induced in rats by giving subcutaneous injections of sodium thyroxinate. Control and experimental animals from each group were injected with P^{32} in dilute HCl and specific activities of plasma inorganic phosphate, and skeletal muscle inorganic phosphate, creatine phosphate, and ATP phosphate determined. The S. A. of the plasma inorganic phosphate was unchanged by Vit. E deficiency and was decreased by hyperthyroidism. Skeletal muscle inorganic phosphate S. A. was increased by Vit. E deficiency and the difficulties in interpreting these data were discussed. The S. A. of phosphates of creatine phosphate and ATP phosphate were high in both Vit. E deficient rabbits and hyperthyroid rats. The significance of these findings was discussed in relation to turnover of creatine phosphate and ATP. 2) These experiments indicate that there is an elevated turnover of skeletal muscle intracellular inorganic phosphate and a normal or decreased turnover of skeletal muscle creatine phosphate and ATP in Vit. E deficiency. In contrast there appears to be no depression in turnover of creatine phosphate and ATP in hyperthyroid rats.

1. Dinning, J. S., *Arch. Biochem. Biophys.*, 1956, v60, 501.
2. Wilkins, L., Fleischmann, W., *J. Clin. Invest.*, 1946, v25, 360.
3. Hoch, F. L., Lipmann, F., *Proc. Nat. Acad. Sci.*, U.S.A., 1954, v40, 909.
4. Tapley, D. F., *J. Biol. Chem.*, 1956, v222, 325.
5. Young, J. M., Dinning, J. S., *ibid.*, 1951, v193, 743.
6. Dinning, J. S., *ibid.*, 1955, v212, 735.
7. Sacks, J., *Am. J. Physiol.*, 1940, v129, 227.
8. Threlfall, C. J., *Biochem. J.*, 1957, v65, 694.
9. Venkataraman, P. R., Venkataraman, A., Schylman, M. P., Greenberg, D. M., *J. Biol. Chem.*, 1950, v185, 175.
10. Ennor, A. H., Rosenberg, H., *Biochem. J.*, 1954, v56, 302.
11. Ferdman, D. L., *Proc. Internat. Conf. on Peaceful Uses of Atomic Energy*, 1956, v10, 482.

Received November 10, 1958. P.S.E.B.M., 1959, v100.

Experimental Colloid Goiter in the Hamster.* (24573)

RICHARD H. FOLLIS, JR.
V. A. Central Laboratory for Anatomical Pathology and Research, Armed Forces Inst. of Pathology, Washington, D.C.

Fifty years have passed since David Marine demonstrated the relationship of iodine to goiter and expressed the belief "that goiter must be classed with nutritional disturbances (diseases) and that as such it will take its place in nosology along with chlorosis, rachitis, osteomalacia, etc.,"(1). This statement was based on morphologic and chemical studies of various types of goiter occurring naturally in man and other species, together with effects produced by administration of iodine to dogs with hyperplastic or colloid types of goiter(2,3). Thus, in a very short time Marine and his collaborators were able to lay the foundations for rational treatment and prophylaxis of endemic goiter, which he and Kimball had conclusively demonstrated by 1920(4). In his experimental studies Marine depended on dogs in which endemic goiters had already developed. He formulated the pathogenesis of goiter as follows: iodine deficiency acting on a normal gland leads to hyperplasia, which continues unless iodine is administered; whereupon colloid accumulates in the thyroid, a state which represents "the nearest approach to normal glands that active hyperplasias can become"(2). Colloid goiter, with varying degrees of nodular hyperplasia and scarring, is the form which the endemic disease takes in man. This morphologic picture has, to our knowledge, never been produced in laboratory animals whose thyroid glands were normal initially. To be sure, thyroid enlargement has been observed: 1) in puppies born to females whose thyroids had been extirpated before

* Supported by a grant-in-aid (H-2999) from USPHS.

