Reports

Pigmy Marmoset as an Experimental Animal

Abstract. The pigmy marmoset has been maintained under laboratory conditions for approximately 1½ years. A synthetic diet has been prepared, which includes milk, eggs, and lard, which may cause a fourfold increase in serum cholesterol levels. This species is proposed as an experimental subject for the study of aging and age-associated diseases.

It is desirable to determine the biochemical and physiological characteristics of every species. A particular trait in one may help to illuminate an area of investigation which is more complex or difficult to study in others. For example, for the study of aging and of age-associated disease, it would be desirable to locate a short-lived primate in which problems arise that are similar to those observed in man.

The pygmy marmoset, *Cebuella pygmaea* (also termed *Callithrix pygmaea*) (Fig. 1), is a New World monkey which grows to about 4 in. in length (exclusive of the tail) and a maximum weight of less than 200 gm. It comprises two subspecies, one of which, the Western (*C. pygmaea pygmaea*), is found in the forests of Equador, Colombia, and Peru; the other, the Eastern (*C. pygmaea niveiventris*), is found in Brazil (*1*). Although definite evidence is not yet available, reliable information suggests a longevity of 6 to 8 years (*2*). This is considerably less than the average life span of other primates for which adequate data are available (*3*). It thus has seemed worth while to investigate the possible use of this animal as an experimental subject for studying problems of aging. Our findings over a period of 1½ years are reported here.

Our pygmy marmosets, all of unknown age, were obtained from Iquitos, in eastern Peru; hence they are of the subspecies *niveiventris*. They were maintained in rabbit cages, each of which contained a small wooden box with an opening just large enough to contain all of the occupants of the larger cage, in relatively close proximity. As many as 20 pygmy marmosets were maintained in a cage, and they usually remained in the wooden box. This proved to be fortunate, for the cages could then be opened readily for cleaning and feeding without risk of the animals' escaping. Furthermore, when specimens were to be obtained, the box, with its occupants, could be put into a sack from which one marmoset was taken at a time. The boxes were not cleaned, since small primates do not do well in unfamiliar quarters; the presence of some of the excreta from their group is apparently necessary (*4*). A second box was introduced when it became necessary to remove the first, and the first was taken out when familiarity with the second had been acquired. In order to obtain specimens of blood, the marmosets were wrapped in a towel so that only the tail protruded. This seemed to tranquilize them. Microspecimens could be taken, as with rats.

It was essential that the temperature be kept uniform and high, and that the humidity should be high. A minimum temperature of 82°F was maintained with an automatic heater. The temperature rose above this with the heat of the summer. The air was maintained near water-saturation by means of a steamer.

Losses in newly acquired animals were due to infection accompanied by pulmonary diseases or diarrhea, or both. Losses of animals in the colony were due to the introduction of infected new arrivals which had not been properly quarantined, to fighting, to refusal to eat, and to accidental exposure to adverse environmental conditions.

Although the marmosets thrive on a diet of various fruits and meal worms, it was of first interest to determine whether they could be maintained on a diet more like man's, including milk, eggs, and lard. A liquid diet was prepared as follows: condensed milk, Carnation brand, 400 ml; corn syrup, Karo brand, 25 ml; water, 400 ml; four eggs, 175 ml; vitamin mixture, Vipenta No. 2, 1.2 ml; vitamin mixture (*5*); salt mixture (*6*).

It was calculated that 15 ml of this mixture, the average daily consumption, provided adequate nutritional intake. There was no other source of water. In addition, the marmosets were provided with Wheat Chex saturated with a mixture of lard and Karo syrup, each 50 percent by volume. This preparation was readily consumed. Though numerous attempts were made to introduce meat into the diet in various forms, it was not accepted.

It was necessary to wean the marmosets from the old, basal diet by presenting the new diet along with the basal diet and then gradually reducing the components of the latter one by one. Basal serum cholesterol levels (*7*) of new arrivals or of those on the basal fruit and meal-worm diet were 167 to 250 mg per 100 cm^3 (210 ± 28). Values were lower for young animals (those judged to be young by their smaller size). After introduction of the synthetic diet, the response of the animals' serum cholesterol was varied, apparently depending at first upon the degree of adaptation to the diet. If the animal did well on the diet, weight was maintained or increased and the serum

