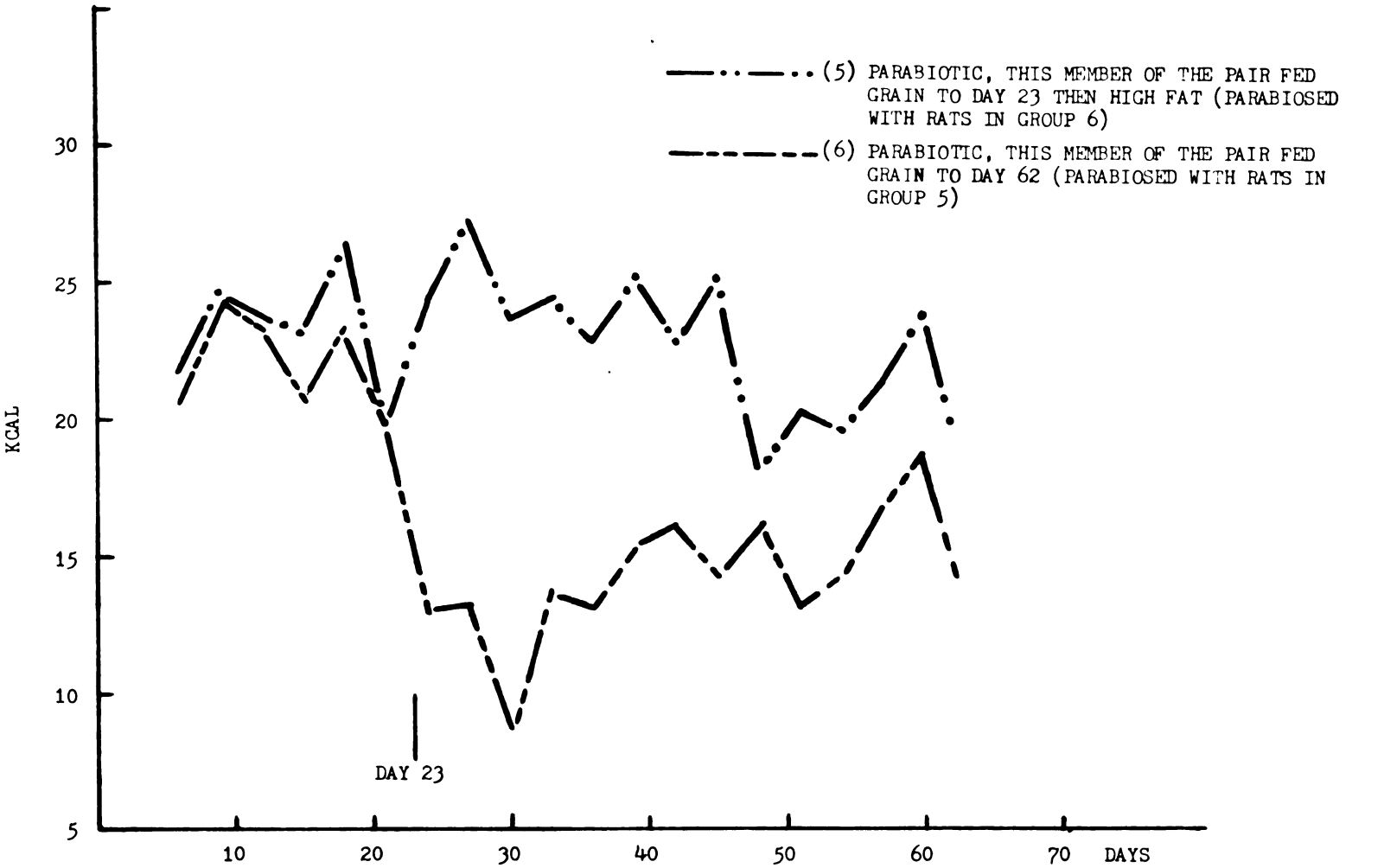

Figure 40. Kcal intakes per 100 grams body weight of parabiotic female Sprague-Dawley rats. (Av./Animal/Day)

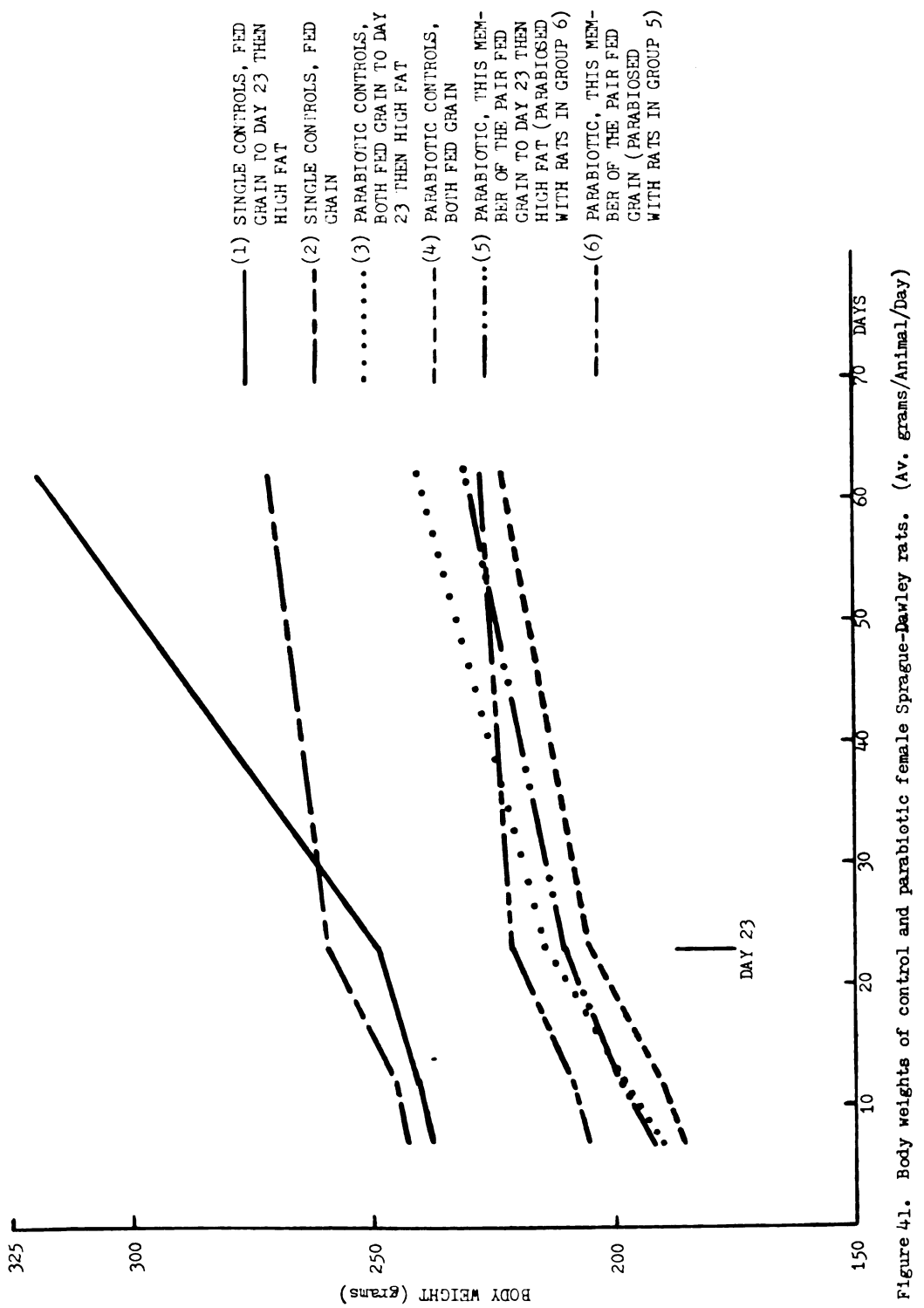

Figure 41. Body weights of control and parabiotic female Sprague-Dawley rats. (Av. grams/Animal/Day)

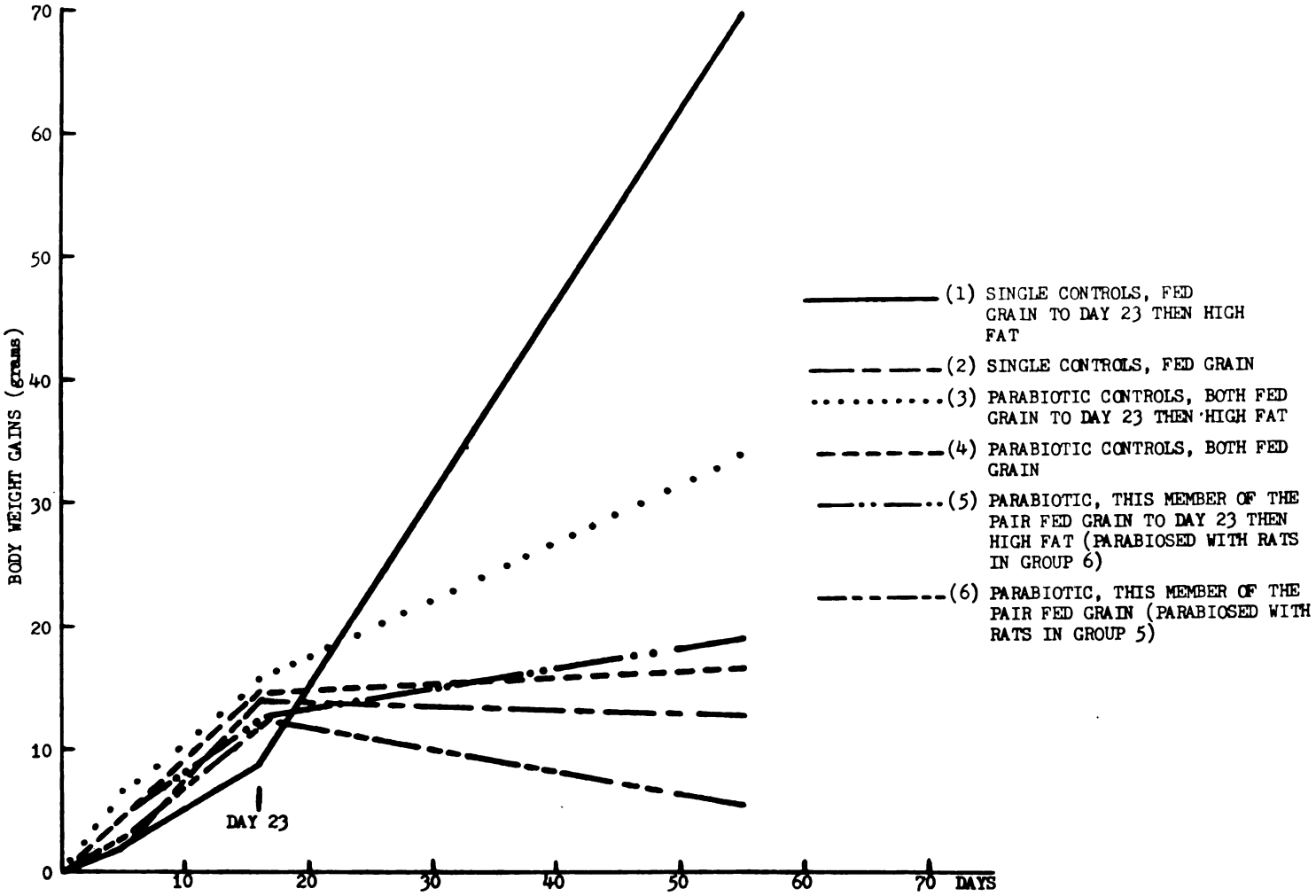

Figure 42. Body weight gains of control and parabiotic female Sprague-Dawley rats. (Av. grams/Animal/Day)

INTRODUCTION

Some of the "assay data" presented in this monograph were obtained on animals lightly anesthetized with metofane inhalation anesthetic.[1] The parabiotic weighing technique requires a light anesthesia and, in order to obtain heart blood for insulin assay, a light anesthetic must be used for cardiac puncture.

It is important to know how an anesthetic affects experimental variables or if the anesthetic stresses an animal more than would be desired in experiments where light anesthesia is necessary.

Ether is a very potent anesthetic and muscle relaxing agent. It is extremely volatile and dangerous to use without proper precautions. The vapor is irritating and the smell quite pungent (362). During ether anesthesia, there is an increase in blood catecholamine concentration and in deep anesthesia ACTH secretion is enhanced. Emesis may occur after ether anesthesia causing a post-surgical stress (363).

The depth of anesthesia does not change rapidly when diethyl ether is employed. There are also favorable cardiovascular and respiratory effects partly as a result of the increased circulating catecholamines (363).

The concern in using anesthetics (especially diethyl ether) is justified by the hyperglycemia associated with ether (362), the marked

[1] Pitman-Moore, Division of Dow Chemical Co., Fort Washington, Pa. 19034. Chemically, methoxyflurane is (2, 2-dichloro-1, 1-difluoroethylmethyl ether) with butylated hydroxytoluene added at a 0.01% level as preservative.

increase in circulating free fatty acids (358)and, as described above, an increase in ACTH which would elicit the "ACTH-corticoid" stress system (363).

