4. Dow, R. P., Coleman, P. H., Meadows, K. E., Work, T., *Am. J. Trop. Med. and Hyg.*, 1964, v13, 462.

5. Chamberlain, R. W., Sudia, W. P., Coleman, P. H., Beadle, L. D., *ibid.*, 1964, v13, 456.

6. Theiler, M., PROC. SOC. EXP. BIOL. AND MED., 1957, v96, 380.

7. Clarke, D. H., Casals, J., *Am. J. Trop. Med and Hyg.*, 1958, v7, 561.

Received April 7, 1964. P.S.E.B.M., 1964, v116.

Experimental Production of Colloid Goiter in the Cebus Monkey.* (29422)

S. B. ANDRUS, A. M. ROACH AND L. C. FILLIOS† (Introduced by F. J. Stare)
Department of Nutrition, Harvard School of Public Health, and Department of Pathology, Harvard Medical School, Boston, Mass.

It has been demonstrated in rats that dietary uracil augments the hypercholesteremia and cardiovascular sudanophilia incident to cholesterol feeding. These effects are associated with a distinct hyperplasia of the thyroid(1,2). It was also shown that orotic acid counteracts these uracil effects, especially in females. In an attempt to reproduce these findings in Cebus monkeys, striking thyroid changes were seen, quite different from those in rats.

Materials and methods. Four *Cebus albifrons* monkeys, 2 males and 2 females, were placed on a purified basal diet of the following composition: casein, 15%; corn oil, 15%; sucrose, 65.5%; salt mixture (Hegsted IV, Nutritional Biochemicals Corp.), 4%; and choline, 0.5%. To each kg of this diet were added 10 mg of thiamine, 10 mg of riboflavin, 10 mg of pyridoxine, 49 mg of niacin, 30 mg of calcium pantothenate, 1 mg of folic acid, 0.2 mg of biotin, and 20 drops of oleum percomorph (Mead Johnson & Co.). Ascorbic acid (50 mg) was added daily to each monkey's ration.

Cholesterol (0.5%), uracil (0.5% or 0.75%), and orotic acid (0.5% or 1%) were added to this basal diet as seen in Fig. 1, these ingredients being included at the expense of sucrose. Fig. 1 also illustrates the serum total cholesterol(3) responses, the animals being bled at biweekly intervals.

After 72 weeks of uracil ingestion, the animals were killed and complete autopsies performed. Tissues were fixed in 10% neutral formalin and alcian blue, hematoxylin, and eosin (AB, H & E) were used as a general stain. Special techniques used will be mentioned as well as biochemical determinations performed on blood and liver. Control thyroid data to be used for comparison were derived from 27 additional Cebus monkeys, of both sexes, which were comparable to the present animals in age and duration of cholesterol feeding. In addition 7 of these had orotic acid feeding trials identical to those above.

Results. Addition of cholesterol to the basal diet resulted in a moderate increase in serum cholesterol levels, which was augmented by addition of dietary uracil (Fig. 1). No differences in this latter response could be seen between the two levels of uracil used. Elevations in total lipids(4) and beta lipoproteins(5) were observed, as expected (6). In regard to other serum components, the serum urea level(7,8) was elevated in all 4 monkeys; the highest level was 78 mg/100 ml for monkey No. 3; the lowest was 47 mg/100 ml for monkey No. 2; control values ranged from 33 to 41 mg/100 ml. Similar observations have been noted in rats(2).

* Supported in part by grants-in-aid from Nat. Inst. of Health (HE-04208) and (H-7013); John A. Hartford Memorial Fund; Nutrition Foundation, New York; and Fund for Research and Teaching, Dept. of Nutrition, Harvard School of Public Health, Boston.

† Established Investigator of the American Heart Association. Present address: Dept. of Nutrition & Food Science, Massachusetts Inst. of Technology, Cambridge, Mass.

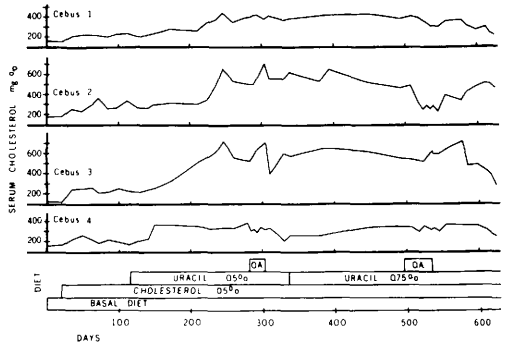

FIG. 1. Serum total cholesterol responses to dietary supplements of cholesterol, uracil, and orotic acid (O.A.).