pregnancy(5), 2) following ingestion of cabbage in rabbits(6), 3) after feeding iodine-deficient diets to rats(7), 4) as a result of administration of thiourea and other compounds to several species(8) and 5) following TSH administration(9). All such goiters are characterized by varying degrees of hyperplasia without the diffuse colloid change which is found in naturally-occurring disease. Colloid cysts have been described in rats treated with thiouracil for prolonged periods(10). The pathogenesis of colloid goiter as formulated by Marine has needed laboratory confirmation for some time. Undoubtedly this gap in our knowledge has contributed to the confusion which has surrounded the subject of endemic goiter in the human for so many years. One of our programs has been to attempt to reproduce certain human deficiency disease syndromes in experimental animals (rats, rabbits, guinea pigs, hamsters and monkeys). During our studies, utilizing diets composed predominately of corn, goiter has been observed(11). Among species thus far studied the hamster appears to be the most suitable experimental subject. This communication records the successful production of colloid goiter in this animal.

Materials and methods. Hamsters weighing 75 to 140 g at beginning of experiment have been placed on 2 diets: (1) ground whole unenriched corn and (2) a modified Remington type mixture(7) consisting of ground corn, 76; wheat gluten, 20; sodium chloride, 2 and calcium carbonate 2; 500 mg of a complete vitamin mixture were added to each kilo of diet No. 2. These diets contain 1.0 and 2.5 μg iodine/100 g, respectively.* For controls, potassium iodide was added to each diet in concentration of 10 mg/kilo. This concentration in the diet was used for repletion studies as well. Food and tap water were allowed *ad lib.* Animals were kept in screen bottom cages in a well-ventilated room at constant temperature (78°F). Male and female animals were maintained on these diets for varying periods to 350 days. The thyroid glands of 54 deficient and 12 control animals have now been studied. Thyroid glands and other tissues were fixed in neutral 4% formalin, dehydrated in alcohols, imbedded in paraffin, cut and stained with hematoxylin-eosin or the periodic acid-Schiff technic.

Results. Response of thyroid gland to the 2 diets is similar save that alterations appear sooner and are more extensive on diet No. 2, since growth of animals is more satisfactory. The normal thyroid gland of the hamster weighs about 7 mg and is composed of follicles having an average diameter of approximately 45 μ. The cells lining the follicles are low cuboidal; eosinophilic colloid is abundant. Blood vessels are not prominent. Accumulations of fatty tissue are found within the gland (Fig. 2).

After 1 to 2 weeks on the deficient diet no thyroid enlargement is found, though grossly both lobes are more prominent because of increased vascularity. By the end of 2nd week, microscopic examination reveals that colloid has disappeared from the follicles, which are lined by cuboidal epithelium; dilated blood vessels separate the follicles. As time goes on, such vascularity becomes even more prominent; sometimes follicles appear to be virtually floating in a pool of blood. So, too, the epithelial cells increase in number and become more columnar, projecting into the lumens of the colloid-depleted follicles. By 125 days the enlarged glands consist of hyperplastic follicles surrounded by prominent vascular channels. The epithelium projects into the follicle as papillary tufts. In places, formation of intrafollicular follicles (12) is seen. At this stage a few follicles may contain eosinophilic colloid. From then on to 325 days, hyperplasia becomes extensive with more infolding of the epithelium. In addition more follicles contain colloid; these are lined by less columnar epithelium. In none of the glands do more than about 10% of the follicles contain colloid.

The reappearance of colloid in follicles with time led us to attempt to accelerate this reaction by administration of iodine. When animals which have been on diets for 125 days are given iodine, the follicles fill up with colloid; the epithelium naturally becomes cuboidal or flattened and the gland grossly and

* Analysis performed by Dr. J. V. Princiotto, Chemo-medico Consultants, Arlington, Va.