Methoxyflurane or Penthrane, in contrast to ether, has a very pleasant odor and does not stimulate buccal, pharyngeal or bronchial secretions as ether. It is also non-flammable and non-explosive. Its vapor pressure is far lower than that of diethyl ether and the volume percentage in air seldom goes above 4; consequently, over dosage is more easily controlled.

Awaking from methoxyflurane is usually quiet with prolonged analgesia. This advantage was considered a major one for parabiosis since post-surgical stupor is advantageous allowing animals to become slowly conscious of their parabiotic state. Unlike ether anesthesia, methoxyflurane considerably suppresses respiratory and cardiac functions (362).

Reports could not be found as to the effects of methoxyflurane on "stress" and blood metabolites such as glucose and free fatty acids.

TECHNIQUES

Adult, male Sprague-Dawley rats were fed a grain ration until the day before experimentation at which time they were fasted for 17 hours. Animals were housed in single cages in an environmentally controlled room. The temperature was 73 $\pm$ 2 degrees Fahrenheit and a 12 hour, 12 hour light, dark cycle was used.

Five animals were decapitated without anesthesia and blood collected into disposable centrifuge tubes with no anticoagulant. Another group of 5 animals was handled exactly the same as the previous group. Their heads were placed into the guillotine for a few seconds but were not

decapitated. They were then placed into a container and methoxyflurane added. The only variable between the two groups was exposure to methoxyflurane. After loss of consciousness, the animals were decapitated and blood collected as in the previous group. All samples were collected and kept in ice water at all times.

Blood samples were centrifuged to further contract the clot and the serum was placed in several small bottles for subsequent assay. Aliquots for each assay were frozen individually to avoid thawing and refreezing.

The adrenal glands were removed, weighed and then frozen in 5 ml of 6% trichloroacetic acid to await adrenal ascorbic acid assay.

All assay procedures for adrenal ascorbic acid, serum free fatty acids and serum glucose were performed as described in this thesis in the section entitled "Energy Metabolism."

RESULTS

As shown in Tables 60-64, all parameters measured were statistically the same between rats treated with methoxyflurane and those that were not. Adrenal weights, adrenal ascorbic acid, and serum free fatty acids were actually slightly lower and blood glucose slightly higher for the methoxyflurane treated animals.

DISCUSSION

Methoxyflurane did not produce any additional stress as evidenced by statistically identical adrenal weights as compared to controls and

by normal fasting adrenal ascorbic acid. Fasting serum glucose was also normal (approximately 94 mg% for treated animals and 87 mg% for controls). No hyperglycemia was apparent as with ether. Ether may increase blood sugar as much as two-fold (362). All t-values for the above responses were also low.

The only parameter that approached significance was that of serum free fatty acids. The fasting levels for both groups were normal (0.69 ueq/ml for methoxyflurane treated animals and 0.88 ueq/ml for the untreated rats).

There does not seem to be any contraindication to the use of methoxyflurane. As an added precaution, control animals were always treated identically to parabionts whenever methoxyflurane was used.

Table 60

ADRENAL WEIGHT (mg) - SPRAGUE-DAWLEY MALE RATS

A Metofane	B No Metofane
53.0	61.9
43.0	51.1
31.8	47.6
39.1	46.5
52.9	54.1

SIGNIFICANCE

Comparison of Means	n	df	Mean ± S. D.	Variance	t	P
AB	5, 5	8	44.0 ± 9.1, 52.2 ± 6.2	83.5, 38.1	1.679	NS

Table 61

ADRENAL WEIGHT / 100 g BODY WEIGHT (mg) - SPRAGUE-DAWLEY MALE RATS

A Metofane	B No Metofane
23.36	20.98
18.86	20.20
14.59	19.19
16.64	20.95
21.50	21.64

SIGNIFICANCE

Comparison of Means	n	df	Mean ± S. D.	Variance	t	P
AB	5, 5	-+	18.99 ± 3.54, 20.59 ± .93	12.55, .87	.977*	NS

*Approximate t-test

+Weighted critical values calculated

Table 62

VITAMIN C (ug) / mg ADRENAL WEIGHT - SPRAGUE-DAWLEY MALE RATS

A Metofane	B No Metofane
1.9813	1.8989
1.9222	1.9506
1.8540	2.9709
2.5045	1.7764
1.2534	2.3851

SIGNIFICANCE

Comparison of Means	n	df	Mean ± S. D.	Variance	t	P
AB	5, 5	8	1.9031 ± .4452, 2.1964 ± .4901	.1982, .2402	.991	NS

Table 63

SERUM FREE FATTY ACIDS (ueq. / ml) - SPRAGUE-DAWLEY MALE RATS [1]

A Metofane	B No Metofane
.488	.940
.728	.770
.885	.893
.563	.790
.768	.990

[1] Animals were fasted for 17 hours.

SIGNIFICANCE

Comparison of Means	n	df	Mean ± S. D.	Variance	t	P
AB	5, 5	8	.686 ± .160, .877 ± .095	.023, .009	2.288	.100

Table 64

GLUCOSE IN ASSAY MEDIUM (conc. mg %) - SPRAGUE-DAWLEY MALE RATS

A Metofane	B No Metofane
9.00	7.50
6.55	6.75
8.00	7.35
7.40	6.55
8.20	7.55

SIGNIFICANCE

Comparison of Means	n	df	Mean ± S. D.	Variance	t	P
AB	5, 5	8	7.83 ± .92, 7.14 ± .46	.84, .21	1.506	NS

PART 10

METABOLIC CONSEQUENCES OF AD LIB. DIFFERENTIALLY FEEDING HIGH FAT AND GRAIN RATIONS TO PARABIOTIC RATS

INTRODUCTION

It has been demonstrated that the food consumption of a grain fed parabiotic rat, cross-circulating with a rat eating a high fat ration, is markedly depressed. It is indicated that some "systemic factor" from the high fat fed animal might be causing the inhibition since only blood factors can cross the parabiotic union. Since the grain fed animal ate less food, one can eliminate any gastrointestinal distension element in causing the inhibition.

Circulating factors affecting food intake have been described in the literature. Glucose and an unidentified lipostatic factor have been the bases for two widely publicized and controversial theories, viz., the glucostatic (173) and the lipostatic theories (256).

As reviewed previously in the general literature review of this thesis, several factors affecting food intake, that can be perfused, have been demonstrated in brain tissue. Islets of Langerhans were dissected from the mouse pancreas and were bathed with effluent which had passed over pieces of brain either from the medial or lateral hypothalamus. Beta cells released insulin when bathed with effluent from the ventrolateral hypothalamus only (260).

In the cross-perfused cat, electrocortical activation was produced in one animal after bulbar reticular formation stimulation in the other. This effect has been attributed to the release of a humoral factor (266). A factor released by the brain has been demonstrated which

stimulates gastric contraction (267). Neurohumoral factors were also demonstrated as being released from the hypothalamus of cross-perfused monkeys. Perfusate was collected from hypothalamic brain areas in starved monkeys and perfused into similar brain areas of sated monkeys. After perfusion, the satiated monkeys began eating. If the donor monkey was fed, there was no effect. Perfusate from the ventromedial area of a fed monkey inhibited feeding in a deprived monkey when perfused into its ventromedial nucleus. Perfused areas that stimulated eating were from the periformical regions. Adrenergic like compounds may be involved in the response (268, 269).

The involvement of general systemic hormones in the control of food intake was suggested in the early 1900's (271). The experimental evidence upon which the hypothesis was based is as follows: Blood taken from food deprived dogs increased stomach contractions in fed dogs after intraperitoneal injection. The converse has also been reported (272). Even as late as 1964, Jefferson et al. (267) have demonstrated a brain humoral factor that activates gastric motility. The problem is that gastric motility is a poor index of hunger and subsequent food intake.

Siegel and Taub (273) in 1952, tested once again the hormone hypothesis. Rats were trained to eat their food in two-hour intervals. After intraperitoneal injection of blood serum from food deprived or satiated rats, food intake was unaltered. This obviously was a better means of measuring hunger than gastric contractions. In 1954, Siegel and Dorman (274) administered orally to 24 hour starved rats, blood serum from food deprived donors or food sated rats. Food was then offered. Intakes were not different from controls in either group.

At this time the hormone hypothesis was seriously doubted. In 1965, Davis (275) reported that humoral satiety factors are not present immediately after the termination of a meal and, therefore, are not involved in limiting meal size. Siegel did not get a response possibly because he collected blood too soon after feeding his rats.

In 1967, Davis and coworkers (276) reported the existence of a food controlling hormone on the basis of rat experiments. When he injected a mixture of blood obtained from hungry and satiated rats, there was a 50% reduction in the intake of the hungry rats. Food intake was not reduced if the donor rat was also deprived or meal fed for 30 minutes immediately before transfusion. No evidence for a hormonal factor increasing intake was found. Davis and his colleagues concluded that satiety factors gradually accumulate in animals fed ad lib. and disappear with fasting (276, 277). Davis was also able to obtain a dose response by fasting his fed rats for one to five hours and injecting this increasingly "deprived blood" into starved rats and observing a slow increase of food intake to normal levels (278). The electrical cortical graph of hungry rats transfused with "satiated blood" displays the high amplitude, low frequency typical of sated animals (279).

In continuous or intermittent cross-circulation of monkeys, using arterial-venous shunts, blood mixing of a deprived monkey produced no change in intake of an eating monkey and the intake of a deprived monkey was not affected by blood from a feeding monkey. The fed monkeys lost only slight amounts of body weight, whereas, the starved monkeys lost significant amounts (280).

At the adipose tissue level, inhibitors of epinephrine stimulated

lipolysis have been reported in hypothalamically lesioned rats (281, 282).

Experiments with parabiosis have been somewhat contradictory but also hampered by problems in methodology. Hervey (283), in 1959, studied food intake in parabiotic pairs of rats with one member of each pair being hypothalamically lesioned in the ventromedial nuclei. The rats with lesions became obese and hyperphagic while their partners exhibited signs of anorexia and became thin. It was concluded that the normal rat's hypothalamus responded to factors circulating over from the overfed animal and demonstrated a feedback system in food intake regulation. Wei Han et al. (284) and Fleming (285) carried out similar experiments and found only slight decreases in intake of the non-lesioned partners. Hervey's rats were fed ad lib., whereas, Wei Han's and Fleming's animals were placed in a very restricted feeder and conditioned to eat in a limited time. When one rat in a pair was starved, the intake of its partner did not increase (284, 286). These results agree with those of Davis in that there does not seem to be a hormone initiating eating but one that signals satiety (276, 277).

Many genetically obese mice and rats have been parabiosed to lean littermates. The obese hyperglycemic mouse has been parabiosed by Haessler and Crawford (287). The lean member of the pair lost weight and the obese gained, but the lean animals of this experiment did not refuse food as in Hervey's work. Adipose tissue from these animals still resembled "lean" and "obese" characteristics in their respective animals. The obese hyperglycemic mouse is hyperphagic (288) without electrolytic brain lesions, but individual food intake could not be measured.

Yellow obese mice are also hyperphagic (288) and have been parabiosed to lean littermates by Weitze (289) and later by Wolff (290). Weitze found that the lean member of a pair kept the Yellow mouse from becoming fat and concluded that the etiology of the obesity was endocrine. Wolff found opposite results with parabiosis having no effect on the weight gains of either the Yellow obese or the lean mouse. Neither investigator reported food intake data.

One of the most interesting parabiosis studies was done with the genetically diabetic mouse (db/db) (291). The db/db mice are the most hyperphagic of any of the genetic obese strains eating approximately twice the intake of normal, lean mice. In this respect the hyperphagia resembles very closely the hyperphagia of the hypothalamic obese mouse or rat. There was no effect on the diabetes when the diabetic was parabiosed to a normal littermate and the normal did not contract the disease but, as in many other studies mentioned (280, 281, 283) the lean normal partners lost weight, were hypoglycemic and died. The obese, hyperphagic gained weight and eventually died as a result of the death of its partner.

George Bray (292), in 1968, parabiosed the Zucker strain of obese rat and found no effect of parabiosis on the weights of the partners. Only three pairs were done, however, and no mention was made as to testing the efficiency of the unions. Also, parabiosis was performed at six weeks of age when the obese rats could be identified and, quite conceivably a circulating substance could have been involved in the syndrome prior to this time. The Zucker rat is hyperphagic (288) but Bray made no mention of food intake in his study.

Hausberger (293, 294), after parabiosing obese hyperglycemic mice,

also reported weight suppression in the obese mouse with the complete prevention of obesity in some. After separation, all obese mice gained weight quickly. Adipose tissue from obese animals, when successfully grafted to lean animals, acquired characteristics of lean adipose tissue and vice versa.

Fleming (285) reported, in a limited number of pairs, similar results as Hausberger. One member of a parabiotic pair was ventromedially lesioned but did not become obese or hyperphagic until after separation from the normal rat. Fleming termed these animals "inhibited hyperphagics." Fleming was also able to show inhibition of feeding in normal parabiotic pairs. One rat was fed for 2 hours. Both rats were fed for a third hour and for the remaining 2 hours of the 5 hour period the second rat was fed alone. The animal fed last decreased its intake in about a 3 week period. The effect was also reversible (285). Three investigators were able to inhibit the development of obesity in three different experimental models, viz., Weitze (289) in 1940, using the Yellow obese mouse; Hausberger (293) in 1958, using the obese hyperglycemic mouse; and Fleming (285) in 1969, using hypothalamically lesioned obese rats.