There were no remarkable changes in serum uric acid(9), serum total protein(10), albumin(11), or hemoglobin. Total lipids(12) in the livers of these monkeys were slightly elevated and the liver total cholesterol(12) was approximately twice the normal level. Such changes were consistent with previous experiences(13). Dietary orotic acid had a clear-cut effect on only one animal, No. 2, whose serum cholesterol level was depressed during the 5 weeks' trial at the 1% level.

The most pertinent findings at autopsy consisted of marked enlargement of the thyroid glands (Fig. 2). These were symmetrically enlarged and on section appeared firm, translucent, and light tan in color. The weights varied from 0.897 to 4.020 g, this range representing a 3- to 20-fold increase above the normal (Table I). Histologically, the increase in thyroid size was associated with two quite different-appearing processes. The two largest glands, those of monkeys No. 1 and 2, showed excessive colloid storage (Fig. 3). The follicles were markedly enlarged; the largest approached 1000 μ in greatest diameter. Control Cebus thyroids show a maximum follicle diameter in the range of 350 μ (Fig. 4) and the mean follicle diameter is far smaller (unpublished data). The epithelium was flat, and intracytoplasmic droplets were not apparent. The colloid,

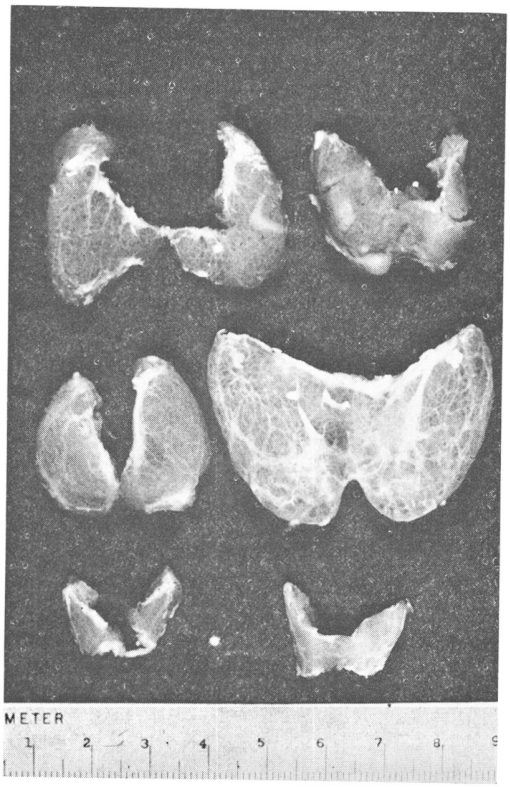

Fig. 2. Thyroid glands from 4 experimental Cebus monkeys (top) and 2 controls (bottom). Experimental animals had been maintained on cholesterol (86 weeks) and uracil (72 weeks) and had 2 brief feeding trials with orotic acid (3 and 5 weeks). The enlarged glands are translucent, tan in color, and show gross follicles. Scale in cm.

TABLE I. Thyroid Changes.

Monkey, No. and sex	Thyroid wt, g	Body wt, kg	g of thyroid* per kg of body wt	Colloid goiter	Hyperplastic goiter
Monkey 1 ♀	4.020	1.42	2.83	+	0
" 2 ♀	1.886	1.83	1.03	+	0
" 3 ♂	1.508	1.09	1.38	+	Focal
" 4 ♂	.897	1.51	.59	Focal	+

* The mean relative thyroid weight of 7 Cebus monkeys receiving cholesterol and orotic acid as above was .13 $\pm$(.03) g of thyroid per kg of body wt. Comparable figures for 20 Cebus receiving cholesterol alone were .14 $\pm$(.01) g of thyroid per kg of body wt (unpublished data—figures in parentheses represent SEM).

