

ABSTRACT

THE ROLE OF EXPERIENCE IN THE BEHAVIORAL/
PHYSIOLOGICAL ADAPTATION OF PEROMYSCUS
MANICULATUS BAIRDII TO EXTENDED
PERIODS OF A PROTEIN FREE DIET

By

Peter Karch

The present investigation was concerned with the behavioral/physiological strategies used by the non-ruminant, herbivorous deer-mouse, Peromyscus maniculatus bairdii, in compensating for the complete removal of a dietary source of protein. Experiments showed that, given an appropriate experience at an earlier time in their development, these mice could endure the subsequent removal of all dietary protein (zero-protein diet) for extended periods of time, ranging from 15 to greater than 100 days, with no loss in body weight or change in general appearance/activity. Without this experience, mice could not endure the zero-protein (O-P) regime, demonstrating an immediate, dramatic weight loss, and death within one week in some cases and shortly thereafter in others. These results were invariably true. The body of this thesis was concerned with specifying those characteristics

conferring on the animals the ability to endure the O-P diet.

Animals were presented with a diet composed of 20% protein (pure lactalbumin), 10% corn oil, 64% sucrose, and adequate vitamins and minerals. The diet was divided and fed to the animals in two separate portions during the "selection" phase: one dish contained only the protein (P) source of the completed diet, the other contained the remaining ingredients (O-P) of the completed diet, in their proper percentages. During the "dilution" phase, only the protein source was diluted with 30% cellulose; during the O-P phase, the protein dish was removed, leaving only the O-P dish in the cage.

The basic experimental procedures involved an experience phase consisting of selection and/or dilution followed by the O-P (experimental) phase. Certain requirements were found to be necessary in order for these mice to exist on the O-P regime:

- Experimental conditions designed to prevent coprophagy prevented the expression of the phenomenon.
- Experiments suggested that coprophagy was providing the animals with sufficient protein during the O-P phase, since body weight and appearance remained unchanged during this phase.

- The deterioration resulting from experiments designed at preventing coprophagy could be reversed only by resupplying dietary protein.
- It was found that coprophagy must be experienced at a specific time in the lives of these mice; all animals were at least two months of age, and it was shown that the ability was lost sometime after five months of age.
- Coprophagy must be experienced for between five and eight days during a phase of experimentation where the dietary source of protein presented the animals was diluted with cellulose, thus presumably placing them in metabolic need in terms of nitrogen balance.
- Two separate experiments demonstrated that females of this species could not withstand the O-P regime. They could however, maintain their body weights and general appearances unchanged during the dilution phase of the experiment.

Results are discussed relevant to the significance of the time period required for coprophagy and the age at which the phenomenon prevails, as well as to the events possibly occurring enabling properly experienced mice to withstand the O-P regime, while naive mice die.

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INTRODUCTION

The environment places two types of stresses on an organism: a more long-term, prevailing set of conditions within which the organism must exist, and a more short-term, immediate set of conditions which the organism must be able to deal with effectively for survival on a day-to-day basis. An example of the former situation might be one geographic area into which an animal was born compared to any other area. The northern deciduous forests present a different array of conditions to an animal than does the tropical jungle, or a desert. Those animals indigenous to the northern forests (or the desert, etc. for that matter) are well adapted to the area's prevailing conditions.

Within any of these geographical areas however, environmental conditions also fluctuate on a shorter term basis, sometimes quite dramatically. For example: the prevailing conditions in a northern forest of moderate rainfall and temperatures, controlled winds, etc. might be superceded one year by severe drought, excessive flooding, severe frost, or a deluge of unusual tornadoes.

These principles even apply at a more specific level: within the course of an animal's lifetime it

probably endures numerous instances of changing dietary protein concentrations, especially dietary deficiencies of protein. Examples of factors producing changes in dietary protein concentrations would be peak population periods for a species, areas where soil, water, minerals, etc. are not adequate to insure proper plant food development, seasonal changes in plant composition, ageing of food sources, or simply the normal feeding pressures on a given area.

As mentioned, the animal must be prepared to deal effectively with these shorter term fluctuations if survival is to be achieved. As Claude Bernard said, a prerequisite to animals' being able to occupy the wide array of environmental conditions that they do is the development of mechanisms assuring the maintenance of homeostasis. That animals do indeed occupy such diverse geographical habitats, and are able to handle such dramatic short-term fluctuations as well, attests to the existence of these homeostatic mechanisms. Accordingly, the array of mechanisms available to an animal would enable it to compensate for both short- and long-term environmental stresses.

This thesis is concerned with those strategies enabling the animal to deal with deficiencies in dietary protein content, representative of a short-term environmental stress. It should be recognized that the present

study is a laboratory study; it does not profess to be otherwise. While in several instances reference will be made to the applicability of the experimental situation, results, etc. to field conditions, these will be at best hypothetical, since none of the reported data were collected in the field.

LITERATURE REVIEW

The alternatives, or strategies, available to an animal faced with a deficient food supply, or a food supply deficient in only one component, are behavioral and/or physiological. Mention will be made, even if only briefly, of those strategies generally accepted as comprising these two main areas, the point being the appropriate placement of the subject of this thesis in the total array of strategies.

Strategies Altering the Amount of Food Available to an Animal

By decreasing competition for a particular food supply, an animal could increase, or at least maintain constant, the amount of food available to itself. Maintaining a territory can reduce competition sufficiently that adequate food is kept available to the territory holder (Hinde, 1956).

It has also been suggested that agonistic encounters among animals resulting from reduction in space available and/or food relative to the number of animals present tends to decrease competition over time by reducing the number of animals present. According to this theory, high population pressures are relieved by the

destruction of the social structure (Calhoun, 1950) important to successful reproductive behaviors, or by excessive stimulation of the pituitary-adrenal axis with its concomitant increase in reproductive failure (Christian, 1964). That the results of Calhoun and Christian were arrived at using populations exceeding the densities occurring in the natural situation is an oft-repeated and debated point, and as such is beyond the scope of this discussion.

While the work of Calhoun and Christian attempted to demonstrate the effectiveness of high population densities per se in the control of animal numbers, Cheatum et al. (1950) showed that a decrease in the quality of deer food due to high population concentrations, plant successional stages, etc. produced decreases in the production and survival of fawns. There was a high correlation between the range quality and fertility (in terms of decreases in corpora lutea and embryos). When food becomes limited because of factors already mentioned, the age of sexual maturity is delayed, fertility is lowered, and older animals dominate the population (Klein, 1965). Klein (1968) also found that growth in deer of North America appears to be related to the quality and quantity of the food supply. This may or may not be related to the density of the population.

Physiological Compensations for Deficient Food Supplies

The present section will deal with some of the changes taking place within the animal allowing it to deal more effectively with a given amount of food, rather than ways whereby the animal can increase the amount of food available, as discussed above.

The normal homeostatic mechanisms of the body dictate that certain amounts of given ingredients are needed for the integrity of the organism. As with any homeostatic system, regulation of these levels is maintained by compensations at the input and/or output end, as well as by means of the efficiency of utilization of the ingredients, all of which is dictated by the metabolic state of the organism, this being controlled or regulated by the endocrine system.

Especially via the pituitary, but also via many other channels, the endocrine system is intimately related to the nervous system, that system responding directly to internal and external stimuli. In fact, there is some evidence indicating that perhaps the two systems are really highly specific modifications of one another, i.e. the nervous and endocrine systems are one and the same, the former being modified for rapid, direct, short-acting communication between two or more points, the latter being modified for slower, indirect, sustained stimulation. Without delving any further into this topic, suffice it

to say that the two systems are closely related, each one having the potential to stimulate the other into activity when it itself has been stimulated, thus manifesting far-reaching physiological ramifications in an organism.

Selective Feeding Tactics

"Selective feeding tactics" are included under the "physiological" heading rather than the "behavioral" since it will be shown that selection of a food serves to satisfy physiological needs, even though overtly it is a behavioral act. The relationship between the overt behavior and the physiological/metabolic state satisfied by the behavior will be shown.

Any discussion of feeding selectivity necessitates some mention of factors affecting the development of a dietary repertoire, i.e. factors used in recognizing foods to be selected, and perhaps, factors used in identifying foods to be avoided as well. Recognizing acceptable and/or not acceptable foods probably involves physical cues associated with the foods such as location, color, shape, size, texture, etc.; perhaps even taste and odors as well.

Animals can learn to accept or reject a food after only one trial, even if this trial involves only minute amounts of the food (Bell et al., 1971). Revusky (1968) showed that a rat can learn to avoid a particular food even if it receives an adverse physiological stimulus 6.5

hours after consuming the food; stronger adverse stimuli increase the time duration of the learning event.

Barnett et al. (1961) showed that activity around a food source can contribute to the learning that a food is edible by a rat; they did not observe any imitative feeding. Zentall et al. (1972) suggests that learning in the rat may be hastened if one animal sees another making the appropriate response in a learning situation.

Rats have demonstrated an ability to avoid nutritionally poor diets as with amino acid deficiencies, vitamin deficiencies, or mineral deficiencies. Similar results have been shown with deer (Longhurst et al., 1968); Rattus rattus (Norman, 1970); voles (Freeland, 1973); and the kangaroo rat (Dipodomys microps) (Kenagy, 1972), with diets known to contain toxins at concentrations demonstrated to be lethal.

Nutrients Selected

It would appear that even if the physical cues do attract the animal, these become important only insofar as they represent hindrances to (or conversely, facilitations in) achieving specific nutrients contained therein.

The literature assumes two approaches to this subject: (1) laboratory experiments showing that animals can (or cannot) sense a particular dietary ingredient, and in fact, presented with a dilution or overabundance or this ingredient in the diet, can make appropriate

compensatory changes in its intake, (2) field studies demonstrating selective feeding tactics for a specific dietary ingredient.

Regarding the laboratory approach, Richter (1936, 1938) found that adrenalectomized rats increased their intakes of all sodium salts with no appetite shown for chlorides. Adrenalectomy disturbs sodium metabolism producing large amounts of sodium in the urine. Similar behaviors have been reported on parathyroidectomy for calcium (McCance et al., 1965; Richter, 1937, 1939), pancreatectomy for water (Richter, 1941), and neurohypophysectomy for water (Richter 1935 a, b; 1936, 1941).

Similarly when complete diets were diluted with a variety of dilutents, animals compensated for the dilution by increasing their dietary intake, achieving a constant caloric intake (Adolph, 1947). The corollary to this was to force-feed animals; their voluntary intake decreased so as to maintain a constant caloric intake as well (Richter, 1941).

Leshner (1970) found that animals selected a high protein diet over a high carbohydrate diet under non-stressful conditions, but under stress (cold) a carbohydrate diet was preferred to a protein diet. Lat (1956) found that excitable animals preferred a high carbohydrate diet, while less excitable animals preferred

high protein. In fact, when rats of equal excitability were fed either the high carbohydrate or the high protein diet, the former diet increased the excitability of those animals feeding on it, while the latter diet decreased the excitability of those animals feeding on it. Excitability was measured by several behavioral criteria including speed of acquisition of a conditioned reflex and intensity of exploratory activity. Rozin (1968) however, found that rats will compensate for the dilution of the protein of a diet by increasing their protein intake alone, while they appear not to be able to compensate for the dilution of the carbohydrate of the diet.

Mitchell et al. (1921) showed that rats will combine a high protein diet and a low protein diet in the proper proportions to give a medium protein diet. They felt that animals could select the amount of protein necessary for their physiological needs (maintaining a proper weight curve). Harper (1967) showed that a high protein diet is preferred to a low protein diet, and a medium protein diet is preferred to a high protein diet.

The above suggests that animals eat to satisfy their physiological needs; it also suggests that the food eaten should provide the correct type and proper amount (or proportion) of the particular ingredients. This would suggest that animals selectively eat foods disproportionately to the relative abundance of the food in the

environment as Feeny (1970), Bell (1969), Einarsen (1964), Klein (1969), Eadie (1969), and Gardarssen (1969), to name a few, have shown.

This selection ability appears to take the form of an ability to sense something wrong in the body, to sense that something is missing from the diet, to make the association between these two events, as well as the association between eating a particular food and solving the problem of "ill-feeling."

It is possible that there is some connection between taste and the physiological state of the animal such that satisfying a "taste-craving" will simultaneously be satisfying a "physiological-craving." Taste receptors, being modified dendritic ends, are sensitive to concentrations of substances in solution; they have an alterable firing threshold (satisfaction). (Compensation for the dilution of dietary ingredients has been achieved when all oropharyngeal detection was bypassed--Epstein et al., 1962. Although this is valuable to know, it does not however mean that normally taste is not involved, but rather that it is also possible to regulate body levels of food substances without the use of oropharyngeal senses.)

Those field studies involving selective feeding tactics appear to be concerned with a relatively small number of nutrients: calcium, phosphorus, carbohydrates,

and nitrogen (included as protein or as nitrogen per se, depending on the analysis presented).

Of these, calcium and carbohydrates have the least influence (as determined by volume of literature available). Hunter (1962) found that grazing intensity was more closely related to the calcium oxide and crude fiber content than any other factor; these components tending to produce neglect of the food on the part of the grazers. Arnold (1964), Bell (1969), Gardarssen (1969), and Gwynne (1969) have shown that preference for a plant decreases with increasing fiber content (age).

The only nutrient even approaching nitrogen's importance in feeding selectivity is phosphorus. In fact, most of the literature concerning nitrogen's influence on food selection also includes reference to the phosphorus content of the food. Arnold (1964) with sheep, Klein (1969) and Severinghaus et al. (1956) on deer, Moss (1968) on Icelandic Ptarmigan, Krebs et al. (1965) on Microtus, and Miller (1968) on sheep, all of which will be mentioned in more detail regarding their statements on nitrogen content, are among those supporting this view.

That nutrient having the greatest influence on food selection by animals is the protein content of the food. As mentioned earlier, the preference referred to here is highly correlated in every case with either the protein content per se or the nitrogen content; the

difference lies in the particular analytical procedure used by one or the other author. Animals appear to be seeking out, even following, those foods affording them certain necessary quantities of protein.

Sheep, being selective grazers, prefer leaves to the stem; young plants to old, dry ones. Comparing the food ingested with the types available to the sheep demonstrates that those foods higher in nitrogen, phosphorus, and energy, and low in fiber are selected by the sheep (Arnold, 1964).

Gardarssen (1969) working with Icelandic Ptarmigan, noted that these birds are very selective feeders. In Summer: Polygonum viviparum is the most important food nutritionally (as determined by chemical analysis) and is preferred by the birds. In Winter: birds feed on Salix herbacea, a plant growing at high altitudes, especially where snow accumulates in the winter. Nutritionally this latter is a better food than all other shrub species in Iceland. It is therefore not surprising that the Ptarmigan attempt to feed on it for as long as possible in the winter. In fact, the birds endure what appears to be considerable hardship and inconvenience in the sense that they will continue to select this plant species over those more accessible, but less nutritious, even when S. herbacea is buried under up to 30 cm of crusty snow. Gardarssen has shown that these birds move from high to

low ground in September for feeding purposes, i.e. following the more preferred food types. It is at this time that the S. herbacea is under snow.

In 1964, Einarsen showed that the crude protein content of the food was an important consideration in food selection by deer and therefore should be considered in management technique for deer populations. Klein (1964, 1969) considered good deer habitats as those areas having preferred forage species in abundance well into the winter months this preference being highly correlated with the protein content. Similarly Thomas et al. (1964) found deer to be eating greater amounts of forage from areas fertilized with nitrogen than from unfertilized areas.

Although Swift (1948) showed that the wheat and clover deer were preferentially feeding on contained 12% more ether extract, 38% more calcium, and 34% more phosphorus than the wheat and clover avoided, Longhurst et al. (1968) concluded that deer select forage on the basis of smell first, which is then followed by taste. The ether extract contains high quantities of volatile fatty acids which have been suggested as acting as olfactory cues in attracting or repelling deer from food types. Volatile fatty acids were also found to be an attractive olfactory compound of the "soft" feces of rabbits (absent from the "hard" feces) and served to attract the rabbits to these feces, probably not for

their intrinsic nutritional value per se, but rather for the other nutritional substances contained within these soft fecal pellets (Myers 1955; Reddy et al., 1968; Henning et al., 1972). It is therefore possible that the volatile fatty acids of the deer forage serve in the same capacity as those in the rabbit feces. Rabbit feces contain high vitamin and protein contents.

Even invertebrate population numbers have been correlated with the nitrogen content of their food sources, the implication being that population size reflects its nutritional status and is strongly correlated with the available nitrogen. For example:

(1) Hughes et al. (1969) suggested that it seemed reasonable to accept the percentage of nitrogen as an index of food quality for their work with the Australian bushfly. They related annual variations in the nitrogen content of cattle dung piles on grass/clover pastures in the southeastern Australian Tablelands (as published by Heath, 1966) to population fluctuations of the bushfly in the same area which feed on the dung (as published by Norris, 1966). Heath showed that the nitrogen content of the dung reaches a peak in the spring and autumn and a minimum in the summer; Norris' curve for the bushfly population changes followed suit (allowing 1-2 weeks lag for needed events). (2) According to Lindemann (1948), Mittler (1958), and Ziegler (1956), the phloem

composition of plants changes with the progress of growth and maturation of the leaves and shoots. Active Growth or Senescence (Spring/Autumn): phloem high in amino acid nitrogen with a large variety of amino acids present. No Growth (Summer): phloem poor in amino acid nitrogen with a limited variety of amino acids present. As the concentration of amino acids of the phloem decreases, aphids feeding on the phloem become smaller, and their birth rate declines. This relationship was also confirmed by Dadd et al. (1965) in lab experiments using synthetic diets.

Although the work of Vesey-Fitzgerald (1960) and Bell (1969) on ungulates in the Serengeti National Park of Tanganyika was primarily meant to demonstrate animal movements in relation to their food resources, their data are quite pertinent to the present study, and will therefore be included.

Vesey-Fitzgerald noticed that large herds and/or heavier animals altered the environment favorably for other species, thereby establishing a dependency among animals. Bell actually measured the extent of this dependency among five species of ungulates and measured the type of alteration of the environment by one animal which made it more or less favorable for another. He showed that the order of grazing succession corresponds

to diet selectivity as well as to body weight. For example:

1. Protein content: lowest requirement in Zebra; highest in gazelle.
2. Mechanical properties: toughest with zebra; delicate with gazelle.
3. Spacing of preferred diet: commonest and most accessible for zebra; rarest and least accessible for gazelle.

His theory for grazing succession among the animals he studied is worth quoting:

When growth ceases at the dry season, the catena top becomes depleted by grazing pressures, forcing animals down the slopes to the longer grasses. Zebras lead since they have a high tolerance for stems due to the colon bacterial digestion, thus opening up the herb layer by trampling. By selectively eating stems, they increase the amount of leaf material available, making it more suitable for the Wildbeest to follow. These reduce the vegetation such that the quantity of dicotyledenous plants available increases; Gazelle prefer these. At the next wet season, the early grass is leafy and abundant, and is therefore ideal for the intake of protein. The grazers become independent of each others effects on the herb layer. Movements lag three weeks behind the rainfall. The order of descent in the dry season is the reverse of the order of ascent at the start of the wet season.

Minimizing Losses of a Required Nutrient

It was suggested above that Richter's (and others) work on dietary self-selection provides one method of studying compensations for dietary deficiencies. This method has even been used by Richter and others to ascertain the dietary requirements of animals. When the diet

is deficient in a particular ingredient, the animal has the ability to seek out this ingredient preferentially, consuming it until it no longer "senses" the deficiency i.e. until the deficiency has been compensated for. Similarly, if the diet level of an ingredient remains constant but the metabolic needs for that ingredient change for whatever the reason (e.g. pregnancy, lactation), the animals will also modify the dietary intake of the ingredient adjusting it to be appropriate to its needs at the time. Compensations for a change in the needs of a dietary ingredient occur solely during feeding; behaviors associated will be those allowing the animal to achieve the correct amounts of the specific ingredient in question at the proper time.

As in any homeostatic system, intake must equal output for conditions to remain the same. If conditions are such that the intake decreases due to the reduced availability of a substance, an organism still has the potential to reduce wastage (losses) through the normal excretory channels. Indeed, increased reabsorption is frequently the case. A few brief examples are in order:

1. Excess sodium loss is a stimulus for the adrenal cortex's release of aldosterone, which stimulates the active reabsorption of sodium from the renal tubules.
2. Dehydration due to lack of dietary water (castaways at sea or on the desert) is a stimulus to decrease urinary losses of water by decreasing the urinary volume. (The countercurrent system and ADH are implicated here.)

3. Starvation initiates many physiological changes in an organism, one of which is to minimize the catabolism of nutrients in general and thus their excretion in the urine, sweat, and digestive juices (Ganong, 1965). Karch (1968) showed that there was a decrease in basal metabolic rate, as measured by oxygen consumption when rats were placed on a 50% caloric restriction.

These examples demonstrate that it becomes important for the body's integrity to minimize losses of essential nutrients during times of dietary deficiencies.

Similar changes in the excretory levels of nutrients would be expected if the metabolic demands increased without the means available for increasing the dietary intake of these nutrients; the object being to maintain the correct amounts of the specific ingredients in question at the proper time, as already mentioned.

Recycling Waste Products

Another effective method of compensating for relative deficiencies of a nutrient is to reuse those substances excreted from the body containing what would be considered waste material under normal circumstances. Urine represents large amounts of non-protein nitrogen (NPN) and water (to name but a few of its valuable substances), these being some of the waste products of metabolism. The feces represents some undigested food material, and a passage for metabolic wastes but is composed mostly of the colon microorganisms themselves as will be discussed in more detail later. It therefore contains

proteins, NPN, carbohydrates, lipids, minerals, vitamins, and water, i.e. all categories of nutrients.

While the subject of uriposia (urine drinking) is somewhat neglected in the literature (a few references to castaways, etc. resorting to drinking their own urine as a last desperate attempt to change their particular misfortune), the subject of coprophagy (eating of fecal material), comparatively speaking, is almost ubiquitous.

Losses of nitrogen in the urine may represent significant quantities especially during survival on low protein diets or starvation, thus it is not surprising to find some measure of conservation at this site even though direct recycling through drinking is apparently not usual. Akester et al. (1967) showed with radiographic studies, direct visual evidence for the passage of radio-opaque urine from the kidneys via the cloaca and colon to the caecum in the domestic fowl. The use of the urine by the fowl is unknown, however Houpt (1963) has demonstrated urea utilization by rabbits fed a low protein diet; the urea first being secreted into the rabbit's caecum then decomposed into CO_2 and NH_3 by caecal bacteria, some of which is incorporated into the bacterial cells for essential and nonessential amino acid production. Houpt et al. (1971) showed similar results regarding nitrogen conservation and usage by ponies. Gallina et al. (1971) suggest the use of NPN as human dietary supplement

since when the diet is adequate in essential amino acids, even humans can replace all non-essential amino acids in the diet by NPN.

Direct recycling of feces seems to be more beneficial than the recycling of urine. Coprophagous populations include such diverse animal species as rats, mice, rabbits, guinea pigs, dogs, swine, poultry, innumerable marine species, and undoubtedly many more. These animals exhibit coprophagy as a normal practice.

Coprophagy has both beneficial and detrimental aspects. While the beneficial aspects are more widely discussed in the literature, most involving nutritional significance as will be discussed shortly, the primary negative aspect of coprophagy is its contribution to the expediency of parasite transfer in coprophagic populations, as well as to the transfer of toxic and radioactive substances in food chains (Chandler et al., 1930; Laird, 1961).

The laboratory rat recycles approximately 30-50% of its total fecal output on a nutritionally adequate diet; given a deficient diet, 100% of the fecal output may be recycled for short periods of time (Barnes, 1962). The rabbit recycles 54-82% of its feces (McBee, 1971). For animals to recycle this quantity of feces, and if it may be assumed that other coprophagous populations recycle anywhere near this amount of feces, then it must suggest

that the animals are deriving something useful from the feces which replaces the deficient substance from the deficient diet and supplements that substance in the adequate diet.

Studies on the metabolism of riboflavin have shown that rabbits selectively consume the mucous-covered fecal pellets ("soft") rather than the dried ("hard") pellets when fed on remains of normal rabbit chow. Analysis of the pellets showed that the riboflavin content of the former was greater than that of the latter (Fukahara).

Kulwick et al., (1953) showed that the soft pellets contained 3-4 times the quantity of riboflavin per gram than the hard, representing 100% more riboflavin to the rabbit.

Nogueira (1963) showed in rabbits that there was a decrease in the biotin activity of the tissues tested (for biotin) when coprophagy was prevented suggesting that since there was no recycling of fecal material, the biotin was permanently lost with the feces, thereby producing a decrease in the tissue quantity. Germ-free rats require biotin, while normal rats on biotin deficient diets continue to live well until coprophagy is prevented; biotin deficiency symptoms then develop (McBee, 1971).

Thiamine metabolism in rats is also affected by coprophagy (Barnes et al., 1960). Thiamine, as are the other B-vitamins, is synthesized in the intestinal tract

of the rat and many other animals. They are synthesized in the lower intestine by the micro-organisms present; absorption from this area of the rat's tract is poor. No significant quantity of thiamine is made in the upper intestine where proficient absorption occurs. Therefore, to be effective, fecal thiamine must be recycled via coprophagy to place it in the upper intestine for proper absorption to occur.

Similarly, the rabbit derives outstanding vitamin benefits from coprophagy: their soft feces, which are recycled, contain 3-4 times more niacin, 6 times more pantothenic acid, and 2-3 times more B_{12} than the hard feces which are not recycled to any great extent. These quantities represent 83% more niacin, 165% more pantothenic acid, and 42% more B_{12} than the hard feces. (The amount of B_{12} in the feces of rabbits fed a B_{12} deficient diet was 221 times greater than the intake; Kulwick et al., 1953.)

In addition to the fecal pellets being good sources of vitamins and minerals, Henning et al. (1972), analyzing the gut contents of rabbits caught in both the day and night phases of their excretory cycle, found that both groups had high concentrations of volatile fatty acids in their caeca and proximate colons. However, the hard pellets (distal colon and rectum--night) had much less VFA than the soft pellets (caecum and proximate colon--

day). Also, due to the ingestion of the soft pellets (Fukahara,), showed that the VFA content of the stomach was greater in the day than the night.

Myers (1955) found a positive correlation between the length of time spent in the soft phase of the excretory cycle and those times of the year when food is scarce and the grasses are dry suggesting a relative importance of one phase over the other in response to environmental conditions.

Describing the soft pellets of the rabbit in more detail: Griffith (1963) found them to be composed of a membranous protein coating and bacterial cells made of 24.4% protein with 81% in the form of the bacterial cells. Huang (1954) estimated the protein content to be 37.1%, while Kulwick et al. (1953) suggest 28.5% protein for the soft compared to 9.2% for the hard feces. Thacker (1955) found that the nitrogen balance in the rabbit was lowered 50% if the soft feces were not eaten. The source of this protein is the bacterial synthesis from NPN in the caecum (Yoshida, 1968). Germ-free rabbits had 1/5 the protein content in their feces and two times the NPN content as did conventional rabbits.

In addition, Barnes (1962) and Barnes et al. (1969), have shown that decreased growth rates in rats (15-25%) occurs when coprophagy is prevented. Coprophagy is also needed for the proper conversion of methionine

to cystine when natural trypsin inhibitors (e.g. raw soybeans) are present in the diet (Barnes et al., 1965, 1967 a, b).