Other time lapse feeding trials were conducted by Schmidt (286) and Schmidt and Andik (295). One rat of a pair was fed for 2 hours. For the next 2 hours no animal received food and for the fourth to sixth hour the second animal was fed. Contrary to Fleming's results, no significant depression of intake was seen in the animal fed last, but a slight decrease was evident from the data. Fleming and Schmidt used Han's restrictive feeder (284).

As stated above, food intake depression has been demonstrated in

grain fed female parabionts cross-circulating with high fat fed partners. It then became of interest to determine the blood metabolite pattern of these grain fed animals. This was done as a means of evaluating the possible role played in this phenomenon by various blood components.

TECHNIQUES

Adult, male Sprague-Dawley rats were used. Males were employed in order to avoid any effects of the estrus on the various biochemical parameters measured. Since it is difficult to obtain good male parabiotic pairs (see Causes of Death in Parabiotic Rats, this thesis), a limited number were available for experimentation; consequently, the control parabiotic pairs (both members of a pair fed either high fat or grain ration) had to be eliminated to increase sample size in other groups. Animals were operated on when 1-2 months old and were used in experimentation upon reaching 3-4 months of age. All single controls were sham operated at the time of parabiotic surgery.

The four remaining experimental groups included: 1) Single high fat fed controls; 2) Single grain fed controls; 3) High fat fed parabionts attached to grain fed partners which comprised group 4. All animals were fed grain ration from day 1 through 10 to allow parabiotic adjustment to the differential feeders. The high fat ration was offered to group 1 from days 11 to 35. Groups 2 and 4 were fed grain continuously. Group 3 was fed high fat from day 11 to day 17; grain from day 17 to 24; and high fat from day 24 to 35. These days are indicated on Figures 43-50.

A group was pair-fed to group 4 (grain fed parabionts cross-circulating with high fat fed animals) since intake in this group was inhibited as in previous experiments. These animals were indicated as group 5 on data tables. The pair-fed group, by necessity, was started after the depressed levels of intake in group 4 were known. Five pair-fed animals were used, one pair-fed to each of the animals in group 4. These rats approximated the same weights as those in group 4 (261 grams vs. 272, respectively). Controls were included with the pair-fed group since pair feeding started after the "main" experiment. Food intake values for group 4 from days 24 to 35 (11 days) were used for pair feeding. These were the 11 days when the partners of group 4 animals (group 3) were fed the high fat ration. The same absolute amounts of food for each of these 11 days as consumed by each animal in group 4 was offered to the corresponding pair-fed animal in group 5. This would approximate the same suppressed levels without parabiosis and the effect of parabiosis should readily be seen.

Even with the extremely small amount of food fed each day to the pair-fed group and even with the use of food saver rings, tiny amounts of food were still spilled but statistically the amounts of food consumed were the same as the amounts offered.

Animal room conditions, surgical techniques and feeding techniques were those previously reported for parabiotic animals (see Parabiotic Rats Differentially Fed Grain and High Fat Rations; An Ad Lib. Differential Feeder for Parabiotic Rats; and Parabiotic Surgical Procedures; respectively, for these techniques).

Animals were differentially fed until a suppression of intake was clearly evident in group 4 and maintained over a reasonable length of

time (8 days). Rats were then anesthetized with methoxyflurane inhalation anesthetic.[1] A blood sample was taken by heart puncture, with ammonium heparin as anticoagulant, for the assay of insulin, followed by separation of the parabionts. All animals (single controls and separated parabionts) were then weighed and decapitated. Animals were alternated when sacrificed so that every other pair would be parabiotic. Blood was collected in disposable centrifuge tubes immersed in ice water. All assay work except insulin was done on serum.

The blood clot was loosened from the sides of the centrifuge tube by a wooden probe. The blood was then centrifuged for approximately 10 minutes at 3000 rpm. Aliquots of serum for each assay were stored in one dram bottles at -40 degrees Fahrenheit.

Thyroid, adrenal and testicular weights were taken with the adrenals stored in 5 ml of 6% trichloroacetic acid at -40 degrees Fahrenheit for subsequent ascorbic acid analysis.

Adrenal ascorbic acid, serum free fatty acids, serum urea nitrogen, serum glucose, plasma insulin and fecal chromium sesquioxide analyses were modified and performed as described elsewhere in this thesis (see Energy Metabolism for all procedures except chromium sesquioxide for which one should check the section entitled An Ad Lib. Differential Feeder for Parabiotic Rats).

RESULTS

Food intake plots are shown in Figures 43-50. Figure 43 is unadjusted for body weight while Figures 44 through 50 are adjusted.

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[1] Purchased under the brand name Metofane (Pitman-Moore, Div. of Dow Chemical Co., Fort Washington, Pa. 19034) Methoxyflurane chemically is 2, 2-dichloro-1, 1-difluoroethylmethyl ether with 0.01% butylated hydroxytoluene as preservative.

Figures 43 and 44 show all four groups while Figures 45-50 show all 6 possible combinations of 2 groups in order that individual relationships can more easily be seen. Pair-fed intakes were not plotted as they are the same as group 4 and would complicate the figure. All points on food intake plots represent food intake averages per animal per day.

As can be seen from Figure 44, parabiotic animals adjusted to the differential feeder within 4-5 days. During the control period from days 4 through 10, all group comparisons were statistically the same, except comparisons 1, 2 and 2, 3, because group 2 was slightly high. It is not known why the feed intake for group 2 was high but, as seen in Figure 44, consumption does decrease on day 14 and remains fairly steady for the rest of the experiment with the usual slightly downward slope for adjusted intakes. Group 2 was the grain fed control group and a good baseline was only needed after day 14 (specifically from days 24 to 35 during the final differential feeding). Adjusted control intakes (days 4-10) (average grams/animal/day) for the four groups are as follows: group 1 (4.65 g), high fat, single, control; group 2 (5.26 g), grain, single, control; group 3 (4.66 g), high fat, parabiont; group 4 (4.91 g), grain, parabiont.

Through day 10, all rats were fed the grain ration. On day 11, the high fat diet was fed to groups 1 and 3. Group 4's intake began to fall and reached very low values for days 14 and 15. Group 3 (high fat fed parabionts cross-circulating with grain fed partners) fell far more rapidly than group 1 (single high fat controls).

It was of interest to see what would happen to group 4's intake if their partners (group 3) were placed back on grain ration. Day 17

represents the first intake value for group 4 after group 3 had been placed back on grain ration. For days 17 to 23, a slight augmentation above control values did occur in group 4's intake (Figures 43 and 44). Control values (per 100 grams body weight) for group 4 (days 6-10 inclusive) compared to values from days 17 through 23 were not different statistically ($P > .05$). The "means" for the control and recovery periods are 5.00 and 5.82 grams per 100 grams body weight, respectively. This is slightly over a 16% increase but significance was lost due to the large variance. Similar food intake augmentation after deprivation has been reported in non-parabiotic rats (26-28). Food intake suppression in the present study could have been due to an "anorexigenic factor" (possibly a nutrient) crossing-over from parabionts in group 3 (high fat fed) to their grain fed partners (group 4).

Group 3 was then placed back on high fat and day 24 shows this first high fat intake. This time group 3 was fed high fat until the end of the experiment. During the last 8 days of the experiment, statistical analyses were done in order to compare the different levels of intake for the four groups. As with female animals, group 4 (grain fed animals cross-circulating with a high fat fed animal) showed a similar food intake depression (36.5%). All curves from day 28 through day 35 were statistically different (Figures 43-50) for all 6 comparisons except 1, 3.

A couple of days prior to scarifice, the differential feeders were checked for proper usage with a 1% dietary tag of chromium sesquioxide (for technique description, see section on an Ad Lib. Differential Feeder for Parabiotic Rats, this thesis). There was, statistically, no crossage of the marker from one side of the feeder to the

other indicating that the parabionts were consuming their correct diets ($P > .05$).

Since intakes looked quite stable for about a week, the animals were sacrificed on day 35. After euthanasia, thyroid, adrenal and testicular weights were determined. Organ weight data are expressed absolutely and as a percentage of body weight. Absolute organ weights probably are more accurate than relative weights since there are disproportinate body weight to organ weight changes in parabiosis and pair feeding (Tables 65-67). For example, Table 67 shows thyroid weights, both adjusted and unadjusted, for the pair fed group and its controls. The absolute thyroid weights are statistically the same for both groups but, on a body weight percentage, the pair fed animals had significantly higher weights. The pair fed group lost an average body weight per animal of 67 grams during the paired feeding period. This loss in body weight, disproportionate to loss in organ weight, explains the significance at the adjusted levels. The subsequent discussion will focus on group 4 (grain fed parabionts cross-circulating with high fat fed partners) in which food intake was suppressed.

In no case is any comparison of means between thyroid weights different for either unadjusted or adjusted values (Tables 65-67). On an absolute weight basis (Table 65) all mean comparisons were non-significant except comparisons 1, 4 and 3, 4, because of a slight but non-significant elevation of thyroid weights in the high fat fed animals and because the thyroids for group 4 were at the light end of the normal range. This assumption was verified by the observation that group 4's thyroid weights were not different from group 2 (the single grain fed controls) and are not different from either the pair fed rats

(group 5) or the controls for group 5 as shown on Table 67. The average absolute thyroid weight for group 4 is 16.2 mg and is almost identical to the pair fed animal's (group 5) at 16.0 mg and the pair fed controls, 16.4 mg. This, in addition to group 4's thyroid weights being in the normal range (328), indicates that the experiment produced no detrimental effect on thyroid weights and that thyroid function was not altered severely enough to change thyroid weight.

The only adrenal weight comparisons that were different, expressed absolutely or as a percentage of body weight, were 1, 3 (single high fat vs. parabiotic high fat) and 2, 3 (single grain vs. parabiotic high fat) (Tables 68-71). The highest adrenal weights were found in the parabiotic high fat animals. Perhaps the parabiosis combined with the high fat to produce an additive effect. These adrenal weights were not hypertrophied, however, and were in the normal range (364).

In the food intake suppressed rats (group 4), the adrenal weights were statistically the same as all other groups whether expressed absolutely or as a percentage of body weight. Absolute adrenal weights for the pair fed group versus their own controls (Table 70) were statistically the same but were different expressed on a percentage of body weight. This difference is basically the result of a loss in weight of the pair fed group.

Even with the slight difference noted in adrenal weights, adrenal ascorbic acid comparisons were all non-significant (Tables 72-73); consequently, "stress" was not a component in food intake responses. Adrenal ascorbic acid values are in agreement with those previously reported for male Spargue-Dawley rats (359). Slight variances in adrenal ascorbic acid will be noted between different investigators as a result

of differences in analytical procedures and experimental design, e.g., in animal room environment.

Testicular weights were the same on an absolute basis with comparisons 1, 3 and 2, 3 showing differences per 100 grams body weight. Both of these comparisons were different because the testicular weights of the high fat parabionts (group 3) were slightly elevated. Group 4 exhibited testicular weights the same as controls (comparison 2, 4).

As seen from Table 76, high fat fed animals had significantly higher levels of serum free fatty acids as compared to grain fed rats. This confirms other observations on the effect of lipemia and lipoprotein lipase on free fatty acid levels in blood (357, 365). Group 4 (the food depressed rats attached to high fat fed rats or group 3) (see Table 76) had almost exactly the same levels of post-prandial serum free fatty acids as group 2 (single grain fed controls). Group 4 had one-half the free fatty acid levels as group 5 (the rats pair fed to group 4). The elevated free fatty acids, in group 5, may be partially explained by the low blood insulin levels, since that hormone inhibits the release of fat depot free fatty acids. Carbohydrate intake was also very low in group 5 and, with the decreased L-α-glycerol phosphate, free fatty acid release from depot fat should be enchanced (366).

Growth hormone, glucagon, glucocorticoids, ACTH, TSH, epinephrine andnorepinephrine all stimulate free fatty acid release in hypoglycemia (366-368). Once high levels of free fatty acids are obtained, stimulated initially by low levels of blood glucose, free fatty acids can produce an anti-insulin effect by inhibiting the entry of glucose into muscle including the heart (366). The net effect is to supply energy

for muscle in the form of free fatty acids and to spare glucose for use by the nervous system. There are many other hormone interactions in the metabolic response to hypoglycemia, e.g., many of the above hormones stimulate gluconeogenesis as well as glycogenolysis. This is shown by epinephrine's inhibition of insulin secretion (366). Minute amounts of epinephrine are effective in that system since its effect is greatly amplified by the adenyl cyclase system (389). Growth hormone can be anti-insulin by decreasing glucose transport and utilization (glycolysis) in muscle (366, 370). Typical hypoglycemic responses were not seen in group 4 even with the food intake decreased by almost one-half. Free fatty acid levels for group 4 were "normal" for a grain fed post-prandial state (371) and were not elevated even with cross-circulation from a hyperlipemic rat (see comparison 2, 3; Table 76). The relatively low blood exchange rates in parabiotic animals (283) (see also Dye Dilution Studies, this thesis) and the rapid clearance rate of free fatty acids from blood (371), probably account for the failure of free fatty acids from the hyperlipemic rat to appear in the grain fed rat.

