2. Title: "Ecology of the Tree Shrew (Tupaia glis)

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OBJECTIVE: To evaluate the potential of the tree shrew for use as a laboratory primate. To colonize the species, define biological norms, determine prevalence of endoparasitic infestation and define bacterial flora of the intestinal tract.

DESCRIPTION: The Tupaidae, represent an important link with the Insectivora. Dentition and general skeletal anatomy are typical of Insectivora. The estrous cycle and central nervous system are more characteristic of the primates. Much can be learned by studying a group of animals that possess characteristics of more than one order. In the natural habitat, highest densities (15-30 per acre) are found in coconut groves where they eat the young coconuts and are considered pests. They can climb with agility and live much like squirrels. Both in the wild and in captivity, they display an extraordinary combination of placidity and violent activity. Tupaia glis are readily available in Thailand, therefore studies of this species were more easily done in this country.

Investigations included observations in the native habitat and in a laboratory environment. Aspects pertinent to diet, caging, bedding and reproductive activity were also investigated. These studies have resulted in the successful colonization of this species in a laboratory environment.

PROGRESS: Eight tree shrews were purchased initially to be utilized in investigations of various commercially available laboratory diets. The four diets investigated were baked simian diet, baked rat and mouse diet, kibbled canine diet and fresh fruit and vegetables. Two animals, one male and one female were caged under similiar conditions in each of the four groups. The diets were fed ad-lib with multivitamin preparations added to the drinking water. Animals fed baked simian diet or baked rat and mouse diet did not survive. Deaths occurred on days 6 and 12, 4 and 13 respectively. Cause of death was malnutrition due primarily to difficulty in biting and chewing the hard baked biscuits. One animal maintained on the fresh fruit and vegetable diet did survive through the 20 th day but had weight loss and alopecia. Animals maintained on the kibbled canine diet remained in excellent condition for the full thirty days and were sacrificed.

The kibbled canine diet being economical, readily available and acceptable, has been fed to all shrews using self feeders. Adults in the colony have been maintained on this diet for as much as one year and are in excellent physical condition. No evidence of malnutrition or dietary deficiency has been noted Multivitamin preparations containing vitamins A, B, C, D and E are added to the drinking water at the rate of 8 cc of preparation per five gallons of drinking water. Pregnant and lactating females

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receive reconstituted whole milk containing added vitamins ad lib in addition to the canine diet thirty days prior to parturition and until the young are weaned. Water and milk are supplied using methods similiar to those used for other laboratory animals such as mice and rats. These tupaiids have little trouble drinking from these sipper tubes, and no dehydration has been encountered.

Various cage sizes and types have been tried. None appeared to affect the animals either physiologically or psychologically. Cage height did play a major role in the amount of exercise observed in the various groups. Animals housed in cages nine inches high did not exercise as much as those housed in higher and larger cages. The overall effect of exercise on colonization of the species is undetermined but may play a very important role. Matig and conception has occurred in all types of caging. Nursing and rearing of young has occurred only in the higher cages. Many types and sizes were investigated ranging from cages 3' high 4' wide and 2' deep to cages 1' high 1' wide and 18" deep. The optimum cage developed for colonization is 17" high 12" wide 24" deep. Each cage contains a perch four inches wide across one end which folds up when the cage is collapsed. The top, floor, and collecting pan are removable making the cage easily handled and cleaned. Each end will support an individual nest box (8" x 7½" x 8"). A self feeder and water bottle holder are installed on one side. The cage as well as the nest boxes are light, collapsible and easily stored.

Various types of bedding for the nest boxes have been investigated. These have included sawdust, shavings, cloth, cotton and shredded paper. Best results have been obtained with shredded paper. This product has acceptable moisture absorption properties and is suitable nesting material for pregnant females nearing parturition. Since high humidity is encountered in nest boxes during the nursing period, the acceptable product must be soft, provide insulation against the metal sides and have high absorption properties. A dermatitis of the tail followed by hair loss was noted in the first group of young born in the colony. This condition could be related to humidity and has not been noted since institution of the use of shredded paper.

Various mating combinations ranging from 1 male and 6 females to 1 male and female were investigated. Mating with conception has occurred at all combinations. After pregnancy is determined by palpation, females are removed from the higher density groups and placed in individual cages. They remain in these cages until the young are weaned. Recent reports relative to post partum mating has led to investigation of permanent monogamous matings. This technique, when followed in the cage described appears to be the most efficient production method.

Table I provides information on the status of the colony comprised of 40 females and 20 males from March 1965 to the present.

