was counted for radioactivity as $BaCO_3$ by standard methods. In experiments in which this arsenolysis method was used, results equivalent to those presented in Table 2 were obtained.

The limitation of both citrulline and carbamyl aspartate synthesis by the same reaction and the incorporation of bicarbonate carbon into the ureido carbon of the citrulline molecule strongly suggests that carbamyl phosphate is the intermediate in carbamylation reactions in the earthworm (*1*). The requirement of *N*-acetyl-L-glutamate and the operation of the reaction at low ammonia concentrations are characteristic of the carbamyl phosphate synthetase system found in mammals and amphibians. The failure of L-glutamine to stimulate citrulline synthesis distinguishes this system from that in certain fungi (*9*). Thus carbamyl phosphate synthesis in the earthworm is mediated by an enzyme system more closely related to that in ureotelic vertebrates than to that in either bacteria or fungi.

The earthworm carbamyl phosphate synthetase is only detectable in the soluble fraction of the cell in contrast to the synthetase in the vertebrates which is found mainly in the mitochondrial fraction. The intracellular localization in the earthworm gut tissue is presented in Table 1. The only variation encountered in this localization was in two of five fractionations where as much as 24 percent of the total units was found in the residue sedimenting at 600*g*. Of 14 different solutions tested, the combination of 0.4*M* mannitol, 0.1*M* K_2SO_4, and 0.015*M* potassium glycylglycinate, *p*H 7.5 was found to be most effective in protecting the quite labile activity during fractionation. The supernatant from tissue homegenates prepared in this solution and centrifuged at 15,000*g* was routinely used as the enzyme source.

A high "carbamyl phosphate phosphatase" activity is present in mitochondrial preparations of the earthworm as well as of other invertebrates. This may account for previous failures to demonstrate carbamyl phosphate synthetase activity with such preparations. We found a marked inhibition of carbamyl phosphate synthesis by water-lysed liver mitochondria from an amphibian (*Rana pipiens* or *Bufo valliceps*) when they were mixed with water-lysed mitochondrial preparations from three species of invertebrates (the earthworm, the land snail *Otala lactea*, and the flatworm *Hymenolepis diminuta*).

The adenosine triphosphatase activity of both the invertebrate and amphibian mitochondria was of the same order of magnitude when measured under the conditions of the synthetase assay. Under conditions where the reaction proceeded to completion on the basis of the ATP present, an increase in the amount of amphibian mitochondria did not result in inhibition of carbamyl phosphate synthesis. An increase in the amount of ATP-generating system did not overcome the inhibition given by the invertebrate mitochondria. The inhibition was not due to the metabolism of *N*-acetyl-L-glutamate since the effectiveness of this cofactor in the amphibian system was not changed after its incubation for up to 3 hours with the invertebrate mitochondria.

During the inhibition of the reaction, there was a liberation of inorganic phosphate which was directly proportional to the amount of inhibition of carbamyl phosphate (as citrulline) synthesis resulting from the breakdown of carbamyl phosphate by these mitochondria. This phosphatase activity was assayed in a system containing, in micromoles per milliliter, dilithium carbamyl phosphate, 10; tris-HCl buffer, *p*H 7.4, 50; and $MgCl_2$, 5. Controls consisted of the complete system containing boiled enzyme. The activities expressed in micromoles of inorganic phosphate (corrected for control values) liberated per hour per mitochondria from 1 g of tissue at 28°C were as follows: *Lumbricus*, 27.6; *Hymenolepis*, 21.6; and *Otala*, 20.4. The specificity of the phosphatase activity is unknown (*10*). Although the phosphatase activity is in excess of the synthetase activity in the earthworm, it appears to be an important factor only under conditions of tissue homogenization which result in mitochondrial disruption. This activity did not interfere with the detection of carbamyl phosphate synthesis in crude homogenates containing intact mitochondria as is shown in Table 1.

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Tree Rat, *Thamnomys surdaster surdaster*, in Laboratory Research

Abstract. *The breeding of successive generations of* Thamnomys *in the laboratory made possible a study of its bionomics and an evaluation of its importance in parasitological and malarial research.*

The tree rat, *Thamnomys surdaster surdaster,* is the natural mammalian host and reservoir of *Plasmodium berghei* (*1*), the malarial parasite most commonly used in laboratory research because it is easily transmitted by inoculation of infected blood into animals such as white mice, albino rats, hamsters and voles. However, until recently, the difficulty of obtaining live specimens of *Thamnomys* from Katanga, and the failure, as reported, of these animals to mate and reproduce in captivity (*2*) prevented their use in malaria and other parasitology research. In the words of the late J. Rodhain, the tree rat has remained *"un obscure representant parmi le tres nombreux rongeurs de l'Afrique Centrale."*

On 12 April 1962, we received a number of *Thamnomys* caught in the wild (*3*). The animals have been mated successfully, and five successive generations of laboratory-bred *Thamnomys,*

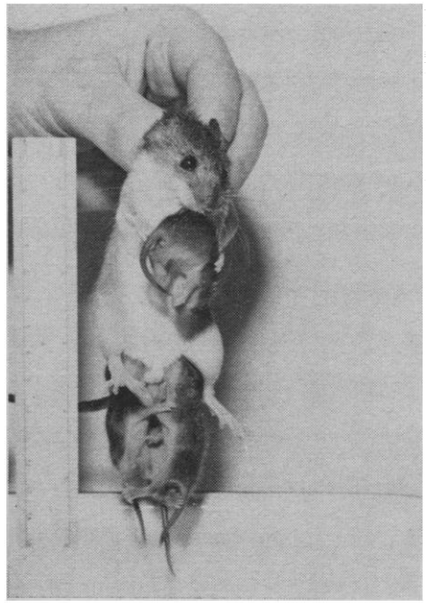

Fig. 1. Laboratory-bred female *Thamnomys* with two 5-day-old young attached to her breasts and one in her mouth.

totaling 254 animals (from 63 litters), have been reared to maturity. A permanent colony has been established, and it is planned to provide other scientific laboratories with specimens for breeding and research.