Pair fed animals exhibited serum free fatty acid levels far higher than their controls[2] (Table 77) but, as expected, the variability was so high that significance was lost, i.e., since animals were fed widely varying amounts of food, the serum free fatty acid levels would reflect the level of food deprivation and, consequently, the variance between different rats would be extreme.

Significance was lost due to this "essential" variance. Since the statistical test used did not realistically reflect the difference

[2] By necessity the pair fed group was started after the beginning of the experiment when levels of food intake for pair feeding were known; consequently, a specific control group was employed with the pair fed group.

between the two means which was quite large (Table 77), the data were paired and statistically analyzed by a "sign" test or corrected chi-square. The assumption was made that pair feeding or a decreased intake would increase serum free fatty acid levels, consequently, the pair fed values (Table 77) were designated "treatment 1" and the control values in Table 77, "treatment 2." Pairing was done so that pair one would constitute the largest number in each group, the second largest in each group pair 2, etc. The five pairs were then substracted (treatment 1 minus treatment 2). In every pair, the pair fed group exhibited higher free fatty acid levels, i.e., the sign values were 5 (+) and 0 (-). On a sign test, pair fed animals exhibited a marked increased tendency or trend in serum free fatty acid levels ($P < .05$).

Table 76 shows the comparison of group 4 and group 5 ($P < .10$). Group 4 (the food depressed group) has about one-half the level of serum free fatty acids. Both of these groups of animals received the same amounts of food but since each group was fed at a different point in time in addition to other variables extremely hard to control such as, exact animal weights and ages at the beginning of the experiments, the best comparison between groups 4 and 5 is the difference for each group between experimental and control values. Group 2 values (the single M-1 fed controls for group 4) were subtracted from group 4 and the specific pair fed control values were subtracted from the pair fed group. These differences were taken after pairing as previously described, followed by statistical analysis with a sign test. The serum free fatty acids were elevated to a greater extent in the pair fed group ($P < .05$) than in the controls.

To summarize, free fatty acid levels in the food depressed group

(group 4) did not follow the typical trends of food deprived animals. Serum free fatty acids of group 4 were statistically as well as almost absolutely identical to the ad lib. grain fed single control animals (group 2). If group 4 had been metabolically deprived by a low food intake, serum free fatty acid levels would have been elevated.

Several sources of error could not be avoided in pair feeding: 1) It was not evident that a pair fed group would be needed until after feeding experiments had begun; consequently, new animals had to be ordered. They were obtained from the same breeding farms but the original stock was still not represented in the pair fed group; 2) In order to pair body weights with group 4, younger animals were ordered; 3) With present equipment it is not possible to pair feed with the same periodicity as the intake in group 4; and 4) Control intakes of the pair fed group were a little higher than control intakes for group 2 or group 4. This was noticed only after data tabulation at the termination of the experiment. Data were only reviewed at the end of experiments in order to avoid "investigator bias," while collecting data. Absolute intakes and body weights were paired quite well between the pair fed group and group 4 during the experimental phase of the feeding trials. It still seems reasonable, though, to expect the free fatty acid levels to be higher in group 4 based only on their absolute food intake.

Serum urea nitrogen was normal for all groups with group 4 having statistically the same level as group 2 despite the depressed food intake (Table 78). Starvation in rats greatly increases urine urea excretion within approximately a day (372).

Comparisons of groups 2 and 3 and 3 and 4 for blood urea levels

are statistically different because group 3 has a relatively low value compared to other groups possibly due to transfer of either urea or amino acids to the food depressed group (group 4) as indicated by group 4 having higher levels compared to group 3. As noted on Table 78, serum urea in group 4 was the same as in the pair fed group. The difference between group 4 and their controls (group 2) compared with the difference between the pair fed rats and their controls was also non-significant whether tested by paired or pooled t-statistics or a sign test as described earlier. With any major involvement of amino acid gluconeogenesis, serum urea levels would have been much higher. There was no difference between the pair fed group and their controls. The decreased urea production caused by a decreased intake possibly was offset equally by an increased urea production associated with gluconeogenesis (Table 79). Gluconeogenesis from glycerol probably was of greater importance since glycerol levels would be elevated with an increased fat depot mobilization of free fatty acids.

The pair fed group was food deprived, consuming less than half their normal intake; nevertheless, a reasonable degree of dietary foodstuffs were still present with metabolic changes offsetting an energy or glucose deficiency not nearly as pronounced as in starvation. The length of deprivation was just ten days. It is conceivable that during this time gluconeogenesis, from glycerol, with subsequent glycogen synthesis and glycogenolysis would have been adequate to maintain these animals in reasonable balance without the involvement of ACTH-corticoid activation. It is evident from the adrenal vitamin C levels (Tables 72-73) that the ACTH-corticoid system was not activated with ascorbic acid levels within normal ranges. With the mobilization of

depot fat for added energy by growth hormone, glucagon and, to a lesser extent, catecholamines, the protein consumed would be spared. Growth hormone also increases free fatty acid movement into muscle. An increase in serum urea nitrogen would be contraindicated by the lack of the ACTH-corticoid system and by dietary protein sparing; consequently, all serum urea values were statistically the same except for the two comparisons discussed.

Plasma insulin (Tables 80-81) tended to be lower in the parabiotic animals fed the high fat diet, although, the comparison between single control groups (comparison 1, 2) was non-significant. Other investigators have reported no statistical difference in plasma insulin between high fat fed rats compared to grain fed controls (325). Injection of a fat emulsion in humans, intravenously, caused no change in insulin levels (373). Plasma insulin levels in parabiotic high fat fed rats (group 3) were not statistically different from control high fat fed rats but were statistically lower than grain fed rats (comparison 2, 3). Insulin levels for group 4 approximated very closely the levels in group 3. There was a tendency for lower levels in high fat fed animals, probably due to lower carbohydrate intakes, the lower food mass in the gastro-intestinal tract and the greater retention of the high fat diet in the stomach. Groups 3 and 4 were slightly lower, for reasons to be discussed later, while group 2 was higher than all other groups. The extent of this difference reached significance in comparisons 2, 3 and 2, 4. All other comparisons were non-significant. The values in group 2 were high probably due to the ad lib. consumption of a high carbohydrate diet with the grain ration containing 53.5% carbohydrate compared to only 6.5% for the high fat ration (374).

Insulin levels in groups 3 and 4 approximated each other. The lower initial secretion in group 4 was expected since oral carbohydrate intake was decreased. Gastro-intestinal hormone secretion would also be decreased with the lower food intake. Gastro-intestinal hormones have been shown to be stimulators of insulin secretion (161, 162, 375). There was no statistical difference in plasma insulin levels between the pair fed values with those of group 4 but group 5 did have lower absolute values. By comparing the differences between the control group for group 4 and group 4, with the difference between the control group of the pair fed animals and the pair fed group (paired as previously described) a significant difference was obtained both by paired-t and sign tests ($P < .05$), the pair fed group having a lower trend in plasma insulin levels.

In summary, insulin levels in group 4 were higher than in pair fed animals but lower than single grain controls (group 2). Plasma insulin in group 4 approximated the levels in their high fat fed partners, group 3. Insulin could cross from group 3 to group 4 or vice versa since insulin can leave the pancreas by the lymph (activity is very high in the thoracic duct) and crosses to the other parabiont without passing through the liver. Insulin present in the interstitial fluid could also cross more readily from one parabiont to the other (366). Plasma insulin for the pair fed group was lower than their controls as a result of a lower carbohydrate or protein intake (Table 81). The insulin levels for groups 1, 3 and 4 are all normal values for moderate to low carbohydrate intakes (349).

All glucose values are expressed in mg% for a constant volume of Somoygi filtrate (Tables 82-84). To approximate mg% in serum, all

values are multiplied by 12 but for statistical comparison of means this is not necessary. Fasted blood glucose levels were significantly lower than controls (Table 83). This comparison was made as a check on the glucose assay system. Fasting serum levels were calculated to approximately 94 mg%. Rats pair fed grain had levels of 175 mg% with single animals fed grain ad lib. averaging 201 mg%. These glucose values agree well with those previously reported (349, 350). There was a decrease in serum glucose in the pair fed group, as shown above, that approached significance (Table 84); whereas, the food depressed group (group 4) had almost identical glucose levels as both the single grain controls (group 2) and the controls for the pair fed group.

By comparing the difference between the controls for group 4 and group 4 with the difference between the controls for the pair fed group and the pair fed animals, one obtains a paired-t statistic of 2.258 which misses $P < .05$ by only .048 and one obtains by a sign test ($P < .05$) indicating a lower trend in blood glucose in the pair fed group as opposed to group 4. It does appear that serum glucose levels are higher in group 4 (food depressed group) than what would be expected by looking at the pair fed data.

DISCUSSION

The ad lib. differential feeder was very efficient in permitting each animal to consume its own diet as evidenced by chromium sesquioxide dietary markers.

Analysis of adrenal ascorbic acid indicated that changes in food intake were not a result of "stress" as resulting from parabiosis or

use of the differential feeder.

Food intake was suppressed in grain fed animals (group 4) attached to high fat fed animals (group 3) compared to control values for group 4. This suppression occurred with blood urea, glucose, and free fatty acid levels statistically the same as single grain ad lib. fed controls. Two factors can probably be ruled out as producing this effect: 1) Insulin and 2) Gastro-intestinal distension. Insulin levels were normal for group 4 but, since the absolute amount of carbohydrate consumed was about half of that consumed by group 2, insulin levels were depressed. Insulin levels in groups 1 and 3 were statistically the same as those in group 4 with groups 1 and 3 consuming more calories. Lower insulin levels (compared to group 2), therefore, would not explain the depressed consumption in group 4.

Gastro-intestinal distension would seem unlikely as having produced the depressed intake in group 4 since these animals were consuming one-half or less of their control consumptions.

Several factors could explain the intake suppression in group 4. Glucose levels were the same in group 4 as group 2 indicating a possible cross-over from group 3. Free fatty acids, crossing over from group 3, could have affected the energy balance of group 4. Since free fatty acids and glucose are cleared so rapidly relative to cross-over rates, it is difficult to demonstrate elevated levels but these compounds could cross the union, be absorbed quickly, and alter energy balance.

An anorexigenic substance liberated by the gut, stimulated by the presence of fat, could have crossed over from group 3 to group 4 inhibiting intake in the grain fed animal. If this were the only mechanism, the "blood pattern" in group 4 should have approximated a

semi-starved state as opposed to the well fed condition observed.

As evidenced by the experiments presented, there does seem to be an independent regulation of body fat as well as food intake but this involves a very complex interrelationship. When both parabionts in a pair are eating a low fat grain ration, their food intake is statistically identical to single sham operated controls but their body fat content is lower. One must conclude that body fat was acted upon independently of food intake. Grain fed animals cross-circulating with high fat fed rats, have a further reduction in body fat probably caused by the reduced food intake. It seems reasonable to conclude that the depressed food intake caused the reduction in body fat (and not the converse) since the response was so rapid. The system becomes very complex if the anorexigenic factor coming from the high fat fed animal is one that contains calories which would tend to counter the reduction in body fat already present in parabiosis. It has also been shown that in non-parabiotic preparations, alterations in body fat content may produce changes in food intake or food efficiency (261-263, 257).

Table 65

THYROID WEIGHT (mg) - SPRAGUE-DAWLEY MALE RATS

1 Single High Fat	2 Single M-1	3 Parabiotic High Fat	4 Parabiotic M-1
28.0	19.6	22.4	18.8
27.3	24.7	19.1	19.7
18.4	18.3	19.1	13.1
20.0	21.4	20.1	13.7
	19.9	25.1	15.9

SIGNIFICANCE

Comparison of Means	n	df	Mean ± S. D.	Variance	t	P
1,2	4, 5	7	23.4 ± 4.9, 20.8 ± 2.5	24.3, 6.0	1.059	NS
1,3	4, 5	7	23.4 ± 4.9, 21.2 ± 2.6	24.3, 6.7	.895	NS
1,4	4, 5	7	23.4 ± 4.9, 16.2 ± 3.0	24.3, 8.7	2.728	.050
2,3	5, 5	8	20.8 ± 2.5, 21.2 ± 2.6	6.0, 6.7	.239	NS
2,4	5, 5	8	20.8 ± 2.5, 16.2 ± 3.0	6.0, 8.7	2.290	.100
3,4	5, 5	8	21.2 ± 2.6, 16.2 ± 3.0	6.7, 8.7	2.803	.050

Table 66

THYROID WEIGHT (mg) / 100 g BODY WEIGHT - SPRAGUE-DAWLEY MALE RATS

1 Single High Fat	2 Single M-1	3 Parabiotic High Fat	4 Parabiotic M-1
6.167	4.465	7.344	5.612
5.594	5.393	5.134	8.312
4.360	4.197	5.685	5.901
4.175	5.784	6.979	4.875
	5.129	7.131	5.579

SIGNIFICANCE

Comparison of Means	n	df	Mean ± S. D.	Variance	t	P
1,2	4, 5	7	5.074 ± .963, 4.994 ± .655	.928, .429	.149	NS
1,3	4, 5	7	5.074 ± .963, 6.455 ± .982	.928, .965	2.113	.100
1,4	4, 5	7	5.074 ± .963, 6.056 ± 1.317	.928, 1.733	1.242	NS
2,3	5, 5	8	4.994 ± .655, 6.455 ± .982	.429, .965	2.767	.050
2,4	5, 5	8	4.994 ± .655, 6.056 ± 1.317	.429, 1.733	1.615	NS
3,4	5, 5	8	6.455 ± .982, 6.056 ± 1.317	.965, 1.733	.543	NS

Table 67

THYROID WEIGHTS (mg) - SPRAGUE-DAWLEY MALE RATS

A and B are unadjusted; C and D are adjusted per 100 g body weight.