TABLE I

SUMMARY OF PARTURITIONS

	P_1	P_2	P_3	P_4	P_5	Total
Total Pregnancies	50	27	16	4	1	98
Total litters born	20	16	8	4	1	49
Total number young born	40	33	15	7	2	97
Total weaned	12	20	11	4	2	49

A large number of pregnant animals either aborted or resorbed the fetuses. Data suggest an average lapse of 60 days between introduction to the male and parturition. The gestation period is approximately 45 days. Litter sizes range from one to three with a mean of two at birth.

Following parturition primiparous females frequently fail to suckle their young and in some cases eat them. Multiparous females more readily suckle their young and cannibalism has not been observed. Since our approach was one of practical laboratory animal production, hand rearing was not undertaken. It appears that puberty may be reached at 5-6 months of age. Offspring reared in the colony have been mated at this age and females have conceived. Two litters have been born and one is being reared.

Live trapping of tree shrews was initiated near Pra Padaeng in coconut plantations. Shrews were live-trapped through December. Reproductive data from these animals indicate breeding continues through August, but reproduction appears to have ceased in September and early October. Stomach contents during this period indicate a strong shift of food habits from fruit to incects and green plant material, doubtless due to the scarcity of fruit at this season. Breeding activity did not start again until the last week in December. At that time 11 females trapped were in early stages of pregnancy. Two weeks earlier, nest building activity was observed in the field. It appears that complete cessation of breeding in the natural population occurred for a period of about 4 months, from August to December Our colonized tree shrews in the laboratory showed no detectable decrease in breeding activity during this period. Although a careful population census has not been made, trapping results suggest that initially the population density in the coconut plantations may have been as high as 25.30/acre. It would appear that home ranges are very small (less than 1 acre) and that there is relatively little tolerance among individuals, even at high densities. Field observations of tree shrew activity suggest that small family groups tend to remain together in discrete acres of the habitat, indicating that family groups, at least, are quire sociable. On the basis of trapping success and observations in the field, the population density seems to have decreased markedly during October, November, and December. There also appeared to be a total lack of immature tree shrews in the field populations.

Fecal specimens were obtained from eighty - eight shrews. Since these were obtained from animals sacrificed for biological definition portions of the intestinal tract were also removed, minced and examined. Both direct smear and formalin ether concentration techniques were employed. Forty - one of the animals examined were infested with one or more species of endoparasite. As noted in the following Table, strongyloides infestation occurred most commonly. Tapeworm hookworm and ascarid infestation were of secondary importance.

TABLE II

PARASITES OBTAINED FROM TUPAIA GLIS

Strongyloides (larva, ova)	32
Tapeworm	5
Hookworm	5
Ascarid	4
Oxyurids	1

A preliminary study of various anthelmentics suggests that oral administration of either Diethylcarbamazine citrate 30 mg/pond for 3 days or Dithiazanine iodide 5 mg/pond for 12 days are effective in eliminating infestation. Spargana sp. are commonly observed in the subcutaneous tissue of the inguinal and subscapular regions. These were recognized only at necropsy and do not appear to affect the general physical condition. The parasite was found chiefly in animals obtained from southern Thailand. A much lower prevalence was encountered in those animals trapped near Bangkok or the northern areas of the country.

The accumulated hematology, biochemistry and electrophoresis results of animals sacrificed during the report period are summarized in Tables III, IV and V.

TABLE III

HEMATOLOGY

	Female 62		Male		Total 94	
	Ave	Range	Ave	Range	Ave	Range
Hct	41	27-29	46	29-57	43	27-57
Hgb	12.7	8.4-15.4	13.7	7.6-18.9	13.0	7.6-18.9
WBC	1.88	0.3-7.2	2.47	0.7-8.4	2.07	0.3-8.4
RBC	6.56	4.0-9.24	6.90	3.58-9.7	6.68	3.58-9.8
N	57	25-90	62	22-90	59	22-90
L	28	0-57	28	2-65	28	0-65
B	1-2	0-6	1-2	0-9	1-2	0-9
E	9	0-37	5	0-21	8	0-37
Baso	2-3	0-15	1.0	0	1-2	0-15
M	2.0	0-7	2.0	0-6	2.0	0-7

