ing after 5-HTP further sustantiates this inhibition of synthesis concept as the mechanism of 5-HTP induced NE depletion.

Summary. The 5-HTP (600 mg/kg, i.p.) reduced rat brain NE to 70% of control levels after 2 hours. The decrease in brain NE after large doses of 5-HTP may be due to inhibition of the biosynthesis of this biogenic amine. In support of this suggestion 5-HTP reduced both incorporation of tyrosine-^{14}C into rat brain NE, and specific activity of the isolated NE. Data are also presented to show that the decrease in brain NE is not due to release such as that produced by reserpine or *d*-amphetamine.

1. Brodie, B. B., Comer, M. S., Costa, E., and Dlabac, A., J. Pharmacol. Exptl. Therap. **152**, 340 (1966).

2. Lovenberg, W., Weissbach, H., and Udenfriend, S., J. Biol. Chem. **237**, 89 (1962).

3. Carmichael, E. A., Feldberg, W., and Fleischhauer, K., J. Physiol., (London) **173**, 354 (1964).

4. Shore, P. A. and Olin, J. S., J. Pharmacol. Exptl. Therap. **122**, 295 (1958).

5. Von Euler, U. S. and Floding, I., Acta Physiol. Scand. **33**, (suppl. 118), 45 (1955).

6. Johnson, G. A. and Boukma, S. J., Anal. Biochem. **18**, 143 (1967).

7. Udenfriend, S., Zaltzman-Nirenberg, P., and Nagatsu, T., Biochem. Pharmacol. **14**, 837 (1965).

8. Anden, N.-E., Corrodi, H., Dahlstrom, A., Fuxe, K., and Hökfelt, T., Life Sci. **5**, 561 (1966).

9. Neff, N. H. and Costa, E., Life Sci. **5**, 951 (1966).

10. Carlsson, A., Lindqvist, M., and Magnusson, T., Nature **180**, 1200 (1957).

11. Rosengren, E., Acta Physiol. Scand. **49**, 364 (1960).

12. Levitt, M., Zheljekov, D., and Udenfriend, S., Biochem. Pharmacol., in press.

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Ascorbic Acid Deficiency in the Squirrel Monkey* (33053)

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Evidence that primates require exogenous ascorbic acid has not been established for all species. The general statement that monkeys require exogenous vitamin C is based in large part on evidence for such a need in the rhesus monkey, *Macaca mulatta* (1). A requirement for exogenous ascorbic acid has also been reported for *Cebus fatuellus* (2), *Macaca cyclopsis swinhoi* (1), and *Cercopithecus aethiops* (3). Elliot *et al.* (4) reported that liver slices of the prosimian primates, *Tupaia glis* and *Nycticebus coucang*, can synthesize ascorbic acid from gulonolactone *in vitro*, and presumably do not require an exogenous source of this vitamin. Chatterjee *et al.* (5), however, have noted that D-glucurono reductase, which converts D-glucuronolactone to L-gulonolactone, is specifically absent in those species which cannot synthesize L-ascorbic acid. If these prosimians can make the conversion of D-glucuronolactone to L-gulonolactone they presumably would not require an exogenous source of this vitamin. The present study was done to determine whether the squirrel monkey, *Saimiri sciureus*, requires an exogenous source of ascorbic acid.

Materials and Methods. Six juvenile Brazilian squirrel monkeys, obtained from Leticia, Colombia were used in this study. The monkeys were fed a diet devoid of ascorbic acid (Table I). After 3 months on the ascorbic acid free regimen, 3 of the animals were supplemented with ascorbic acid at a level of 10 mg/kg of body weight per day. The ascorbic acid was given intramuscularly as an aqueous solution, prepared just prior to administration. The remainder of the animals were continued on the ascorbic acid free regimen for the 4-month period of study. Body weight, packed cell volume, and serum ascor-

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TABLE I. Ascorbic Acid Free Diet.

Item	(%)
Vitamin free casein	15.0
Peanut butter	20.0
Corn oil (with 146,000 IU of vitamin D_3/liter)	5.0
Salts mixture USP IV	2.0
Complete vitamin fortification mixture (except devoid of ascorbic acid)[a]	2.2
Sucrose	20.0
Wheat flour	35.8

[a] Nutritional Biochemicals Corporation, Cleveland, Ohio.

bic acid were examined at monthly intervals. Serum ascorbic acid was determined by titration of the serum with dichlorophenolindophenol (6). After the fourth month all animals were killed and the tissues were examined.

Results. The monkeys remained normal in appearance for 3 months. At this time all of the animals developed large fluctuant swellings over the parietal areas of the head, which proved to be subperiosteal hemorrhage. The average body weight of the monkeys decreased from 659 gm at 2 months to 611 gm at 3 months. Also, the average packed cell volume of the monkeys decreased from 42% at 2 months to 26% at 3 months. The average serum ascorbic acid level decreased steadily from 0.67 mg/100 ml at the beginning of the study to 0.13 mg/100 ml at 3 months. One of the monkeys died after 3 months on this regimen.

Of the remaining 5 animals, 3 were supplemented thereafter with 10 mg/kg of body weight/day with ascorbic acid given intramuscularly. The other 2 monkeys were continued on an ascorbic acid free regimen. During the next month, the supplemented animals greatly improved in outward appearance, became more active, their packed cell volumes and serum ascorbic acid returned to baseline levels, while their body weight decreased from an average of 650 gm at 3 months to 620 gm at 4 months. The nonsupplemented animals became more debilitated, their packed cell volumes decreased to 14%, no ascorbic acid could be measured in their serum, and their average body weight decreased from 535 gm at 3 months to 400 gm at 4 months. At the end of the fourth month all of the animals were killed.