Pair Fed		Controls	
A	C	B	D
15.2	5.00	18.4	5.41
15.6	6.72	17.6	4.62
17.6	6.74	17.0	5.00
17.3	6.29	13.6	3.44
14.3	6.11	15.4	3.90

SIGNIFICANCE

Comparison of Means	n	df	Mean ± S. D.	Variance	t	P
AB	5, 5	8	16.0 ± 1.4, 16.4 ± 1.9	2.0, 3.7	.376	NS
CD	5, 5	8	6.17 ± .71, 4.47 ± .80	.50, .64	3.546	.010

Table 68

ADRENAL WEIGHT (mg) - SPRAGUE-DAWLEY MALE RATS

1 Single High Fat	2 Single M-1	3 Parabiotic High Fat	4 Parabiotic M-1	5 Pair Fed to 4
53.2	49.7	59.4	46.6	50.9
53.7	49.9	59.8	62.8	45.3
52.0	50.5	56.3	46.0	42.3
48.9	46.9	53.7	42.4	45.4
	52.8	54.3	47.9	

SIGNIFICANCE

Comparison of Means	n	df	Mean ± S. D.	Variance	t	P
1,2	4, 5	7	51.95 ± 2.15, 49.96 ± 2.11	4.64, 4.45	1.394	NS
1,3	4, 5	7	51.95 ± 2.15, 56.70 ± 2.80	4.64, 8.00	2.770	.050
1,4	4, 5	-+	51.95 ± 2.15, 49.10 ± 7.90	4.64, 62.50	.771*	NS
2,3	5, 5	8#	49.96 ± 2.11, 56.70 ± 2.80	4.45, 8.00	4.279	.010
2,4	5, 5	-+	49.96 ± 2.11, 49.10 ± 7.90	4.45, 62.50	.235*	NS
3,4	5, 5	-+	56.70 ± 2.80, 49.10 ± 7.90	8.00, 62.50	2.024*	NS
4,5	5, 4	7	49.10 ± 7.90, 46.00 ± 3.60	62.50, 12.80	.735	NS

*Approximate t-test
+Weighted critical values calculated
#Degrees of freedom calculated

Table 69

ADRENAL WEIGHT / 100 g BODY WEIGHT (mg) - SPRAGUE-DAWLEY MALE RATS

1 Single High Fat	2 Single M-1	3 Parabiotic High Fat	4 Parabiotic M-1	5 Pair Fed to 4
11.72	11.32	20.63	13.91	16.74
11.00	10.90	17.80	26.50	19.53
12.32	11.58	15.13	20.72	15.38
16.03	12.68	17.61	15.09	19.40
	13.61	15.43	16.81	

SIGNIFICANCE

Comparison of Means	n	df	Mean ± S. D.	Variance	t	P
1,2	4, 5	7	12.77 ± 2.24, 12.02 ± 1.11	5.02, 1.23	.662	NS
1,3	4, 5	7	12.77 ± 2.24, 17.32 ± 2.22	5.02, 4.91	3.048	.020
1,4	4, 5	7	12.77 ± 2.24, 18.61 ± 5.11	5.02, 26.11	2.107	.100
2,3	5, 5	8	12.02 ± 1.11, 17.32 ± 2.22	1.23, 4.91	4.786	.010
2,4	5, 5	-+	12.02 ± 1.11, 18.61 ± 5.11	1.23, 26.11	2.818*	.100
3,4	5, 5	8	17.32 ± 2.22, 18.61 ± 5.11	4.91, 26.11	.516	NS
4,5	5, 4	7	18.61 ± 5.11, 17.76 ± 2.04	26.11, 4.18	.308	NS

*Approximate t-test
+Weighted critical values calculated

Table 70

ADRENAL WEIGHT (mg) - SPRAGUE-DAWLEY MALE RATS

A Pair Fed	B Control
50.9	47.1
45.3	48.9
42.3	48.1
45.4	54.4
	54.7

SIGNIFICANCE

Comparison of Means	n	df	Mean ± S. D.	Variance	t	P
AB	4, 5	7	46.0 ± 3.6, 50.6 ± 3.6	12.8, 13.2	1.918	.100

Table 71

ADRENAL WEIGHT / 100 g BODY WEIGHT (mg) - SPRAGUE-DAWLEY MALE RATS

A Pair Fed	B Control
16.74	13.85
19.53	12.83
15.38	14.15
19.40	13.77
	13.85

SIGNIFICANCE

Comparison of Means	n	df	Mean ± S. D.	Variance	t	P
AB	4, 5	-+	17.76 ± 2.04, 13.69 ± .50	4.18, .25	3.890*	.050

*Approximate t-test

+Weighted critical values calculated

Table 72

VITAMIN C (ug) / mg ADRENAL WEIGHT - SPRAGUE-DAWLEY MALE RATS

1 Single High Fat	2 Single M-1	3 Parabiotic High Fat	4 Parabiotic M-1	5 Pair Fed to 4
3.0470	3.6419	3.0370	2.5730	2.2770
3.3836	2.4749	3.1839	2.7675	2.6468
3.3538	3.2733	3.2860	3.0174	2.2104
2.5133	3.4968	2.6294	2.5212	2.2952
	2.3958	2.7514	2.4656	

SIGNIFICANCE

Comparison of Means	n	df	Mean ± S. D.	Variance	t	P
1,2	4, 5	7	3.0744 ± .4038, 3.0565 ± .5827	.1631, .3396	.052	NS
1,3	4, 5	7	3.0744 ± .4038, 2.9775 ± .2800	.1631, .0784	.426	NS
1,4	4, 5	7	3.0744 ± .4038, 2.6689 ± .2256	.1631, .0509	1.921	.100
2,3	5, 5	8	3.0565 ± .5827, 2.9775 ± .2800	.3396, .0784	.273	NS
2,4	5, 5	-+	3.0565 ± .5827, 2.6689 ± .2250	.3396, .0509	1.387*	NS
3,4	5, 5	8	2.9775 ± .2800, 2.6689 ± .2250	.0784, .0509	1.919	.100
4,5	5, 4	7	2.6689 ± .2256, 2.3574 ± .1964	.0509, .0386	2.175	.100

*Approximate t-test

+Weighted critical values calculated

Table 73

VITAMIN C (ug) / mg ADRENAL WEIGHT - SPRAGUE-DAWLEY MALE RATS

A Pair Fed	B Control
2.2770	2.2548
2.6468	2.7055
2.2104	2.2952
2.2952	2.0092
	2.4333

SIGNIFICANCE

Comparison of Means	n	df	Mean ± S. D.	Variance	t	P
AB	4, 5	7	2.3574 ± .1964, 2.3396 ± .2554	.0386, .0652	.114	NS

Table 74

RIGHT AND LEFT COMBINED TESTICULAR WEIGHT (g) - SPRAGUE-DAWLEY MALE RATS

1 Single High Fat	2 Single M-1	3 Parabiotic High Fat	4 Parabiotic M-1
4.0355	3.8273	3.0464	2.0251
4.2080	3.9564	3.8148	3.5231
3.5896	4.0703	4.0298	3.2576
3.5348	3.6768	3.9797	3.6755
	3.8334	3.4782	3.4447

SIGNIFICANCE

Comparison of Means	n	df	Mean ± S. D.	Variance	t	P
1,2	4, 5	7	3.8420 ± .3314, 3.8728 ± .1484	.1098, .0220	.188	NS
1,3	4, 5	7	3.8420 ± .3314, 3.6698 ± .4099	.1098, .1680	.679	NS
1,4	4, 5	7	3.8420 ± .3314, 3.1852 ± .6658	.1098, .4432	1.786	NS
2,3	5, 5	-+	3.8728 ± .1484, 3.6698 ± .4099	.0220, .1680	1.041*	NS
2,4	5, 5	-+	3.8728 ± .1484, 3.1852 ± .6658	.0220, .4432	2.254*	.100
3,4	5, 5	8	3.6698 ± .4099, 3.1852 ± .6658	.1680, .4432	1.386	NS

*Approximate t-test
+Weighted critical values calculated

Table 75

RIGHT AND LEFT COMBINED TESTICULAR WEIGHT (g) / 100 g BODY WEIGHT
SPRAGUE-DAWLEY MALE RATS

1 Single High Fat	2 Single M-1	3 Parabiotic High Fat	4 Parabiotic M-1
.889	.872	1.058	.605
.862	.864	1.135	1.487
.851	.934	1.083	1.467
.738	.994	1.305	1.308
	.988	.988	1.209

SIGNIFICANCE

Comparison of Means	n	df	Mean ± S. D.	Variance	t	P
1,2	4, 5	7	.835 ± .067, .930 ± .062	.004, .004	2.229	.100
1,3	4, 5	7	.835 ± .067, 1.114 ± .119	.004, .014	4.150	.010
1,4	4, 5	-+	.835 ± .067, 1.215 ± .360	.004, .130	2.313*	.100
2,3	5, 5	8	.930 ± .062, 1.114 ± .119	.004, .014	3.055	.020
2,4	5, 5	-+	.930 ± .062, 1.215 ± .360	.004, .130	1.741*	NS
3,4	5, 5	-+	1.114 ± .119, 1.215 ± .360	.014, .130	.595*	NS

*Approximate t-test
+Weighted critical values calculated

Table 76

SERUM FREE FATTY ACIDS (ueq/ml) - SPRAGUE-DAWLEY MALE RATS

1 Single Control Fed High Fat	2 Single Control Fed Grain (M-1)	3[1] Parabiotic High Fat attached to Parabiotic M-1	4[1] Parabiotic M-1 attached to Parabiotic High Fat	5[2] Single Control Pair Fed Grain to Group 4
.305	.213	.435	.390	.330
.380	.225	.333	.210	.870
.412	.263	.598	.198	.328
.328	.343	.338	.178	.338
	.215	.535	.298	.483

COMPARISON BETWEEN MEANS OF THE VALUES LISTED ABOVE

Comparison of Means	n	df	Mean ± S. D.	Variance	t	P
1,2	4, 5	7	.356 ± .049, .252 ± .055	.002, .003	2.986	.050
1,3	4, 5	7	.356 ± .049, .448 ± .118	.002, .014	1.436	NS
1,4	4, 5	7	.356 ± .049, .255 ± .088	.002, .008	2.044	.100
2,3	5, 5	8	.252 ± .055, .448 ± .118	.003, .014	3.371	.010
2,4	5, 5	8	.252 ± .055, .255 ± .088	.003, .008	.064	NS
3,4	5, 5	8	.448 ± .118, .255 ± .088	.014, .008	2.926	.020
4,5	5, 5	8	.255 ± .088, .470 ± .233	.008, .054	1.927	.100

[1] Groups 3 and 4 represent 5 pairs of parabiotic rats. One member of each pair was fed the high fat ration (group 3) and its partner was fed grain (M-1) diet (group 4).

[2] Since food intake was depressed in group 4, it was necessary to have a group of single rats pair fed, with the same depressed intakes, to group 4.

Table 77

SERUM FREE FATTY ACIDS (ueq/ml) - SPRAGUE-DAWLEY MALE RATS FED A GRAIN (M-1) RATION

A[1] Pair Fed	B[2] Control
.330	.413
.870	.278
.328	.295
.338	.298
.483	.310

COMPARISON BETWEEN MEANS OF THE VALUES LISTED ABOVE

Comparison of Means	n	df	Mean ± S. D.	Variance	t	P
AB	5, 5	-[+]	.470 ± .233, .319 ± .054	.054, .003	1.399[*]	NS

[1] This group of animals is the same as group 5, Table 76, viz., single animals pair fed to group 4.

[2] Since the pair fed group could not be started until a later time when initial intakes for group 4 were known, an ad lib. group of single animals was included as specific controls for the pair fed group.