The caecum, as the rumen, is a rich source of microorganisms (8×10^{11} cells per gram wet weight) representing 25-50% of the caecal contents. Fifty to sixty percent of these have to date been cultivated and identified; many are the same as those of the rumen, and many are different. Rumen microorganisms are known to be rich sources of protein (Reed et al., 1949; Weller, 1957; Hoogenrood et al., 1970) and of water soluble vitamins (Hungate, 1966). Gustaffson et al. (1964) found that prevention of coprophagy in rats produced major changes in the number of fecal and caecal lactobacilli, enterococci, and coliform bacteria (the lactobacilli decreased while the enterococci and coliform increased relative to their counts in non-prevented animals). Along with the bacteria are many types of protozoans, flagellates, and ciliates.

Endocrinological Compensations to Nutrient Deficiencies

As mentioned earlier, the role of the endocrine system along with the nervous system, is in maintaining integrated homeostasis of the various subsystems of the organism. It would therefore be appropriate for the endocrine system to respond to changes in the dietary nutrient level. The theoretical and controlled events

concerning the endocrinological compensations for altered nutrient intakes are covered in any textbook on physiology or biochemistry. Although these changes will be briefly mentioned in outline form for completeness' sake it is by no means meant to be comprehensive. Interest centers with the section following this concerning the field studies demonstrating the ability of animals to exist in nutrient impoverished environments by virtue of their capacities to compensate for the nutrient deficits endocrinologically.

Carbohydrates--most of the hormones affect carbohydrate metabolism.

Insulin--Insulin is released by the pancreas in response to high blood levels of glucose and acts to lower the blood glucose level by increasing the transport of glucose from the blood to the cell, and by decreasing hepatic gluconeogenesis. (Other effects of insulin on metabolism are consequential to these two.)

Glucagon--Glucagon is released from the pancreas in response to low blood glucose levels, and acts to raise these levels by: (1) stimulating adenyl cyclase thus activating phosphorylase which increases hepatic glycogenolysis; (2) increasing gluconeogenesis from the glucogenic amino acids present in the liver; (3) increasing the metabolic rate (along with thyroxine and the adrenal cortical hormones) due to the deamination of amino acids and the increased utilization of these deaminated amino acids in gluconeogenesis.

Epinephrine--(Norepinephrine is less effective in this capacity)--In the liver, epinephrine activates phosphorylase for glucose catabolism, thus increasing the blood glucose levels. It stimulates the one-way production of pyruvate from glucose 6 phosphate due to the absence of glucose 6 phosphatase in the muscle. Pyruvate is then changed to lactate which is channelled to the liver to be changed to pyruvate again, and then

to glycogen (Ruch, 1960); the liver does contain the necessary enzyme to accomplish this conversion.

Thyroxin--Thyroxin produces an increased intestinal absorption of glucose, increased hepatic glycogenolysis, and increased rate of insulin degradation thus producing hyperglycemia. (These effects are all conducive to a diabetic-like effect, hence thyroxin is said to be diabetogenic.)

Adrenal Glucocorticoids--The glucocorticoids increase the blood glucose level also and are therefore also diabetogenic. They accomplish this by altering several metabolic events, enzymes, and substrate concentrations, the net effect being hyperglycemia.

Somatotropin--STH has an anti-insulin effect by decreasing the glucose uptake in the tissues, increasing the hepatic glucose output, and increasing the blood glucose level. STH and the glucocorticoids inhibit the phosphorylation of glucose (Morgan et al., 1959).

Hormones and other regulatory factors affect regulation by their actions at certain key "directioned" reactions of a metabolic pathway. Some of them for carbohydrate metabolism are presented in White et al. (1964).

Proteins--The hormonal effects on protein metabolism are best summed up by the following:

ACTH--This substance is best included in the carbohydrate section since this is where its main effects are, but as a consequence of gluconeogenesis, protein synthesis is retarded.

Glucagon--Miller (1961) showed that glucagon administration to rats produced urinary excretion of nitrogen, phosphorus, and a loss in body weight due to liver and muscle mass losses.

Insulin--Insulin decreases blood amino acid levels, increases entry of amino acids into the cell, and increases incorporation of amino acids into protein within the cell.

STH--STH lowers plasma amino acid levels, suggesting an increased incorporation into the cells.

The substances also stimulate RNA synthesis, thus further verifying their effects on protein synthesis.

Fats--Norepinephrine and epinephrine increase the hydrolysis of triglycerides of adipose tissue thereby increasing the free fatty acid levels in the blood. These hormones activate cAMP which activates lipase in the adipose tissue for the hydrolysis. This action of the catecholamines is facilitated by ACTH, STH, the glucocorticoids, and thyroxine; it is inhibited by insulin and possibly LTH. Glucagon increases the plasma-free fatty acid levels of the blood, but its stimulation of insulin release due to the hyperglycemia soon lowers it again.

Endocrinological Compensations for Reduced Nutrient Intake-- Field Studies

Non-Calorigenic Nutrients.--Most of the work in this area has been with mineral deficits for reasons which will become evident later. Blair-West et al. (1968) have shown that severe sodium (Na^+) deficiencies do occur in wild animals in their natural environment as verified by Na^+ excretion and hormone secretion processes which are concerned with Na^+ balance. They found animals engaged in behavioral, physiological, and morphological steps attempting to correct the situation. Animals living in low Na^+ areas, or areas having seasonally low Na^+ contents in forage (as compared to animals in Na^+ replete areas) had raised circulating aldosterone levels, raised renin levels, were attracted to, and ate voraciously, sticks impregnated with a variety of salts (especially those containing the Na^+ cation), and exhibited numerous

morphological changes in the salivary glands suggesting increased glandular activity in Na^+ reabsorption.

Some of the more prominent changes in the salivary glands exhibited were: more extensive duct systems, greater height of duct cells, greater vascular supply, greater number of mitochondria, greater epithelium size, greater number of acinar granules, and a greater number of cristae/unit volume of mitochondria. Whether or not these morphological changes were the direct result of the dietary Na^+ deficiency, or of the raised level of the circulating aldosterone due to the deficiency of dietary Na^+ , is a question raised by the authors, though, as yet, is unresolved.

In spite of all these compensatory mechanisms observed, the authors also noticed that if the dietary Na^+ content were nil, the conservatory processes would probably not be adequate to avoid progressive Na^+ depletion.

Laboratory experiments have shown that aldosterone acts to increase the reabsorption of Na^+ from all places Na^+ is lost from the body: urine, sweat, saliva, feces. That these same mechanisms are operative in the field situation where animals are living in Na^+ poor areas has been shown by several studies. Blair-West et al. (1968) have shown Na^+ conservation by kidney reabsorption as already mentioned. They have also noticed that anything

changing the efficiency of Na^+ reabsorption from the gut (microbial or nematodal disturbances) also affects the stress of dietary Na^+ deficiency; this stress being the severity of the deficiency as well as the extent of the operation of the compensatory mechanisms.

Bott et al. (1964) have reported that low forage Na^+ results in Na^+ conservation by the kidneys and low salivary Na^+/K^+ ratios in cattle, this latter being indicative of a significant Na^+ deficit and a hypersecretion of aldosterone by the adrenal cortex.

Coffre et al. (1967) demonstrated that the toad colon is responsive to aldosterone, endogenously and exogenously, in vitro and in vivo, in terms of Na^+ transport. Bentley (1971), commenting on Coffre's paper, mentions that "it appears likely that such effects also occur in the mammal's gut, though for technical reasons, it is more difficult to demonstrate." Bott et al. (1964) suggest that "the reduction of Na^+ content in the feces during Na^+ deficiency has been demonstrated in sheep, dogs, and man, and it seems probable that fecal Na^+ conservation in cattle is also determined by raised secretions of aldosterone."

Herbivorous non-ruminants lose little Na^+ in their sweat on Na^+ depletion according to McFarlane (1964). In man, Johnston et al. (1947) have shown that a decrease in the Na^+/K^+ ratio in sweat has been related to the

action of the adrenal cortical hormones. There is no reason, they suggest, that these steroids should not also contribute to the acclimatization in salt levels of the sweat seen in other species.

McFarlane (cited in Blair-West, 1968) reported that the primitive mountain tribes of New Guinea subsisting on vegetarian diets have a K^+ intake greater than the Na^+ intake; a Na^+ deficiency exists in the diet. The peripheral aldosterone levels are greater in these people than in the same type people eating European foods. The people of the New Guinea Highlands have been described by anthropologists as preparing salt cakes, and also engaging in salt trade, factors suggesting the importance of salt in the lives of these people.

Aumann et al. (1965) demonstrated in field observations, and substantiated in laboratory experiments, that there is a relation between the population density and the Na^+ availability and selection by Microtine rodents. Low, medium, and high Na^+ levels of the soil were correlated with low, medium, and high densities of animals living in these respective areas. Also, reproductive capabilities (number of offspring produced) and salt selection were highly correlated to Na^+ levels of the diets offered animals in the laboratory experiments. The authors hypothesized that the increased salt intake due to crowding in the laboratory experiments was due to Na^+

deficiencies as a result of inadequate adrenal cortical regulation of Na^+ metabolism. The population growth levels decrease as a result of decreased dietary salt levels. "With unrestricted access to salt, the suppressive effects of crowding on population growth are all alleviated. Given sufficient salt in the forage, populations grow unrestrictedly until food, or some other factor, becomes critical in limiting the population."

The Great Lakes area and many other areas of the world are notorious as a "Goiter Belt" for humans and other animals, the reason being iodine deficiencies in the food available in these areas. Iodine is an essential constituent for T_3/T_4 production. (T_3/T_4 represents the 3- and 4-iodine-protein complex of thyroid hormone.) The thyroid gland, in attempting to maintain a constant production of T_3/T_4 for metabolic and other reasons, increases its tissue mass in order to trap maximal amounts of this mineral . . . thus the goiter.

It appears however, that opposite effects are seen in deer existing in low iodine areas (Ulrey, personal communication, 1973). Studies comparing the circulating T_3/T_4 levels of wild deer eating forage alone with those of penned, experimental deer fed synthetic diets (containing adequate iodine levels) and wild deer fed forage with and without iodine supplementation have indicated that iodine deficiencies in forage are responsible for

low circulating T_3/T_4 levels. The size of the thyroids were not measured directly, however, if low circulating levels of T_3/T_4 were found, the suggestion would be that morphological compensations had not occurred in these animals.

Calorigenic Foods.--Because minerals can be leached from the soil, their lack affects the mineral content of the vegetation growing there, thus, the possibility of low mineral content areas exists. This deficiency is therefore passed on to herbivorous animals alone; carnivores are not Na^+ deficient, nor are any compensatory mechanisms observed in them, since the flesh they eat comes from herbivores in which all of the above mentioned Na^+ compensatory mechanisms had been operative. The flesh is then no longer Na^+ deficient.

Similar circumstances do not hold for calorigenic foods, since carbohydrates, lipids, and proteins are not present in the soil; the differences between herbivores and carnivores also do not exist. Thus, while there is some literature available for endocrine compensations applying to wild animals enduring mineral deficiencies, the available literature for calorigenic nutrients in this respect appears to be limited to proteins alone, and even that is minimal.

Carbohydrates

There is no specific response to low dietary carbohydrate levels other than the altered preference for carbohydrate mentioned given that carbohydrate is offered as such in the experimental situation; carbohydrate is an unnecessary dietary ingredient (White et al., 1964; Ganong, 1973). Regarding low circulating levels of carbohydrate, especially glucose (for reasons other than dietary), the body has means available to raise the blood glucose levels from other food sources (e.g. gluconeogenesis) including an appetite mechanism stimulating feeding behavior in general. This invariably raises the blood glucose levels since it is virtually impossible to eat natural foods not containing carbohydrates, let alone sufficient carbohydrate to raise the blood glucose to acceptable levels.

Thus, given a dietary deficiency of a carbohydrate (if that is possible), all of the theoretical compensatory tactics available to the animal would probably be exhibited, but to find a natural situation where low carbohydrate levels exist in the forage is not probable and has therefore not been dealt with experimentally.

Fats

It is currently debatable whether or not a source of fats is a necessary dietary ingredient (Bell et al., 1961; Wright, 1965). Animal species in question must be

specified, and even then controversy exists (Prosser, 1973). The primary roles played by fats are to provide a rich, storable reserve supply of energy for the organism, to supply the essential fatty acids (in the case of man this is linoleic alone, but for other animals it may be linoleic and arachidonic) from which the other fatty acids can be made by the body, and to provide a vehicle for the absorption of the fat-soluble vitamins. This latter role is not a necessity intrinsic to the properties of the fatty acids themselves; the role of "energy provider" is a debatable necessity since carbohydrates can, and usually do, substitute adequately in this capacity. This leaves the role fats play in providing the essential fatty acids, which is presumably the debatable point.

However, all concerned agree, as with carbohydrates, it is practically impossible to ingest any natural food without achieving sufficient fatty acids of the proper type. Thus an animal facing a fatty acid deficiency in the natural situation is all but impossible, is academic, and therefore has not been considered in the literature as far as I know.

Proteins

Suggesting why dietary protein is so important to the animal, and therefore why so many animal species (see above) appear even to be using the protein content of foods as the object of diet selection, may be valuable

in understanding the following section on compensations for protein deficiencies.

1. Protein must be in the diet for life to continue. The essential amino acids which the protein contributes, must be in the proper proportion to each other to be of benefit to the animal (Rose, 1957; Sanahuja, 1963, 1967; Beaton, 1964). Although Williams (1961, 1963, 1964, 1971) and Williams et al. (1966) have shown rats to be able to remain alive on protein-free rations for greater than 72 days, this is not without consequence to the rats; body weight was lost due to the protein deficiency alone as well as the inanition resulting from the protein deficiency. In addition, alterations in whole enzyme systems were demonstrated; these effects (and others mentioned in the papers) were reversed on protein repletion.
Fats and carbohydrates do not necessarily have to be supplied to the animal for survival, although controversy exists, as mentioned.
2. Amino acids are not stored to any great extent (in a specific storage form) in the body as are carbohydrates (liver and muscle glycogen) and fats (periorgan, subcutaneous). Therefore the amino acids must be continuously supplied by the diet.
3. Proteins are multipurpose substances, serving in such vital capacities as cell structure, chromosome structure, hemoglobin structure, enzyme and hormone structure and synthesis, and energy. The real importance of fats and carbohydrates is in the energy these substances provide the animal.
4. The metabolism of protein (including its digestion and absorption) requires approximately 30% of the caloric potential of the protein ingested, i.e., 30% of the protein moiety of a meal is channeled away from utilizable energy for the animal's basal (and other) activities for the digestion, absorption and metabolism of the protein per se. Carbohydrate requires approximately 6%; fat 4% (White et al., 1964). In other words, loss of the potential energy from a fat or carbohydrate source for their metabolism is really quite negligible compared to the great loss encountered when protein is metabolized. The diet must be able to compensate for this loss.

5. The caloric output of the protein burned in the body differs from that burned in a calorimeter. It is less in the former instance by approximately 1.56 cal/gm due to nitrogen losses in the urine (as urea primarily) since the nitrogen removed from the protein on deamination is not oxidizable and therefore does not contribute to the caloric output in an organism.

In the course of an animal's lifetime it probably endures numerous instances of changing dietary protein concentrations, especially dietary protein deficiencies, e.g. in overpopulated areas, areas where soil, water, minerals, etc. are not adequate for proper food production, during seasonal changes, or simply during normal feeding activities on a given area. Given the situation of dietary protein deficiency and no means of migrating away from the situation to "better" areas, what does the animal do? Dietary protein deficiency constitutes a stress to the organism. There are several alternatives available to the organism to deal with the stress.

1. One important mechanism called upon in stressful situations is the stimulation of the adrenal cortex to release the glucocorticoids (mineralocorticoids and sex steroids are simultaneously released, but will not be considered here). The actions of the glucocorticoids are diabetogenic in nature; the changes mentioned earlier produce hyperglycemia. Thorn et al. (1959) enumerates profound metabolic changes associated with carbohydrates, lipids, and proteins. Included among these latter are increased production of amino acids from protein in the liver and periphery, increased hepatic uptake of the amino acids, and increased transamination.
2. Acute stress provides the stimulus for the catecholamine secretion, and their potent cardiovascular-pulmonary-metabolic responses to the stress. It would be important to point out here

that the cortical secretions from the adrenal gland, while they are independently regulated from the pituitary and hypothalamus, may also be the result of catecholamine stimulation to the hypothalamus.

3. If one were to apply Richter's self-selection principles to the existent situation of deficient dietary protein, one would have to presume that alterations in protein metabolism induced by the dietary deficiency would become important in dictating limits of protein selectivity and sensitivity. The organism becomes more sensitive to the protein levels in the food, more aware of its own situation, and actively begins to search out protein, ceasing when the "feeling of deficiency" has been alleviated.

As mentioned, this could possibly involve some type of projection of the organism's metabolic state with regard to a particular ingredient onto the threshold of stimulation and/or resting membrane potential of the neurons associated with detecting that particular ingredient.

4. Since the intestinal absorption of dietary ingredients is another one of the key places for regulation of the body's constituents, and the kidney's reabsorption/excretion is similarly employed, there is no obvious reason why these areas should not also be involved in the regulation of amino acid levels in the body on protein deprivation. Altered urinary nitrogen losses are common in states of minimal protein deprivation; in total starvation there are initial indications of nitrogen retention by decreasing nitrogen losses in the urine, however, obligatory nitrogen losses do occur as the body's protein content is metabolized providing the needed energy and nitrogen for enzyme synthesis, etc. This is nonetheless, still considerably reduced due to the reduction in metabolic rate concomitant to starvation and semistarvation.
5. Coprophagy increases as the quantity of protein in the diet decreases. Barnes showed that the recycling of feces increased from 30-50% normally to 100% for short periods of time during dietary deficiencies. (Further information on coprophagy was presented earlier.)

Given no dietary source of protein, what role, if any, does coprophagy play in the maintenance of the animal? Protein, because of its vital roles in repair, replacement, blood proteins, etc. will continue to be utilized by the body. Plasma proteins are quite labile but are in equilibrium with other structural proteins such that these would decrease in quantity subsequent to the decrease of the plasma proteins. This would be indicated by a loss of body weight.

While it would be possible to conserve protein's utilization in the situation described, this would have limited value both in quantity of protein conservable as well as length of time conservation of protein is possible; the animal would of necessity be operating at a lower plane of metabolism.

It would be more efficient if the normal metabolism was maintained with coprophagy providing the means of recovering the lost proteinaceous substances. A greater efficiency of protein absorption from the intestinal tract would be one of the most important factors permitting a situation such as this; the body weight would be expected to remain stable. The better an animal is at extracting a greater amount of protein from the recycled feces for a greater length of time, the longer he would be expected to maintain his weight, and other physiological processes stable. Those animals at the other end of the spectrum

of efficiencies would have stable body weights for a short while, then begin a gradual decline to death from protein starvation. (All animals would eventually reach this point since metabolism continues during life; the difference between animals would therefore be in their ability to recapture and reuse this protein of metabolism.)

While coprophagy may be restoring lost proteinaceous substances to the animal on a protein deficient diet enabling it to sustain the lack of protein for prolonged periods of time, one may not ignore the great losses of nitrogen in the urine. Urine normally contains less than 0.15 gm of protein/100 ml, this being the mucin and cell sloughings from the urogenital tract, but greater than 2.3 gm of nitrogen/100 ml, most of which (85%) is in the form of urea. This nitrogen is the direct result of protein metabolism in the body. (The composition of the urine varies with the animal and the diet.)

In contrast, fecal material contains only 1-2 gm of the total solid content as nitrogen, with greater than 1/3 of the total solid content as the intestinal bacteria themselves, a rich source of protein. Other sources of protein are the intestinal secretions and small amounts of undigested protein (this latter is normally quite minimal). In short, feces contain fairly rich sources of protein as well as some non-protein nitrogen (NPN),

while the urine contains "no" protein, but is a relatively well endowed source of NPN.

In terms of nitrogen balance and efficiency of utilization while enduring a protein deficient diet, losing the urinary source of nitrogen is wasteful. In terms of energy balance it is also wasteful; approximately 1.56 Cal/gm of urea are lost, as mentioned earlier. Elimination of this wastage must not only entail a means of recycling urine but also some means of using the nitrogen obtained. This would necessitate the ability to make amino acids from non-protein nitrogen sources as does the ruminant. Can non-ruminant herbivores use urea as do ruminants? Are other tactics used to ensure survival on protein deficient diets?

The subject of this thesis then involves the question: can Peromyscus maniculatus bairdii, a non-ruminant, herbivorous mouse, endure prolonged periods of time on a protein-free diet? What factors are necessary, and what strategies does this animal utilize in assuring its survival on such a regime, if survival on this regime is indeed possible?

GENERAL MATERIALS AND METHODS

Peromyscus maniculatus bairdii males were used throughout most of this study; females of this subspecies were used in two experiments, as will be described. The ages and weights of the animals used depended on the experimental design. Mice were F_1 generation from wild caught parents. The animals were weaned at approximately 21 days and placed on Purina Mouse Breeder Chow (the exact composition will be given in Appendix C) until they were of appropriate age for a particular experiment. Water was provided ad libitum. The environmental temperature was maintained at $21 \pm 2^\circ\text{C}$ with a 14/10 light/dark cycle. The cages were of two types:

Regular Cages--Cages were made of clear plastic on four sides with wire screen tops containing a food hopper measuring 8.5 cm deep x 7 cm diameter; the water bottle rested on this top with the spout penetrating into the living area. The hopper also extended into the living area to 6.5 cm from the floor. Food was not provided in the hopper. Dimensions of the cages were 28 cm x 13 cm x 15 cm (deep).

Screen Cages--These cages were the same as described for the "regular cages" except that the floor was of wire screening (0.85 cm mesh).

Cages were provided with one or two food dishes (depending on the phase of experimentation) and only part of one "nestlett." "Nestlettes" are fiber nesting material 5 cm x 5 cm x 1 cm. This provided a more attractive nest site than did their food dishes. At no time did this ever remain a problem during data collection.

Diets

Pre-experimental.--Wild caught parents were fed Purina Mouse Breeder Chow from the time they were brought into the colony. Thus newborn animals had chow available throughout their suckling period. Young were kept on chow until they were of proper experimental age.

Experimental.--On achieving the proper experimental age (as determined by design), animals were fed a 20% protein diet* composed of 20% lactalbumin, 10% corn oil, 64% sucrose. The caloric value of the food was determined to be 3.93 K cal/gm in a bomb calorimeter.

*Diets included adequate provision of vitamins and minerals; a more precise composition is provided in Appendix C.

Experimental Feeding Phases

Selection Phase ("selection").--The constituents of the 20% protein diet were divided into two parts and fed to the animals in two separate dishes of equal size, color, composition, etc.

Dish 1 contained only the protein source of the completed diet, i.e. pure, powdered lactalbumin. It was termed the "P" dish.

Dish 2 contained the remaining ingredients of the completed diet in their proper percentages, i.e. the fat, carbohydrate, vitamins, minerals, and the cellulose. It was termed the "O-P" (zero-protein) dish.

(Positioning of the P and the O-P dishes relative to each other was randomized initially. This proved unnecessary as it did not affect intakes from the dishes and was later eliminated.)

Dilution Phase ("dilution").--The lactalbumin alone was diluted with 30% cellulose; no cellulose was added to the O-P dishes. Thirty percent dilution was considered a compromise adequate in allowing expression of the phenomenon in question (this will be clarified later), while at the same time not so excessive in amount as to produce rejection or inability to handle it on the part of the mouse.

Zero-Protein Phase ("O-P").---That phase of the experiment where the dietary protein source was removed, thus providing the animal with a diet totally devoid of protein. The O-P phase was considered to be the experimental, or test, phase of the procedure. (A Biuret test was performed on the O-P fraction of the diet, verifying the diet's lack of protein. This test was performed on two separate occasions, using two different batches of the diet, as a check on experimental procedure and constancy. In each instance testing indicated the O-P diet to be free of protein.)

The Phenomenon

The experimental phenomenon consisted of maintaining body weight and general appearance constant while eating a diet totally lacking in protein. While no animal could live "forever" without a source of dietary protein, the procedures included herein are meant to demonstrate and characterize the capabilities of certain animals receiving selected "experiences" to prolonged survival times on a O-P diet.

On indication that an animal would not be able to endure the O-P regime any longer, a dish of protein was always added to the cage (selection) and measurements continued until body weight returned approximately to the pre-experimental level. While this procedure eliminated any possible deaths by continued weight loss

after data collection had ceased, it also served to re-establish at the end of each experimental procedure that it was the addition of protein to the diet which would bring the animal's body weight and/or general appearance back to "normal." This showed that it was the lack of dietary protein, or the inability to handle the O-P regime in any manner, and therefore the lack of protein intake (as dictated by the particular experimental procedure) that placed it in the situation it was in.

The criterion arbitrarily established to prevent excessive dying among animals was a 20% body weight loss from the day the O-P regime was initiated. This "20% criterion" was established as part of the experimental design during the second O-P regime of Group II animals (see explanation of group designations in Experiment I) and therefore included all experiments except those represented in Figures 1, 2, 2a, 3, and 5. Achieving this level of weight loss on a O-P regime meant that the animal could no longer endure the lack of dietary protein; the experiment was considered ended. Protein was then added to bring the weight back to the pre-experimental level, as already described.

Measurements

Throughout all phases of this study food and water were provided ad libitum; food and water intakes and body weight were measured to the nearest 0.1 gm on a daily

basis. (All intake data will be related to body weight data in the tables appearing in Appendix B.) Metal lids with holes in them were affixed over the feeding dishes thus minimizing spillage. Food was weighed in its dishes, the difference in weight per day representing food intake for that 24 hour period. Should spillage have exceeded the sensitivity of the scale used to weigh the dishes, and this could be fairly accurately estimated with experience, food intake data for that period were disregarded. This was not usual.

"General Appearance"

A moribund mouse, even several days before actual death, could be predicted with absolute accuracy. Its coat became matted; constriction in the lateral abdominal area became apparent; the animal would huddle in a corner of the cage, shaking; its equilibrium was usually poor towards the final day or two, and its reaction time (to be picked-up or chased for weighing) was greatly increased. Exhibiting these symptoms, there was never any doubt that within 1-2 days the animal would be dead unless resupplied with the protein portion of the diet. This is not to say that an animal would not die without exhibiting these symptoms; one or two did die under these circumstances, but they were definitely exceptions to the rule. (Usually, however, animals reached the 20% weight loss

criterion before exhibiting symptoms of general appearance
degredation.)

EXPERIMENTAL PROCEDURES

The presentation of experimental procedures will be in two main sections, the reference point being the establishment of the phenomenon in question. Experiments leading up to and following from this point will be presented in their entirety, i.e. specific methods, results, and discussions for each experiment performed will be presented. A "general discussion" will follow.

Leading to the Establishment of the Phenomenon

A. Experience vs. Non- Experience

Introduction

Pilot work (described in more detail in Appendix A) suggested that P. m. bairdii could survive on less dietary protein during the dilution phase than during the selection phase. Since this was not compatible with those concepts discussed in the literature review on dietary self-selection, the question became how much less could they endure and for how long?

Methods

Eighteen male P. m. bairdii between 45 and 145 days of age were divided into two groups of nine animals each. Group I, held 20 days initially on the selection regime, was then divided into three subgroups for "dilution" (22 days), where the protein alone was diluted 15, 30, and 45% with cellulose (the O-P diet remained unchanged). Subsequent to the dilution, the animals of Group I, as well as the nine animals of Group II*, were placed on the O-P regime. The O-P regime was continued for as long as the animals could sustain their pre-experimental body weight (i.e. exceed the 20% criterion) and/or appearance.

