Development of a Semipurified Diet for the Adult Pocket Mouse (*Perognathus*)[1]

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The pocket mouse (Perognathus) is one of the smallest known mammals, and is typical of a group of desert rodents that do not drink water. Because of their small size, their ability to survive for long periods of time on dry food (1), their resistance to radiation (2) and their ability to hibernate and estivate(3), these animals are of great interest to the physiologist and biologist. They may be suitable tools for the study of mammalian physiology in long space flights, for understanding many aspects of the control mechanism of hibernation and for studies of electrolyte and water economy.

Because it produces only small amounts of excreta, this animal is exceptionally clean, is easy to handle in a laboratory, and requires minimum care. Most laboratories (1,4) maintain these animals and related desert rodents on mixtures of dry seeds. Apparently no semipurified diet has been developed for them, and no report has come to our attention regarding their nutritional requirements. The development of a semipurified diet adequate for reproduction, growth and maintenance of the pocket mouse appears necessary before most of the studies outlined above can be undertaken. Recently, Zeman (5) developed a semipurified diet for the Mongolian gerbil (Meriones unguiculatus), but our studies indicate that the nutritional requirements of the pocket mouse differ from those of that rodent.

In this communication we are describing a diet adequate for maintenance of adult pocket mice, together with certain unusual mineral requirements of the animal, found during development of the diet.

METHODS AND RESULTS

Male and female adult pocket mice of two subspecies were used in our experiments, all trapped in the Sonora desert region of the United States: *Perognathus penicillatus*[a] (weight at maturity, 15-25 g; trapped in the high desert around Tucson, Arizona, elevation 3,000 ft) and *P. longimembris*[a] (weight at maturity, 7-11 g; trapped in Antelope Valley, California, elevation 3,000 ft).

The mice were kept in the laboratory for at least 30 days after arrival, on a mixture of seeds that had proved satisfactory for their maintenance (1,4). The mixture is made of equal parts of sunflower seeds, millet, canary seeds and oats. Some fresh carrots and lettuce were added twice a week.

The animals were housed either in one-gallon, clear glass jars[4] with perforated tops, or in plastic cages[4] with screen tops. The bottom of each jar or cage was covered with 1 to 2 inches of washed sand[5] and a small amber jar was provided for a nest. The provision of sand and nest proved to be essential for the well being of the animals. Clean cages and sand were provided monthly. The animals have to be housed individually because they are very territorial, and do not accept other mice except during estrus of the female.

Lighting in the animal room was kept on a schedule similar to the outdoor day-and-night cycle, and temperatures were maintained between 21° and 23°.

All diets had to be pelleted. The pocket mouse does not handle powders or moist mixtures well. Pellets $\frac{1}{2}$ inch to $1\frac{1}{2}$ inches long and approximately $\frac{1}{2}$ inch wide proved satisfactory. They were made by pressing the wet mixture of ingredients through a large commercial Hobart Model M-80 meat grinder and drying on screens under an air fan. All diets were kept under refrigeration, in closed containers.

The first step in the development of the diet was the attempt to feed a semipurified, pelleted diet used for regular laboratory mice. Table 1 lists the composition of this basal diet, which is a modification of that described by Bell (6). This diet failed. The mice lost weight very rapidly and died unless they were placed back on the standard seed diet before the weight loss reached about 25% of their original body weight (table 2). Various modifications were tried, to find out which portion of the diet was causing the problem. Diets were prepared containing one of the following changes: increases of fat, protein or vitamin content, decreases of fiber or mineral content, replacement of casein by soy protein, of corn oil by sunflower oil, of cornstarch by glucose, or of sucrose by cornstarch. All of the modified diets failed.

When, however, the basal diet was supplemented with fresh carrots ad libitum, the mice did well. We are assuming that the carrots served as a vehicle of water because carrots, extracted with petroleum ether and water and autoclaved to remove as many of the micronutrients as possible, served as well as untreated carrots. Lyophilized carrots were ineffective in improving the performance of the basal diet to any noticeable extent. The addition of 5 to 10% carrot powder to the basal diet was also ineffective. Only very large amounts of carrot powder (approximately 50% of the diet) were effective. The composition of this diet was, however, so different from that of the original, that it can no longer be considered as modification of the basal diet.

Since pocket mice thrive for long periods of time on sunflower seeds, it was decided to study which fraction of this seed was responsible for the success of the natural diet. Sunflower meal was fractionated into: 1) ether extract (lipids and oil-soluble ingredients); 2) protein-mineral-carbohydrate residue of ether extraction; 3) fraction 2 after heating to destroy heat-labile vitamins; 4) protein (fraction 2 extracted by means of diluted NaCl solution); 5) ash. Each fraction was then used separately as the appropriate part of the basal diet (table 1) with the other ingredients left unchanged.