[+] Weighted critical values calculated

[*] Approximate t-test

Table 78

UREA NITROGEN IN SERUM (conc. mg %) - SPRAGUE-DAWLEY MALE RATS

1 Single Control Fed High Fat	2 Single Control Fed Grain (M-1)	3[1] Parabiotic High Fat attached to Parabiotic M-1	4[1] Parabiotic M-1 attached to Parabiotic High Fat	5[2] Single Control Pair Fed Grain to Group 4
13.44	17.40	14.18	14.75	20.42
13.14	18.75	14.51	16.56	18.34
18.89	15.27	13.38	16.13	14.56
15.96	19.29	13.61	15.90	15.10
	18.75	9.30	17.44	18.11

COMPARISON BETWEEN MEANS OF THE VALUES LISTED ABOVE

Comparison of Means	n	df	Mean ± S. D.	Variance	t	P
1,2	4, 5	7	15.36 ± 2.67, 17.89 ± 1.62	7.15, 2.64	1.768	NS
1,3	4, 5	7	15.36 ± 2.67, 13.00 ± 2.11	7.15, 4.47	1.485	NS
1,4	4, 5	-+	15.36 ± 2.67, 16.16 ± .98	7.15, .96	.569*	NS
2,3	5, 5	8	17.89 ± 1.62, 13.00 ± 2.11	2.64, 4.47	4.107	.010
2,4	5, 5	8	17.89 ± 1.62, 16.16 ± .98	2.64, .96	2.046	.100
3,4	5, 5	8	13.00 ± 2.11, 16.16 ± .98	4.47, .96	3.031	.020
4,5	5, 5	8	16.16 ± .98, 17.31 ± 2.44	.96, 5.95	.978	NS

[1] Groups 3 and 4 represent 5 pairs of parabiotic rats. One member of each pair was fed the high fat ration (group 3) and its partner was fed grain (M-1) diet (group 4).

[2] Since food intake was depressed in group 4, it was necessary to have a group of single rats pair fed, with the same depressed intakes, to group 4.

+ Weighted critical values calculated

* Approximate t-test

Table 79

UREA NITROGEN IN SERUM (conc. mg %) - SPRAGUE-DAWLEY MALE RATS

A[1] Pair Fed	B[2] Control
20.42	17.85
18.34	19.28
14.56	18.00
15.10	17.47
18.11	17.36

COMPARISON BETWEEN MEANS OF THE VALUES LISTED ABOVE

Comparison of Means	n	df	Mean ± S. D.	Variance	t	P
AB	5, 5	-[+]	17.31 ± 2.44, 17.99 ± .77	5.95, .59	.595*	NS

[1] This group of animals is the same as group 5, Table 76, viz., single animals pair fed to group 4.

[2] Since the pair fed group could not be started until a later time when initial intakes for group 4 were known, an ad lib. group of single animals was included as specific controls for the pair fed group.

[+] Weighted critical values calculated

[*] Approximate t-test

Table 80

INSULIN IN PLASMA (micro-units / ml) - SPRAGUE-DAWLEY MALE RATS

1 Single Control Fed High Fat	2 Single Control Fed Grain (M-1)	3[1] Parabiotic High Fat attached to Parabiotic M-1	4[1] Parabiotic M-1 attached to Parabiotic High Fat	5[2] Single Control Pair Fed Grain to Group 4
85.86	101.30	32.72	46.24	42.17
47.84	47.97	47.97	56.13	12.10
49.31	50.14	33.29	25.32	24.41
28.45	84.46	34.25	42.29	33.01
	44.69	32.15	23.02	31.83
	71.68			
	100.09			
	64.66			
	54.32			

COMPARISON BETWEEN MEANS OF THE VALUES LISTED ABOVE

Comparison of Means	n	df	Mean ± S. D.	Variance	t	P
1,2	4, 9	11	52.87 ± 23.96, 68.81 ± 22.02	574.22, 484.88	1.176	NS
1,3	4, 5	-[+]	52.87 ± 23.96, 36.08 ± 6.69	574.22, 44.81	1.359[*]	NS
1,4	4, 5	7	52.87 ± 23.96, 38.60 ± 14.13	574.22, 199.60	1.121	NS
2,3	9, 5	-[+]	68.81 ± 22.02, 36.08 ± 6.69	484.88, 44.81	4.130[*]	.01
2,4	9, 5	12	68.81 ± 22.02, 38.60 ± 14.13	484.88, 199.60	2.746	.02
3,4	5, 5	8	36.08 ± 6.69, 38.60 ± 14.13	44.81, 199.60	.361	NS
4,5	5, 5	8	38.60 ± 14.13, 28.70 ± 11.22	199.60, 125.94	1.226	NS

[1] Groups 3 and 4 represent 5 pairs of parabiotic rats. One member of each pair was fed the high fat ration (group 3) and its partner was fed grain (M-1) diet (group 4).

[2] Since food intake was depressed in group 4, it was necessary to have a group of single rats pair fed, with the same depressed intakes, to group 4.

[+] Weighted critical values calculated

[*] Approximate t-test

Table 81

INSULIN IN PLASMA (micro-units / ml) - SPRAGUE-DAWLEY MALE RATS

A[1] Pair Fed	B[2] Controls
42.17	44.69
12.10	71.68
24.41	100.09
33.01	64.66
31.83	54.32

COMPARISON BETWEEN MEANS OF THE VALUES LISTED ABOVE

Comparison of Means	n	df	Mean ± S. D.	Variance	t	P
AB	5, 5	8	28.70 ± 11.22, 67.09 ± 21.10	125.94, 445.20	3.591	.010

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[1] This group of animals is the same as group 5, Table 76, viz., single animals pair fed to group 4.

[2] Since the pair fed group could not be started until a later time when initial intakes for group 4 were known, an ad lib. group of single animals was included as specific controls for the pair fed group.

Table 82

GLUCOSE IN ASSAY MEDIUM (conc. mg %) - SPRAGUE-DAWLEY MALE RATS

1 Single Control Fed High Fat	2 Single Control Fed Grain (M-1)	3[1] Parabiotic High Fat attached to Parabiotic M-1	4[1] Parabiotic M-1 attached to Parabiotic High Fat	5[2] Single Control Pair Fed Grain to Group 4
19.50	21.50	16.50	16.15	11.40
20.00	17.35	20.45	18.70	14.30
18.25	12.55	17.70	17.15	15.35
19.40	13.50	16.05	16.85	16.00
	16.05	16.65	14.70	16.00

COMPARISON BETWEEN MEANS OF THE VALUES LISTED ABOVE

Comparison of Means	n	df	Mean ± S. D.		Variance		t	P
1,2	4, 5	-+	19.30 ± .74,	16.19 ± 3.54	.55,	12.51	1.913*	NS
1,3	4, 5	7	19.30 ± .74,	17.47 ± 1.77	.55,	3.14	1.902	.100
1,4	4, 5	7	19.30 ± .74,	16.71 ± 1.46	.55,	2.13	3.188	.020
2,3	5, 5	8	16.19 ± 3.54,	17.47 ± 1.77	12.51,	3.14	.723	NS
2,4	5, 5	8	16.19 ± 3.54,	16.71 ± 1.46	12.51,	2.13	.304	NS
3,4	5, 5	8	17.47 ± 1.77,	16.71 ± 1.46	3.14,	2.13	.740	NS
4,5	5, 5	8	16.71 ± 1.46,	14.61 ± 1.92	2.13,	3.70	1.944	.100

[1] Groups 3 and 4 represent 5 pairs of parabiotic rats. One member of each pair was fed the high fat ration (group 3) and its partner was fed grain (M-1) diet (group 4).

[2] Since food intake was depressed in group 4, it was necessary to have a group of single rats pair fed, with the same depressed intakes, to group 4.

+ Weighted critical values calculated

* Approximate t-test

Table 83

GLUCOSE IN ASSAY MEDIUM (conc. mg %) - SPRAGUE-DAWLEY MALE RATS

A Single M-1 Fed	B Single M-1 Fasted
21.50	9.00
17.35	6.55
12.55	8.00
13.50	7.40
16.05	8.20

COMPARISON BETWEEN MEANS OF THE VALUES LISTED ABOVE

Comparison of Means	n	df	Mean ± S. D.	Variance	t	P
AB	5, 5	-+	16.19 ± 3.54, 7.83 ± .92	12.51, .84	5.116*	.010

*Approximate t-test

+Weighted critical values calculated

Table 84

GLUCOSE IN ASSAY MEDIUM (conc. mg %) - SPRAGUE-DAWLEY MALE RATS

A[1] Pair Fed	B[2] Control
11.40	16.85
14.30	16.00
15.35	19.30
16.00	15.65
16.00	16.10

COMPARISON BETWEEN MEANS OF THE VALUES LISTED ABOVE

Comparison of Means	n	df	Mean ± S. D.	Variance	t	P
AB	5, 5	8	14.61 ± 1.92, 16.78 ± 1.48	3.70, 2.18	2.001	.100

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[1] This group of animals is the same as group 5, Table 76, viz., single animals pair fed to group 4.

[2] Since the pair fed group could not be started until a later time when initial intakes for group 4 were known, an ad lib. group of single animals was included as specific controls for the pair fed group.

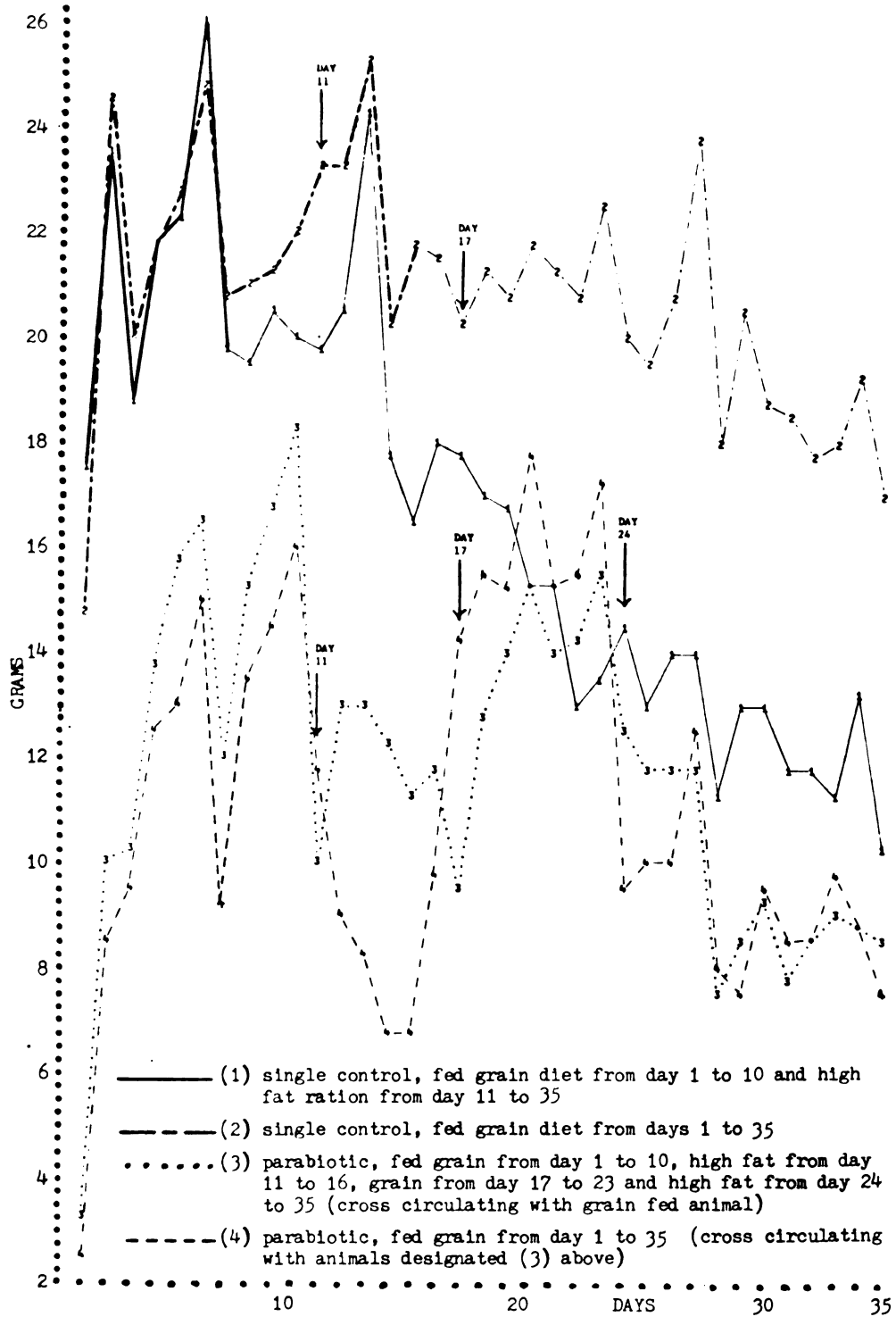

Figure 43. Food intakes of control and parabiotic male Sprague-Dawley rats. (Av./Animal/Day)