Pathologic changes in the supporting tissues of the body as well as extensive hemorrhages were noted at necropsy in the nonsupplemented animals. One animal had a freely rotatable tibia due to a proximal metaphyseal fracture. The epiphyses and periosteum of the long bones were easily detachable from the shafts of these bones. The bones of the skull were easily separable by gentle traction. Several teeth had been lost and the remaining were loose and easily extractable by gentle traction. The skin of one animal was exceedingly thin and easily torn. Extensive hemorrhages were evident beneath the periosteum of the diaphyses of the long bones, of the parietal bones of the skull, and of the ribs (Fig. 1). One animal had a large endosteal hemorrhage of the frontal bones which displaced the frontal lobes of the brain. One animal had petechial hemorrhages in the skin.

Microscopically, the subperiosteal hemorrhages were only poorly organized. This was true also for the hemorrhage over the skull which had been noted 1 month earlier. At the

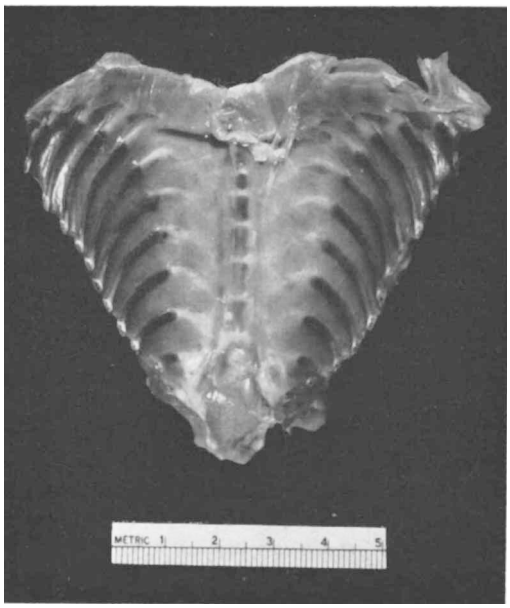

FIG. 1. Subperiosteal hemorrhage in the ribs of a squirrel monkey on an ascorbic acid free regimen for 4 months. 0.85 ×.

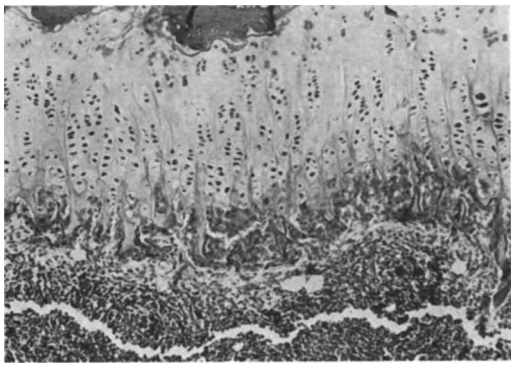

FIG. 2. Photomicrograph of the proximal tibia of a squirrel monkey fed the ascorbic acid free diet for 4 months. Long, delicate cartilagenous spicules extend into loose connective tissue containing spindle-shaped cells. There is a paucity of osteoid tissue. Decalcified H & E, 100 ×.

FIG. 3. A photomicrograph of the proximal tibia of a squirrel monkey on an ascorbic acid free regimen for 3 months, and then supplemented with ascorbic acid, 10 mg/kg of body wt./day, for the last month of the study. Bone appears normal. Decalcified H & E, 100 ×.

epiphyses of the long bones, lack of bone deposition on the cartilagenous spicules was evident. The osteoblasts in the area just beneath the epiphyseal cartilage were spindle shaped and had the appearance of fibrocytes (Fig. 2). The cortices of the bones exhibited rarefaction.

Animals supplemented with ascorbic acid for the last month of study were essentially normal. Resorption of subperiosteal hemorrhages was almost complete, and the bone lesions had healed (Fig. 3).

Discussion. The simultaneous development of large subperiosteal hemorrhages of the parietal bones of the skull in all six monkeys occurred after moving the monkeys to a different cage. These hemorrhages were apparently manifestations of ascorbic acid deficiency and trauma. Marked decreases in packed cell volumes coincided with the development of these massive hemorrhages. Although food consumption was not accurately measured, reduced food intake was observed after other signs of deficiency were manifested. Reduced food consumption was especially evident in those two animals continued on the ascorbic acid free regimen for the fourth month, and was reflected in the marked loss of body weight. Inanition undoubtedly was a complicating factor in the histologic changes observed in these animals. Serum ascorbic acid decreased gradually during the period of ascorbic acid deprivation. Titration of small volumes of serum with dichlorophenolindophenol is only a crude estimate of the ascorbic acid level, however, decreases in the serum ascorbic acid over a period of time is of some significance. The induction of a syndrome typical of ascorbic acid deficiency by a regimen devoid of ascorbic acid, and reversal of these pathologic changes by providing the vitamin suggest that squirrel monkeys require an exogenous source of this vitamin.

1. Day, P. L., Vitamins Hormones **2**, 71 (1944).
2. Shaw, J. H., Federation Proc. **8**, 396 (1949).
3. Follis, R. H., Jr., Proc. Soc. Exptl. Biol. Med. **96**, 523 (1957).
4. Elliot, O., Yess, N. J., and Hegsted, D. M., Nature **212**, 739 (1966).
5. Chatterjee, I. B., Kar, N. C., Ghosh, N. C., and Guka, B. C., Nature **192**, 163 (1961).
6. "Laboratory Manual of Pediatric Micro and Ultramicro-Biochemical Techniques." O'Brien, D. and Ibbott, F. A., eds. 3rd ed. pp. 39–41. Harper (Hoeber), New York, 1964.

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