The diets containing the fat extract (fraction 1) instead of corn oil, and in which casein was replaced by the protein (fraction 4), failed. The residue of the fat extraction, both before and after heat treatment (fractions 2 and 3), produced a very satisfactory diet when used in the basal diet. Ash,(fraction 5) used at a level of 5% in place of the original salt mix, produced a diet that was more satisfactory than the basal diet. The animals did fairly well on this diet for two or three times as long as on the basal diet, but after 7 to 10 weeks, this diet also failed. These results indicated that the mineral composition of the diet was responsible for its success or failure.

An analysis of the sunflower meal showed that the seeds have a high potassium and a low sodium content, and contain a large amount of magnesium and little calcium as compared with Bell's mouse diet. The total phosphorus is also quite high (table 3).

Salt mixes imitating this natural mineral composition were prepared, and used in the basal diet (table 4). Even though the correct K/Na and Mg/Ca ratios and concentrations, as well as a high percentage of phosphorus were used, weight losses and death occurred in the same way as with the basal diet.

Various modifications of the ~~diet~~ salt-mix described in table 4 were tried. The ratios and concentrations of calcium, magnesium, potassium and sodium were varied and different proportions of chlorides, sulfates and citrates were used. All these modifications failed.

Finally success was achieved when the inorganic phosphates were replaced by organic phosphates in the form of glycerophosphates of calcium magnesium and sodium.

Table 5 shows the composition of the diet that has proved successful in maintaining weight, good appearance and a normal behavioral pattern in adult P. penicillatus and P. longimembris. Over 100 animals have been kept on this diet in our laboratory for over 6 months.

Two groups of animals, one on the seed diet and one on the semipurified diet, were autopsied after 2 months. The gross appearance of the organs was normal, and organ size was the same in the two groups (table 6). Microscopic examination showed that the kidney, liver and intestinal mucosa of animals in both groups were histologically essentially normal. The analyses of the carcasses showed that the animals in these two groups did not differ in respect to moisture, protein, fat and ash content (table 6). A rather great variability of fat and moisture content within each group was noted, which was due to a higher fat and lower moisture content in the females compared with the males.

DISCUSSION

Although the diet we have described appears to be adequate for the maintenance of adult Perognathus, preliminary results indicate that it is not adequate to support growth without supplementation with lettuce and carrots. These supplements probably provide a source of water which appears to be necessary for weanling Perognathus.

We have limited our studies to the use of glycerophosphates as they are easily available and the purpose of this research was to develop a practical diet. Other organic phosphates, such as the calcium and magnesium inositols, may or may not be equally effective.

We do not yet know why adult Perognathus can thrive on a diet containing calcium and magnesium as glycerophosphates, but not on the same diet with these minerals present as inorganic salts. Nor is it clear which of the minerals—calcium, magnesium or phosphorus—is responsible for the difficulty.

The results obtained when carrots were added to the otherwise lethal basal diet suggest two possible alternate explanations. The minerals (or one of them) in the form of glycerophosphates may be more readily available to the animals in the absence of water than are inorganic salts, and the additional moisture in the carrots may increase the availability of the inorganic salts. Alternately, too much of the minerals may be absorbed when they are present in the inorganic form, and the mice may be able to utilize or excrete the exesss only in the presence of additional water. Experiments are now under way to study these problems.

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FOOT NOTES

1 Supported in part by NASA Grant NGE 05-003-118.

2 Supplied by The Pet Coral, 446 Oracle Road, Tucson, Arizona.

3 Supplied by Ernest Carl, 38960 Yucca Tree Street, Palmdale, California.

4 Scientific Products Company, Menlo Park, California.

5 Del Monte Properties, Pebble Beach, California.

6 ElMolino Mills, Alhambra, California.

ABSTRACT A semipurified diet has been developed for the pocket mouse (Perognathus longimembris and P. penicillatus), small desert rodents that do not drink water. The key difference between this diet and a standard semipurified mouse diet is the mineral composition. The ratio of K/Na and Mg/Ca is high and the inorganic phosphates are replaced by the calcium, magnesium and sodium salts of glycerophosphates. The adequacy of this diet has been shown by the maintenance of over 100 pocket mice for 6 months without weight loss, with a normal behavioral pattern and in apparent good health. Carcass composition and the size and microscopic appearance of organs were the same for animals on this diet as compared with animals fed their customary mixed seed diet.