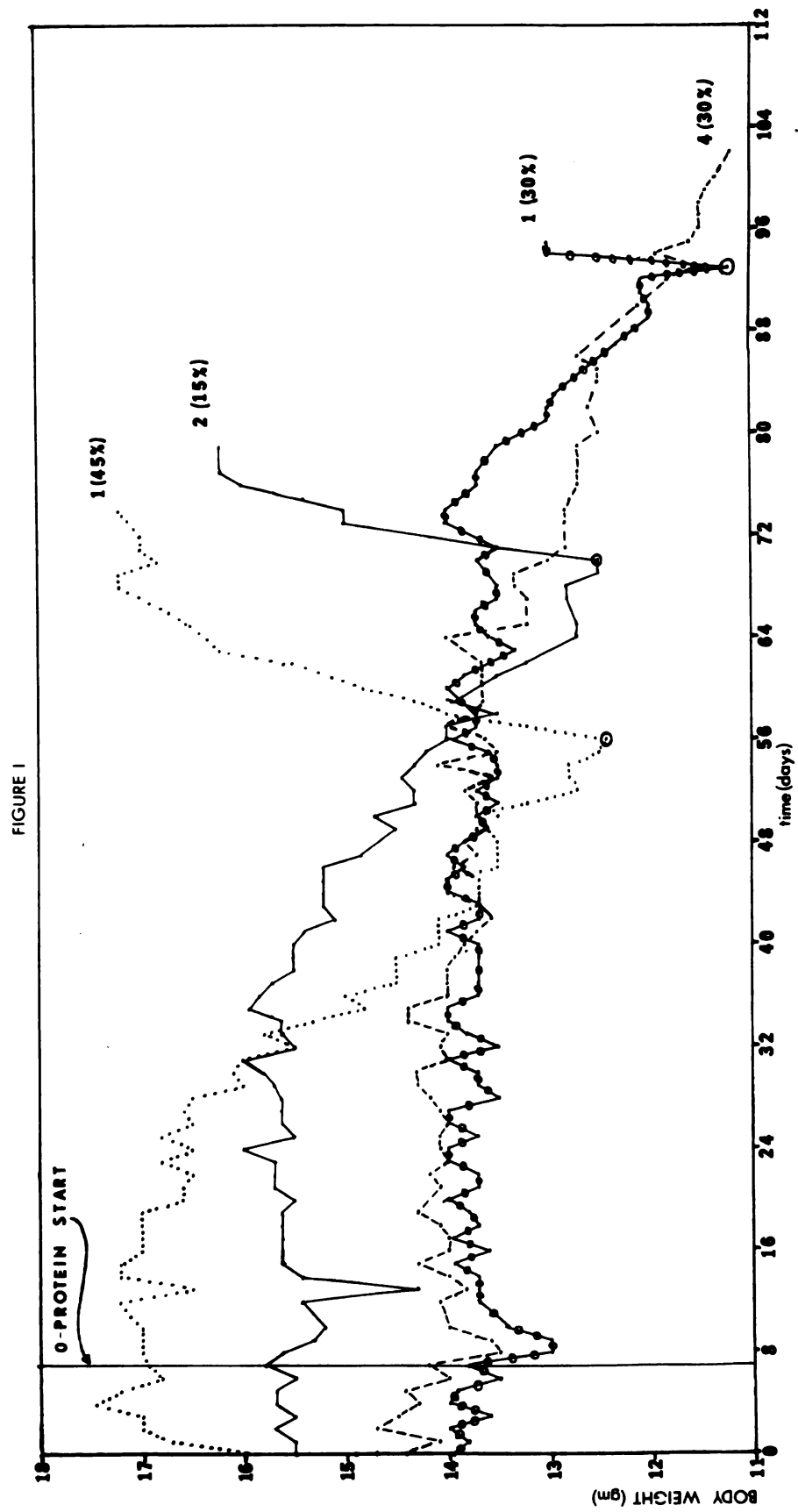
Results

Figure 1 (on 2 pages) compared with Figures 2 and 2a illustrate the results of this experiment. It can be seen that while all of the animals of Group I maintain their pre-experimental body weights for between 45 and 100 days during the O-P regime, all of those in Group II (including the five additional animals mentioned above) began an immediate, dramatic decline in body weight as

*This experiment was repeated with five more animals to substantiate the fact that animals of this group did not sustain body weight, since the water bottles were faulty for the first four days of the original Group II procedure, and may have been the cause of the deaths, as well as the poor performance, on the subsequent O-P regime.

Figure 1.--Group I Animals: Selection/Dilution Experience
Prior to Zero-Protein.

- The graphs represent the body weight changes in Group I animals from the start of protein's removal.
- The graph is meant as a comparison with Figures 2 and 2a (Group II animals of the text), thus the reason for starting only seven days prior to protein's removal.
- Prior to what is presented these animals experienced 20 days of selection followed by 22 days of dilution; day "0" of the graph represents seven days before the end of the dilution phase (day 15 of dilution).
- Animals' group and group number are listed in parentheses near the symbol representing the animal on the graph.



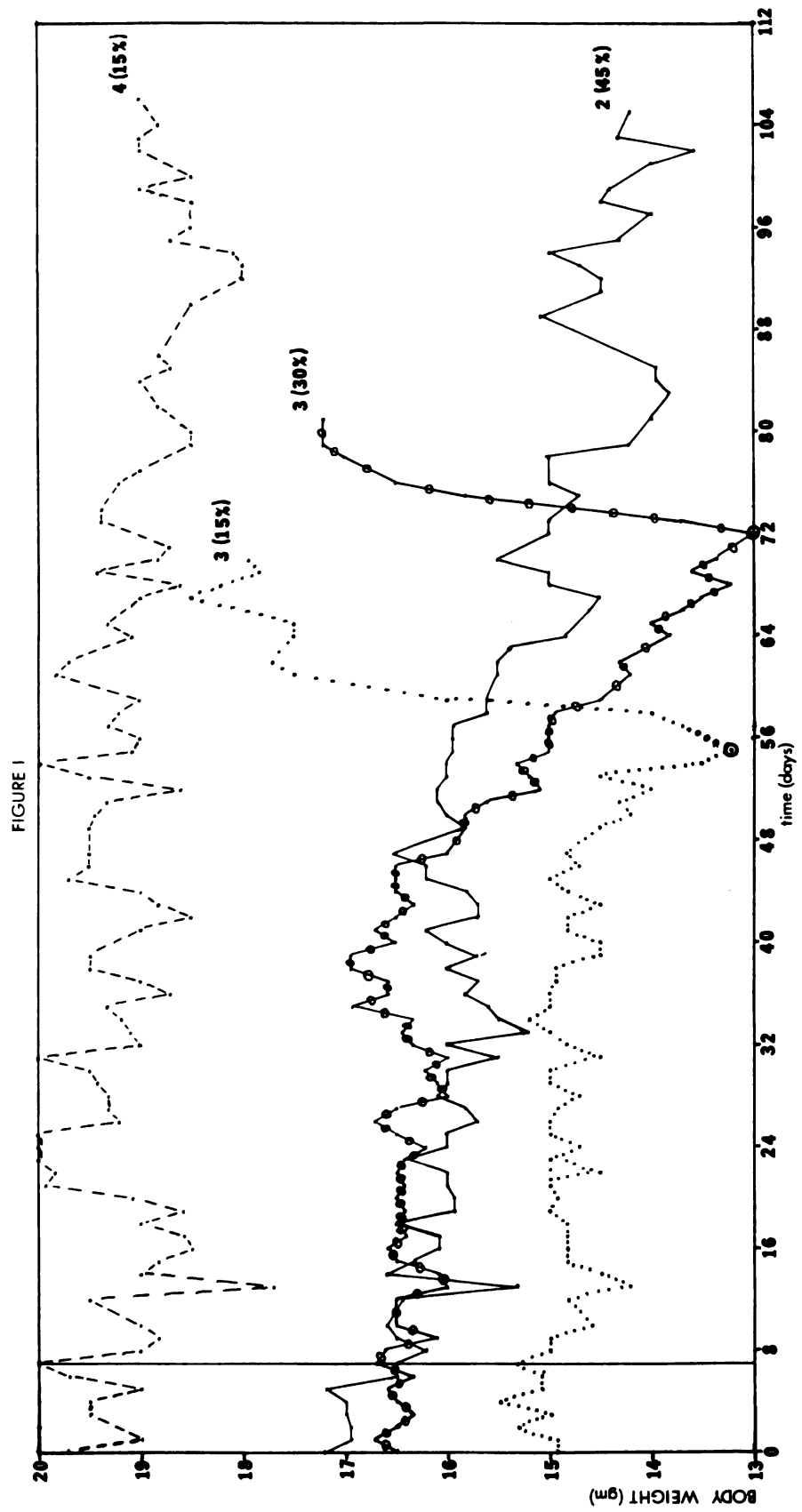


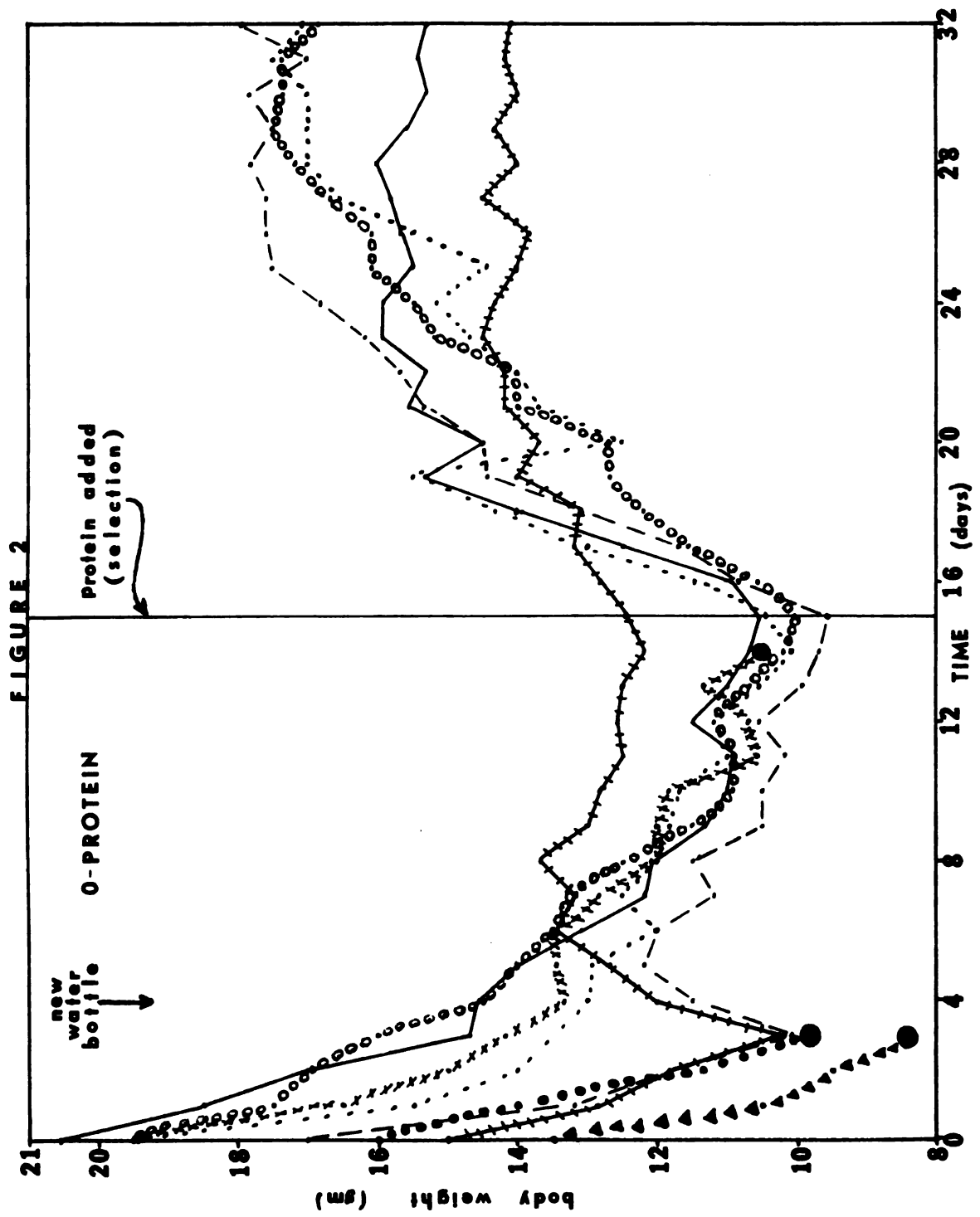
Figure 2.--Group II Animals: Zero-Protein Immediately.

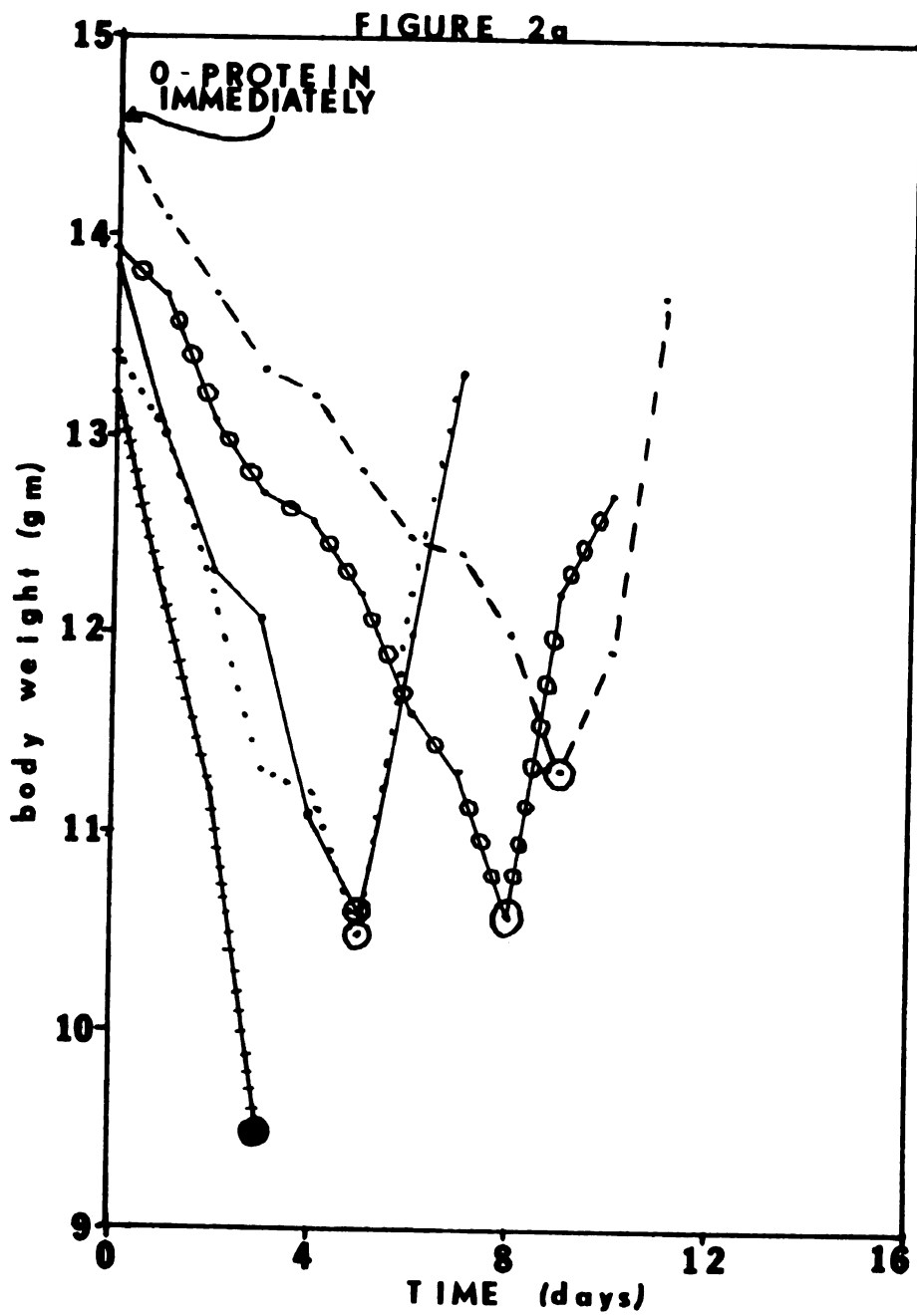
- The graph represents the body weight changes for the nine animals of Group II from the start of the O-P phase. These animals had no experience, i.e. they were placed on the O-P diet immediately. This graph should be compared with Figure 1.
- Four animals died ("●"); five were salvaged from a similar fate by the addition of protein at "○".
- "Water?"--The water bottles were suspected to be faulty and therefore may have contributed to the dramatic weight loss exhibited. Most animals did respond to the new water bottles (on Day 4), two quite sharply for several days. Their body weights however, began to decline again after that. (The arrow represents the day a new water bottle was added.)
- The symbols for animals in Figures 2, 3, and 5 are the same and as follows:

<u>Animal</u>	<u>Symbol</u>
1	_____
2	- - -
3	
4	+ + + +
7	o o o o

Figure 2a.--Repeat Group II Animals: Zero-Protein Immediately.

- The graph represents a replication of Group II's results (O-P immediately). The experiment was repeated since the faulty water bottles may have contributed to the body weight changes illustrated in Figure 2 above.
- One animal died ("●").
- All animals still living after the 20% weight loss responded positively to the addition of protein (from "○").





soon as they were placed on O-P. Four of the nine animals of this group died; the remaining five were salvaged readily from a similar fate by the addition of a dish of protein to the cage.*

Discussion

The results of this comparison showed that since Group II animals differed from those of Group I only in being naive to the experimental situation, then the experimental situation per se, i.e. prior experience at selection and/or dilution, must have imparted an ability to survive the extended periods of time without a source of dietary protein available.

*It should be pointed out here that statistical analysis of experimental data was not performed; it was felt that such analyses were unnecessary, adding nothing positive to the results as reported, in the light of the type of response elicited by the animals to the various experimental situations. The present experiment exemplifies results typical of all other experiments. Placed on a O-P regime, an animal either maintained its body weight and/or general appearance constant from Day 1 of the regime, or it did not, i.e. body weight and/or general appearance declined immediately. There was never any intermediate position assumed. In addition to this, within any given experimental situation, all animals performed the same way, i.e. they all either did, or did not, maintain body weight and/or general appearance constant on the O-P regime.

The only factor affecting whether or not a group of animals would or would not exhibit the phenomenon was the experimental situation per se; under certain circumstances the ability prevailed, under others, it did not. The intent of this thesis, in fact, was to characterize those circumstances conducive to the expression of the phenomenon, distinguishing them from those preventing such expression.

It should therefore be possible to give Group II animals the experience necessary, thus conferring on them abilities similar to those of Group I animals regarding survival.

B. Substantiating the Need for Experience

Methods

Following the above experiment, 5 of the animals of Group II were saved from their moribund course by being placed on the selection regime, i.e. by having protein added to their diet in a second dish. Dilution (at a 30% level) followed after 20 days of selection and lasted for 25 days, after which the Group II animals were again placed on the O-P regime.

Results

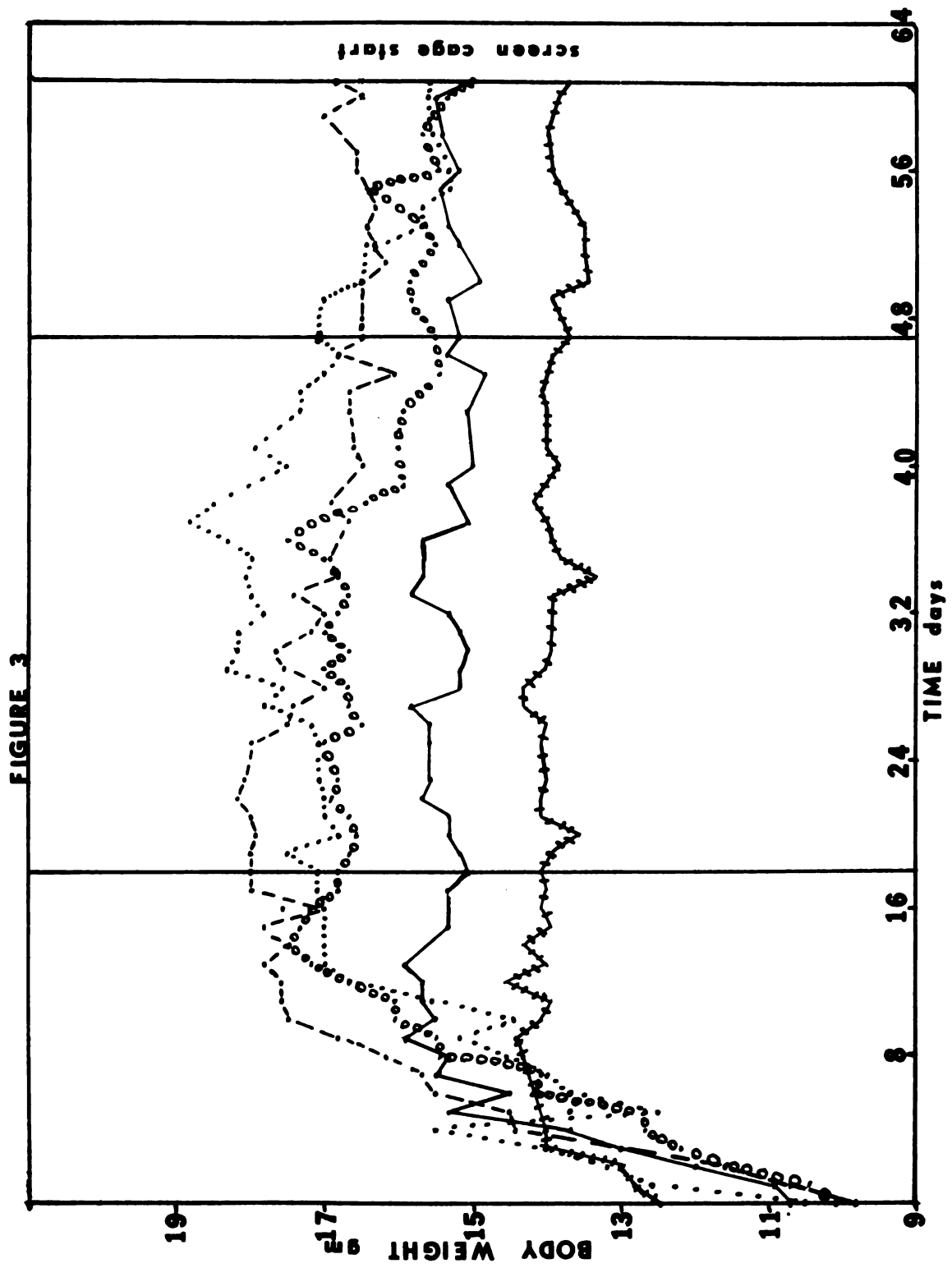
Figure 3 shows the results of this experiment. All five surviving animals of Group II sustained their body weight and general appearance with no supply of dietary protein being available.

Discussion

Results of experiments performed so far show that given prior experience at the selection/dilution regime, animals demonstrated an ability to sustain their body weight for extended periods of time (ranging from 15 to greater than 75 days), after which their body weight began

Figure 3.--Group II Survivors.

- The graph represents the body weight changes of the five survivors of Group II after being resupplied with protein (selection regime).
- It should be noted that the animals represented in Figure 2 are the same animals continued through to Figure 3 (and Figure 5: see later).
- The symbols used in representing performance of animals for Figures 2, 3, and 5 are the same; see Figure 2 for symbols used.
- The selection regime of Figure 3 is the same phase as that in Figure 2 occurring after protein's addition.



a gradual decline. The demonstration of sustained body weight exhibited by Group II animals after experiencing the selection/dilution regime was terminated after 14 days; it was considered unnecessary to carry it further. The point had been made within the first few days of the O-P regime, namely, that these animals had acquired an ability differing from that demonstrated by them during their first encounter with the O-P regime. There was no indication by any of the five animals of Group II that results would be different from results demonstrated by Group I animals.

Subsequent to the Establishment of the Phenomenon

A. Selection and/or Dilution as the Needed Experience

Introduction

Clarification of the exact type of experience required to confer on the mice the ability to survive the O-P regime was necessary to minimize the length of, and to hasten the conclusion of, future experimental determinations, as well as to serve as possible explanation as to what is taking place with wild P. m. bairdii in the field situation. Attempting to imagine how or when the wild P. m. bairdii would ever experience a choice situation involving pure protein on the one hand, and the other ingredients of a completed diet on the

other, i.e. the conditions intrinsic to the selection regime alone, were futile. This simply would not occur in the field situation.

However, it was entirely plausible for a mouse to be experiencing graduated dilutions of its source of dietary protein, either absolutely or relatively to the other ingredients of the diet. In fact, absolute quantities of the protein component of a plant for example, can vary in their relative availability by virtue of the plant's fiber (cellulose) content, that same ingredient used in the dilutions of these experimental diets. Thus the probability that the dilution phase was the more significant of the two was suggested.

Methods

Five male P. m. bairdii between three and four months of age were presented with the dilution regime for eight days, after which they were placed on the O-P regime.

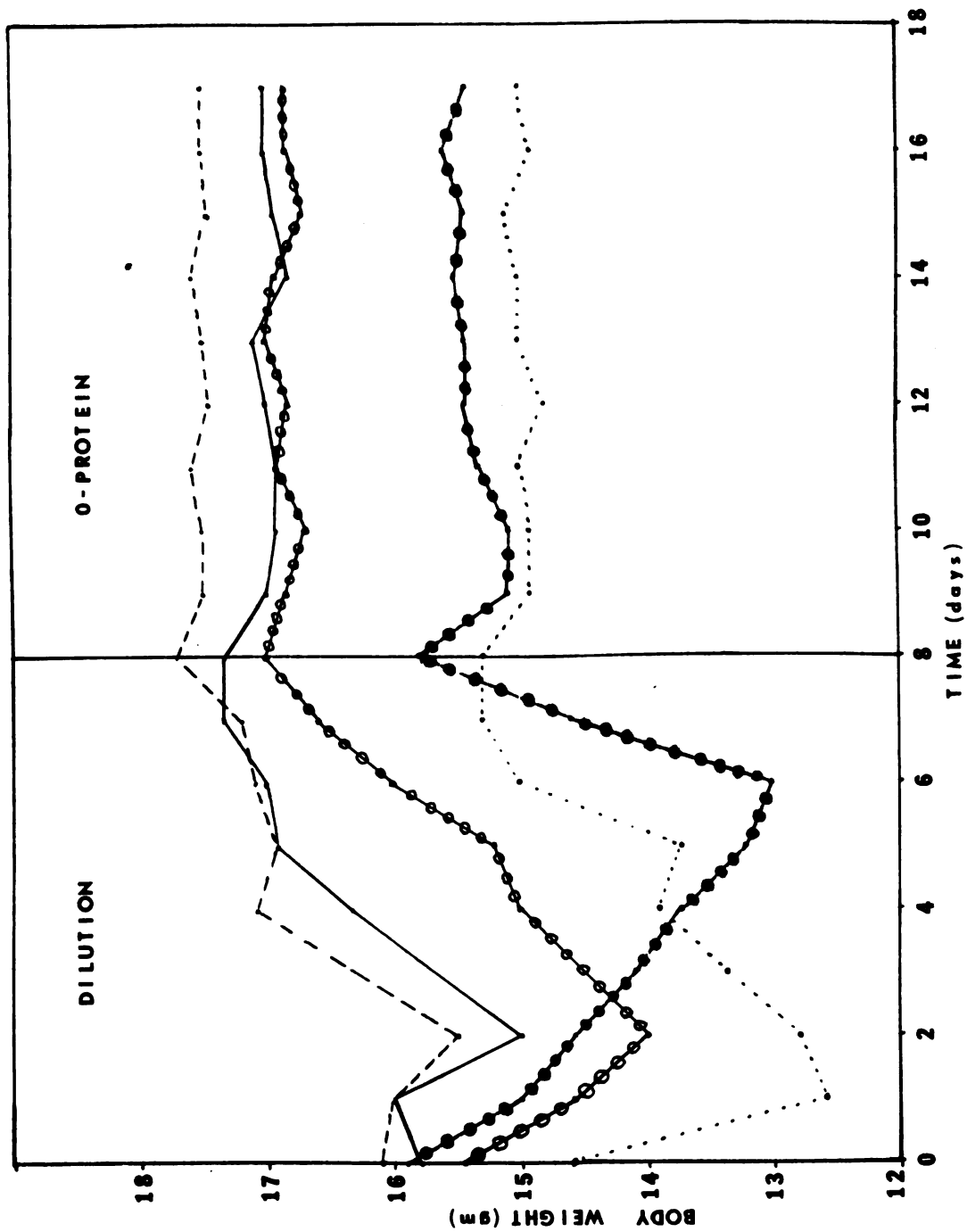
Results

Figure 4 illustrates the results of this procedure. All five animals sustained their body weight for nine days of the O-P regime. The initial decline in body weight is attributed to an adjustment period as discussed on page 82.