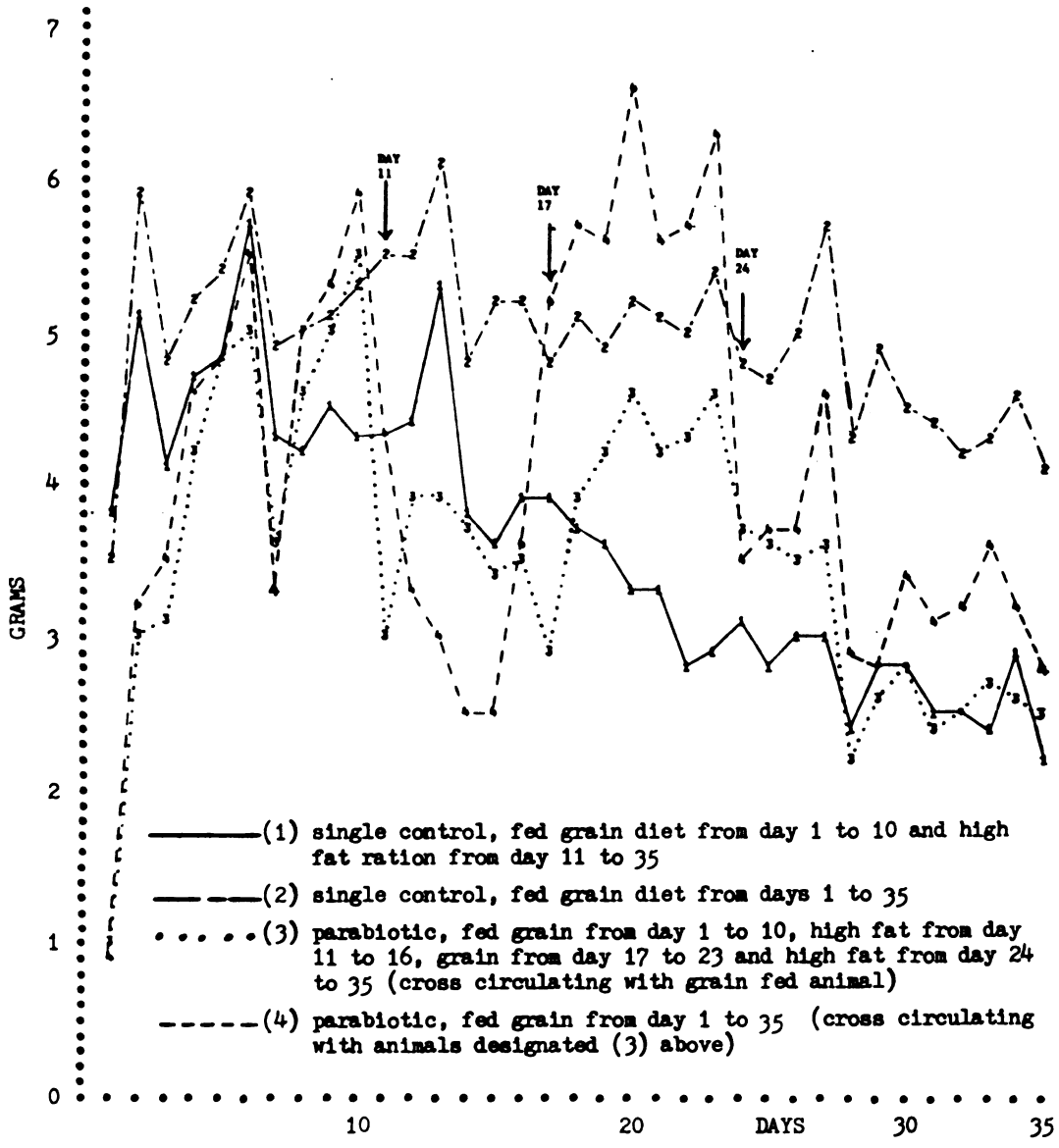

Figure 44. Food intakes per 100 grams body weight of control and parabiotic male Sprague-Dawley rats. (Av./Animal/Day)

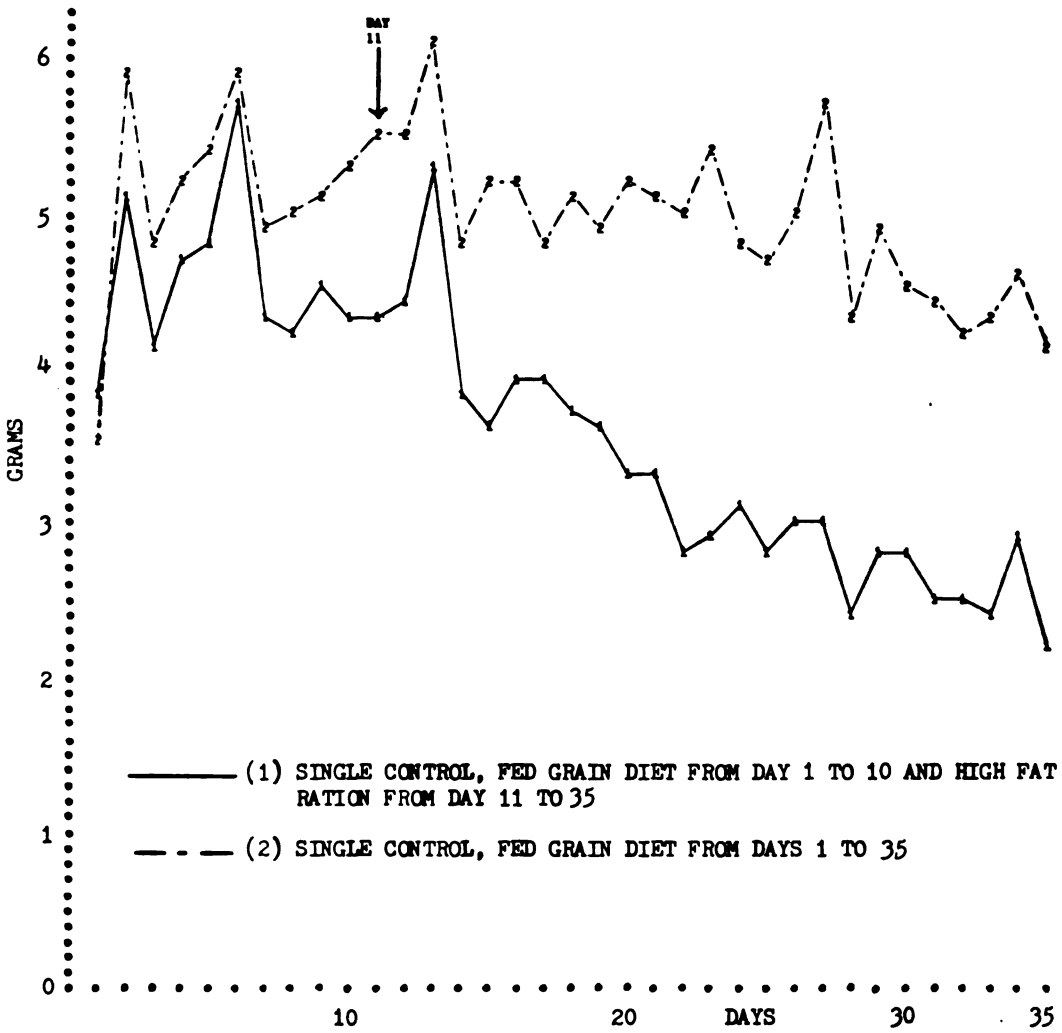

Figure 45. Food intakes per 100 grams body weight of control male Sprague-Dawley rats. (Av./Animal/Day)

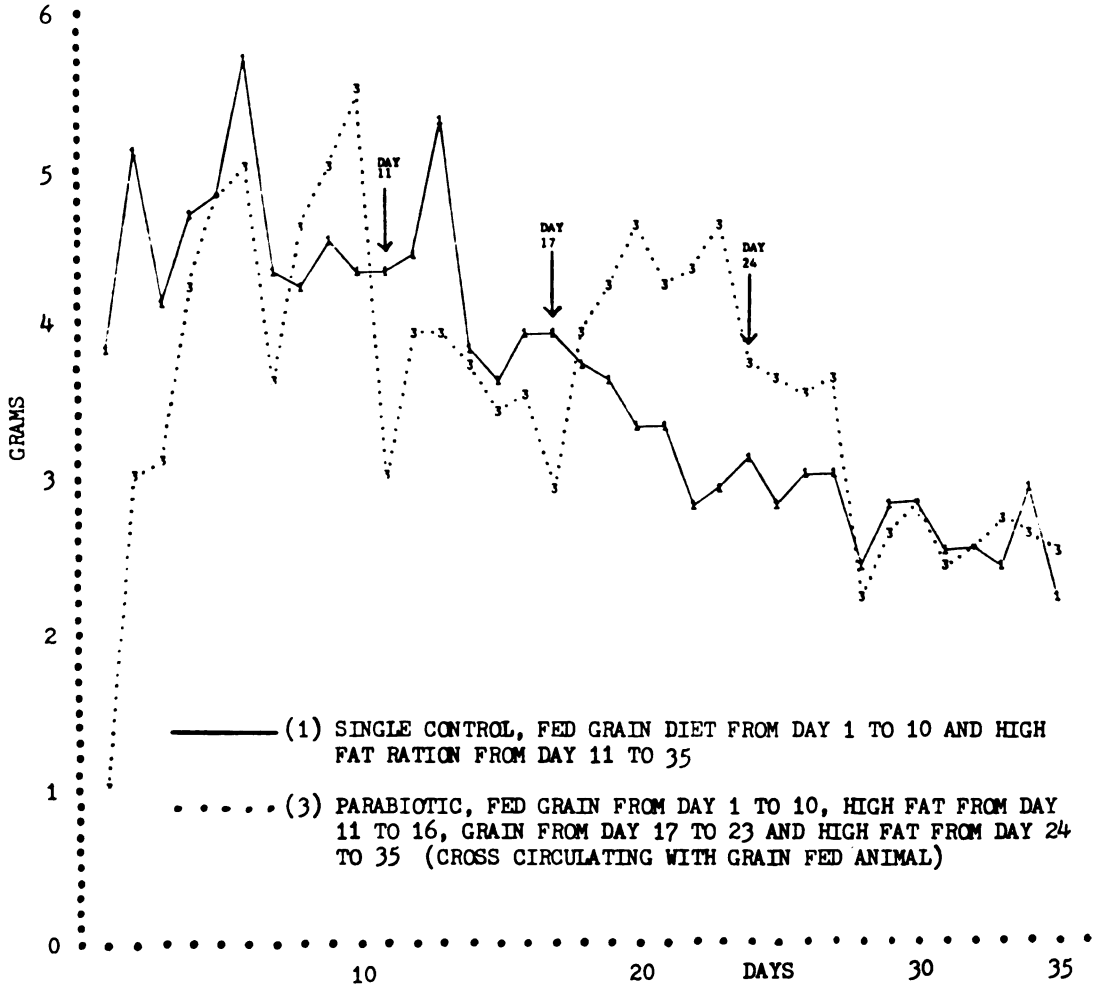

Figure 46. Food intake per 100 grams body weight of control and parabiotic male Sprague-Dawley rats. (Av./Animal/Day)

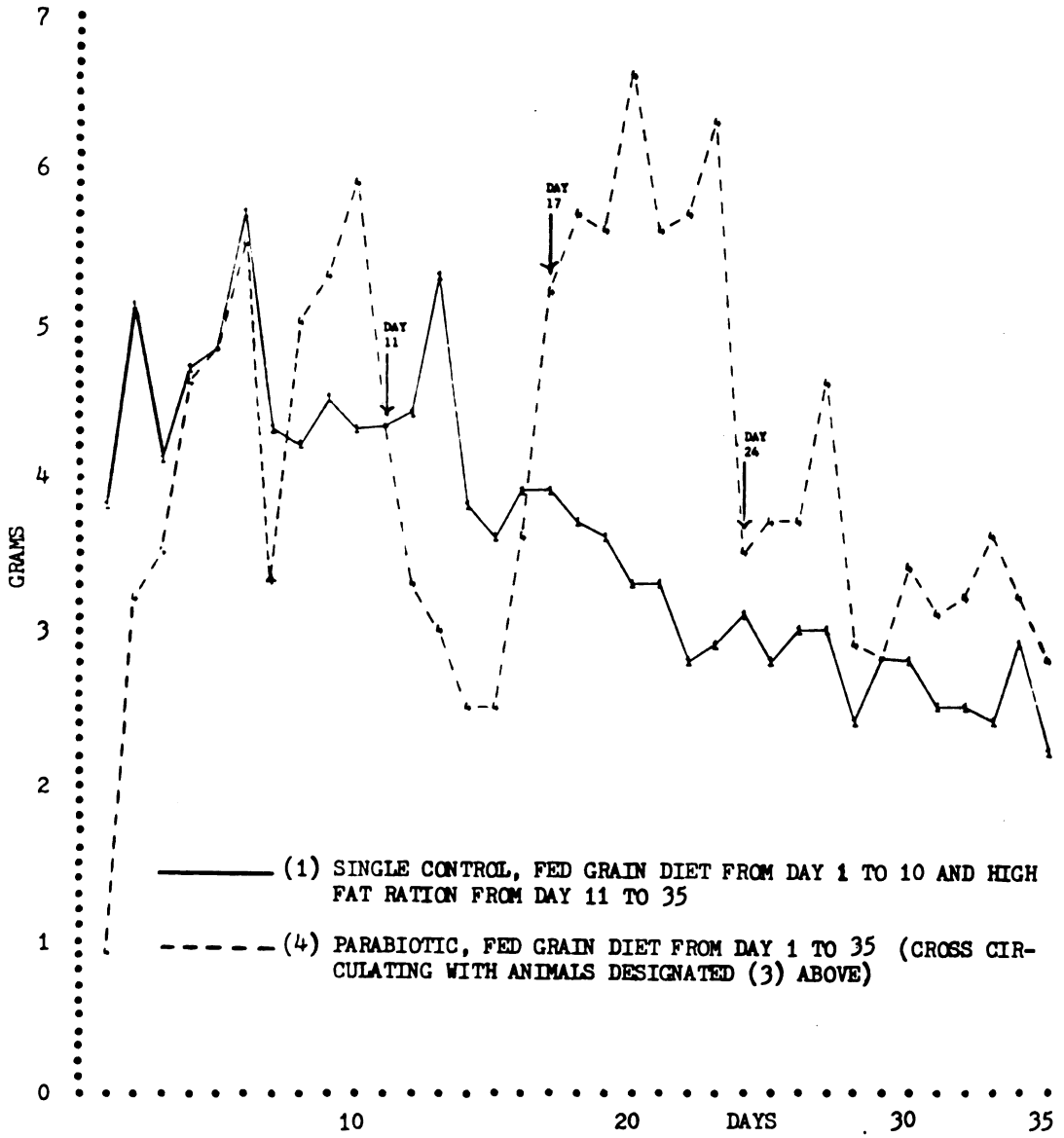

Figure 47. Food intakes per 100 grams body weight of control and parabiotic male Sprague-Dawley rats. (Av./Animal/Day)