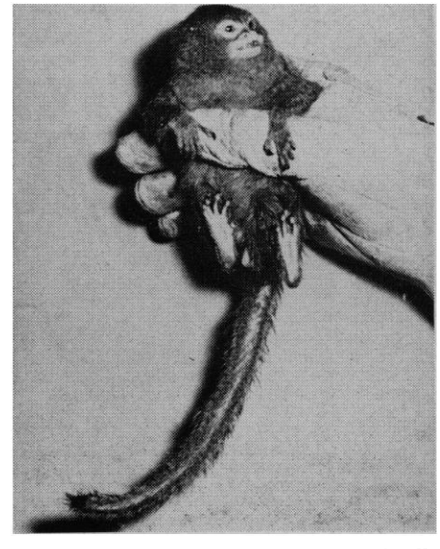

Fig. 1. The pygmy marmoset (*Cebuella pygmaea*).

Instructions for preparing reports. Begin the report with an abstract of from 45 to 55 words. The abstract should *not* repeat phrases employed in the title. It should work with the title to give the reader a summary of the results presented in the report proper.

Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to Contributors" [*Science* **125**, 16 (1957)].

cholesterol levels rose to a maximum value in 4 to 8 months. The greatest value obtained to date is 880 mg per 100 cm^3. In a number of animals, subsequent to the period of maximum level of serum cholesterol, which was maintained for several months, the value fell precipitously along with decrease in body weight. In many cases this was followed by death of the animals, in which general emaciation or evidence of pulmonary hemorrhagic edema, or both, was observed.

We are firmly convinced that this species will become a useful laboratory animal. To date there have been no successful matings, however. This may be due to the failure to separate pairs. We would appreciate any information which other investigators may have on the matter of propagation of this species (*8*).

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References and Notes

1. I. T. Sanderson, *The Monkey Kingdom* (Doubleday, Garden City, N.Y., 1957), pp. 49–56; W. C. O. Hill, *Primates (Comparative Anatomy and Taxonomy)*: vol. 3, *Pithecoidea: Platyrrhini (Families Hapalidae and Callimiconidae)* (Interscience, New York, and University Press, Edinburgh, 1953–57); W. Fiedler, "Übersicht über das System der Primates," in *Handbuch der Primatologia*, H. Hofer *et al.*, Eds. (Karger, Basel, Switzerland, 1956), vol. 1, pp. 1–266.
2. This was a concensus obtained after consultation with personnel of several zoological gardens and individuals who obtain and sell animals from South America.
3. A. H. Schultz ["Postembryonic age changes," in *Handbuch der Primatologia*, H. Hofer *et al.*, Eds. (Karger, Basel, Switzerland, 1956), vol. 1, pp. 887–964], gives the following "rough averages of unpublished data by the writer, and data from reports of zoological gardens and other sources": lemur, 14-year life span; macaque, 24 years; gibbon, 30 years; orangutan, 30 years; chimpanzee, 35 years. Unfortunately, no adequate data for any New World monkey seem to be available; hence, whether the pygmy marmoset is typical of New World monkeys in general, or whether it is aberrant, cannot be said.
4. We are grateful to Dr. J. R. Hendrickson, University of Malaya, for supplying this information.
5. The vitamin mixture contained vitamin C, 250 mg; vitamin B_1, 4 mg; vitamin B_2, 5 mg; vitamin B_6, 20 mg; niacin, 20 mg; pantothenol, 20 mg; *p*-aminobenzoic acid, 20 mg; inositol, 1 gm; choline, 1.2 gm.
6. The salt mixture contained KCl, 24.4 gm; $FeSO_4 \cdot 7\,H_2O$, 13.6 gm; $MgSO_4 \cdot 7H_2O$, 14.4 gm; $CuCl_2 \cdot 2\,H_2O$, 0.42 gm; $ZnSO_4 \cdot 7\,H_2O$, 0.7 gm; $CoCl_2 \cdot 6\,H_2O$, 0.4 gm; $MnSO_4$, 0.2 gm; KI, 0.2 gm, in a volume of 125 ml.
7. The procedure of J. J. Carr and I. J. Drekter [*Clin. Chem.* **2**, 353 (1956)] was modified so that tests could be carried out on 0.025 ml of serum.
8. This work was supported by grants from the U.S. Atomic Energy Commission and the U.S. Public Health Service.