Figure 4.--Dilution Alone, Then Zero-Protein.

- The graph represents the body weight changes of five animals demonstrating that the dilution phase was sufficient for the phenomenon to appear.
- Animals were placed on the dilution regime immediately and kept on it for eight days.
- The O-P phase commenced on the eighth day.

FIGURE 4



Discussion

In view of the immediacy of the weight loss in every instance when the animals did not have the ability to endure the O-P diet, it seemed unnecessary to carry this experiment out longer than the nine days; again, there was no indication that these animals would be any different from those of Group I (Experiment A, Set I) in this regard.

It should be noted that this experiment demonstrated that the dilution phase was sufficient for the appearance of the phenomenon and thus was the more apropos of the two phases relative to the earlier discussion of the events occurring in the field. Whether it is also necessary depends on whether or not the selection phase is also shown to be sufficient; this possibility was not excluded by the present experiment. Preliminary testing however, suggested that this latter alternative would not, by itself, have this relationship to the phenomenon. Testing was fairly uncontrolled, hence further work is necessary here.

How the dilution phase became significant to the mouse in imparting the needed experience was suggested by the use of screen cages.

B. The Effects of Screen Cages

Introduction

It is well known that rodents (among other animals) exhibit degrees of coprophagic behavior varying with the species and the extent of the diet's deficiency in one or more ingredients relative to the body's requirement for these ingredients, as discussed in the literature review. It was felt that this could be a contributing factor to the animal's ability to survive on the O-P regime.

Methods

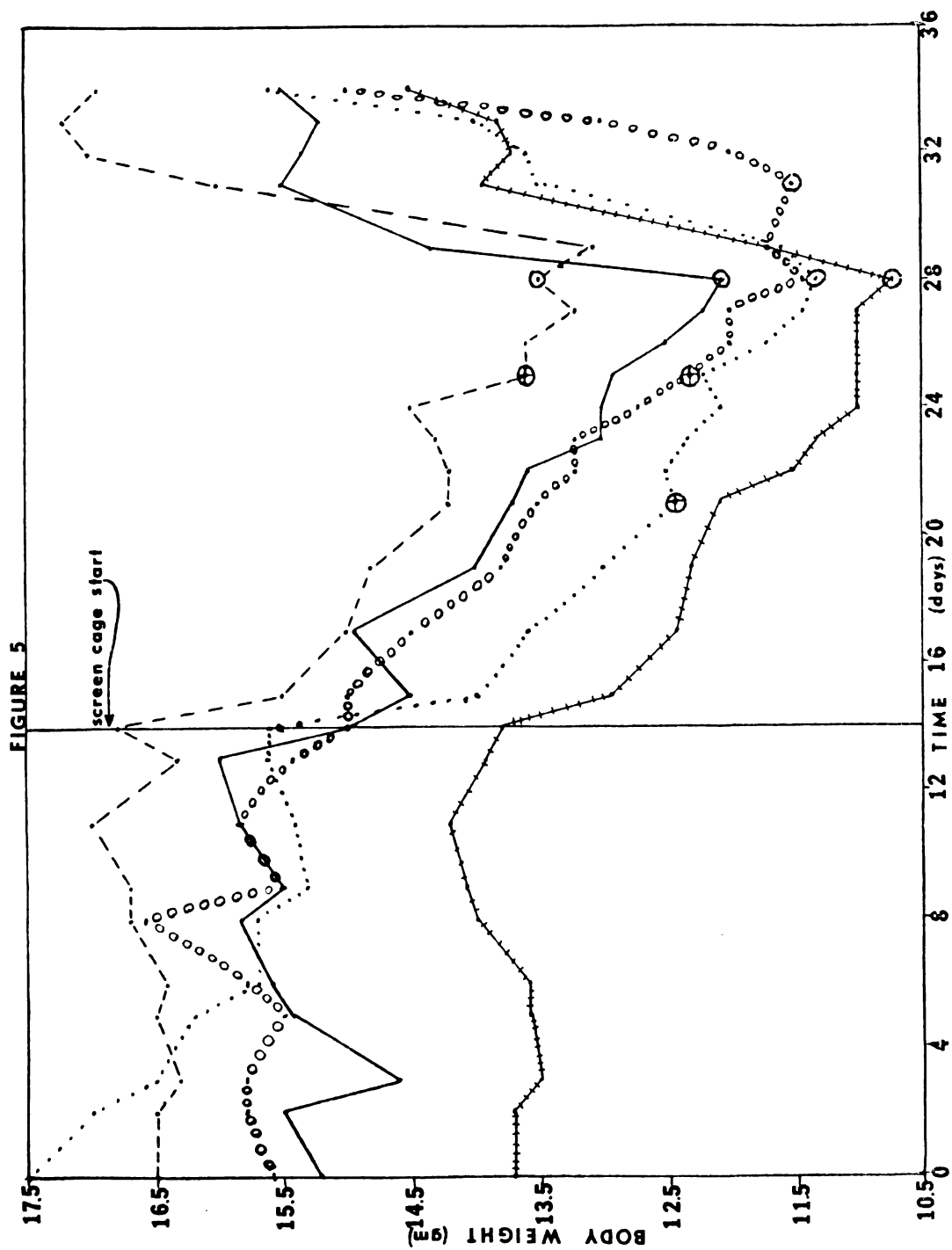
The five mice of Group II (Experiment A, Set I), after having demonstrated their ability to sustain body weight on a O-P diet (having been given the needed experience beforehand), were placed in screen-bottom cages in an attempt to reduce the quantity of coprophagy. The animals were continued on the O-P regime through the transition from regular cages to screen cages.

Results

Figure 5 shows that the body weight of all animals declined immediately when they were placed in the screen cages. It was also observed that the decline in body weight was not altered by placing three of the animals back into regular cages (animals 2, 3, and 7) after several days on the screen cages; restoration of the

Figure 5.--The Effect of Screen-Bottomed Cages on Body Weight.

- The graph represents the effects of screen-bottomed cages on the body weight changes for the five salvaged animals of Group II (Figures 2 and 3).
- This graph follows from Figure 3; it begins at the second O-P phase of Figure 3.
- The placement of the animals back into the regular cages (at "●") did not alter the decline in body weight.
- In every case, reversal of the weight loss was not accomplished until a protein dish was added (at "○").
- See Figure 2 for symbols used.



pre-screen cage body weight was not accomplished until a dish of protein was added to the cage in every instance.

Discussion

This experiment, using screen cages with Group II animals, showed that even minimal reduction in coprophagy lowered the animal's ability to sustain body weight on the O-P regime by removing a useful ingredient from the animal. It was recognized that total prevention of coprophagy could not be accomplished without the use of tail cups or collars as mentioned in the literature (Geyer et al., 1947; Morgan et al., 1961; Yukkin, 1963), but the reduction in coprophagy was accomplished by the use of the cages. Feces fell through the cage floor and were therefore inaccessible to the mouse.

The potential nutritional importance that fecal material can provide the animal has already been elaborated upon. That Group II animals were deriving protein from the recycled feces, this protein being the factor enabling them to maintain their body weight constant on the O-P diet, is suggested by the fact that the decline in body weight could not be reversed until the dietary protein source was replaced to both those animals in screen cages as well as those in regular cages.

This experiment, establishing that coprophagy was the probable means whereby the animal was recovering lost proteinaceous substances from the body for use in

body weight maintenance during periods of dietary protein deficiency, also suggested that the better an animal is at extracting a greater amount of protein from this recycled feces for a greater length of time, the longer this animal would be expected to maintain its body weight and other physiological processes stable. Animals at the other end of the spectrum of efficiencies would have stable body weights for a short time but then begin a gradual decline to death from protein starvation. (All animals would eventually die of protein starvation; the difference between animals would therefore be in their ability to recapture and reuse this fecal protein.)

These expectations were entirely consistent with the observed results. Animals did vary in their ability to maintain body weight constant (some being better than others). All animals would have eventually died had they been allowed to do so, the reason suggested: excessive reuse of fecal protein to the point of exhaustion, as well as the continued requirement and therefore drain of body protein sources. The most direct test of these statements would obviously be a measure of fecal protein content over time. This was attempted but proved technically unsuccessful; it should be repeated.

C. Screen Cages During Dilution

Introduction

It was becoming increasingly apparent that there was a connection between the requirement of the dilution phase as being the needed experience and coprophagy per se. It was shown that some experience at dilution was required for the phenomenon to appear. It was also suggested that coprophagy has the ability to supply the animal with protein.

Where then did the difference lie between Group I and Group II animals? Simply in their experiencing the dilution phase? It was felt that coprophagy was taking place during the dilution phase, and in fact this was the significance of the dilution, not simply the experiencing of dilution per se. Testing this hypothesis occurred by preventing coprophagy during the dilution phase and allowing it during the subsequent O-P phase.

Method

Six male P. m. bairdii* approximately two to three months of age were placed in screen cages during an immediate dilution phase, the length of which depended upon

*As a check on the accuracy of this experiment since only six animals were involved (this being an important part of the thesis), six more animals were given similar treatment, the results of which are shown in Figure 6b, concurring with those of Figure 6a.

the length of time required for the animals' body weight to become stabilized. At that time, the animals were placed in regular cages and the protein portion of the diet was removed.

Results

Figures 6a and b illustrate the results of this experiment. It can be seen that when coprophagy was prevented during the dilution phase, animals could no longer endure the O-P diet, even if this latter phase took place in regular cages, with their feces available for recycling. The results show that the general appearance of two animals was grossly deteriorated, one almost dying.

Discussion

Experiments have shown that dilution was important to the appearance of the phenomenon, and also that coprophagy could resupply useful protein to the animal. The relationship between these two events became apparent with the present experiment: coprophagy was occurring of necessity during the dilution phase.

It was therefore felt that the difference between Group I and II's (Experiment A, Set I) response to the O-P regime was due to the fact that Group I had experience at dilution as stated, but moreover, it was experiencing coprophagy per se, or the metabolic attributes of

Figure 6a.--Screen Cages: Dilution; Regular Cages:
Zero-Protein.

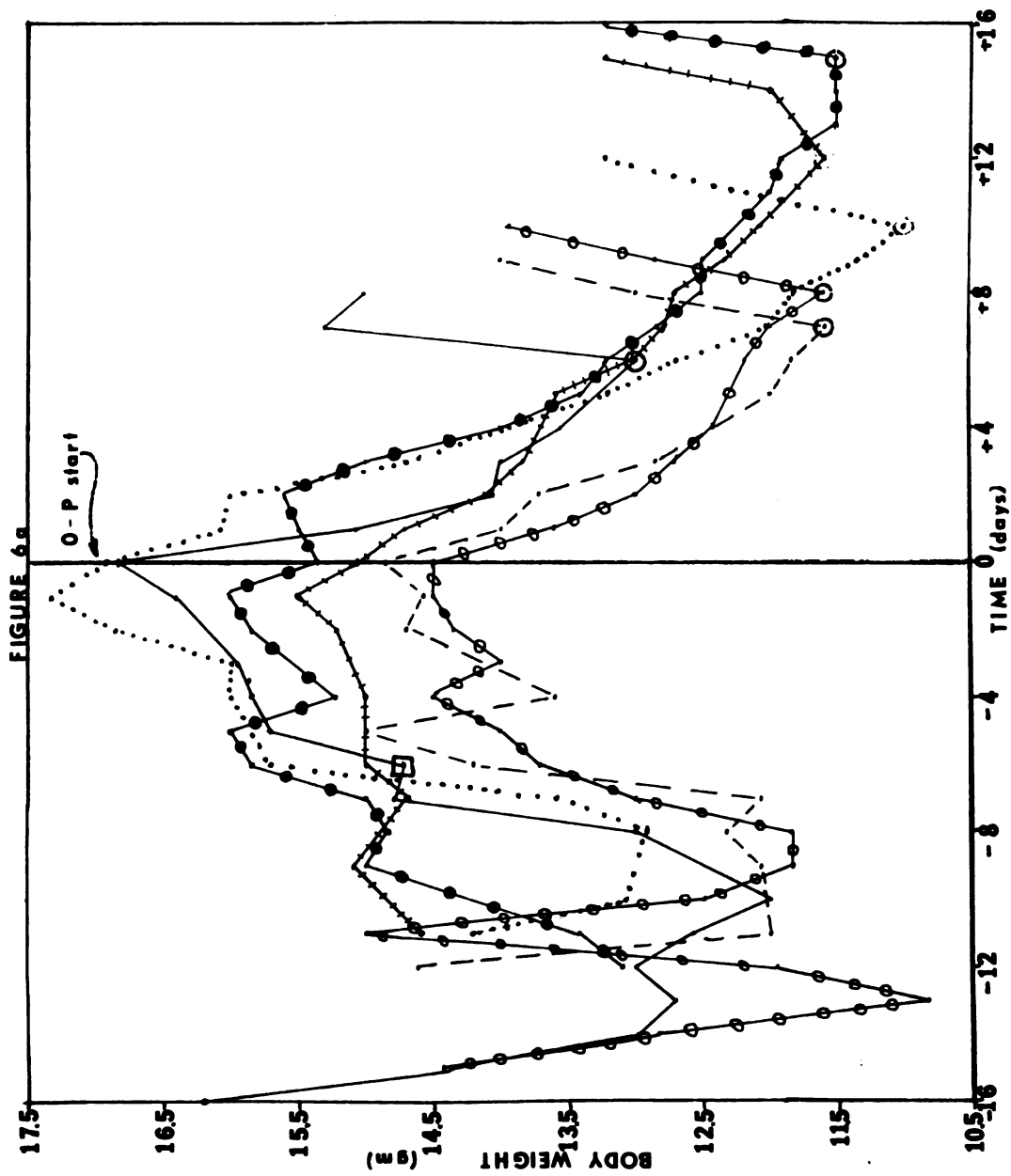
Figure 6b.--Repeat--Screen Cages: Dilution; Regular
Cages: Zero-Protein.

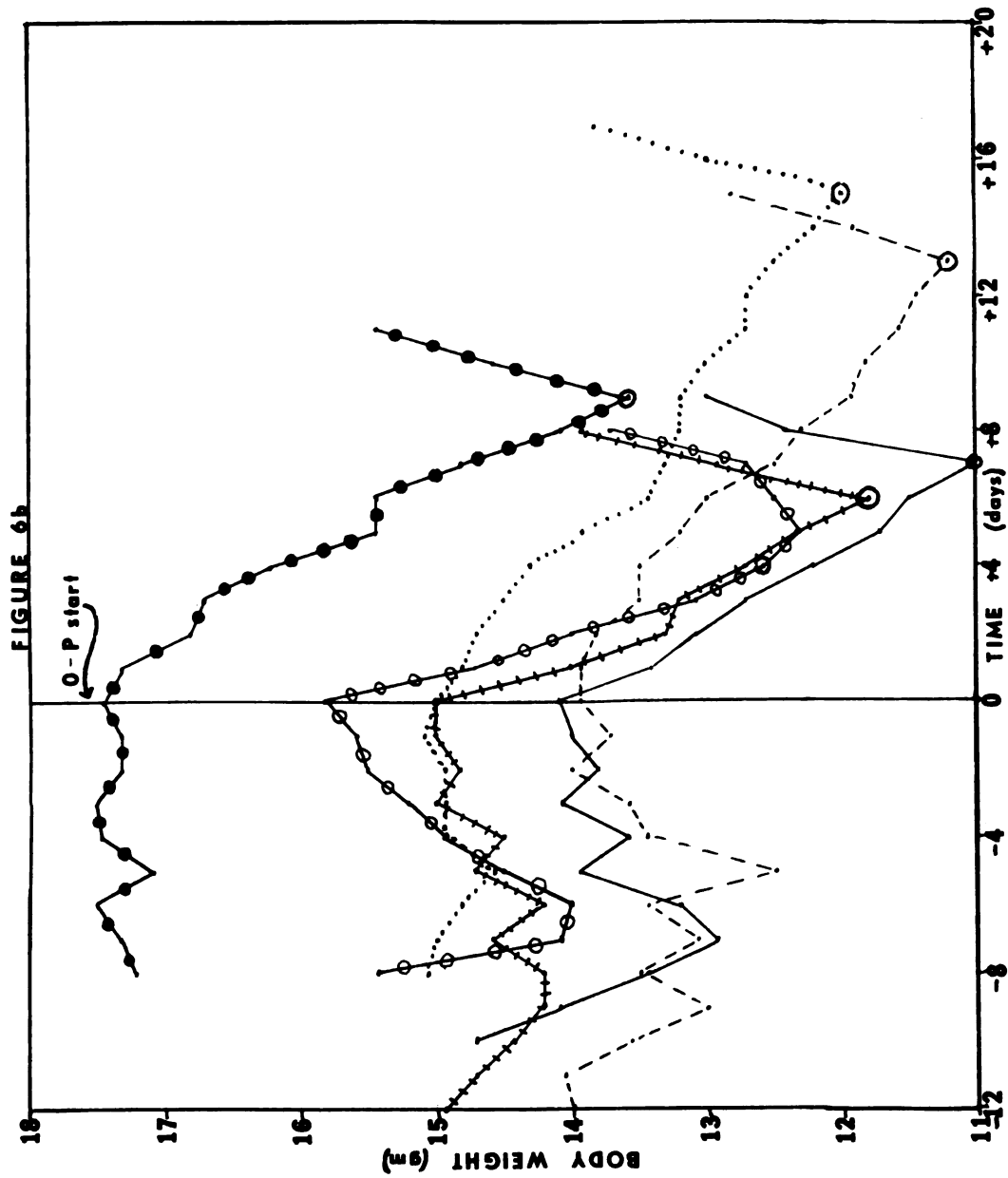
--The graphs represent the body weight changes occurring
when animals were placed on the dilution regime in
screen then the O-P regime in regular cages.

--Figure 6b is a replication of Figure 6a.

--In every case body weight decreased when the O-P
regime was initiated (at line).

--In every case the body weight change sharply reversed
itself when protein was resupplied ("o").





coprophagy, at a time when the animals were in need of an additional source of protein. When these animals were then placed on the O-P regime, having already experienced coprophagy and the benefits this behavior could provide them during times of dietary deficiency (this association being made when some protein was available), there was no problem in making the smooth, rapid transition to coprophagy when the protein was removed.

Group II animals on the other hand, had no experience at coprophagy prior to being placed on the O-P regime where knowledge about this behavior would have been useful. They first had to learn about coprophagy during the O-P phase. Although they may very well have achieved this given sufficient time, time was not in their favor; they were losing weight rapidly while they were learning (if indeed they were learning) to the point where their fate had become irreversible.

The above was preliminary thinking to this point in time. The logic, as it applied to the present experiment was that, without the feces available during the dilution phase, the animals would be lacking experience at coprophagy during this phase of dietary deficiency, effectively placing them in a similar situation as were Group II animals on experiencing the O-P diet for the first time. Therefore, when placed on the O-P diet (regular cages), their body weights should have decreased

as did the animals' of Group II. The results of this experiment show this to be true.

D. Length of Time for Application of Dilution Phase

Introduction

Exactly how long the animals had to have experienced the coprophagy during the dilution phase for the phenomenon to appear became apparent by experiments allowing graded periods of time on the dilution regime. The original experiment allowed approximately 22 days on the dilution regime, after which the O-P regime was implemented.

Method

Two experiments were performed to determine the minimal length of time for the animals to remain in the dilution phase for the phenomenon to appear. Ten male P. m. bairdii of approximately three months of age were used in these experiments; they were divided into two groups of five animals each.

The criterion chosen for the length of time in dilution was whether or not the pre-dilution body weight had been re-established before the O-P diet was presented. This was eight and five days, respectively, i.e. the O-P diet was instituted after only five days or eight days of dilution, this latter representing a length of time

on the dilution regime for the pre-diluted body weight to be re-established.

Results

The results of these experiments are illustrated in Figures 7a and b. It can be seen that after only five days of the dilution experience none of the animals involved could endure the O-P regime; their body weights declined to the 20% criterion. In addition to the decline in body weight, one animal died long before the 20% criterion had been achieved for it, and two others were quite bad in appearance; they were found shaking in a corner of the cage.

It should be noted that the decline in body weight for four of the five animals of this group differs from other groups reported in this thesis in that the decline did not begin immediately after the initiation of the O-P regime but rather remained stable for approximately three to six days of a seven day experimental period.

Those animals experiencing the dilution phase for eight days (representing a length of time sufficient to permit their body weights to return to the pre-dilution weight), were all able to endure the O-P regime with no loss in body weight.

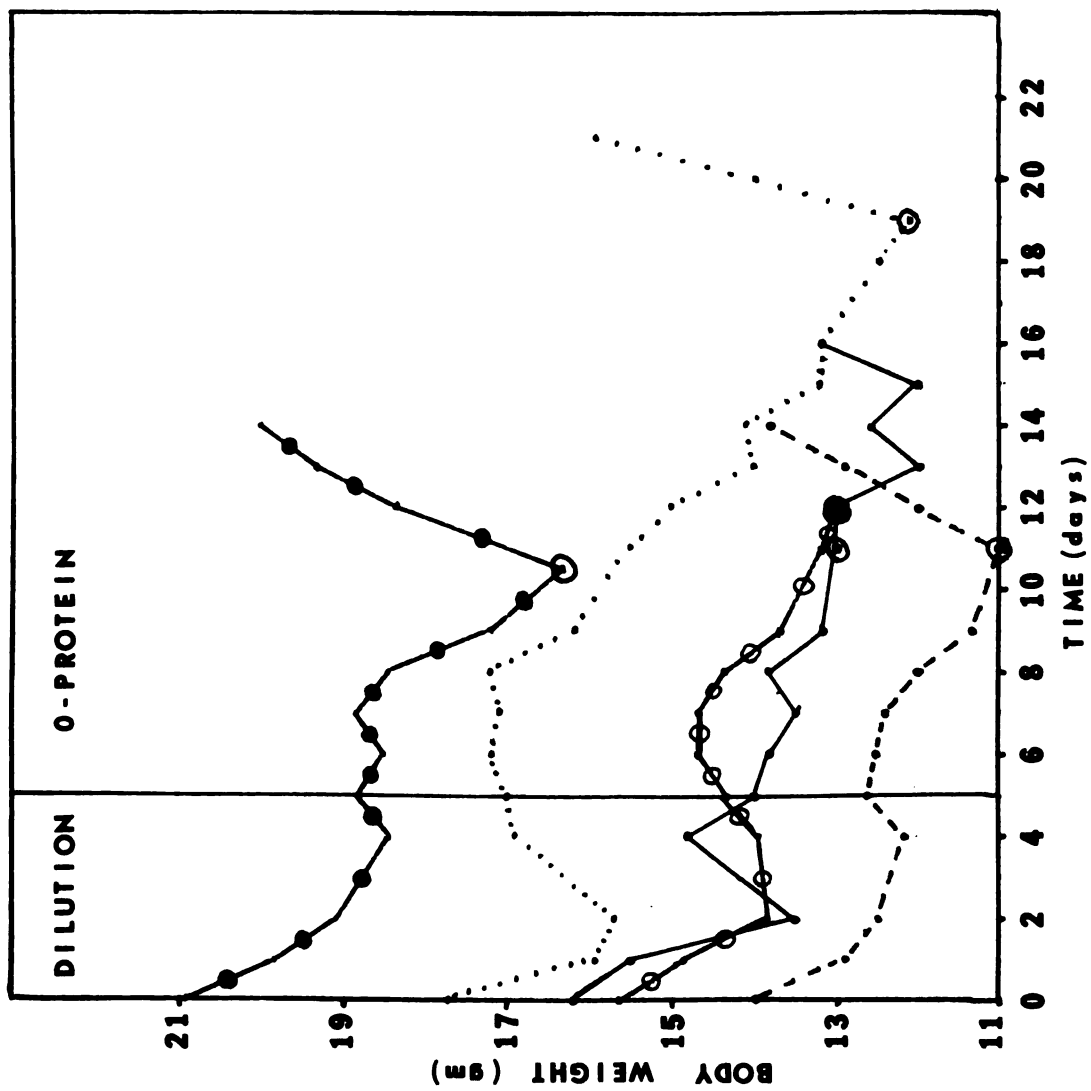
Figure 7a.--Duration of Dilution (5 days).

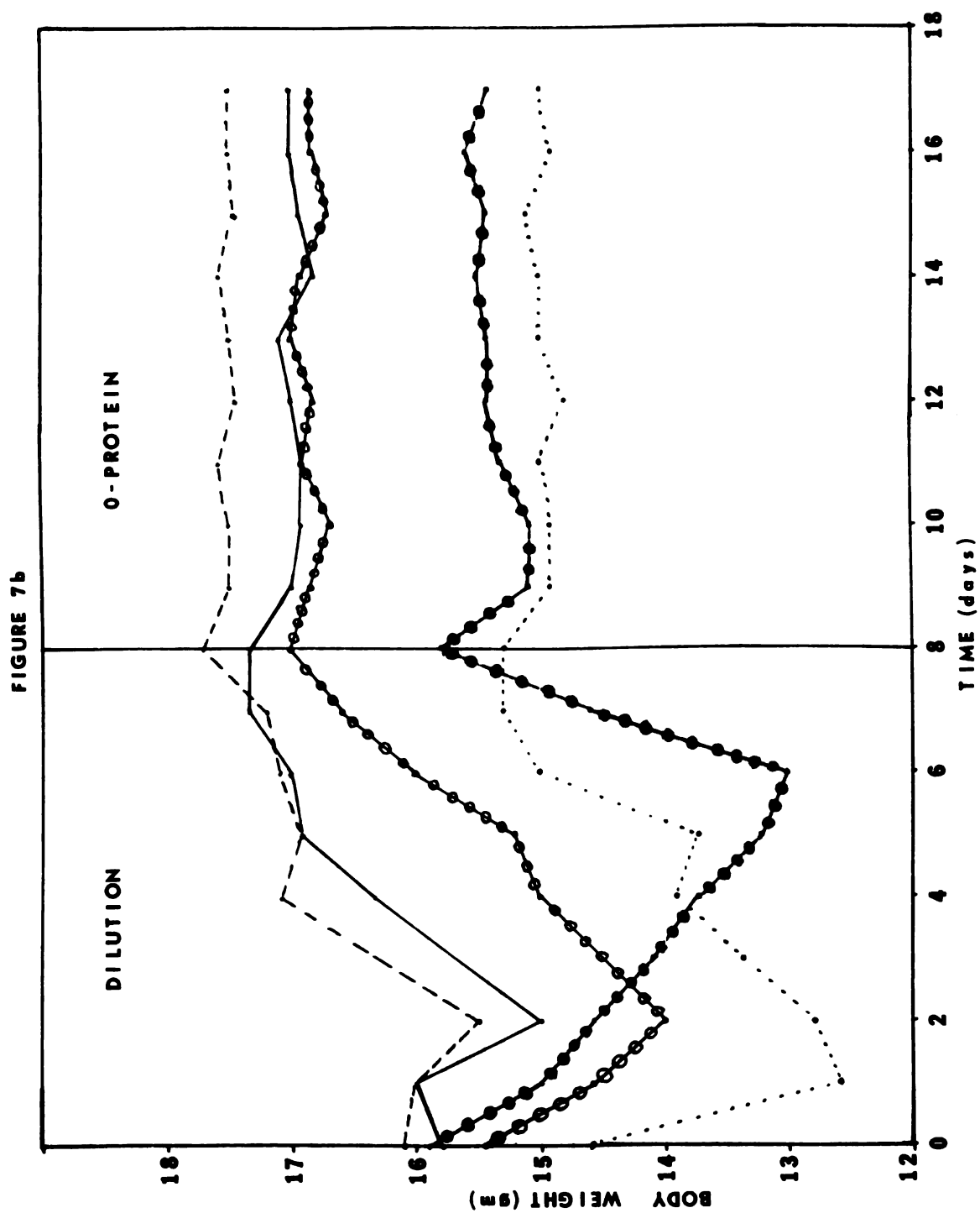
--The graph represents the changes in body weight occurring when dilution was in effect for only five days. Five days was less than sufficient time to permit the reestablishment of the pre-dilution body weight.