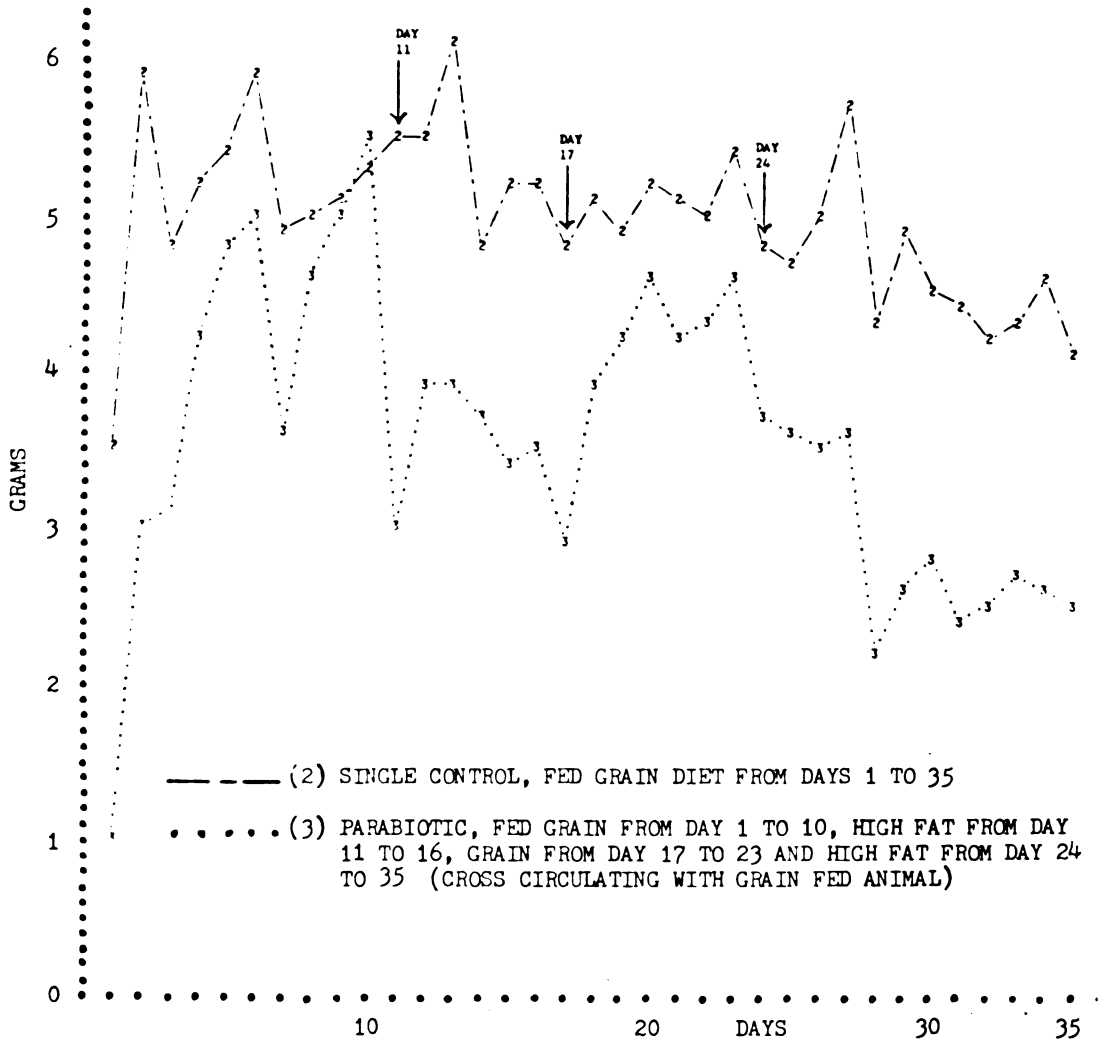

Figure 48. Food intakes per 100 grams body weight of control and parabiotic male Sprague-Dawley rats. (Av./Animal/Day)

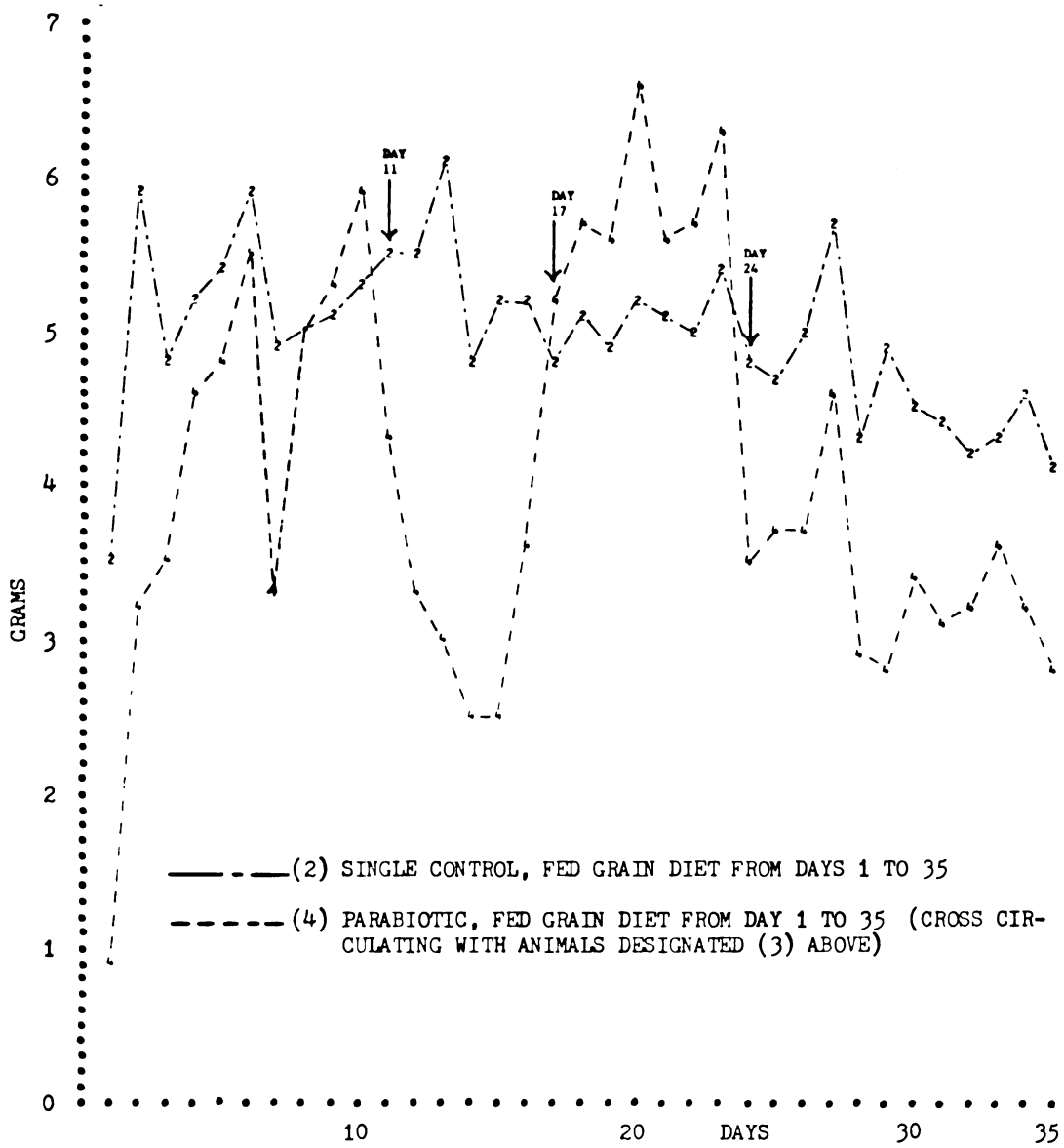

Figure 49. Food intakes per 100 grams body weight of control and parabiotic male Sprague-Dawley rats. (Av./Animal/Day)

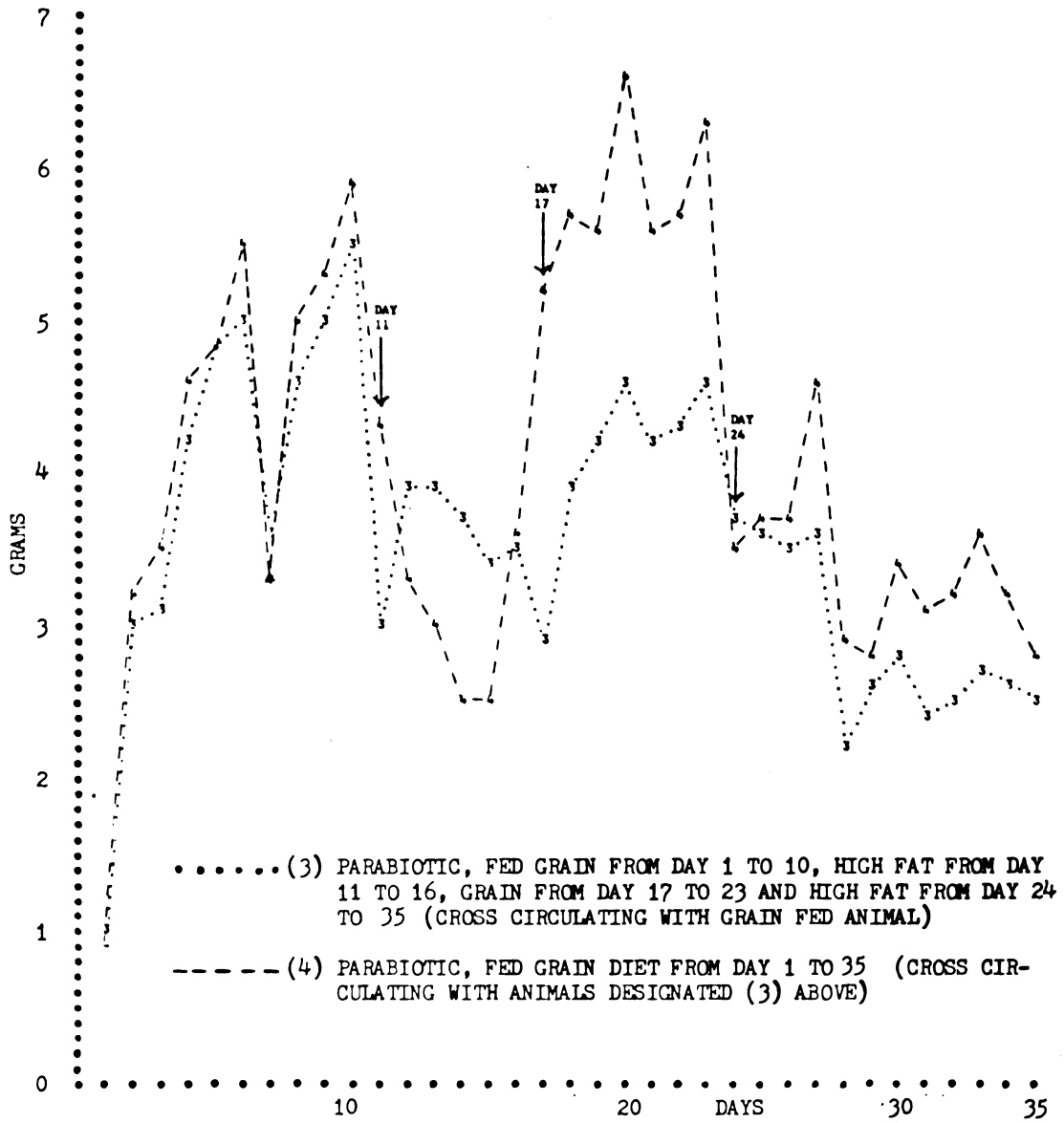

Figure 50. Food intake per 100 grams body weight of parabiotic male Sprague-Dawley rats. (Av./Animal/Day)

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