TABLE 1

Composition of basal diet

(Modified from Bell (6))

Ingredient	g/100 g diet	Vitamins	mg/100 g diet
Casein[1]	22.3	$ZnCO_3$	25.0
Corn starch	44.3	$CuSO_4$ (anhydrous)	25.0
Sucrose	5.2	$MnSO_4$	25.0
Corn oil	9.0	KI	5.0
Fiber[2]	11.2	Choline chloride	135.0
$CaHPO_4$ (anhydrous)	1.2	Riboflavin	0.4
$CaCO_3$	2.5	Thiamine hydrochloride	0.3
NaCl	1.3	Niacin	0.3
KCl	0.4	Pyridoxine hydrochloride	0.1
$MgSO_4 \cdot 7H_2O$	0.1	Folic acid	2.5
Fe citrate	0.1	Biotin	0.01
		Inositol	0.1
		PABA	1.2
		Pantothenic acid	0.9
		Menadione	10.0
		DL-α-Tocopherol acetate	6.0
		Vitamin B_{12}	0.5 μg/100 g diet
		Vitamin A	50 IU/100 g diet
		Vitamin D	20 IU/100 g diet

[1]Vitamin-free casein, General Biochemicals, Chagrin Falls, Ohio.

[2]Solka-Floc, Brown and Company, 733 Third Avenue, New York.

TABLE 2

Typical weight loss for *Perognathus penicillatus* and *P. longimembris* on basal diet

Species	Weight after days on diet				
	1	7	14	21	28
			(g)		
P. penicillatus	20.0	16.0	13.8	13.8	died
P. longimembris	8.8	8.0	6.2	5.2	died

TABLE 3

Mineral composition of sunflower meal

	(g/100 g)	
	Ash[1]	Whole meal
Na	0.2[2]	0.01
K	17.2	0.8
Ca	2.8	0.1
Mg	10.1	0.5
Fe	0.1	0.005
Cl	--	0.1 [2]
SO_4	--	0.2
P_2O_5	--	1.9

[1]Ash approximately 5% of meal;

[2]the cations were determined in the ash, the anions were determined in the meal because of possible losses during ashing.

TABLE 4

Typical salt mix used to simulate the composition of sunflower meal

	(g/100 g diet)
NaCl	0.03
KCl	0.20
K_2HPO_4	1.36
$MgSO_4$ (anhydrous)	0.65
$MgHPO_4$	3.1
$CaHPO_4$	0.45
Fe-citrate	0.025

Same trace minerals as in Bell's salt mix (Table 1)

TABLE 5

Composition of semipurified diet for *Perognathus*

Diet ingredients	
	(g/100 g diet)
Soy protein[1]	22.0
Corn starch	52.2
Corn oil	10.0
Fiber[2]	7.0
Gum arabic[3]	3.5
Vitamin mix[4]	2.0
Na-glycerophosphate[3]	0.30
KCl	0.60
Ca-glycerophosphate[3]	0.60
Mg-glycerophosphate[3]	1.48
$MgSO_4$	0.30
Fe-citrate	0.04
	(mg/100 g diet)
$ZnCO_3$	25.0
$CuSO_4$ (anhydrous)	25.0
$MnSO_4$	25.0
KI	5.0

[1]Soya assay protein, General Biochemicals, Chagrin Falls, Ohio.

[2]Solka-Floc, Brown and Company, 733 Third Avenue, New York.

[3]S. B. Penick and Company, 100 Church Street, New York.

[4]See Table 1.

TABLE 6

Organ weights and carcass composition of *Perognathus* penicillatus fed seeds or the semipurified diet[1]

Diet	Body wt. at autopsy	Carcass[2] wt.	Moisture[3]	Protein	Fat	Ash	Liver	Heart	Kidney	Lung
	(g)	(g)	(g/100 g carcass)				(g/100 g body wt)			
Seeds[4]	21.2±1.1	12.8±0.6	60.7±4.1	19.3±1.3	20.9±4.5	3.3±0.3	3.4±0.4	0.5±0.04	0.9±0.08	0.6±0.06
Semi-[5] purified	22.4±1.1	13.4±0.7	57.2±4.3	18.5±1.3	19.9±6.1	2.7±0.2	3.1±0.1	0.5±0.03	0.9±0.09	0.5±0.03

[1] Four animals (two males, two females) each after two months on the respective diet (mean ± SE).

[2] Carcass represents body after removal of head, tail, and all viscera except perirenal fat pads.

[3] Moisture was determined by drying at 110°C to constant weight; fat was determined by Soxhlet extraction of the dried carcass with petroleum ether for 24 hours and weighing of extracted crude fat; proteins and ash were determined on aliquots of the powdered dry, fat-free carcass by the Kjeldahl method of nitrogen determination and ashing at 800°C, respectively.

[4] Equal parts of sunflower seeds, millet, canary seeds and oats.

[5] See Table 5.