Figure 7b.--Duration of Dilution (8 days).

--The graph represents the changes in body weight occurring when dilution was in effect for eight days. Eight days was sufficient time for the reestablishment of the pre-dilution body weight.

FIGURE 7a





Discussion

This experiment provided either five or eight days experience on the dilution regime. These lengths of time were selected because they represented a period of time before which, or after which, respectively, body weight had returned to the pre-dilution level. On initiating the dilution regime, a small drop in body weight was observed. The animals usually recovered from this initial decline within 1 to 1 1/2 weeks, although there were exceptions in either direction. (This decline probably represented at least a period of adjustment to a new diet regime which was reflected in the decrease, followed by the re-establishment of the body weight.)

The intent of this experiment was to wait until all animals of a group had recovered the lost weight, whatever the time required for this to occur, before initiating the O-P regime. This turned out to be eight days for all the animals of Group B, hence the eight day period mentioned above. The animals of Group A were placed on the O-P regime before their body weight had returned to pre-dilution levels. At approximately five days on the dilution regime, two animals were beginning to reverse their weight loss, hence the five day time here.

Fortunately the groups comprising this comparison were fairly close in time durations, thus a fairly accurate specification of the time required for dilution to be

experienced could be stated. These experiments show that animals must experience the dilution phase for at least eight days for the phenomenon to appear. Whether or not the criterion is actually time in terms of days, or rather that the pre-dilution body weight had or had not been re-established prior to the O-P regime, this being eight or five days respectively, was not clarified by this experiment.

Considering the unusual manner of body weight loss exhibited by Group A animals of this experiment, one cannot help speculating that if the five and eight day interval on which this experimental comparison is based was not fortunate in yet another way. It is possible that the five day animals were on the borderline between demonstrating the phenomenon and not. It would be interesting to repeat this experiment using four days on the one hand, and six or seven days on the other. Would the four day group show a more immediate weight loss than the five day group while the six (or seven) day group be more like the eight day animals?

The reader should be reminded at this stage that the significance of the dilution phase to the animal is as a means of first experiencing the attributes of coprophagy. Therefore, coprophagy must be experienced for at least eight days for the phenomenon to appear.

D. Sex of the Animal

Introduction

It was of interest to ascertain whether or not female P. m. bairdii would also exhibit the phenomenon.

Method

Two separate experiments were performed using five animals in one group, six in the other. The animals were between two and four months of age. They were placed on the selection, dilution, and then O-P regime, this latter phase not being initiated until an animal had stabilized its body weight. In most cases this was either at, or very near, the pre-experimental weight. After reaching the 20% criterion, all animals were placed back on a selection regime.

Results

The results of these two separate attempts (total of eleven animals) at determining whether or not female P. m. bairdii also exhibit the phenomenon are shown in Figures 8a and b. In every case females were not able to sustain their body weight on the O-P regime. In every case, after achieving the 20% criterion, the mice responded positively to the addition of protein to the cage.

Figure 8a.--Testing Females (Original).

--The graph represents body weight changes occurring in female Peromyscus maniculatus bairdii when subjected to the selection, dilution, and O-P regimes.

--The selection, dilution, O-P, and protein addition phase initiations are indicated by vertical lines.

Figure 8b.--Testing Females (Repeat).

--This graph represents the same information as presented in 8a.

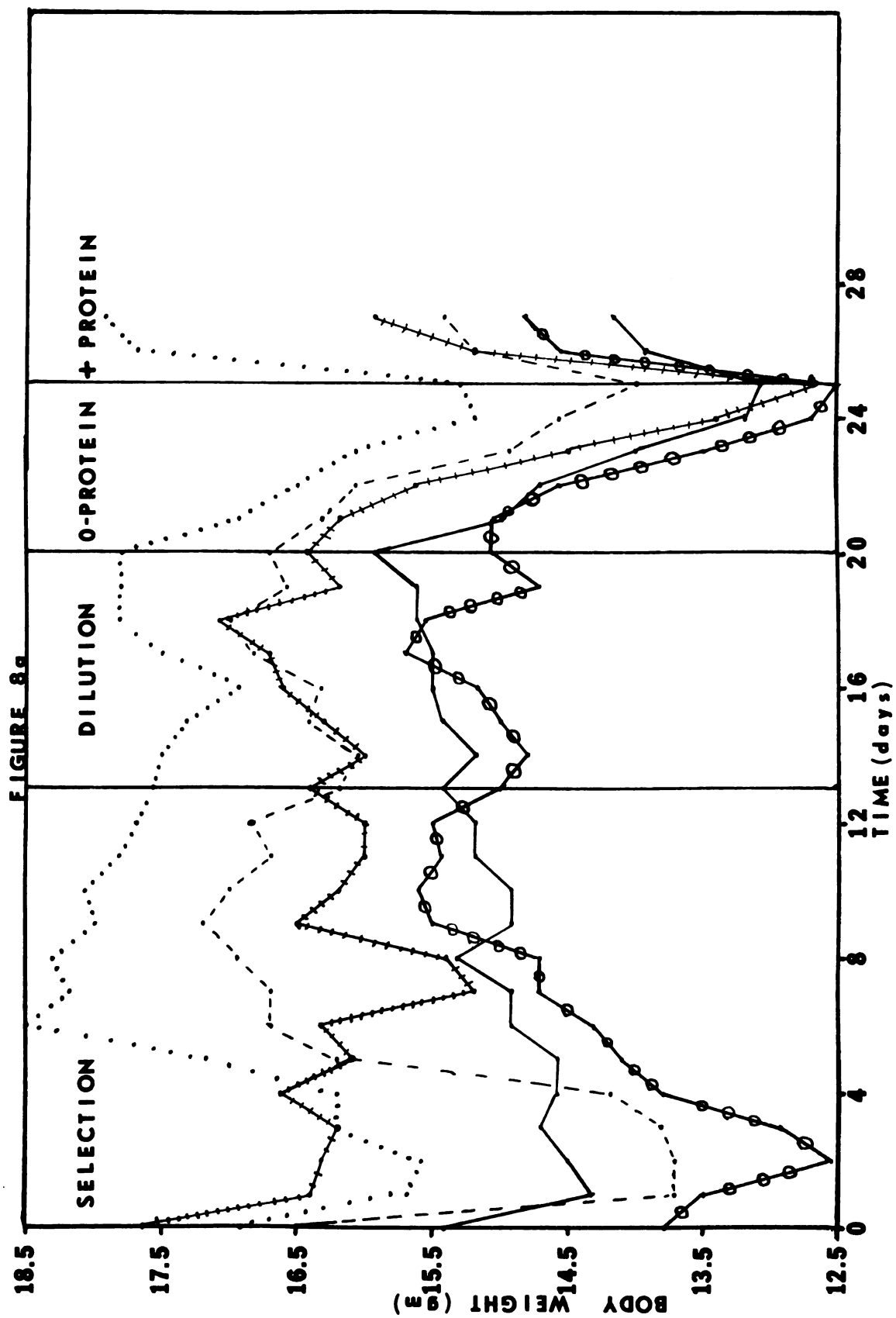
--Due to the variable lengths of time involved for the commencement of the various phases for each animal, the following symbols were used:

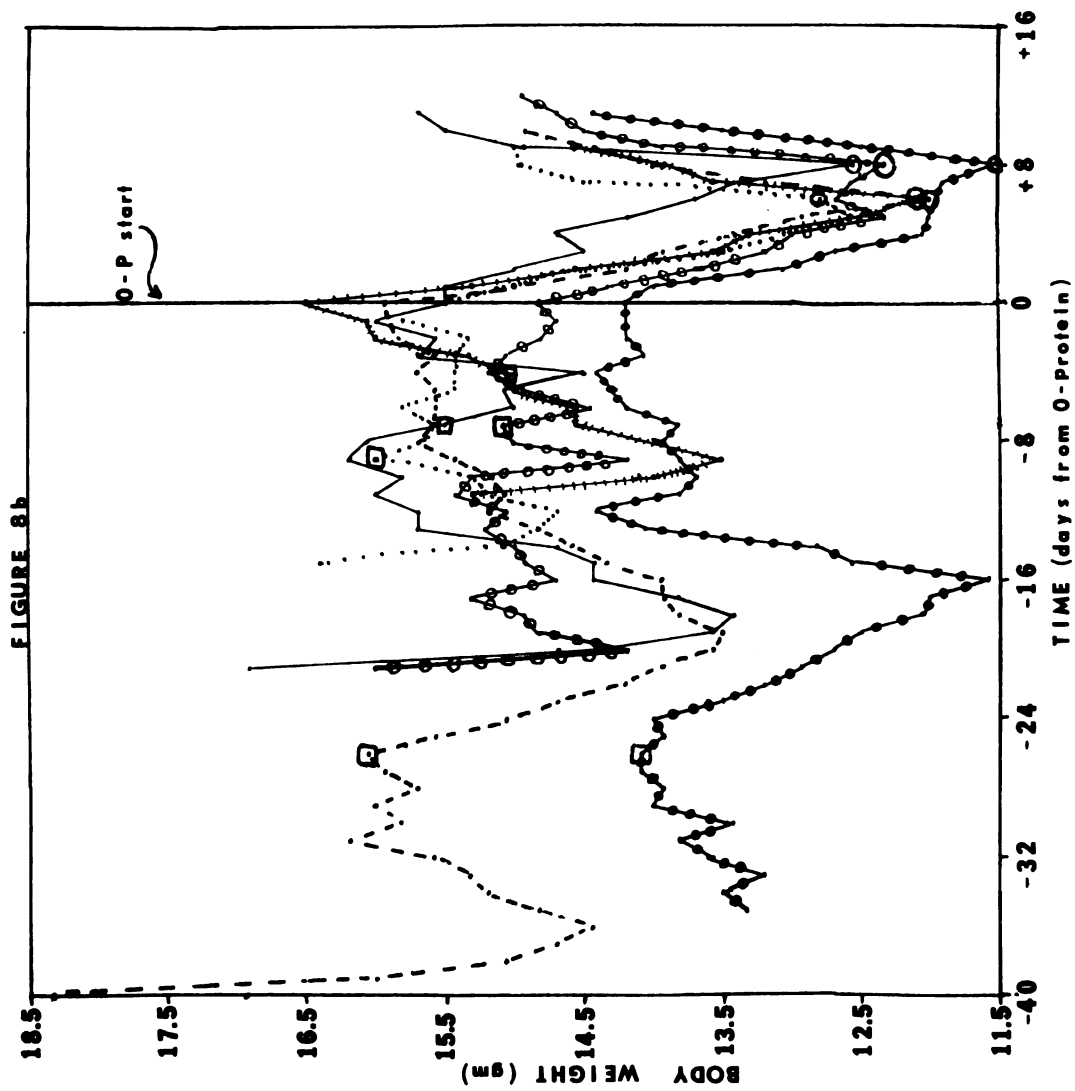
--animals were started off on the selection regime; selection was continued until the dilution phase began.

--commencement of the dilution phase = 

--commencement of the O-P phase = |

--commencement of the protein addition = ⊙





Discussion

Traditionally, females have not been used in food intake experiments because their cyclic changes in behavior coinciding with their reproductive cycle tended to interfere with interpretation of food intake data. Reasoning for the use of females in this thesis stemmed primarily from curiosity, but also for reasons of completeness. If the phenomenon observed had real applicability under field conditions, no reason could be found to suppose why females should not also have the same need to demonstrate similar abilities as the male; both sexes would be subjected to the same fluctuations in dietary protein levels.

If females did not handle the problem in a manner similar to males, then either they have their own, unique manner of dealing with protein fluctuations, or they must die off in greater numbers during periods of protein depletion. This latter would be indicated in the sex distribution of wild populations. Blair (1940), Howard (1949), Linduska (1942), using live traps, nest boxes, and snap traps, respectively, indicate males comprising 51% of the population (significance not demonstrated for this figure). Although males are more active than females, thereby having a greater trap exposure (Townsend, 1935), the distribution ratio of males to females is not sufficiently different in any species of Peromyscus

reported in King (1968) to justify concluding that females were dying off in excessively large numbers due to an inability at handling dietary protein deficiencies.

This suggests that females must have an alternative method for handling the situation, one which was not revealed by these experiments as designed. No suggestion as to what this alternative method might be can be made at this time.

E. Age of the Animal

Introduction

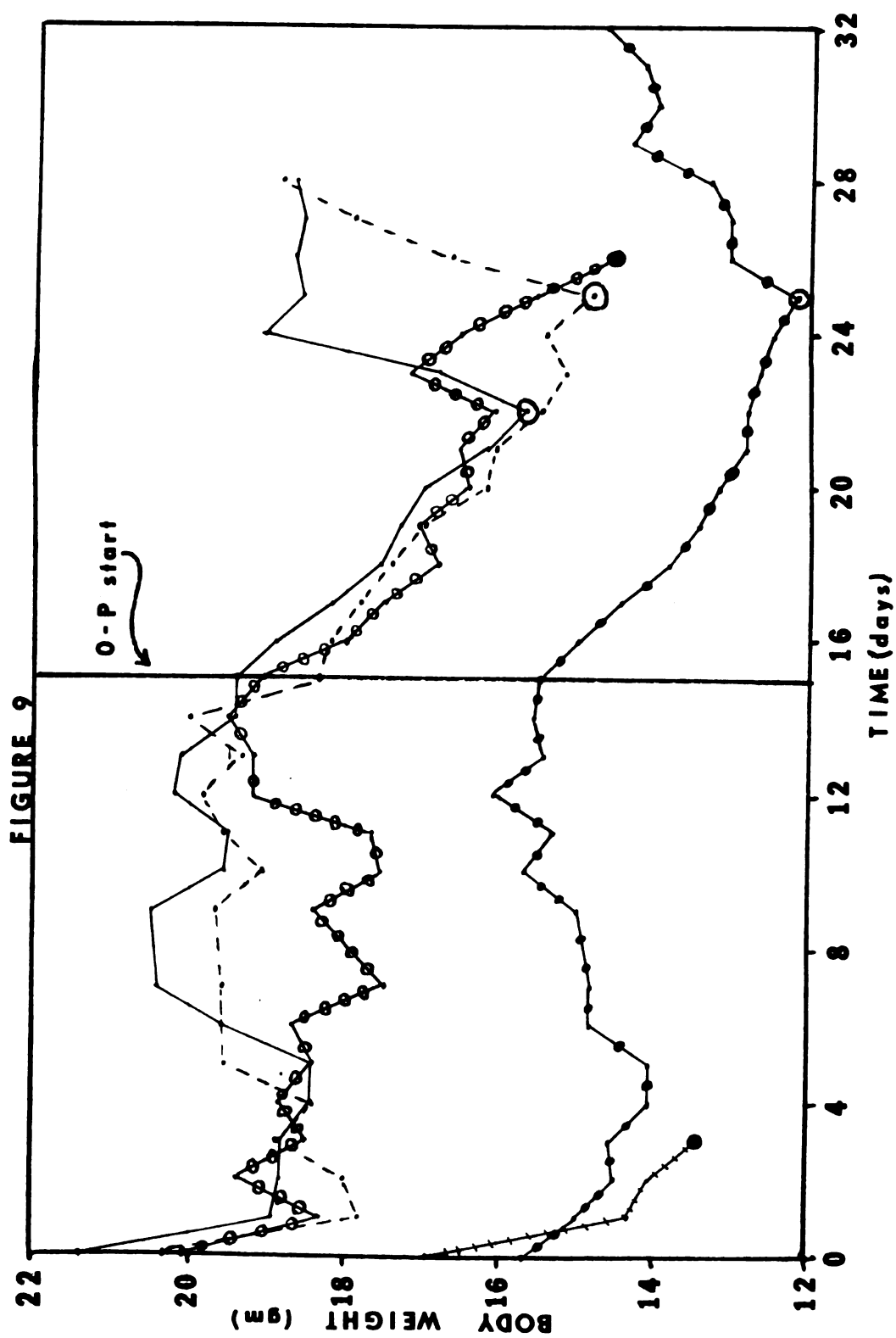
The original experiments were performed using mice ranging from two to four months of age. Unknowingly, one experiment was performed using mice of seven to nine months of age, the results of which are shown in Figure 9. These older animals could not endure the O-P regime. In fact, one animal died while in the dilution phase; another died on the O-P regime after exhibiting extreme degradation of its general appearance.

Method

Attempting to elucidate these apparently bizarre results by repetition, and also to specify more precisely the age at which the animals no longer demonstrated the ability to endure the O-P diet (were these preliminary results truly accurate) another experiment was performed. Four male P. m. bairdii between the ages of six and

Figure 9.--Effect of Age (Seven to Nine Months).

- The graph represents the body weight changes resulting from the age of the animals involved.
- All animals (5) were between seven and nine months of age.
- The graph starts at the dilution phase; O-P starts as indicated at the vertical line.
- Two animals died "●" (one during dilution; one during O-P).
- All survivors responded positively to the addition of protein at "○".



seven months of age were placed on the dilution regime for a period of eight days, after which the protein dish was removed.

Results

The animals maintained body weight constant during the dilution phase, but could not endure the O-P diet once initiated. Results illustrated in Figure 10, appear quite similar to those of the original age experiment (Figure 9). One animal died after four days of O-P; two had seriously deteriorated in appearance, requiring the addition of protein to prevent their deaths.

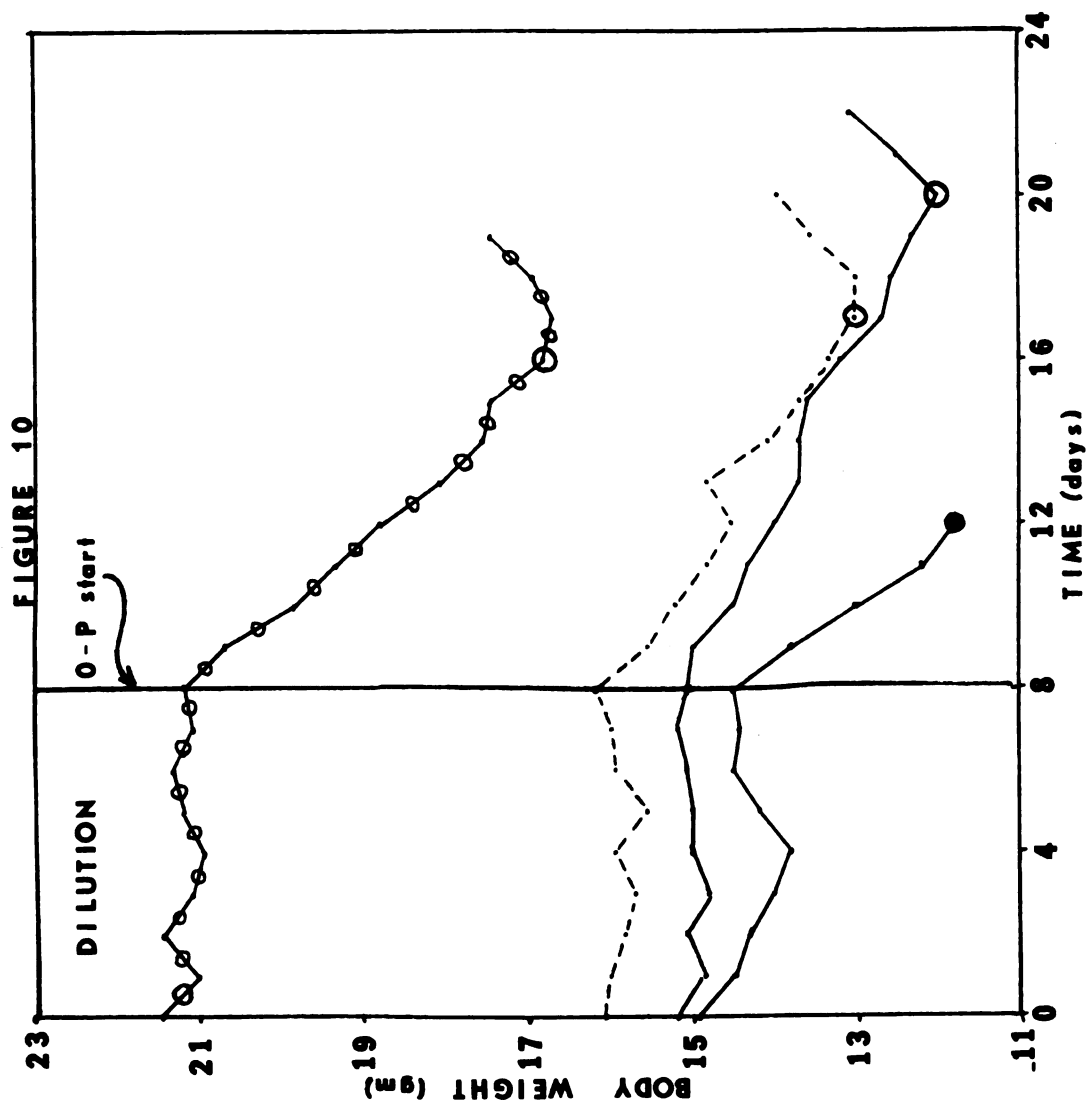
Discussion

Animals older than seven months of age were subjected to the dilution, then O-P regime, and were found not to be able to sustain body weight on the O-P diet, while animals five months of age or less (presumably to two months of age--the earliest age tested) could endure the regime. A second experiment using animals six months of age and older narrowed the required age for the appearance of the phenomenon to between five and six months of age.

Under conditions used in these experiments, results indicate that animals appear to lose the ability to endure the O-P regime with age (approximately six months of age), i.e. those animals between two and five

Figure 10.--Effect of Age (Six to Seven Months).

- The graph represents a repetition of the age experiment (Figure 9) using four animals six and seven months of age.
- The graph starts in the dilution phase; O-P phase starts as indicated by the vertical line.
- One animal died during the O-P phase "●".
- All survivors responded positively to the addition of protein at "○".



months will demonstrate the phenomenon, provided with the appropriate experience, while those of greater than six months of age will not.

GENERAL DISCUSSION

The original intent of these experiments was to demonstrate the ability of P. m. bairdii at compensating for what was assumed to be a common occurrence in its natural history, namely, a deficient quantity of dietary protein (whatever the extent of this deficiency).

The literature shows that rats both have the ability to, and actually do, compensate for dilution of dietary protein as well as a multitude of other dietary components. The literature also shows that a wide variety of animal species appear to be selectively seeking out (and consuming) foods of higher protein content. In fact, the literature as a whole could easily be interpreted as suggesting that movements of animals in response to those foods high in protein content are actually means of assuring a constant protein intake during periods when environmental protein levels are low. This would be in lieu of stimulating into action the entire battery of physiological compensatory mechanisms available to the animal. In conjunction with the above, the literature on self-selection specifies that animals eat quantities of specific food types according to their metabolic needs for those foods.

Added to what has already been said, the importance of protein in such diverse, vital physiological roles as synthesis of enzymes, hormones, hemoglobin, DNA, RNA, as well as the structure of the cell itself, and as a source of energy to the body, would indicate that maintenance of a constant supply of dietary protein in response to fluctuating environmental conditions would be desirable, even necessary, to the continuation of life.

It was therefore expected that all animals* should demonstrate protein compensatory abilities.

Several possibilities as to why these mice did not compensate as expected are mentioned in Appendix A, one of these suggesting the experimental series presented in this thesis, namely, that given the appropriate experience at one time in their life, mice can endure a subsequently presented O-P regime for extended periods of time with no loss in body weight or changes in general appearance. Without this experience, mice cannot endure the O-P regime, demonstrating an immediate, dramatic weight loss,

*It is accepted that compensatory abilities for body weight maintenance (and perhaps other parameters, including dietary protein) have been bred out of the domestic animal's repertoire of abilities; selective pressures are in the direction of high productivity. This is not to say that no domestic animal has protein compensatory abilities (some do), but rather that if the ability to maintain body weight in some animals has been lost due to selective breeding, it is possible that other compensatory abilities may also be lost, or at least be severely reduced.

and death (in some cases within one week, in others, shortly thereafter). These results were invariably true.

The body of this thesis was concerned with specifying those characteristics conferring on the animals the ability to endure the O-P diet.

The significance of the dilution phase as being the sufficient experience for the appearance of the phenomenon was demonstrated, although exclusion of the selection phase from being similarly sufficient was not confirmed. Preliminary testing however, did not indicate that this latter would be a plausible alternative, although testing was fairly uncontrolled.

Further, experiments showed that coprophagy was the means whereby the animals could endure the O-P regime, the suggestion being that coprophagy was supplying the needed quantity of protein during periods of dietary protein deficiency.

Experiments suggested that the better an animal was at extracting a greater amount of protein from recycled feces for a greater length of time, the longer the animal would be expected to maintain its body weight and other physiological processes stable. Animals at the other end of the spectrum of efficiencies would have stable body weights for a short time but then begin a gradual decline to death from protein starvation; the difference between animals would be in their ability to

recapture and reuse fecal protein. These expectations were entirely consistent with the observed results as already mentioned.

Data showed a connection between the dilution phase and coprophagy such that this was the phase when the animal was metabolically in need of some additional protein since it was not compensating for the dilution by increasing its protein intake. Further experiments showed that the dilution phase required between five and eight days of application for the phenomenon to prevail, the combined data of which began to suggest that those animals able to survive on the O-P regime with no loss in body weight were experiencing coprophagy per se, or the attributes of coprophagy, at a time when they were in need of an additional source of protein. The benefit this experience provided these animals at this time was discussed earlier.

An alternative explanation is derived from Freeland et al. (1974). On discussing feeding strategies of herbivores in response to toxic substances in their foods (termed secondary compounds), they suggested two methods whereby the ruminant could handle these substances: (1) the development of appropriate enzyme systems to effectively detoxify these substances; (2) the alteration of the rumen microflora (by selective pressures) to more effectively detoxify these substances. Both measures

require a period of time (approximately ten days) before they are maximally operative.