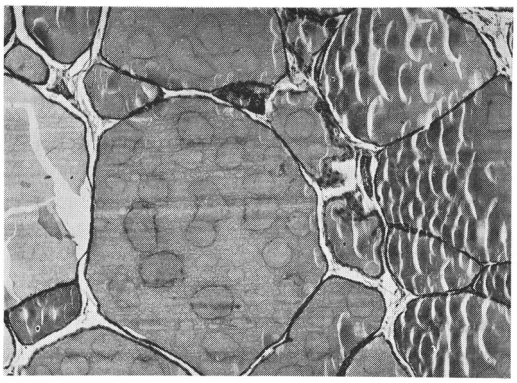

FIG. 3. Excessive colloid storage seen in Cebus monkey No. 1, diet as in Fig. 2. Follicles are markedly distended by intensely stained colloid and epithelium is inconspicuous. Compare with Fig. 4. AB, H & F; 40 ×.

which was more extensively reticulated than that of the controls, was inconstantly stained by alcian blue and intensely stained by the periodic acid Schiff procedure. Fibrosis, hemorrhage, and inflammation were not seen. The thyroid of Monkey No. 3 for the most part showed a similar process, but in addition contained at least one focus of hyperplasia, consisting of much smaller follicles often devoid of colloid, a more prominent epithelial cell type, and occasional papilla formation (Fig. 5). In the fourth thyroid, the smallest of the series (from monkey No. 4) there was a rim of thyroid tissue showing excessive colloid storage as above, but the great bulk of the parenchyma was composed

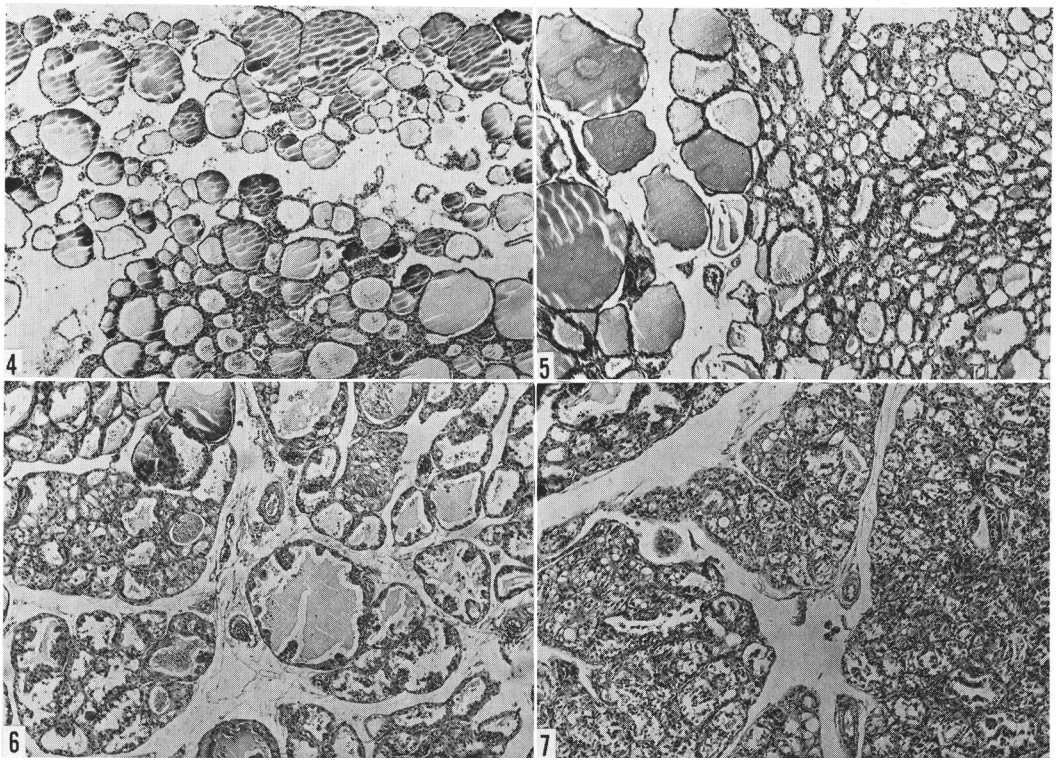

FIG. 4. Thyroid gland from control Cebus monkey showing variation in histologic appearance of peripheral follicles (top) and centrally placed follicles (bottom). Compare with Fig. 3. AB, H & E; 40 ×.