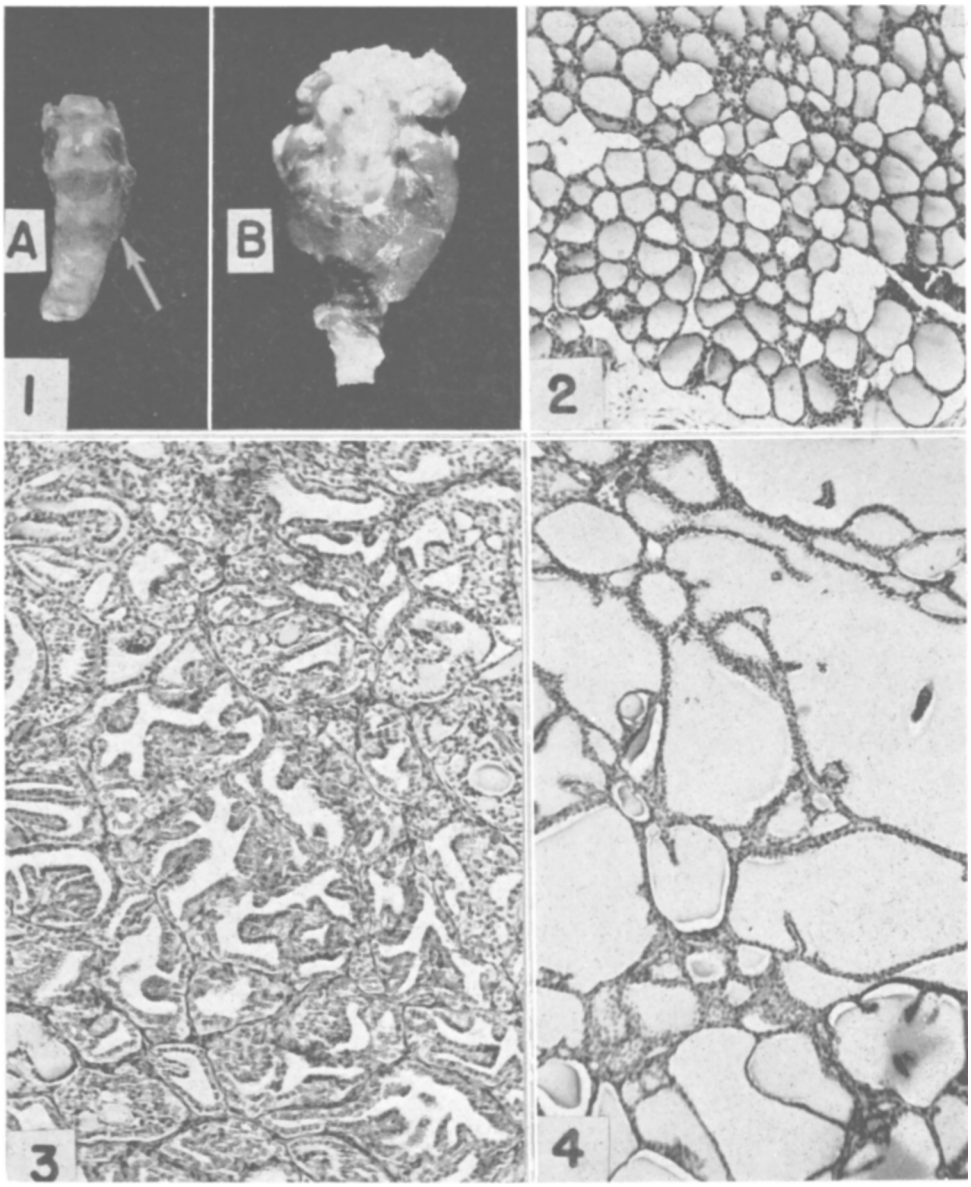

FIG. 1. A. Larynx and trachea of normal hamster. Thyroid lobes are represented by small structures (arrow) beneath laryngeal muscles. B. Colloid goiter developing after 325 days on iodine-deficient diet followed by 2 wk of iodine administration.

FIG. 2. Microscopic section of normal thyroid gland (×100).

FIG. 3. Hyperplastic gland after 325 days on iodine-deficient diet (×100).