As these may be the ways by which the ruminant handles detoxification of plant secondary compounds, so might they also be the methods whereby the mouse handles the diluted diet in conjunction with coprophagy. The population of microorganisms in the rabbit and rat caecum and proximate colon has been identified to the degree that changes in relative numbers of specific types of bacteria are known to occur on experiencing coprophagy, prohibition of coprophagy, and the alteration of dietary components. It would be within the realm of possibilities to suggest that similar changes may also be occurring in the colon or caecum of the mouse on experiencing the diluted regime along with coprophagy.

In addition to what has just been said, and in conjunction with Freeland's enzyme system hypothesis just outlined, Williams (1961, 1963, 1964, 1971) and Williams et al. (1966) have demonstrated alterations in enzyme systems related to the maintenance of energy metabolism in the liver cells in an extensive investigation concerning the changes occurring in the rat liver resulting from total protein depletion and subsequent repletion.

Further, Houpt (1963) and Houpt et al. (1971) have shown that rabbits and ponies can utilize urea as a means of conserving nitrogen on a low protein diet. Urea

is secreted into the caecum, decomposed to CO_2 and NH_3 by the caecal bacteria, some of the NH_3 being incorporated into the bacterial cells themselves as essential and non-essential amino acids. In fact, Akester et al. (1967) have shown direct visual evidence for the passage of urine from the kidneys via the cloaca and colon, to the caecum in domestic fowl. This statement on the passage of urine from the kidney to caecum of the fowl is not meant to suggest a similar passage in the mouse, but rather to emphasize that the excessive urinary nitrogen losses are at least reduced by this unusual compensatory system in the fowl, as well as by other techniques in other animals.

Thus the problem of excessive nitrogen losses via urine suspected of occurring (as mentioned in the literature review) may not necessarily be taking place during the O-P phase. The experience at coprophagy taking place during the dilution phase, giving Group I animals an advantage over Group II animals in handling the O-P diet, may indeed have been a period of time needed to initiate the channeling of urinary urea into the colon for bacterial growth. Coprophagy would therefore be providing these animals with large quantities of protein derived from the bacterial synthesis of NPN into their body tissues. This phase, and the time required to be in this phase for the phenomenon to appear (eight days), may also serve to

develop those enzymes required of the animal's metabolic machinery to use the NPN in the hepatic synthesis of amino acids as suggested by Houpt.

Measurements of the urinary and fecal nitrogen contents during the dilution and O-P phases, with and without coprophagy being allowed, would add much to the elucidation of this problem.

Further Characterizations of the Phenomenon

Age

An exploratory experiment indicated that age and/or species may be factors in preventing the expression of the phenomenon; three P. melanophrys, greater than one year of age, could not handle the O-P regime (one died, the other two demonstrated immediate, rapid weight loss). At the time both variables (age and species) were suspected as contributing to the difference in behavior noted. Subsequent experimentation suggested age as being the more critical variable in this regard.

Animals older than seven months of age were subjected to the dilution, then O-P regime, and were found not to be able to sustain body weight on the O-P regime, while animals five months of age or less could endure the regime. A second experiment using animals six months of age and older narrowed the required age for the

appearance of the phenomenon to between five and six months of age.

One is readily reminded of experiments on critical period and imprinting for example, bird song development (Thorpe 1961). If isolated at three to four days of age, a young male chaffinch produces an incomplete song later in life, but if he hears adults singing as a fledgling two to three weeks old or in early juvenile life before he sings himself, he will produce the species characteristic song the following year even if kept in isolation after exposure. Fine details of the song are added during the year within a two to three week period of time (from competitive neighbors) after which the bird will never learn any other songs.

Similarly the imprinting experiments of Lorenz (1937) and Hess (1959), as well as many others, suggest that imprinting is effective in producing maximal following during a brief and relatively specific period of ontogeny. For most precocial birds this period extends from about eight hours to about twenty hours after hatching with the incidence of subsequent following decreasing sharply if initial exposure to the test object occurs at a later age.

While Hess states that the critical period is the "direct result of a highly invariant sequence of maturational events which has as a counterpart any of the

innate responses at a reflexive or tropistic level,"

Schneirla (1959) suggested that the

bird's readiness to respond to a moving object at one particular stage of its ontogeny and its lack of responsiveness when initial exposure occurs at a later stage are the result of changes brought about through the progressive interaction between the developing organism and its sensory environment.

The imprinting concept, as applied to the present experiment, is perhaps a bit premature, however, it is not impossible to imagine a period of time early in the life of the mouse when dietary "habits" are being established having as their stimuli the diet type available along with the metabolic needs of the animal and an ability to develop, or modify enzyme types or micro-organism species in response to the diet available and/or the metabolic necessities of the animal at the time.

That these same conditions would not be present at all, or would not be present to the same degree of modifiability in an older animal may be the result of an already established dietary repertoire (from a younger age) assuring the most efficient handling of this already established array of foods.

Drickamer (1970), attempting to show the genetic and learned components of mouse feeding habits, found that young mice will adopt a different feeding strategy, and are more adventuresome in terms of attempting new diet types than adult animals. Wecker (1963), attempting

similar demonstrations for mouse habitat selection, found that mice had a capacity for learning one type of environmental preference if exposure took place at an early age.

Sex

Female P. m. bairdii of the same age range and experience as their male counterparts did not demonstrate the phenomenon. These experiments did not reveal how females handle the situation of dietary protein deficiency. The most that can be concluded from these data is that females of this species must compensate for dietary protein deficiencies in a manner different from males, if they indeed do compensate. If they do not compensate for the deficiency in some way, and if it could be presumed that the quantity and/or availability of protein in the natural foodstuff of Peromyscus does vary at all, then the females either continue to live in spite of the deficiency, losing whatever body weight occurs during the extent of the deficiency, or they die off because of the deficiency. These alternatives should be investigated.

APPENDICES

APPENDIX A

APPENDIX A

I. The original intent of these experiments was to demonstrate the ability of Peromyscus maniculatus bairdii* at compensating for what was assumed to be a common occurrence in its natural history, namely, a deficiency of dietary protein (whatever the extent of this deficiency). The results indicated that the animals did not compensate for the three levels of dilution (15, 30, and 45%) as would have been expected. The body weights remained unchanged.

For the three dilution levels used it would have been expected that animals in the 30% group would compensate noticeably more than those of the 15% group; those in the 45% group more than both the 30% and 15% groups. (It was for this reason that the dilution levels were originally selected during the planning of the experiment.) This however, was not the case: one animal (45%) raised its protein intake during the dilution phase from approximately 0.13 to 0.21 gm/day, an increase of only about

*Three Peromyscus melanophrys were part of this pilot experiment but did not demonstrate an ability to sustain their body weight and appearance without dietary protein and were therefore eliminated from future designs.

6.2%, while another (15%) raised its protein intake from 0.25 to 0.35 gm/day, an increase of about 40%. The former should have increased its intake 45%, the latter only 15%. What was the stimulus for the 40% increase in a 15% diluted diet? The point is that the degree of compensation, indeed whether or not compensation to any degree took place at all, was not a certainty as the literature would lead one to expect. The fact that no compensation took place was continually re-supported in subsequent experiments.

It appeared as if the animals could get along on less protein than that selected prior to dilution, since the volume of food removed from the protein dish did not increase during the dilution phase. Several possibilities could have explained these results:

1. During the pre-dilution phase, animals were eating more protein than they metabolically required. Animals should be eating, according to the self-selection "philosophy," only amounts adequate to satisfy metabolic needs, not in excess of those needs.
2. It is also possible that the animals were not metabolically deficient, therefore having no need to compensate. Although this is indicated by the fact that their body weights remained constant,

it appears to contradict their protein intake data for this period (see Appendix B).

(Another alternative is that the animals were averse to the taste of the diluted protein, and although they could sense the deficit incurred by the decreasing amounts of protein, they would not eat enough of it to compensate. The data of the experiment do not support this alternative primarily because were this the case, body weight would have declined, and also because of several behaviors: no spillage occurred, animals did not resort to eating from the other dish, etc.)

Of the several explanations available at the time, the one which was most interesting was that in spite of their apparently deficient protein intake (15, 30, and 45% less than before dilution), these animals had no need to compensate since they were not metabolically deficient enough to require this action. As mentioned, this was suggested by their sustained body weights during this period.

It seemed important at this stage to determine how much less protein the animals could endure before they would begin to show the effects of protein deficiency--reduction in body weight. The O-P diet was presented with the intent of first observing the response of the animals (Group I) to the opposite extreme, then

gradually determining a threshold level of dietary protein needed to sustain body weight.

It was at this time that nine other animals (Group II) were also presented the same O-P diet, the reason being that it was felt that the animals of the first group, having already experienced "selection" and "dilution," would somehow affect the objective outcome of the O-P test; these nine new animals were naive to the experimental situation.

II. The selection phase of the experiments served several purposes during the evolution of the experimental design:

- A. Initially it served as a period testing the capabilities of the animals towards survival on a synthetic diet of this nature. Although this was one of the original reasons for this phase during the early pilot work, becoming unnecessary during subsequent major experimental procedures, it did remain useful as such for work with wild caught mice (see later).
- B. Survival initially meant that the animals had the ability to combine the protein and O-Protein fractions of the total diet in amounts suitable to at least maintain body weight (if not growth) and general appearances.

- C. This phase had been considered a necessary part of the "experience" required of the mice to establish the phenomenon. The selection phase was subsequently ruled out as being not essential to the phenomenon's demonstration, and was therefore eliminated from later procedures.
- D. This phase also served as a period of acclimatization to the experimental procedure, as well as a time when baseline food intake data could be collected for use in later comparison studies.

III. The significance of the dilution phase changed when it was learned that the animals did not compensate for the diluted diets as presented. Its new significance was one of being the necessary and sufficient "experience" for the phenomenon in question. It thus became undesirable to maintain the three dilution levels; a 30% level was chosen as a compromise. This level represented adequate dilution of the protein to easily exhibit intake compensations should they exist, as well as what appeared to be sufficient dietary protein deficiency, and therefore metabolic deficiency (if compensation did not take place), assuring the expression of the phenomenon in question.

APPENDIX B

APPENDIX B

Since the thesis is not dependent on the food intake data, but rather on body weight (and general appearance) changes, this section will provide the reader with that food intake information corresponding to the body weight changes presented in the figures of the text.

So as not to have excessive numbers of tables, the two best and two poorest examples of the particular experimental objective will be presented except where otherwise indicated. This will present to the reader a range of results obtained.

The numbers across a column represent the body weight and nutrient intakes for a given day; numbers down the page represent successive experimental days. Missed days of data collection are indicated by omitted values of body weight for the missed day(s); the nutrient intake data for the missed day(s) are presented on the day returned, and are the average intake for those days missed.

SYMBOLS USED (in grams):

+	average nutrient intake for days of data collection missed
*	spilled food or water
O-P	protein removed (O-P regime started)
+P	protein added
dil.	dilution phase started
B.W.	body weight
P.I.	intake from the protein dish
O-PI	intake from the O-P dish
W.I.	water intake

APPENDIX C

Table 1.--Reference to Figure 1 Animals.

Best Examples: demonstrate sustained body weight during the O-P regime for the longest period of time (#4-I; 2-III).

Poorest Examples: demonstrate sustained body weight during the O-P regime for the shortest period of time (#1-III; 3-III).

#3-III: although he sustained body weight for a short time on the O-P regime, he kept spilling food from the O-P dish. It is possible that this was the reason for the extremely short period of weight maintenance (15 days). Notice that this animal kept spilling food from this dish even though the dish was changed and protein was added to the cage in another dish.

Fig. 1. Animal--Sel., Dil., O-P											
B.W.	P.I.	O-P.I.	W.I.	B.W.	P.I.	O-P.I.	W.I.	B.W.	P.I.	O-P.I.	W.I.
4(I)											
23.1	0.9	2.0	2.8			1.8+	2.4+	19.0		2.0+	2.0+
22.4	0.5	2.1	2.4	19.5		1.3	0.8			2.0+	2.0+
21.8	*	2.2+	2.7+	17.7		1.9	4.1	19.8		1.8	2.2
	*	2.2+	2.7+	19.0		1.5	3.2	19.7		2.2+	1.8+
	*	2.2+	2.7+	18.8		1.6	3.1			2.2+	1.8+
22.4	0.2	2.0	3.1	18.5		2.3	2.7	19.1		2.0	1.9
22.7	0.1	2.0	2.2	18.5		1.6	2.9	19.3		1.4+	2.3+
21.9	0.1	1.3	2.1	19.0		1.2	3.2			1.4+	2.3+
21.5	0.0	1.4	2.2	18.5		2.2	3.6	19.0		1.7	2.1
21.1	0.0	1.8	2.9	19.0		2.1	3.1	18.6		1.8	3.0
20.5	0.1	1.3	2.2	19.9		2.3	3.1	19.4		1.9	1.7
20.2	0.0	1.8	2.2	19.8		1.6	2.7	18.8		1.8	1.8
20.7	0.1	1.8	2.2	20.3		1.8	2.2	18.7		2.2+	2.2+
20.1	0.1	1.5	2.7	20.2		1.8	3.0			2.2+	2.2+
20.1	0.0	1.7	2.7	20.0		1.5	2.6	19.5		2.7	2.4
20.1	0.2	1.3	2.0	19.2		1.6	2.4	19.5		2.0+	1.8+
19.5	*	1.5	3.3	19.3		1.6	2.3			2.0+	1.8+
19.6	*	1.2	2.0	19.3		2.0	2.0	19.2		1.7	1.6
18.9	0.2	2.0	2.0	19.4		1.9	2.6	19.0		1.7+	1.9+
19.4	0.4	1.6	1.9	19.5		1.5	2.8			1.7+	1.9+
dil/				20.0		1.6	2.5	18.5		1.2	1.0
19.0	*	1.1	2.5	19.0		2.5	2.3	18.5		1.6+	2.5+
19.0	0.2	1.3	2.2	19.1		1.5	1.9			1.6+	2.5+
19.3	0.4	2.0	2.5	19.2		1.4	?	18.8		1.8+	2.2+
20.3	0.0	2.4	1.3	19.3		1.8	2.8			1.8+	2.2+
20.5	0.1	2.0	2.5	18.7		1.7	2.8	19.0		1.4	2.0
20.5	0.3	2.1	2.3	19.0		2.2	2.7	18.7		1.8	2.4
20.6	0.1	1.3	1.6	19.5		2.1	2.3	18.9		1.6+	2.2+
19.6	0.1	1.0	1.8	19.4		1.4	1.9			1.6+	2.2+
19.0	0.2	1.5	2.5	18.3		2.0	2.7			1.6+	2.2+
19.8	0.1	1.4	2.2	19.0		1.7	1.5			1.6+	2.2+
19.3	0.4	1.5	1.8	18.6		1.6	2.4	18.6		1.7+	1.8+
19.5	0.3	1.5	2.0	18.8		2.2	2.5			1.7+	1.8+
19.3	0.3	1.6	2.4	19.1		1.9	2.2	18.1		1.6	1.8
19.3	0.5	1.2	2.5	19.7		1.9	2.1	18.0		1.7	1.6
19.4	0.5	1.6	2.2	19.5		1.8	2.8	18.1		1.9	1.9
19.6	0.6	0.8	2.3	19.5		1.9+	2.2+	18.7		2.0	2.0
19.0	0.7	1.7	2.4			1.9+	2.2+	18.5		2.2+	2.2+
20.0	0.5	1.2	1.5	19.5		2.3	2.4			2.2+	2.2+
19.5	0.5	1.3	2.4	19.4		2.1	2.1	18.5		2.5	1.9
19.5	0.5	1.4	2.3	19.2		1.6	2.9	19.0		1.4	2.0
19.0	0.2	1.8	3.0	18.6		2.2	2.0	18.5		2.1+	2.0+
19.7	0.5	1.4	2.7	19.5		2.2	3.0			2.1+	2.0+
O-P				19.9		1.6	2.0	19.0		2.2	2.0
20.0		2.0	2.5	19.1		1.8	1.8	19.1		2.0	2.0
19.0		1.8	2.7	19.0		1.8	2.2	18.8		1.8+	1.6+
18.8		1.4	3.2	19.3		1.5	1.8			1.8+	1.6+
19.0		1.8+	2.4+	19.2		1.1	1.8	19.0		2.0	*

This animal continued for nine more days; body weight was 20.5 at the end of this time. Although he could have gone longer, I terminated the experiment.

2(III)											
12.8	*	0.3	1.7			1.6+	1.6+	15.6		1.8+	1.9+
13.0	0.0	1.8	2.0	16.4		1.4	0.0			1.8+	1.9+
13.8	0.1+	2.5+	2.3+	15.3		2.5	2.5	15.5		1.5	1.6
	0.1+	2.5+	2.3+	16.6		1.5	1.8	15.5		1.7+	1.5+
	0.1+	2.5+	2.3+	16.3		1.5	1.6			1.7+	1.5+
15.8	0.4	2.2	2.0	16.1		1.9	1.7	15.4		1.3	1.5

Column continued on next page

Table 1.--Continued.

				Fig. 1. Animal--Sel., Dil., O-P							
B.W.	P.I.	O-P.I.	W.I.	B.W.	P.I.	O-P.I.	W.I.	B.W.	P.I.	O-P.I.	W.I.
15.8	0.0	2.3	2.1	16.0		1.9	1.5	14.8		1.4+	1.5+
15.8	0.2	2.4	3.4	16.4		1.8	2.3			1.4+	1.5+
16.8	0.0	2.3	2.5	15.9		1.6	1.7	14.6		1.8	1.2
16.6	0.2	2.0	2.4	15.9		1.9	1.8	14.5		1.9	2.1
15.7	0.1	1.8	1.7	16.1		1.9	1.6	15.0		2.3	2.5
16.1	0.1	2.6	1.8	16.0		2.2	1.5	15.0		2.4	1.8
17.0	0.0	2.4	2.1	16.4		1.6	1.1	15.5		1.6+	1.7+
17.4	0.1	1.7	1.7	16.0		2.0	3.4			1.6+	1.7+
16.9	0.0	2.0	1.7	16.0		1.5	1.6	14.0		1.6	2.1
17.0	0.2	1.9	2.2	15.7		1.3	1.4	15.0		2.1+	1.8+
16.6	?	?	2.0	15.8		2.0	1.5			2.1+	1.8+
17.2	?	?	2.3	16.1		1.6	1.2	14.8		2.0	2.2
17.1	0.4	2.4	1.4	16.0		1.6	1.5	15.0		2.0+	2.2+
17.6	0.1	2.0	1.5	16.0		1.8	1.8			2.0+	2.2+
dil/				15.5		1.7	2.3	15.0		1.0	1.7
17.5	0.2	2.4	2.0	15.9		1.8	1.0	14.2		1.6+	2.5+
17.5	0.2	2.0	1.6	15.2		1.8	0.9			1.6+	2.5+
17.6	0.4	1.6	1.9	15.4		1.6	?	14.0		1.7+	2.2+
17.0	0.1	2.5	1.7	15.5		1.9	1.5			1.7+	2.2+
17.5	0.3	2.1	1.6	15.8		1.8	2.0	13.8		1.3	2.1
17.8	0.2	2.2	1.6	15.7		2.0	1.8	13.9		1.5	2.1
17.5	0.3	1.9	1.6	15.9		1.9	1.9	13.8		1.6+	2.8+
17.6	0.1	1.9	1.8	15.7		2.0	1.0			1.6+	2.8+
17.5	0.1	1.8	2.0	16.0		1.9	1.5			1.6+	2.8+
17.5	0.0	2.1	2.0	16.2		1.5	1.0			1.6+	2.8+
17.8	0.3	2.8	1.9	15.7		1.8	1.5	15.1		1.9+	2.1+
17.6	0.2	1.8	1.7	15.7		2.2	2.0			1.9+	2.1+
17.4	0.1	1.7	1.6	15.8		2.0	1.7	14.6		1.6	2.8
17.2	0.0	1.9	1.8	16.2		1.8	1.0	14.6		1.6	2.5
17.5	0.2	1.5	1.5	16.2		2.5	1.3	14.7		1.8	2.7
17.2	0.0	1.3	1.7	16.5		1.8+	1.5+	15.0		1.2	2.3
16.9	0.4	1.5	2.0			1.8+	1.5+	14.3		1.1+	2.0+
16.9	0.0	1.9	1.5	15.8		2.0	1.5			1.1+	2.0+
17.0	0.2	1.3	1.7	16.0		2.2	2.0	14.0		1.9	2.2
17.0	0.2	1.8	2.0	16.1		2.1	1.8	14.5		1.5	2.4
17.2	0.3	1.3	1.6	16.1		1.9	1.3	14.3		1.2+	2.6+
16.5	0.2	1.4	1.4	16.0		1.7	1.9			1.2+	2.6+
O-P				16.0		2.0	1.5	14.1		1.4	2.5
16.5		1.7	1.6	15.9		1.9	1.3	13.6		1.8	3.1
16.2		2.0	1.8	15.9		1.7	1.2	14.3		1.5+	2.3+
16.5		1.5	1.7	15.9		1.3	1.9			1.5+	2.3+
16.6		1.6+	1.6+	15.6		1.8	1.2	14.1		2.4	*

The experiment was terminated for this animal at this time.

1(III)											
16.1	0.3	1.8	1.2	17.0	0.0	2.0	1.6	14.5		1.4	1.4
15.2	0.1	0.8	1.9	17.0	0.0	2.0	1.5	14.1		1.7	1.8
15.2	0.0+	1.4+	3.9+	17.3	0.1	2.0	2.4	14.0		1.3	1.0
	0.0+	1.4+	3.9+	17.2	0.1	1.3	2.4	14.0		1.0	1.0
0.0+	1.4+	3.9+		16.8	0.0	1.8	1.2	13.7		1.5	1.3
14.5	0.2	1.2	1.5	O-P				14.0		1.2	1.2
14.0	*	1.5	1.6	16.9		1.9	1.5	14.0		1.5	1.9
14.0	0.0	1.2	1.7	17.0		1.8	1.7	13.5		1.0	1.0
13.8	0.0	1.1	1.8	17.0		1.5	1.2	13.5		3.0	1.0
13.5	0.1	1.3	2.4	17.0		1.5+	1.4+	13.5		1.1+	1.0+
13.0	0.0	0.8	1.5			1.5+	1.4+			1.1+	1.0+
12.8	0.0	1.4	1.2	17.2		1.5	0.8	13.7		1.1	0.9
13.0	0.1	1.4	1.1	16.5		1.9	2.1	13.2		1.6	0.6
12.8	0.0	1.0	1.5	17.0		1.9	1.7	12.7		1.2	0.8
13.0	0.0	1.2	1.4	17.2		1.8	1.3	12.8		1.2	0.4
13.0	0.4	1.3	1.2	17.0		1.8	1.5	12.8		1.1	1.1
12.7	?	?	1.3	17.0		1.6	1.5	12.5		1.0	0.7
13.1	?	?	1.0	17.0		2.0	1.3	+P			
12.5	0.4	1.6	1.3	16.6		1.5	1.3	12.3	*	1.2	1.3
13.0	0.0	1.5	1.5	16.6		1.8	1.5	13.2	0.1	1.7	1.1
dil/				16.5		2.0	1.5	14.0	0.0	1.3	1.4
14.0	*	1.7	1.5	16.8		1.7	1.1	14.3	0.0	1.4	1.4
14.1	0.1	1.5	1.5	16.5		1.7	1.8	14.8	0.1+	1.9+	1.6+
14.5	0.0	1.5	1.5	16.8		1.5	2.0		0.1+	1.9+	1.6+
14.9	0.0	1.8	1.6	16.5		1.5	1.5	15.5	0.0	2.0	1.5
15.0	0.0	1.8	1.6	16.6		1.5	1.3	16.2	0.0+	2.0+	1.6+

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Table 1.--Continued.

Fig. 1. Animal--Sel., Dil., O-P											
B.W.	P.I.	O-P.I.	W.I.	B.W.	P.I.	O-P.I.	W.I.	B.W.	P.I.	O-P.I.	W.I.
15.6	0.1	2.1	1.5	16.5		1.9	1.0		0.0+	2.0+	1.6+
15.5	0.1	1.8	1.8	16.0		1.6	1.0	16.5	0.0	1.6	1.6
15.6	0.0	1.9	1.6	16.1		1.4	1.3	16.7	0.0+	2.0+	1.5+
15.5	0.0	2.1	1.5	16.0		1.4	1.3		0.0+	2.0+	1.5+
15.7	0.0	1.7	1.7	15.5		1.4	1.5	17.2	0.1	1.7	1.3
16.2	0.4	2.2	1.6	15.8		1.1	1.3	17.1	0.0	1.6	1.5
16.0	0.1	2.3	1.7	15.3		1.1	1.3	16.8	0.2	1.8	1.6
16.6	0.0	2.0	1.3	14.8		1.3	?	17.0	0.1	2.0	1.6
16.6	0.0	2.3	1.7	15.0		1.2	1.1	17.0	0.2+	1.8+	1.4+
16.7	0.1	1.8	1.5	14.5		1.0	1.4		0.2+	1.8+	1.4+
16.0	0.1	1.8	1.7	14.5		1.3	1.5	17.2			
16.7	0.2	1.7	1.7								
3(III)											
17.0	*	1.6	3.3	17.9	0.4	4.6	3.5	15.4		*	4.7
15.3	0.7	4.6	3.7	18.3	0.0	2.7	3.0	15.2		*	4.4
15.5	0.8+	3.3+	3.9+	17.7	0.3	2.2	3.3	15.0		1.7	4.6
	0.8+	3.3+	3.9+	17.5	0.0	2.3	3.8	14.5		1.4	5.0
	0.8+	3.3+	3.9+	17.7	0.4	2.8	3.0	14.2		*	4.6
16.5	0.7	3.6	4.5	16.7	0.8	*	4.2	14.0		*	2.6
16.5	0.1	4.0	3.3	17.7	0.7	*	3.2	13.9		*	4.2
16.9	0.0	3.6	3.8	18.0	0.6	3.9	3.5	13.6		*	5.7
17.0	0.9	5.6	4.0	18.2	0.7	3.5	3.3	13.0		*	6.0
17.3	0.6	4.4	4.5	17.5	0.7	2.6	4.1	+P			
17.5	0.5	4.1	3.1	17.5	0.5	6.4	3.7	12.6		*	5.2
17.5	0.6	4.2	3.8	17.6	0.7	*	4.5	15.0	0.3	*	4.8
18.0	1.0	4.0	3.1	17.7	0.4	*	3.2	16.0	0.7	4.8	4.1
17.5	0.6	4.1	4.4	17.5	0.8	4.3	3.4	17.8	1.2	2.6	4.4
17.3	0.5	4.2	4.0	17.3	0.5	3.0	3.5	18.0	0.3	*	4.2
18.0	0.3	3.4	3.7	17.0	0.4	*	3.8	18.3	1.9	*	5.8
17.2	*	3.0	4.1	O-P				19.2	0.5	*	?
17.8	*	5.1	3.4	17.6		*	3.0	20.0	0.4	3.4	?
17.8	0.8	6.3	3.6	16.5		4.4	3.5	18.0	0.7	*	?
18.0	0.4	1.6	3.2	16.0		*	3.5	19.5	0.5	1.7	3.2
dil/				16.0		6.5+	3.5+	18.6	1.0	*	3.2
17.5	1.5	3.0	3.4			6.5+	3.5+	18.4	0.6	*	3.3
17.0	0.0	2.1	3.5	17.5		0.8	3.6	18.5	1.0	*	3.5
17.2	0.2	5.0	3.7	16.7		*	3.2	18.3	0.4	*	3.8
17.3	0.3	4.2	4.5	17.0		5.5	2.5	18.7	0.6	*	4.2
17.8	0.0	5.0	4.0	16.7		*	2.5	19.2	0.7	*	3.2
17.5	0.0	3.5	3.2	16.0		2.3	4.3				

Table 2.--Reference to Figures 2, 3, 5, and 2a Animals.