FIG. 5. Thyroid gland from Cebus monkey No. 3, diet as in Fig. 2. The excessive colloid storage (left) contrasts sharply with the focus of colloid-poor hyperplastic follicles (right). AB, H & E; 40 ×.

FIG. 6. Thyroid gland from Cebus monkey No. 4, diet as in Fig. There is marked epithelial hyperplasia, occasionally involving large follicles containing colloid. Papillary infolding is prominent. AB, H & E; 40 ×.

FIG. 7. Same specimen as in Fig. 6. Marked epithelial hyperplasia all but obscures the follicular pattern of the thyroid gland. AB, H & E; 40 ×.

of remarkably hyperplastic tissue (Fig. 6). Loss of colloid, increased cell height, epithelial papillae, and increased vascularity were prominent. The enlarged epithelial cells were often coarsely vacuolated. In many foci the follicles were much reduced in size and occasionally the glandular pattern was all but lost (Fig. 7). There was variation in both cell and nuclear size. Mitoses were not apparent and vascular invasion was not seen.

On the basis of both the weights and histologic appearances of the gonads, only monkeys Nos. 2 and 4 were sexually mature. Differential stains of the hypophyses (periodic acid Schiff-orange G and aldehyde fuchsin-trichrome, following formol sublimate mordanting) revealed only minor variations in distribution of the various cell types. Monkey No. 3 showed moderate amounts of liver cell fat and decreased adrenal cortical lipid (frozen sections stained with Sudan IV). The remaining histologic findings were not of note. Gross sudanophilic lesions in aortas and larger arteries were similar to those seen in Cebus monkeys receiving dietary cholesterol without uracil or orotic acid (unpublished data).

Discussion. The chronic feeding of uracil and cholesterol in Cebus monkeys produced marked increases in thyroid size, this result being due primarily to massive colloid accumulation. These changes have not been seen in Cebus monkeys fed cholesterol alone or cholesterol and orotic acid (unpublished data). Uracil feeding alone has not been tested in monkeys. These results are in sharp contrast to those in the rat where uracil and cholesterol resulted in only modest increases in thyroid size, the process being that of pure epithelial hyperplasia with increased vascularity and loss of colloid(1,2). Differences in the serum cholesterol responses of the two species were also apparent. Thus the potentiating effect of uracil on serum cholesterol elevation was less marked in the monkey than in the rat(2); and the sex differential in this response, present in the latter species, was not seen. It is of interest that the effect of orotic acid in counteracting the uracil-induced serum cholesterol changes which are seen in the female rat(2) was seen only in the one sexually mature female monkey, No. 2.

In the monkey the picture was essentially that of diffuse colloid goiter, although many of the associated changes seen in man were absent, *e.g.*, fibrosis, cellular infiltration and hemorrhage, changes probably related to chronicity. The thyroids from the 2 females were the largest of the 4 and showed this picture of colloid goiter in pure form. The males, in contrast, demonstrated mixtures of both excess colloid storage and epithelial hyperplasia with loss of colloid. Monkey No. 3 showed predominantly the former, while in No. 4 the process was very largely that of cellular hyperplasia and here the increase in gland weight was least marked. The degree of hyperplasia in this animal was much more excessive than that seen in rats fed uracil (2). In many areas the degree of anaplasia and the distortion of normal architecture suggested a well differentiated carcinoma.

The pathogenesis of these changes can only be speculative. Are the 2 processes different responses to the same stimulus, or do they represent sequential changes? Follis(14,15) has produced experimental colloid goiter in the hamster by first inducing epithelial hyperplasia by means of iodine deficiency, with or without thiouracil, followed by colloid repletion of the hyperplastic gland under the influence of iodine supplementation and removal of the specific goitrogen.

It is tempting to invoke a similar sequence of events in the present animals, since in at least 2 of them both types of thyroid change were present, though it is obvious that iodine deficiency should not be a factor in these diets (4% salts IV supply 24 mg of iodine per kg of diet). If we postulate an initial response of thyroid hyperplasia, such as is seen in rats fed uracil and cholesterol, we are at a loss to know what factor(s) might have converted this reaction into one of involution or colloid storage, the major response of the thyroids at termination of this experiment. The possibility of orotic acid effecting the latter change exists, though in view of the relatively brief duration of this dietary supplementation such a possibility does not appear likely. Orotic acid feeding in rats re-

ceiving uracil and cholesterol reduces the degree of cellular hyperplasia(2) but has not been observed to induce excessive colloid storage even when the dietary protocol of the present experiment was duplicated (unpublished data). Repetition of the present experiment with isolation of the various dietary factors and serial sacrifice is indicated.