TABLE IV

BIOCHEMISTRY

	Female			Male			Total		
	Ave	Range	#	Ave	Range	#	Ave	Range	#
Thymol Thurbidity	0.9	0.6-1.7	5	1.2	0.7-2.0	3	1.0	0.6-2.0	8
Cholesterol	87	36-193	15	105	57-192	12	95	36-193	27
BUN	29.5	9.0-84.4	59	37.1	4.0-184.0	33	32.0	4.0-184.0	101
Protein	6.2	3.3-7.8	62	5.47	3.3-7.8	31	6.0	3.3-7.8	93
Alkaline PO_4	4.7	1.6-26.0	20	22.8	1.1-102.0	12	10.4	1.1-102.0	38
Acid PO_4	0.08	—	1	0.08	—	1	0.08	—	2
SGOT	211	640	21	161	94-250	11	194	640	32
SGPT	38	113	19	32	6-89	9	36	6-113	28
Bilirubin Direct	0.18	0.0-0.5	32	0.14	0.0-0.3	19	0.16	0.0-0.5	51
Bilirubin Total	0.32	0.0-0.8	36	0.35	0.1-0.5	19	0.33	0.0-0.8	55
CO_2	12.8	4.1-23.4	54	13.1	6.1-21.9	29	13.6	4.1-23.4	83
Na^+	155	145-178	34	156	117-178	18	155	117-178	52
K^+	5.0	7.3	34	4.9	3.4-8.5	18	4.9	2.7-8.5	52
CL^-	117	109-135	56	117	106-125	23	117	106-135	79
Uric Acid	1.6	1.5-1.8	2	—	—	—	1.6	1.5-1.8	2

TABLE V

MEAN ELECTROPHORESIS VALUES

Animals	Globulin					Albumin	A/ratio of the
		1	2	B	X		Means
Caged 24	3.1	.29	.39	1.27	1.14	3.77	1.26
Trapped 21	3:7	.23	.59	1.32	1.54	2.52	0.68
Procured 21	3.4	.29	.28	1.69	1.16	1.93	0.61
Total 79	3.4	.27	.39	1.46	1.25	2.63	0.77

TABLE VI

Colony Group	#	Total Protein	Total Globulin	Albumin
60 days	25	6.9±.51 (6.0-7.7)	3.1±.46 (2.4–3.9)	3.8±.45 (2.9-4.8)
30 days	35	5.3±1.12 (3.3-7.4)	3.4±0.91 (1.8-5.6)	1.9±0.57 (0.8-3.0)
Trapped	12	6.4±.56 (5.8-7.8)	3.3±61 (2.6-4.4)	3.1±.52 (2.1-4.3)

Although average hematocrit and hemoglobin values are on the lower side of normal for most mammals, the values did not show an increase with colonization. The total leucocyte count was consistently low and did not seem to be affected by period of colonization or related to any other microbiological, biochemical or pathological feature. Some tree shrews had an unusually high eosinophile count but the presence of a high or low eosiniphile count could not be related to gross or microscopic evidence of parasitism. Some heavily parasitized animals had very low eosinophile counts and conversely in some animals with a high eosinophile counts, no evidence of parasitism could be detected.

The increase in serum protein and albumine from newly procured to trapped and colonized animals is undoubtedly a reflection of the improved state of nutrition of these animals. The native diet readily abundant in Thailand consists of fruits, insects and leaves, and although not well balanced, is quite adequate for their survival and reproduction. The newly procured animals which are trapped, held by animal dealers and transported to market areas are not as well fed either by reason of poor diet or poor appetite related to xecitement and disturbance. The difference in total protein values for males and females (Table IV) is largely a result of the disparity between the male and female ratio in the 60 days colonized group, a group which had high total protein and albumin values. In newly procured animals a significant difference in total protein values does exist between male and female, but seems to be a reflection of $alpha_2$ and beta globulin levels rather than albumin levels.

The $alpha_2$ globulin in males seemed to be either very high (around 1.0) or very low (around 0.2). In the female high values 1.5 were observed in pregnant animals whereas other animals were below 1.0. Many males also had 0.0 beta globulin levels, hence lowering the overall average

The improved albumin levels obtained from animals under colony conditions reflect the balanced nature of the commercial diet. Although 60 days seemed to be the breaking point between high and low serum protein and albumin levels, a steady improvement could be seen from time of purchase to around 60 days (Table V).

SUMMARY: The tree shrew (Tupaia glis) has been colonized and will reproduce in a controlled environment. This species adapts well to the laboratory environment and may contribute significantly to research in areas requiring primates or higher rodents.

The kibbled canine diet supplemented with multivitamin preparation has been fed to all animals in the colony for extended periods and all have remained in excellent physical condition.

Optimum cage requirements and design have been described. Use of these should facilitate efficient production of the species. The age of pubescence and short gestation period provide access to an economical laboratory primate.

Endoparasitic infestation found in the natural habitat may be controlled by anthelmenthics and method of caging.

Hematological, biochemical and electrophorectic data are reported. Improved albumin levels were obtained from animal maintained under colony conditions and reflect the balanced nature of the commercial diet. Hematocrit, hemoglobin values and leucocyte counts were consistently lower than those reported for other mammals.