- There were nine animals in Figures 2, 3, and 5; five for Figure 2a.
- Figure 2a was a check on the O-P phase of Figure 2 due to suspected faulty water bottles. New water bottles were supplied on the fifth day of the experiment.
- Animals #5, 6, 8, 9 (Figure 2) and #3 (Figure 2a) died.
- Animals #1, 2, 3, 4, 7 (Figures 2, 3, and 5) and #1, 2, 4, 5 (Figure 2a) survived, however, only with progressive weight losses and decline in general appearance.
- The addition of a protein dish rapidly and dramatically reversed the decline in body weight and appearance of these animals. This represents the selection regime.
- Animals represented by Figures 2, 3, and 5 were continued on this selection regime until body weights were re-established, then subjected to the dilution regime (30%) followed by the O-P regime again. This table for these animals follows them from immediate O-P, through selection, dilution, O-P, screen cages, and subsequent termination by protein replacement.
- After the screen cage phase of this experiment, these five animals (survivors of first O-P encounter) were divided into two groups:
1. protein replacement while still in the screen cage (#1, 4).
 2. placed back in regular cages, then protein replacement after several days (#2, 3, 7).
- The various stages of this experiment are indicated in the table by the following legend:
- O-P no dietary source of protein provided in cage
 +P protein added in a separate dish (selection)
 s.c. animals in screen cages
 r.c. animals in regular cages
 dil 30% dilution initiated

Fig. 2 Animal--O-P Immediately											
B.W.	P.I.	O-P.I.	W.I.	B.W.	P.I.	O-P.I.	W.I.	B.W.	P.I.	O-P.I.	W.I.
5											
13.5	*	0.0		9.6		2.8+	0.1+	8.4	DEAD		
10.5	*	0.1				2.8+	0.1+				
6											
19.5	*	5.8		13.4		1.0	1.2	11.4		1.0	0.8
16.5	0.0	0.2		13.5		1.0	1.9	10.6		1.2	0.8
15.0	0.2+	0.2+		12.8		0.5	0.6	10.7		1.1	0.8
	0.2+	0.2+		12.0		*	0.6	11.3		0.6	0.4
13.3	*	1.7		12.0		*	0.7	10.5	DEAD		
8											
30.7	*	6.6		24.0		0.0	1.1	19.4		0.0	0.2
27.7	0.2	6.1		23.0		0.1	1.1	18.4		0.0	0.8
26.1	0.0	6.0+		21.8		0.0	0.2	16.7		0.0	0.3
	0.0+	6.0+		20.7		0.0	0.7	16.5	DEAD		
9											
17.0	0.6	0.0		11.3		0.1+	0.1+	10.4	DEAD		
13.4	0.0	0.0				0.1+	0.1+				
1											
O-P				15.3	0.5	*	1.9	O-P			
20.6	2.6	0.4		7	0.8	2.3	1.9	15.2	2.1+	1.8+	
18.5	0.7	0.4		15.3	0.2	2.3	1.2		2.1+	1.8+	
17.0	0.8+	0.3+		dil/				15.4	2.7	2.0	
	0.8+	0.3+		15.1	0.1	2.4	1.8	14.8	2.6+	2.1+	
14.7	0.3	2.2		15.2	0.0	2.0	1.8		2.6+	2.1+	
14.6	0.3	0.7		15.3	0.4	2.1	1.4	15.3	2.6	2.3	
14.0	0.5	1.6		15.3	0.3	1.7	1.6	15.4	2.6+	1.8+	
13.0	0.8	0.4		15.7	0.4	2.0	2.1		2.6+	1.8+	
12.2	0.8	0.9		15.6	0.8+	1.8+	1.8+	15.5	2.2	1.7	
12.1	0.6	1.0			0.8+	1.8+	1.8+	15.4	2.8+	1.8+	
11.3	1.0	1.0		15.6	0.4	1.9	2.2		2.8+	1.8+	
11.0	1.3	1.5		15.6	0.0	1.9	2.2	15.5	1.7+	1.7+	

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Table 2.--Continued.

Fig. 2 Animal--O-P Immediately											
B.W.	P.I.	O-P.I.	W.I.	B.W.	P.I.	O-P.I.	W.I.	B.W.	P.I.	O-P.I.	W.I.
10.8		1.3	1.3	15.8	0.0	2.1	2.1			1.7+	1.7+
11.5		1.0	1.3	15.2	0.2	1.7	2.0	15.6		3.1	1.8
11.0		1.2	1.0	15.2	0.4	2.5	2.0	s.c.			
10.7		1.1	1.2	15.1	1.0	2.1	2.1	15.0		2.6	1.4
+P				15.2	1.3	1.9	1.8	14.8		2.0+	1.4+
10.7	0.1	1.0	1.9	15.3	0.6	2.0	1.5			2.0+	1.4+
10.9	0.1	1.8	2.9	15.8	1.2	2.2	2.0	15.0		1.0	1.0
13.0	0.7	2.0	2.3	15.6	0.8	2.1	1.3	14.1		2.5+	1.0+
13.8	0.8	2.0	2.6	15.6	1.7+	2.1+	1.7+			2.5+	1.0+
15.3	1.3	2.0	1.8		1.7+	2.1+	1.7+	13.8		2.0	1.8
14.6	3.2	2.5	2.3	15.7	1.2	1.9	1.8	13.6		1.5	1.1
15.6	0.9	2.0	2.6	15.1	1.4+	2.1+	1.6+	13.1		1.9	1.6
15.3	2.5	2.3	2.5		1.4+	2.1+	1.6+	13.0		2.0	2.5
15.9	0.1	2.6	2.0	15.4	0.5	1.8	1.6	12.8		1.9	2.7
15.9	0.1	2.0	1.8	15.1	0.7+	1.5+	2.0+	12.5		1.4	2.6
15.5	1.5	1.9	2.2		0.7+	1.5+	2.0+	12.2		5.6	2.5
15.7	0.1	2.2	1.8	15.3	1.4	1.3	1.5	+P			
15.7	1.0	2.3	2.0	15.0	0.2	1.9	1.8	12.0		2.6	3.1
15.9	0.9	3.2	1.9	14.8	0.8	2.0	1.8	14.3	skip a day; no		
?	0.4	2.4	1.9	15.4	0.1	1.9	1.9	15.7	food intakes		

2

O-P				17.9	?	1.8	3.6	16.2		2.1+	4.4+
17.0		0.6	0.2	dil/						2.1+	4.4+
13.2		0.5	0.1	17.9	0.2	1.7	3.7	16.5		3.0	5.2
11.8		1.2+	0.0+	18.0	0.5	1.8	4.6	16.3		1.2+	4.6+
		1.2+	0.0+	17.8	0.7	1.8	4.6			1.2+	4.6+
9.8		1.3	3.1	18.0	0.1	1.5	3.7	16.5		1.8	5.0
11.5		2.0	2.4	18.2	0.2	1.9	5.0	16.5		2.7+	5.5+
12.2		1.3	1.7	18.1	1.0+	1.8+	4.1+			2.7+	5.5+
12.0		1.3	1.4		1.0+	1.8+	4.1+	17.0		3.0+	5.8+
11.2		1.3	2.3	18.0	0.5	1.5	3.8			3.0+	5.8+
11.5		1.2	2.8	17.5	0.8	2.0	4.2	16.0		2.4	5.7
10.5		2.2	4.5	17.4	0.4	2.2	4.3	s.c.			
10.5		1.5	5.0	17.0	0.6	1.8	5.2	16.7		2.3	4.4
10.2		2.5	5.8	17.6	0.6	1.9	4.3	15.4		2.5+	4.0+
10.6		3.7	3.5	17.7	0.4	2.2	4.5			2.5+	4.0+
10.0		*	1.3	17.2	0.6	2.3	4.0	15.0		2.0+	5.0+
9.7		*	3.8	17.0	1.0	2.1	4.2			2.0+	5.0+
+P				17.3	1.4	1.4	4.0	14.8		2.0+	3.0+
9.7	3.0	1.5	4.0	16.7	0.8	2.0	4.8			2.0+	3.0+
10.8	1.5	1.2	4.2	16.8	1.5+	2.0+	4.5+	14.2		2.0	3.7
11.7	1.2	1.2	5.4		1.5+	2.0+	4.5+	14.2		2.2	2.6
13.0	1.0	1.5	4.9	16.7	1.5	1.8	4.3	14.3		2.0	0.3
14.4	1.5	1.4	4.9	16.8	0.7+	2.0+	4.5+	14.4		1.2	2.5
14.5	2.2	1.4	6.0		0.7+	2.0+	4.5+	r.c.			
15.3	1.9	1.2	5.1	16.7	1.0	1.9	4.9	13.5		2.0	3.0
15.7	1.9	1.5	5.6	16.7	0.7+	1.8+	4.2+	13.5		0.0	2.3
16.2	1.8	3.0	6.0		0.7+	1.8+	4.2+	13.2		2.2	3.0
16.8	1.9	2.1	4.2	16.7	1.0	1.9	4.0	+P			
17.5	0.2	1.6	4.7	16.7	0.7	1.4	5.2	13.4	1.5	1.0	2.0
17.6	1.7	1.9	4.7	16.2	1.2	2.0	5.2	13.1	1.1+	1.5+	4.0+
17.6	1.4	1.6	5.4	17.0	0.2	1.7	5.6		1.0+	1.5+	4.0+
17.8	1.2	1.5	4.2	O-P				16.0	1.0	2.1	4.5
17.5	1.4	1.8	5.5	16.5		1.2+	4.8+	17.0	1.2	2.5	3.9
17.8	1.4	1.0	4.2			1.2+	4.8+	17.5	1.0	2.4	3.9
17.0	1.3	1.7	5.4	16.4		0.2	5.2				

3

O-P				17.0	0.1	1.5	1.7	16.5		1.9+	1.4+
19.4		0.8	0.4	dil/						1.9+	1.4+
15.5		0.2	0.0	17.1	0.0	2.8	1.7	16.0		1.4	1.6
14.1		0.2+	0.4+	17.5	0.0	1.8	1.3	15.7		2.4+	1.5+
		0.2+	0.4+	16.8	0.5	1.6	1.9			2.4+	1.5+
11.8		0.1	2.7	17.0	0.1	2.2	1.6	15.7		1.6	1.4
13.1		0.2	1.6	17.0	0.1	0.1	1.8	15.3		1.2+	1.5+
12.9		0.0	1.8	17.1	0.5+	1.7+	2.2+			1.2+	1.5+
12.5		1.0	2.5		0.5+	1.7+	2.2+	15.3		1.9+	1.2+
13.0		1.1	1.1	17.1	0.4	1.3	2.3			1.9+	1.2+
12.5		1.2	1.8	17.2	0.7	2.2	2.2	15.5		1.7	1.6

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Table 2.--Continued.

Fig. 2 Animal--O-P Immediately															
B.W.	P.I.	O-P.I.	W.I.	B.W.	P.I.	O-P.I.	W.I.	B.W.	P.I.	O-P.I.	W.I.	B.W.	P.I.	O-P.I.	W.I.
12.3		1.0	0.8	17.8	0.4	2.6	2.0	s.c.							
11.7		0.8	1.0	17.6	0.7	1.7	2.1	15.5		2.6	1.8				
11.0		1.0	1.0	18.3	0.6	2.6	1.9	14.0		2.0	1.0				
11.2		1.0	1.0	18.2	0.8	1.6	2.2	13.5		2.0+	1.0+				
10.6		1.0	0.6	18.3	0.5	1.8	1.7			2.0+	1.0+				
10.1		1.0	1.2	17.8	0.5	1.8	2.1	13.0		2.8+	1.0+				
+P				18.0	0.8	1.5	1.8			2.8+	1.0+				
10.5	2.0	1.6	2.7	18.1	0.4	1.5	1.9	12.8		*	1.0+				
12.4	1.4	1.0	2.1	18.0	0.8+	2.0+	2.0+			*	1.0+				
13.2	1.0	1.0	2.8		0.8+	2.0+	2.0+	r.c.							
14.3	1.1	1.1	2.6	18.6	0.6	1.6	3.5	12.4		0.2	1.4				
15.5	0.1	0.2	0.7	18.6	0.8+	1.3+	1.7+	12.5	1.	1.5	0.5				
12.5	0.1	1.2	2.3		0.8+	1.3+	1.7+	12.1		1.3	1.0				
13.7	0.3	1.3	2.3	17.5	1.0	2.0	2.2	12.0		1.2	0.9				
14.0	0.1	1.0	1.5	17.8	0.9+	1.4+	1.9+	12.1		*	0.5				
14.7	0.3	2.2	2.0		0.9+	1.4+	1.9+	11.8		*	0.4				
15.2	0.3	1.7	1.0	17.7	0.9	2.0	2.3	11.4		*	0.5				
14.4	0.0	2.4	2.2	17.7	0.9	1.0	2.2	+P							
15.6	0.2	2.4	1.7	17.5	0.9	2.0	?	11.3	0.5	0.6	1.5				
16.5	0.3	2.0	2.0	17.3	0.5	1.4	1.9	11.5	0.1+	1.8+	2.0+				
17.0	0.3	2.3	1.8	O-P					0.1+	1.8+	2.0+				
17.0	0.2	2.1	2.1	17.5		1.3+	1.9+	13.5	0.3	1.7	2.4				
17.0	0.6	2.3	1.6			1.3+	1.9+	13.6	0.4	1.8	2.1				
17.5	0.7	2.3	1.7	17.0		1.2	1.7	13.8	0.4	1.7	2.3				
4															
15.0		2.1	0.3	14.0	0.3	2.1	1.3	13.6		2.0	1.8				
12.8		0.0	0.3	dil/				13.4		2.4+	1.8+				
11.8		0.4	0.1	14.1	?	2.0	2.0			2.4+	1.8+				
		0.4	0.1	14.0	0.0	2.0	1.7	13.5		1.7	2.0				
10.0		0.4	3.5	13.6	0.0	1.8	2.0	13.5		3.1+	2.2+				
12.1		0.7	1.8	14.0	0.1	1.9	1.4			3.1+	2.2+				
12.6		1.2	2.4	14.1	0.1	2.2	2.0	13.8		1.9	2.0				
13.4		1.4	1.5	14.0	0.2+	1.8+	1.8+	13.8		1.9+	2.3+				
13.2		1.6	1.8		0.2+	1.8+	1.8+			1.9+	2.3+				
13.7		1.5	1.4	14.1	0.0	1.5	2.1	14.0		2.0	2.2				
12.0		1.6	1.6	14.0	0.5	2.1	2.1	13.8		2.0	2.1				
12.9		1.6	1.5	14.3	0.8	2.3	2.1	s.c.							
12.5		1.2	1.1	14.3	0.5	1.8	1.8	13.5		2.2	2.0				
12.7		1.0	1.2	14.0	0.4	1.7	2.0	12.8		1.6+	1.5+				
12.5		1.5	1.4	13.9	1.1	1.5	1.7			1.6+	1.5+				
12.2		1.3	1.2	13.0	0.1	1.0	1.5	12.4		1.5+	1.0+				
+P				13.9	1.2	1.5	1.6			1.5+	1.0+				
12.5	0.8	1.0	2.0	13.9	0.5	1.8	1.6	12.3		1.4+	1.3+				
12.8	1.0	1.6	2.0	13.4	0.2	2.1	1.6			1.4+	1.3+				
13.0	0.8	1.8	2.2	13.8	0.5+	2.4+	2.4+	12.0		1.3	0.8				
14.0	1.0	2.0	1.7		0.5+	2.4+	2.4+	11.5		1.4	1.0				
14.0	0.5	2.8	1.5	13.9	0.9	2.2	1.5	11.2		1.5	1.0				
13.7	0.2	2.1	1.8	14.0	0.5+	2.0+	2.0+	11.0		1.5	1.0				
14.2	0.4	2.5	1.7		0.5+	2.0+	2.0+	11.0		1.1	1.2				
14.3	0.6	2.0	1.9	13.7	1.0	2.0	1.5	11.0		1.3+	0.5+				
14.5	0.5	2.5	1.8	13.8	0.2+	1.8+	2.0+			1.3+	0.5+				
14.3	0.3	2.3	1.2		0.2+	1.8+	2.0+	+P							
14.0	0.3	1.2	2.0	13.8	0.3	2.0	2.6	10.5	0.4	1.0	2.3				
13.8	1.0	2.3	1.9	13.8	0.3	2.0	1.5	11.7	0.3+	2.0+	2.5+				
14.5	0.0	1.8	1.5	13.8	0.3	2.0	1.7		0.3+	2.0+	2.5+				
14.0	0.3	1.9	1.9	13.7	0.2	2.1	1.7	13.8	0.5	2.0	2.1				
14.3	0.4	2.2	1.8	O-P				13.6	0.7	2.0	2.5				
13.9	1.0	1.7	2.0	13.6		1.7+	1.7+	13.7	0.5	2.3	2.6				
14.1	0.5	2.0	2.1			1.7+	1.7+								
7															
O-P				16.5	0.5	1.3	1.4			2.3+	2.4+				
19.6		*	1.8	dil/				15.5		2.2	2.3				
17.5		0.6	2.1	16.8	0.5	1.3	2.3	15.7		2.5+	2.9+				
16.5		0.9+	3.0+	16.7	0.0	1.3	2.3			2.5+	2.9+				
		0.9+	3.0+	16.6	0.5	1.4	1.9	16.2		2.5	2.5				
16.0		0.9	0.9	16.7	0.4	1.9	1.6	15.5		1.4+	2.8+				
14.5		0.6	0.6	17.0	0.2	1.7	1.8			1.4+	2.8+				
14.0		1.0	1.4	16.8	0.6+	1.8+	2.3+	15.7		1.6+	2.4+				

Column continued on next page

Table 2.--Continued.

Fig. 2 Animal--O-P Immediately											
B.W.	P.I.	O-P.I.	W.I.	B.W.	P.I.	O-P.I.	W.I.	B.W.	P.I.	O-P.I.	W.I.
13.5		1.1	0.4		0.6+	1.8+	2.3+			1.6+	2.4+
12.5		0.5	0.3	17.0	0.5	1.2	1.8	15.3		1.7	2.9
12.5		0.6	1.0	16.5	0.6	1.8	1.9	s.c.			
11.5		0.5	0.5	16.7	0.5	1.5	2.3	15.0		3.3	2.4
10.9		1.5	1.2	16.7	0.9	1.8	1.9	15.0		2.8+	3.0+
10.9		1.2	1.2	17.0	0.5	2.0	2.2			2.8+	3.0+
11.2		1.7	1.7	16.7	1.4	2.2	2.1	14.5		3.0+	4.2+
10.7		0.4	2.0	17.0	0.0	0.3	2.1			3.0+	4.2+
10.2		1.5	1.5	16.7	0.8	1.5	2.0	13.8		2.5+	4.6+
+P				17.0	0.5	1.3	2.3			2.5+	4.6+
10.2	0.1	0.8	1.5	16.7	0.5	1.4	2.2	13.5		2.0	4.4
10.7	0.6	1.4	2.0	16.6	1.0+	2.0+	2.5+	13.3		2.5	4.4
11.6	0.4	1.3	1.5		1.0+	2.0+	2.5+	13.3		2.3	3.0
12.2	0.2	1.2	1.4	17.0	1.1	2.2	2.3	12.7		2.5	3.4
12.7	0.6	2.0	1.7	17.5	1.5+	2.0+	1.4+	r.c.			
12.7	0.3	1.8	2.5		1.5+	2.0+	1.3+	12.3		2.9	2.5
14.2	0.3	1.6	2.5	16.3	1.0	1.0	1.4	12.1		1.7	2.7
14.1	0.2	1.9	2.7	15.8	1.1+	1.4+	1.5+	11.5		1.2	2.0
15.3	0.5	1.9	2.3		1.1+	1.4+	1.5+	11.7		1.9+	3.5+
15.5	0.3	1.7	2.5	16.1	1.0	2.0	1.8			1.9+	3.5+
16.0	0.5	2.9	2.1	16.1	1.2	1.5	1.9	+P			
16.1	0.5	2.0	2.5	16.8	1.0	1.5	1.9	11.5	3.5	1.0	1.0
16.7	0.5	1.8	2.4	O-P				12.0	0.4	2.9	2.9
17.2	0.6	2.8	2.0	15.5		1.2+	2.5+	13.0	0.0+	3.3+	2.5+
17.5	1.8	2.0	2.2			1.2+	2.5+		0.0+	3.3+	2.5+
17.3	0.6	1.5	2.3	15.8		2.5	2.6	15.0	0.4	2.9	?
17.2	0.5	1.5	2.1	15.8		2.3+	2.4+				

Fig. 2a Animal--Recheck O-P Immediately

1											
13.8		1.3	2.6	11.0		1.3	1.9	13.3			
13.0		0.5	2.1	+P							
12.2		1.0	1.5	10.6	0.3	0.7	1.7				
12.1		1.7	1.5	12.0	0.4	1.3	1.6				
2											
14.5		1.1	3.9	12.9		1.4	2.9	+P			
14.1		0.7	2.3	12.5		1.0	4.5	11.3	0.3	1.0	5.3
13.7		1.0	3.5	12.4		1.6	5.0	12.0	1.0	1.5	4.2
13.3		1.5	5.7	12.0		0.7	3.3	13.7			
13.2		1.2	3.7								
3											
13.2		0.9	2.5	11.1		0.4	2.1	9.5	DEAD		
12.1		1.5	4.0								
4											
13.4		1.1	4.0	11.1		0.8	3.8	13.2			
13.0		1.5	4.5	+P							
12.1		1.9	2.7	10.5	0.9	1.2	3.0				
11.3		0.5	2.5	12.1	0.8	1.1	2.3				
5											
13.9		1.2	2.3	12.5		0.8	1.2	+P			
13.7		1.0	3.0	12.1		1.3	2.7	10.6	0.6	0.8	3.0
13.1		1.6	2.0	11.7		1.0	2.7	12.1	0.9	1.2	3.3
12.7		0.6	1.7	11.3		1.1	1.9	12.7			

Table 3.--Reference to Figure 4 Animals.

--All five animals of the experiment are included in this table.

--Eight days of dilution experience were provided before the O-P regime was initiated.

				Fig. 4 Animal--Dilution Alone							
B.W.	P.I.	O-P.I.	W.I.	B.W.	P.I.	O-P.I.	W.I.	B.W.	P.I.	O-P.I.	W.I.
1											
15.8	1.8	4.2	7.2	17.3	0.4	5.7	6.1	17.0		*	6.5
16.0	0.6	3.5	8.0		O-P			17.1		2.0	9.0
15.0	**	**	7.1+	17.3		5.4	7.4	16.8		2.9	7.3
	**	**	7.1+	17.0		4.5+	5.7+	16.9		3.0+	7.1+
16.3	1.2	4.3	8.8			4.5+	5.7+			3.0+	7.1+
16.9	0.9	4.1	6.4	16.9		1.4	6.8	17.0		2.0	8.7
17.1	0.4	3.7	7.6	16.9		3.2	9.1	17.0			
2											
16.1	1.1	2.9	5.8	17.2	0.8	4.7	5.8	17.4		*	7.3
15.9	0.9	2.2	7.4		O-P			17.5		2.7	5.4
15.5	0.3+	3.9+	6.1+	17.7		5.1	8.7	17.6		3.6	7.0
	0.3+	3.9+	6.1+	17.5		3.6+	6.1+	17.4		3.0+	5.5+
17.1	1.5	5.0	6.8			3.6+	6.1+			3.0+	5.5+
16.8	0.3	*	7.8	17.4		3.4	7.5	17.5		2.2	5.6
17.1	1.0	3.1	4.5	17.6		4.5	7.6	17.5			
3											
15.4	0.2	2.1	4.4	16.6	0.3	3.4	5.0	16.8		2.2	5.0
14.7	0.1	2.8	5.0		O-P			17.0		2.5	4.4
14.0	**	1.5+	4.3+	17.0		3.8	5.7	16.9		2.7	5.0
	**	1.5+	4.3+	16.8		2.2+	4.7+	16.7		3.1+	3.7+
15.0	0.8	3.1	5.3			2.2+	4.7+			3.1+	3.7+
15.2	0.4	3.0	5.2	16.7		1.7	4.9	16.8		1.6	4.3
16.0	0.8	3.2	5.1	16.9		2.0	5.9	16.8			
4											
15.9	0.2	2.0	4.2		O-P			15.4		2.1	6.1
15.0	0.2	2.1	4.6	15.8		4.0	6.1	15.5		2.4	5.7
14.6	0.0+	1.7+	3.8+	15.1		1.6+	4.3+	15.4		3.0+	5.3+
	0.0+	1.7+	3.8+			1.6+	4.3+			3.0+	5.3+
13.7	0.2	2.2	4.3	15.1		2.1	6.1	15.6		1.9	6.6
13.2	0.0	1.7	3.5	15.3		4.0	6.2	15.4		2.9	5.1
13.0	1.1	2.5	5.2	15.4		3.1	6.0	15.5			
14.9	0.3	2.8	4.7								
5											
14.8	0.4	1.6	4.6	15.3	1.7	2.9	7.5	14.8		4.0	8.9
12.6	0.3	3.1	8.4		O-P			15.0		2.0	5.7
12.8	0.1+	3.2+	6.5+	15.3		3.6	10.1	15.0		2.5	8.4
	0.1+	3.2+	6.5+	14.9		3.5+	8.0+	15.1		2.7+	7.7+
13.9	*	2.7	8.0			3.5+	8.0+			2.7+	7.7+
13.7	1.1	3.7	8.0	14.9		3.5	8.3	14.9		2.8	11.4
15.0	*	3.0	8.2	15.0		3.5	9.6	15.0			

Table 4.--Reference to Figure 6a and 6b Animals.

Figure 6a--Animal #1 could not survive on the dilution regime initially; however, after being supplied with pure protein (Day 8) until dilution was again initiated (Day 13), body weight was maintained sufficiently to place on the O-P regime in regular cages (Day 19).

Figure 6b--Represents a check on the procedure and results of Figure 6a.

Only two examples are presented in this table since remarkable consistency among animals was demonstrated. Differences among animals existed only in the length of time required to reach the 20% criterion.