Summary. Four Cebus monkeys, 2 males and 2 females, were maintained on a casein-corn oil diet containing 0.5% cholesterol for 86 weeks. For the greater part of this time the diet contained uracil (0.5-0.75%) and during 2 short feeding trials of 3 and 5 weeks duration orotic acid was also included in the diet (0.5%-1%). Uracil produced a further increment in serum cholesterol elevation beyond that due to dietary cholesterol alone as well as an elevation of serum urea. In one female concomitant feeding of orotic acid at the 1% level lowered the serum cholesterol. At autopsy the 2 females demonstrated large colloid goiters while the thyroids of the 2 males showed a mixed picture of both colloid goiter and that of colloid-poor hyperplasia. In one of the males this latter process was extreme and could not be distinguished from early carcinoma. It is assumed that these thyroid changes resulted from ingestion of uracil (or uracil and cholesterol), though the possible role of orotic acid cannot be completely excluded. Speculations on the pathogenesis of the two types of thyroid change are discussed as well as comparisons of the above results with those seen in rats.

The authors wish to thank Mr. Thomas Faherty for the histologic preparations and Mr. Bruce Dorr for technical assistance.

1. Fillios, L. C., Naito, C., Andrus, S. B., Roach, A. M., *Am. J. Clin. Nutr.*, 1959, v7, 70.
2. ———, *Circ. Research*, 1960, v8, 71.
3. Carpenter, K., Gotsis, A., Hegsted, D. M., *Clin. Chem.*, 1957, v3, 233.
4. Entenman, C., in *Methods in Enzymology*, S. P. Colowick and N. O. Kaplan, eds., 1957, Academic Press, New York, p299.
5. Gofman, J. W., Lindgren, F., Elliott, H. A., Mantz, W., Hewitt, J., Strisower, B., Herring, V., *Science*, 1950, v11, 166.
6. Mann, G. V., Andrus, S. B., McNally, A. M., Stare, F. J., *J. Exp. Med.*, 1953, v98, 195.
7. Ormsby, A. A., *J. Biol. Chem.*, 1942, v146, 595.
8. Kawerau, E., *Sci. Proc. Roy. Dublin Soc.*, 1946, v24, 63.
9. Praetorius, E., Paulson, H., *Scand. J. Clin. Lab. Invest.*, 1953, v5, 273.
10. Gornall, A. G., Bardawill, C. J., David, M. M., *J. Biol. Chem.*, 1949, v177, 751.
11. Fister, H. J., in *Manual of Standardized Procedures for Spectrophotometric Chemistry*, 1950, Standard Scientific Supply Corp., New York, pA-10, 1.
12. Fillios, L. C., Mann, G. V., *Metabolism*, 1954, v3, 16.
13. Portman, O. W., Sinisterra, L., *J. Exp. Med.*, 1957, v106, 727.
14. Follis, R. H., Jr., PROC. SOC. EXP. BIOL. AND MED., 1959, v100, 203.
15. ———, *Nature*, 1959, v183, 1817.

Received April 10, 1964. P.S.E.B.M., 1964, v116.

Radiation-Induced Leukemia in Germfree Mice.* (29423)

MORRIS POLLARD AND T. MATSUZAWA
Lobund Laboratory, University of Notre Dame, Notre Dame, Ind.

The leukemogenic effect of whole-body administered X-rays in mice has been documented abundantly(1-5). Optimal conditions for eliciting leukemic disease in such mice have been defined as being related to age and frequency of exposure(6). Certain strains of mice, which have a low incidence of spontaneous leukemia, carry an occult leukemogenic virus which can be activated by exposure to moderate dosages of X-rays(7,8). However, irradiation does not accelerate the appearance of clinical disease in the high-leukemic C58 and AK strains of mice. Fur-

* Supported by funds from Office of Naval Research, U. S. Public Health Service and Tobacco Research Committee.