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Synxenic and Attempted Axenic Cultivation of Rotifers

Abstract. Three species of rotifers have now been grown synxenically and, to a limited extent, axenically. *Brachionus variabilis* thrives in suitable media containing *Chlorella pyrenoidosa* and a bacterial species. *Lecane inermis* and *Philodina acuticornis* var. *odiosa* are bacteriophagous, the former doing best with two bacterial species (dixenically), the latter doing well with *Escherichia coli* alone (monoxenically).

Relatively few species of invertebrate metazoa have been cultured synxenically [that is, such that a species is grown in the presence of a known number (one or more) of other species or of their living cells (*1*)]. Even fewer have been grown indefinitely under axenic conditions (*2*). During the past 2½ years several species of the aschelminth class Rotifera have been maintained in xenic culture (harboring a mixed, undetermined microbial flora) in our laboratory (*3, 4*). Certain of these have been established synxenically and numerous efforts to initiate axenic cultures have been made (*4–6*).

Our work has been done principally with two species of monogonont rotifers, *Brachionus variabilis* and *Lecane inermis* [the latter of which was erroneously referred to as a tiny, unidentified "bdelloid" in recent notes (*3, 6*)], and with a large bdelloid, *Philodina acuticornis* var. *odiosa (7).*

Xenic cultures of *B. variabilis* were first maintained in Petri dishes containing Seitz-filtered pond water, to which were added enough packed cells of axenic *Chlorella pyrenoidosa* (from a mineral medium) to give a semitransparent green turbidity apparent on inspection under the dissecting microscope. To initiate a culture in this medium one or more egg-bearing females, accompanied by a mixed microbial flora, were inoculated. More recently, *B. variabilis* has been grown well in 13-mm outside diameter, screw-capped test tubes containing the same basal medium except that the chlorellae are better derived from a mineral-glucose medium. *Lecane inermis* flourishes in Petri dishes of 0.1-percent Horlick's malted milk in distilled water. *Philodina acuticornis* grows vigorously in Petri dishes of distilled or pond water containing a ground-up Longlife infusoria table (*8*).

By various techniques the foregoing rotifers have been put into dixenic or monoxenic cultures with certain protists. This has generally been accomplished by first axenizing eggs with hypochlorite followed by antibiotic (penicillin and streptomycin) treatment (*6*) or by antibiotic treatment alone (*6, 9*). More detailed information is being published elsewhere (*4*).

Brachionids so far studied under synxenic conditions (*5, 8–10*) are typically algivorous. Until our culture became contaminated, we grew *B. variabilis* well dixenically for several months with serial subculturing in the presence of *C. pyrenoidosa* plus an unidentified species of gram-negative, rod-shaped bacteria. The basal medium was a 19:1 mixture of Seitz-filtered pond water and concentrated *C. pyrenoidosa* from a mineral-glucose medium. With *E. coli*, instead, as the sole bacterial species, the rotifers died out quickly.

By contrast, sustained monoxenic cultivation of *B. variabilis* has yet to be accomplished. Nathan and Laderman (*9*) have reported limited growth of this species in monoxenic association with *C. pyrenoidosa* in unsupplemented Seitz-filtered pond water; this has also been our finding. However, supplementation with traces of yeast extract and cyanocobalamine has led to flourishing cultures, although conditions permitting successful subculturing from these have not yet been worked out. Bazire (*10*) reported culturing *Epiphanes* [syn. *Hydatina*] *senta* monoxenically or dixenically, with one or two species of algal flagellates.

Our results with *Lecane inermis* and *Philodina acuticornis* indicate that both can live as bacterium-feeders.

Lecane inermis grows well in 0.05-percent Horlick's malted milk (in distilled water and Seitz-filtered pond water, 1:1) in dixenic association with *E. coli* and an unidentified species of gram-negative, rod-shaped bacteria, or with the latter plus a second unidentified species of similar morphology. With either of these unidentified species it also grows well monoxenically. It survives for a while, but does not reproduce in the company of *E. coli* alone. [After our recent report (*5*) of successfully growing *L. inermis* monoxenically with *E. coli*, we found that the second organism had been overlooked earlier because of its initially very slow growth in test media.]

Unlike *L. inermis, P. acuticornis* grows well in the presence of *E. coli* as the sole other living species. The best medium known at present is an empirically developed autoclaved extract of Longlife infusoria tablets.

Our efforts to culture rotifers axenically have so far met with very limited success, despite intensive effort.

The best axenic growth so far observed for *B. variabilis* has been in Seitz-filtered pond water to which were added low levels of whole human blood (about 1 percent), chick embryo ex-