Fig. 6a Animal--Screen/Regular Cage											
B.W.	P.I.	O-P.I.	W.I.	B.W.	P.I.	O-P.I.	W.I.	B.W.	P.I.	O-P.I.	W.I.
1											
16.2	0.0	0.2	6.4	13.0	0.9	3.1	10.1	15.1		2.6	4.8
14.3	0.1+	3.8+	4.9	14.8	0.9	4.0	13.0	14.1		4.3	5.9
	0.1+	3.8+	4.9+	dil/				14.0		4.2	5.0
13.0	0.0	*	7.7	14.7	1.1	3.1	9.0	13.6		3.9+	5.1+
13.0	0.0	5.4	5.8	15.7	1.3	3.6	4.9			3.9+	5.1+
12.7	0.2	5.6	7.2	15.8	0.9	4.2	5.9	+P			
13.0	0.2	2.3	6.1	15.9	1.0+	4.2+	5.2+	13.0	1.4	3.1	7.1
+P					1.0+	4.2+	5.2+	15.3	0.9	2.6	6.2
12.6	0.4	*	9.4	16.4	0.6	3.6	5.0	15.0			
12.0	0.5+	4.2+	7.6+	O-P							
	0.5+	4.2+	7.6+	16.8		4.4	5.5				
3											
14.4	1.1	2.8	8.9	13.7	0.9	3.3	8.1	13.0		2.9	6.9
12.8	0.4+	2.9+	6.9+	14.0	0.5	3.6	8.0	12.7		2.8	6.3
	0.4+	2.9+	6.9+	14.5	0.6	2.9	7.5	12.4		0.7+	5.5+
10.8	0.3	4.1	8.6	14.0	0.2	3.2	9.6			0.7+	5.5+
11.9	0.1	2.9	7.3	14.4	0.9+	3.3+	12.6+	12.2		3.4	5.4
15.0	0.6	3.4	1.9		0.9+	3.3+	12.6+	12.0		4.6	7.1
12.5	0.1	2.3	6.7	14.5	0.4	3.1	8.6	+P			
11.8	0.8	4.2	9.3	O-P				11.6	0.9	2.7	3.5
11.8	0.6+	3.1+	8.0+	14.5		2.9	8.6	12.8	1.0	2.2	6.1
	0.6+	3.1+	8.0+	13.6		2.6	6.8	13.9			
13.0	0.6	2.8	7.9								
4											
13.1	1.2	2.0	11.4	16.0	0.7	2.2	7.4	12.5		1.5	8.4
13.4	0.8+	2.2+	6.4+	O-P				12.5		1.5+	7.9+
	0.8+	2.2+	6.4+	15.3		2.3	6.9			1.5+	7.9+
15.0	0.6	2.4	7.4	15.5		1.8	5.5	12.0		1.2	7.3
14.8	0.7	2.1	5.0	15.6		2.5	7.7	11.9		1.4	8.1
15.0	1.0	2.4	5.9	15.0		1.5+	4.6+	11.5		1.4	7.3
15.8	0.7	2.4	4.9			1.5+	4.6+	11.5		1.2	7.0
16.0	0.8	2.8	6.5	14.0		1.6	10.8	+P			
15.2	0.7+	2.0+	4.9+	13.4		1.9	?	11.5			
	0.7+	2.0+	4.9+	13.2		1.4	7.2	13.2			
15.8	0.8	2.1	6.5	12.8		1.5	8.0				
6											
14.2	0.8	2.7	6.3		1.0+	3.1+	5.6+	13.2		1.4	3.6
13.1	0.4+	2.0+	4.0+	16.8	1.0	2.3	6.5	12.7		1.8	4.3
	0.4+	2.0+	4.0+	17.3	1.0	2.8	6.2	12.0		1.9	4.1
12.9	0.6	2.7	6.1	O-P				11.8		1.7	5.2
13.6	0.9	2.4	5.2	16.9		2.7	5.3	11.3		2.0	4.2
?	1.0	2.9	6.3	16.1		1.5	3.8	+P			
15.7	1.1	3.0	5.0	16.0		1.3	4.2	11.0	0.8+	2.0+	5.0+
16.0	1.4	4.1	6.9	14.7		1.4+	4.0+		0.8+	2.0+	5.0+
16.0	1.0+	3.1+	5.6+			1.4+	4.0+	13.2			

Table 4.--Continued.

Fig. 6b Animal--Screen/Regular Check											
B.W.	P.I.	O-P.I.	W.I.	B.W.	P.I.	O-P.I.	W.I.	B.W.	P.I.	O-P.I.	W.I.
1											
14.7	1.2	2.9	4.7	13.8	0.6	3.8	7.6	12.2		2.9	5.9
14.1	0.5	3.1	7.9	14.0	0.9	3.5	8.4	11.7		2.1	8.2
13.4	0.4	2.6	7.4	O-P				11.5		3.5	5.7
12.9	1.1	2.0	8.2	14.1		3.0	11.5	+P			
13.2	1.0	3.5	6.1	13.4		3.2	9.1	11.0	0.5	3.0	6.3
13.9	1.4	3.0	7.4	13.1		3.1	6.6	12.4	0.8	2.8	7.9
13.6	1.0	4.4	8.0	12.7		3.1	6.5	13.0			
14.1	0.4	3.0	5.8								
6											
15.1	0.7	1.6	5.1	14.8		2.4	8.1	13.0		2.1	6.7
15.0	0.2	2.4	4.9	14.7		2.0	7.7	12.7		2.2	6.9
14.8	0.4	2.8	6.6	14.5		2.9	6.8	12.7		2.1	5.5
14.6	0.6	2.7	6.6	14.3		3.0	7.3	12.5		2.2	6.4
14.9	0.6	2.5	6.7	13.9		2.5	5.7	12.2		2.6	6.3
14.9	0.6	2.0	7.5	13.4		2.4	7.6	+P			
14.9	0.4	2.2	4.8	13.3		3.8	7.8	12.0	0.6	2.2	5.1
15.1	0.8	2.8	6.2	13.2		2.1	7.8	13.0	0.6	2.3	6.3
O-P				13.2		2.4	8.0	13.8			
15.0		2.8	8.2								

Table 5.--Reference to Figure 7a and 7b Animals.

Figure 7a--(five days experience)

--All five animals of the experiment are included in this table.

--#1 was in bad shape on the day of protein addition.

--#2 exhibited extremely slow reflexes towards being caught on the day of protein addition.

--#5 was continued past the day the 20% criterion was reached (out of curiosity). Notice the unusually high water intakes.

--#3 died before reaching the 20% criterion. Its appearance was quite normal. An explanation cannot be offered for this.

Figure 7b--(eight days experience)

--All five animals of the experiment are included in this table.

Fig. 7a Animal--Five Days Experience											
B.W.	P.I.	O-P.I.	W.I.	B.W.	P.I.	O-P.I.	W.I.	B.W.	P.I.	O-P.I.	W.I.
1											
16.2	0.3	3.3	7.5	13.8		3.7	9.5	13.0	0.0	3.0	6.2
15.5	0.5	2.8	10.9	13.5		2.9	7.1	12.0	0.1	2.9	7.7
13.5	**	**	7.9+	13.8		5.2	9.7	12.6	0.0	2.9	5.8
	**	**	7.9+	13.2		3.2+	8.4+	12.0	0.5	2.2	5.0
14.8	0.4	3.7	9.1			3.2+	8.4+	13.2			
O-P				dil/							
14.0		4.1	7.5	13.0	0.0	2.6	9.6				
2											
14.0	?	1.6	4.1	12.5		2.9	5.6	dil/			
12.9	0.1	7.1	5.8	12.4		1.9	4.5	11.0	0.7	2.1	6.0
12.5	0.0+	**	3.3+	12.0		2.4	6.9	12.0	0.3	3.0	9.0
	0.0+	**	3.3+	11.3		2.0+	4.7+	12.9	0.6	2.8	7.5
12.2	0.4	2.9	6.8			2.0+	4.7+	13.8			
O-P											
12.6		1.8	3.9								
3											
15.6	1.9	2.2	7.9	O-P				13.7		2.4+	6.5+
14.8	1.1	1.8	9.4	14.3	*	7.8				2.4+	6.5+
13.8	1.0+	1.8+	7.1+	14.7		2.6	9.8	13.2		0.3	4.5
	1.0+	1.8+	7.1+	14.7		2.6	7.6	13.0	DEAD		
13.9	2.0	2.8	9.7	14.3		2.5	5.9				
4											
20.9	0.2	2.8	6.9	18.5		3.6	9.3	dil/			
19.8	0.4	1.4	8.4	18.8		2.9	8.7	16.3	2.0	1.3	9.8
19.1	0.2+	1.4+	7.4+	18.4		3.2	12.2	18.3	2.2	*	11.5
	0.2+	1.4+	7.4+	17.2		3.6+	15.5+	19.3	1.7	2.8	10.0
18.4	2.7	2.3	10.4			3.6+	15.5+	20.0			
O-P											
18.8		2.7	9.4								
5											
17.7	0.1	3.8	11.6	17.1		3.3	10.0	13.2		5.1	13.6
15.9	0.4	2.7	12.6	17.2		3.9	12.3	13.2		2.6+	17.2+
15.7	1.4+	3.3+	8.0+	16.2		3.6+	13.1+			2.6+	17.2+
	1.4+	3.3+	8.0+			3.6+	13.1+	12.5		3.0	15.8
16.9	2.7	3.2	10.3	15.5		2.0	9.1	+P			
O-P				15.0		*	14.2	12.1	*	3.6	5.5
17.0		3.7	9.5	14.0		2.6	11.9	14.0	0.9	3.8	7.2
17.2		3.6	10.1	14.1		2.9	14.0	15.9			

Table 5.--Continued.

Fig. 7b Animal--Eight Days Experience											
B.W.	P.I.	O-P.I.	W.I.	B.W.	P.I.	O-P.I.	W.I.	B.W.	P.I.	O-P.I.	W.I.
1											
15.8	1.8	4.2	7.2	17.3	0.4	5.7	6.1	17.0		*	6.5
16.0	0.6	3.5	8.0	O-P				17.1		2.0	9.0
15.0	**	**	7.1+	17.3		5.4	7.4	16.8		2.9	7.3
	**	**	7.1+	17.0		4.5+	5.7+	16.9		3.0+	7.1+
16.3	1.2	4.3	8.8			4.5+	5.7+			3.0+	7.1+
16.9	0.9	4.1	6.4	16.9		1.4	6.8	17.0		2.0	8.7
17.1	0.4	3.7	7.6	16.9		3.2	9.1	17.0			
2											
16.1	1.1	2.9	5.8	17.2	0.8	4.7	5.8	17.4		*	7.3
15.9	0.9	2.2	7.4	O-P				17.5		2.7	5.4
15.5	0.3+	3.9+	6.1+	17.7		5.1	8.7	17.6		3.6	7.0
	0.3+	3.9+	6.1+	17.5		3.6+	6.1+	17.4		3.0+	5.5+
17.1	1.5	5.0	6.8			3.6+	6.1+			3.0+	5.5+
16.8	0.3	*	7.8	17.4		3.4	7.5	17.5		2.2	5.6
17.1	1.0	3.1	4.5	17.6		4.5	7.6	17.5			
3											
15.4	0.2	2.1	4.4	16.6	0.3	3.4	5.0	16.8		2.2	5.0
14.7	0.1	2.8	5.0	O-P				17.0		2.5	4.4
14.0	**	1.5+	4.3+	17.0		3.8	5.7	16.9		2.7	5.0
	**	1.5+	4.3+	16.8		2.2+	4.7+	16.7		3.1+	3.7+
15.0	0.8	3.1	5.3			2.2+	4.7+			3.1+	3.7+
15.2	0.4	3.0	5.2	16.7		1.7	4.9	16.8		1.6	4.3
16.0	0.8	3.2	5.1	16.9		2.0	5.9	16.8			
4											
15.9	0.2	2.0	4.2	O-P				15.4		2.1	6.1
15.0	0.2	2.1	4.6	15.8		4.0	6.1	15.5		2.4	5.7
14.6	0.0+	1.7+	3.8+	15.1		1.6+	4.3+	15.4		3.0+	5.3+
	0.0+	1.7+	3.8+			1.6+	4.3+			3.0+	5.3+
13.7	0.2	2.2	4.3	15.1		2.1	6.1	15.6		1.9	6.6
13.2	0.0	1.7	3.5	15.3		4.0	6.2	15.4		2.9	5.1
13.0	1.1	2.5	5.2	15.4		3.1	6.0	15.5			
14.9	0.3	2.8	4.7								
5											
14.8	0.4	1.6	4.6	15.3	1.7	2.9	7.5	14.8		4.0	8.9
12.6	0.3	3.1	8.4	O-P				15.0		2.0	5.7
12.8	0.1+	3.2+	6.5+	15.3		3.6	10.1	15.0		2.5	8.4
	0.1+	3.2+	6.5+	14.9		3.5+	8.0+	15.1		2.7+	7.7+
13.9	*	2.7	8.0			3.5+	8.0+			2.7+	7.7+
13.7	1.1	3.7	8.0	14.9		3.5	8.3	14.9		2.8	11.4
15.0	*	3.0	8.2	15.0		3.5	9.6	15.0			

Table 6.--Reference to Figure 8a and 8b Animals.

Figure 8a--"good"--Animals 1 and 4.

--"bad"--Animals 2 and 3.

(Excessive spillage and erratic intakes were predominant with these animals.)

Figure 8b--"good"--Animals 2 and 5.

--"bad"--Animals 1 and 4.

Presumably there were problems with #4's water bottle for the first two days such that she was in danger of dying. Chow was therefore added to the cage (in addition to the protein) for four days (Day 3-7 of the experiment), then protein selection alone from Day 7 to the dilution phase.

Fig. 8a Animals--Test Females											
B.W.	P.I.	O-P.I.	W.I.	B.W.	P.I.	O-P.I.	W.I.	B.W.	P.I.	O-P.I.	W.I.
1											
15.4	*	*	2.5	15.2	3.4	5.3	2.0	O-P			
14.3	*	*	2.0	15.2	0.9	2.9	1.0	15.9		6.0	2.0
14.5	*	*	1.5	dil/				15.0		1.1	2.0
14.7	*	*	1.5	15.4	2.2	0.6	2.5	14.7		1.4	2.0
14.6	*	*	2.0	15.2	0.0	2.4	3.0	14.0		3.7+	2.6+
14.6	0.5	3.1	1.0	15.4	0.7	2.7	2.5			3.7+	2.6+
14.9	0.3	3.1	2.0	15.5	0.1	2.6	2.0	13.2		0.3	3.0
14.9	*	2.9	3.0	15.5	0.1	2.3	2.0	+P			
15.3	0.4+	4.0+	2.5+	15.6	**	2.3+	2.5+	13.1	0.1	2.4	4.0
	0.4+	4.0+	2.5+		**	2.3+	2.5+	13.9	3.2	5.9	2.0
14.9	0.3	0.4	2.5	15.5	0.3	2.8	7.5	14.2			
14.9	0.0	3.1	2.0								
4											
17.6	*	*	4.5	16.0	4.5	4.2	4.0	O-P			
16.4	0.6	0.7	3.0	16.0	1.3	1.8	3.5	16.4		6.2	2.0
16.3	*	*	5.0	dil/				16.2		1.5	2.5
16.2	1.0	1.2	4.5	16.4	2.9	0.8	3.0	15.6		1.6	2.0
16.6	*	*	1.5	16.0	1.0	1.7	3.5	14.5		3.3+	3.3+
16.1	0.7	0.6	1.0	16.3	1.2	2.4	2.5			3.3+	3.3+
16.3	0.8	1.0	1.0	16.6	1.2	2.0	2.0	13.4		1.2	4.0
15.2	1.0	1.2	1.5	16.7	1.1	2.1	2.5	+P			
16.5	**	**	**	17.1	1.3+	1.5+	3.3+	12.7	1.1	2.4	4.0
	**	**	**		1.3+	1.5+	3.3+	15.2	3.8	5.2	4.5
16.5	*	*	3.5	16.2	1.8	1.5	3.5	15.9			
16.2	0.9	1.6	4.0								
2											
13.8	*	*	5.0	15.4	*	5.6	3.5	O-P			
13.5	*	*	4.0	15.5	0.9	2.1	4.0	15.1		5.9	3.0
12.6	*	*	5.0	dil/				15.1		0.5	4.0
12.9	*	*	5.0	15.0	3.3	0.3	4.0	14.6		2.0	2.5
13.8	*	*	4.5	14.8	0.5	2.7	3.5	13.5		3.4+	3.7+
14.2	0.8	2.0	5.5	15.0	0.7	3.2	4.0			3.4+	3.7+
14.3	0.8	2.2	5.0	15.2	0.5	2.8	4.0	12.7		1.2	5.0
14.7	1.5	2.1	1.0	15.7	0.6	2.7	2.5	+P			
14.7	2.4+	3.9+	3.3+	15.6	0.7+	2.8+	4.7+	12.5	0.5	2.4	4.0
	2.4+	3.9+	3.3+		0.7+	2.8+	4.7+	14.6	3.5	5.1	3.5
15.5	*	*	3.0	14.7	0.8	2.4	4.0	14.8			
15.6	0.7	2.1	3.0								
3											
16.5	*	*	1.5	16.7	3.6	6.6	4.5	O-P			
13.7	*	2.4	0.5	16.8	0.4	3.4	4.0	16.7		6.0	4.5
13.8	*	*	1.0	dil/				16.3		0.7	4.5
13.8	*	*	0.5	16.2	2.6	0.4	5.5	16.1		3.2	4.0
14.2	*	*	11.5	16.1	0.4	3.3	5.5	14.9		4.7+	4.0+
16.2	1.2	4.1	7.5	16.4	0.6	*	5.5			4.7+	4.0+
16.7	0.4	3.5	6.0	16.3	0.4	0.8	5.0	14.6		1.4	0.0
16.7	0.7	4.0	7.0	16.8	0.6	3.6	4.0	+P			
16.9	2.3+	**	4.7+	17.0	0.8+	**	0.3+	14.0	0.5	*	4.5
	2.3+	**	4.7+		0.8+	**	0.3+	15.2	3.0	5.6	4.0
17.2	*	0.6	4.5	16.6	0.6	3.7	5.5	15.3			
17.0	0.4	3.5	5.5								

Table 6.--Continued.

				Fig. 8b Animal--Test Females							
B.W.	P.I.	O-P.I.	W.I.	B.W.	P.I.	O-P.I.	W.I.	B.W.	P.I.	O-P.I.	W.I.
2											
16.0	0.9	0.7	4.0	14.2	1.6	2.9	5.0	14.3		2.6	4.5
14.2	0.6	2.1	5.0	15.0	0.9	4.5	5.5	13.7		2.5	5.0
14.8	*	3.7	6.0	dil/				13.2		2.7	4.0
14.9	0.8	3.0	4.5	15.1	0.0	1.4	4.5	13.0		3.0	5.0
15.3	0.9	2.5	5.5	14.4	0.6	3.3	4.5	12.4		2.0	4.0
14.7	1.0	2.5	4.5	14.9	0.7	2.1	5.0	12.7		2.2	5.0
14.9	0.8	3.3	6.5	15.2	1.0	3.1	5.0	12.6		2.9	3.5
15.0	0.8	3.1	6.5	15.1	0.6	3.4	6.5	+P			
15.2	0.8	2.9	6.0	14.8	0.8	2.1	4.5	12.3	*	2.1	4.5
15.2	0.6	3.1	7.0	14.7	0.8	3.0	4.5	13.9	1.4	2.4	5.5
15.4	0.6	3.0	6.0	O-P							
15.3	0.5	2.2	4.5	14.8		2.7	5.0				
5											
15.3	0.4	1.6	3.6	11.9		1.4	3.4	O-P			
14.0	0.5	1.4	2.8	11.6		1.2	3.6	16.5		1.6	2.8
13.5	0.7	*	2.6	dil/				15.3		1.5	2.6
13.9	0.4	2.7	3.2	11.4	0.9	1.9	3.1	14.6		0.9	2.4
14.6	0.4	3.0	3.1	12.9	0.5	2.3	2.7	13.6		0.7	2.6
14.6	0.5	2.4	2.5	13.7	0.8	2.6	3.3	13.3		0.6	3.2
O-P				14.3	0.4	2.0	3.7	12.4		1.0	3.2
15.0		1.8	2.9	15.1	0.6	2.5	2.9	+P			
14.3		2.2	3.2	15.3	0.8	3.3	4.5	12.1	1.0	0.8	4.0
14.0		0.9	2.8	16.0	1.0	2.0	3.3	13.6	0.4	2.0	2.8
13.5		1.5	3.5	16.1	0.7	2.2	3.2	13.9	0.6	2.5	3.1
12.7		1.2	3.6					14.6			
1											
16.9	0.9	0.9	4.5	16.2	0.1	3.2	5.5	15.5		5.7	3.5
14.4	0.1	1.9	5.0	16.1	*	2.7	6.0	15.0		2.9	4.0
13.6	0.0	2.7	5.5	dil/				14.5		2.3	3.0
13.4	0.2	2.6	6.0	15.5	0.2	2.9	4.5	14.7		2.9	4.0
13.8	0.0	3.8	8.0	15.0	0.4	2.9	4.5	14.2		2.0	3.5
14.4	0.0	3.0	5.0	15.1	0.0	2.6	3.5	13.7		1.9	3.5
14.4	0.2	3.1	7.0	14.5	1.3	3.2	3.5	13.4		2.2	2.0
14.7	0.5	3.6	7.0	15.7	0.7	4.0	5.0	+P			
15.7	0.7	3.7	5.5	15.6	0.7	2.8	3.0	12.6	1.7	2.8	5.0
15.7	0.4	3.9	6.5	16.0	1.2	3.0	4.0	14.9	0.9	3.0	4.0
16.0	0.4	3.3	6.0	O-P				15.5	0.9	3.5	4.0
15.8	1.3	2.8	7.5	15.5		0.3	2.5				
4											
14.5	0.2	0.1	0.1	13.2	0.0	2.2	3.8	14.4	0.4	3.1	3.0
11.9	1.5	0.0	0.1	12.9	0.0	1.5	2.8	14.1	0.1	2.6	3.0
10.6	0.0	1.1	4.0	12.7	0.1	2.1	3.8	14.2	0.5+	2.4+	2.0+
13.1	0.0	0.6	2.9	12.5	0.2	1.2	3.9		0.5+	2.4+	2.0+
13.7	0.1	2.0	4.4	12.1	0.0	1.6	4.1	O-P			
13.3	0.1	2.1	4.0	12.0	0.1	1.3	9.5	14.2		2.7	3.0
13.5	0.3	2.1	3.8	11.6	0.5	1.6	3.5	14.0		1.5	3.5
13.2	0.3	2.5	3.8	12.6	0.7	2.5	4.0	13.1		1.4	3.0
13.6	0.7	3.0	3.6	12.8	0.2	2.8	2.0	12.7		1.4	2.0
13.8	0.2	2.7	4.2	14.1	0.3	2.5	2.0	12.1		1.7	7.5
13.4	0.3	2.6	3.3	14.4	0.2	2.5	2.0	12.0		1.8+	7.0+
14.0	0.5	2.1	3.2	13.8	0.2	2.5	2.0			1.8+	7.0+
13.9	0.2	3.2	2.9	13.7	0.3	2.6	2.0	11.9		2.1	9.7
14.1	0.9	2.2	2.6	13.8	0.3	2.6	2.5	+P			
dil/				13.9	0.3	2.3	2.5	11.4	0.6	1.2	4.0
14.1	0.1	2.9	2.5	13.8	0.3	2.3	2.5	12.5	0.4	2.1	3.0
13.9	0.2	2.8	3.2	14.2	0.2	2.9	4.0	13.5	0.3	2.5	2.0
14.0	0.0	2.0	3.2	14.3	0.3	2.8	3.5	14.4			
13.5	0.3	1.9	3.7								

Table 7.--Reference to Figure 9 and 10 Animals.

Figure 9--"good"--Animals 1 and 4 (Animal 4 appeared very bad near the day of death).

--"bad"--Animal #2--This animal died after only four days of dilution. Appearance changes could be seen by day three.

Figure 10--Only two animals are shown in this table, one which died on the O-P regime (prior to reaching the 20% criterion), and another (#1), typical of the other two animals in this experiment.

Fig. 9 Animal--Too Old

B.W.	P.I.	O-P.I.	W.I.	B.W.	P.I.	O-P.I.	W.I.	B.W.	P.I.	O-P.I.	W.I.
1											
21.4	*	*	3.5	19.5	1.0	3.9	6.5	17.0		2.5	3.0
18.9	*	*	4.0	20.2	1.4	*	11.5	16.2		2.5	3.5
18.8	0.1	2.8	3.5	20.1	1.3	2.9	?	+P			
18.8	0.8	3.7	3.0	19.4	1.1	3.9	8.0	15.7	2.2	1.5	6.0
18.4	0.0	2.9	3.5	O-P				16.8	1.0	2.2	6.5
18.4	1.4	4.2	6.5	19.4		*	4.0	19.1	1.5	*	6.5
19.6	0.9	*	3.5	18.8		0.5	4.5	18.6	1.3	3.3	6.0
20.4	0.8+	1.8+	4.7+	18.2		5.9	3.5	18.7	1.7	*	6.0
	0.8+	1.8+	4.7+	17.6		1.9	5.0	18.6	2.0	2.6	3.5
20.5	1.4	4.3	6.5	17.3		2.5	4.0	18.7			
19.6	1.8	1.9	6.0								
4											
20.1	*	*	4.5	17.6	0.6	3.8	6.0	17.1		3.0	6.5
18.3	*	*	5.0	17.7	0.8	3.9	5.0	16.4		2.9	6.5
19.3	1.2	3.5	5.5	19.2	0.7	4.6	4.0	16.6		*	4.0
18.5	0.3	4.7	6.5	19.2	0.2	*	4.0	16.1		*	4.5
18.8	0.2	4.1	5.5	19.5	0.5	3.0	4.5	17.2		4.1	3.5
18.4	0.3	4.2	7.0	O-P				16.6		*	3.5
18.7	0.6	3.0	4.0	19.1		3.1	5.0	+P			
17.5	0.1+	1.4+	5.7+	18.0		0.0	6.0	15.6	0.3	1.7	0.5
	0.1+	1.4+	5.7+	17.5		5.9	5.0	14.6	DEAD		
18.4	0.0	2.8	5.5	16.8		3.5	6.0				
2											
16.9	*	*	5.0	14.1	-	-	1.0	13.4	DEAD		
14.3	*	*	7.0								

Fig. 10 Animal--Too Old Repeat

1											
15.2	1.1	2.7	6.0	15.0		2.0	8.1	12.7		3.0	5.9
14.9	0.4	*	6.8	14.5		3.9	6.9	12.6		2.1	5.1
15.1	0.3	2.7	6.1	14.3		2.6	5.9	12.3		2.8	6.3
14.8	0.5	2.8	5.6	14.0		3.3	6.0	+P			
15.0	0.6	2.3	5.5	13.7		3.2	5.6	12.0	1.6	1.7	7.6
15.0	0.6	2.1	6.2	13.7		2.1	4.1	12.5	0.9	2.9	6.3
15.1	0.9	*	5.7	13.6		2.1	8.2	13.1			
15.2	0.8	2.9	7.0	13.2		2.7	5.6				
O-P											
15.1		3.1	6.8								
4											
14.9	0.7	2.2	8.9	14.2	0.3	3.6	5.9	13.8		2.2	10.1
14.5	0.0	3.1	4.7	14.5	0.4	3.0	6.1	13.0		0.5	11.9
14.3	0.1	2.6	5.5	14.4	0.5	1.7	5.4	12.2		0.7	2.1
14.0	0.2	3.3	4.5	O-P				11.8	DEAD		
13.8	0.5	2.4	4.9	14.5		3.7	7.6				

APPENDIX C

Composition of the Experimental Diet

<u>Ingredient</u>	<u>Percent Weight</u>
Vitamin mix (General Biochem. 40060)	1.125
Sucrose	70.6
Salt Mix (Phillips-Hart from General Biochem. #170820)	4.0
Non-Nutritive Fiber (cellulose) (General Biochem #160390)	2.0
Corn oil	3.1
Lactalbumin (General Biochem #160310)	19.675

The composition of the Mouse Breeder Chow (Purina) is presented on the following page.

Approximate Chemical Composition
of Purina Laboratory Chow

% protein	20.33
% fat	11.53
% fiber	2.69
NFE (by difference)	46.18
Gross Energy (K cal/gm)	4.47
Ash	6.26
Vitamin	adequate for proper nutrition

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