The adult tree rat is a small, swift, and timid rodent exhibiting in nature typical nocturnal habits. Its fur is various shades of brown but is cream-white on the belly. Its average body length is 10.4 cm. The sturdy and long tail, which shows easily accessible veins, measures about 13 cm. The weight of the adult tree rat averages between 55 g and 66.5 g. In their natural environment of the forest galleries of the Congo, *Thamnomys* are found sheltered in holes in trees up to 2.5 meters from the ground (*4*). But they should not be considered arboreal rodents for they move very frequently on the ground. Their food consists of seeds of wild grains and fruit. Under laboratory conditions, their behavior greatly resembles their habits in nature. Paired and housed in cages, and provided with ample straw and a Mason jar to simulate a nest, they will crowd therein and readily adapt themselves to their new surroundings. All their movements, feeding, and mating occur in the dark hours of night.

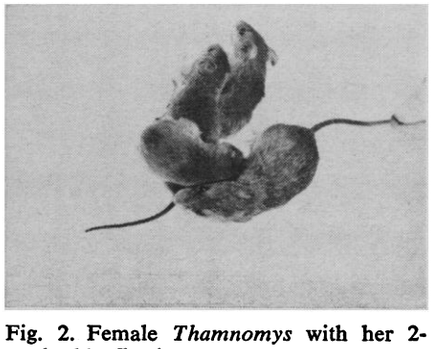

Fig. 2. Female *Thamnomys* with her 2-week-old offspring.

A plentiful supply of sunflower seeds replenished twice a week, supplemented by fresh vegetables, sweet potatoes, apples, corn, and lettuce will ensure their normal growth (*5*).

In their native region of Katanga the fluctuations in temperature between day and night are great. At night, the temperature may be as low as 14.8°C (*6*). *Thamnomys* tolerate these climatic changes well but may die if exposed for long periods to high temperatures and high relative humidity. Tree rats reach sexual maturity at the age of 4 months; litters are produced every 6 to 7 weeks. These consist of 2, 3, 4, or, rarely, 5 young. The newborn attach themselves to their mothers' nipples and will not abandon them during the first 2 weeks of their life. One may lift the mother, even shake her, yet the young will hold on (Fig. 1). The young tree rats grow in size and increase in weight during the first 5 or 6 months of their life. At 4 days their average weight is 4.1 g; at 8 days, 7.8 g. The average weight at 30 days is 25.3 g; at 3 months, 40.5 g; and at 6 months, 58.5 g. The blood of 10 laboratory-bred male and 10 female adult tree rats was examined. The average results for the males were: hemoglobin, 14.33 g/100 ml; erythrocytes, 10,152,000 per cubic millimeter; leukocytes, 14,600 per cubic millimeter. For the females, the average results were: hemoglobin, 12.25 g/100 ml; erythrocytes, 12,550,000 per cubic millimeter; leukocytes, 12,850 per cubic millimeter.

Thamnomys should be paired as early as the 6th week after birth. Older *Thamnomys* tend to live by themselves and resent the intrusion of their mates. The nests and the newborn should be disturbed as little as possible; a female will abandon or even kill her young if pried or disturbed too often. There are no difficulties in the handling of *Thamnomys* in routine work.

The tree rat may be especially useful in parasitological studies. *Thamnomys* has been found to harbor three hemosporidian parasites in nature: *Plasmodium berghei, Plasmodium vinckei* (*7*), and *Babesia rodhaini* (*8*). Though the plasmodia may easily be transmitted to other laboratory animals, only in the tree rat and in the hamster will the development and the natural evolution of the parasite take place (*9*). Newly isolated strains of *P. berghei* kept by continuous blood transfers in white mice or in albino rats lose their ability to produce gametocytes within a period of months. The disappearance of the sexual forms of a parasite, as demonstrated by Sergent for the Theileria (*10*), may remain a permanent feature of an "exhausted" strain propagated in the absence of cyclical transmission. By passing such exhausted strains through young tree rats, their natural hosts, they may be invigorated and their ability to produce gametocytes restored (*11*).

The susceptibility of the tree rat to a number of pathogenic protozoa and helminths is being investigated. Preliminary results show that *Thamnomys* may be infected with *Leishmania donovani*. A light to moderate infection ensues with a tendency to natural regression and disappearance of parasites from spleen and liver within 6 to 7 months.

A strain of *Trypanosoma lewisi* has been established in tree rats and a study of its behavior in this rodent host is now in progress. A study is also being conducted on the use of *Thamnomys* in experimental research on *Entamoeba histolytica, Hymenolepis nana,* and several filarial infections (*12*).

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