FIG. 4. Colloid goiter developing in gland after 2 wk of iodine administration to gland similar to that seen in Fig. 3 (×100).

microscopically is less vascular. After 2 weeks of treatment remnants of papillary projections, some still lined by cuboidal epithelium, are found, but mainly the gland is composed of follicles, some as large as 150 μ in diameter, *e.g.*, 3 times normal. Small normal-sized follicles lined by flattened epithelium are found between these. If animals which have been on the deficient diet for over 300 days (Fig. 3) are repleted in similar fashion even larger colloid lined follicles are found (Fig. 4), some of which are 400 μ in diameter. Such

follicles still exhibit sprig-like projections into their lumens.

As might be expected, pituitary glands of the animals having hyperplastic glands are enlarged and contain thyrotrophic basophilic cells.

Discussion. Marine's fundamental contributions to our understanding of the pathogenesis of colloid goiter have all too briefly been mentioned above(1,2,3). The experimental observations described herein would seem to place Marine's conclusions, which were based on studies of naturally occurring goiters in man and animals, on a firm basis, since changes in thyroid structure from the normal to the hyperplastic gland and thence to the colloid form of goiter following administration of iodine can be followed, in the hamster at least, with ease. Now that colloid goiter can be produced in a small laboratory animal, a number of questions may be asked and in time perhaps can be answered. First and foremost is how permanent is the change since it is well-known that a colloid goiter in man does not ordinarily regress appreciably? Moreover, may the experimental colloid filled gland be made to regress under appropriate treatment? Is the colloid of the colloid goiter different chemically or physically from the normal? Is there a "point of no return" at some stage during the development of the change, *i.e.,* in early stages, at which the gland can no longer revert to its normal structure following iodine administration? May alternating cycles of deficiency and repletion lead to nodular changes such as are seen in human material? These and other questions are currently under investigation in this laboratory. Much of the confusion associated with our understanding of the pathogenesis of endemic goiter has undoubtedly been due to a failure to produce and study the disease in the laboratory(13). It is rather astounding that such has been the case, particularly when it is estimated(14) that the total number of cases of goiter in the world today "is probably not far short of 200 million"! It is hoped that these studies in the hamster may stimulate investigations in this and other species so that many of the unsolved problems can be elucidated.

Summary. Extreme hyperplasia of the thyroid gland has been produced in hamsters by placing them on iodine-deficient diets. When iodine is administered large amounts of colloid accumulate in the thyroid follicles, giving rise to the morphological picture of diffuse colloid goiter. Marine's concept of the pathogenesis of colloid goiter: normal gland ⟶ hyperplasia ⟶ (iodine treatment) ⟶ colloid goiter, appears to have been proved under laboratory conditions, in the hamster at least.

1. Marine, D., Williams, W. W., *Arch. Int. Med.,* 1908, v1, 349.

2. Marine, D., Lenhart, C. H., *Bull. Johns Hopkins Hosp.,* 1909, v20, 131.

3. ———, *Arch. Int. Med.,* 1909, v4, 440.

4. Marine, D., Kimball, O. P., *ibid.,* 1920, v25, 661.

5. Halsted, W. S., *Johns Hopkins Hosp. Rep.,* 1896, v1, 372.

6. Chesney, A. M., Clawson, T. A., Webster, B., *Bull. Johns Hopkins Hosp.,* 1928, v43, 261.

7. Levine, H., Remington, R. E., von Kolnitz, H., *J. Nutrition,* 1933, v6, 325.

8. Mackenzie, C. G., Mackenzie, J. B., *Endocrinology,* 1943, v32, 185.

9. Loeb, L., Bassett, R. B., PROC. SOC. EXP. BIOL. AND MED., 1929, v26, 860.

10. Money, W. L., Fitzgerald, P. J., Goodwin, J. T., Rawson, R. W., *Cancer,* 1953, v6, 111.

11. Follis, R. H., Jr., *Am. J. Clin. Nutrition,* 1958, v6, 459.

12. Kroon, D. B., *Acta endocrinol.,* 1951, v8, 343.

13. Follis, R. H., Jr., *Deficiency Disease,* Charles C. Thomas, Springfield, 1958.

14. Kelly, F. C., Snedden, W. W., *Bull. Wld. Hlth. Org.,* 1958, v18, 5.

Received November 18, 1958. P.S.E.B.M., 1959, v100.