hydroxyl group at position 3 of the sterol during oxidation of cholesterol to bile acid. Mukherjee *et al.*(8) reported on the occurrence of an enzyme sytem in whole rat liver homogenate which esterified cholesterol only in the presence of ATP, CoA, and palmitate or palmityl CoA. This system differs in certain respects from that reported here and in the previous study(1). Activity was obtained in the absence of those cofactors and esterification was markedly stimulated by the presence of DPN. In the light of present information concerning the mechanism of formation of ester bonds in the synthesis of phosphatides and triglycerides, the effect of DPN in the present sytem is not clear. Further studies are underway more fully to characterize the microsomal cholesterol esterifying system.

Summary. Rat liver homogenates were fractionated into mitochondria, microsomes and soluble fraction. Cholesterol-4-C^{14} was found to be esterified in sytems containing whole homogenate, mitochondria plus soluble fraction, microsomes plus soluble fraction and soluble fraction alone. Virtually no esterifying activity was obtained in the presence of heated soluble fraction. A stimulatory effect of DPN on sterol ester formation was noted. The findings suggest that different parts of the liver cell possess the capability of forming sterol esters.

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Iodinated Protein Patterns in Thyroids from Normal and Iodine Deficient Hamsters. (27421)

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When hamsters have subsisted on iodine-deficient diets for some months, many of the hyperplastic follicles of the thyroid gland, after fixation and staining for microscopic examination, are found to contain a poorly coagulable material. This contrasts with the dense "colloid" observed in the follicles of normal animals or of hamsters which have chronic colloid goiters(1). It seemed of interest to characterize the protein patterns of glands from normal and iodine deficient animals to determine differences, if any, which might explain the histological appearances. This communication describes certain dissimilarities which have been observed.

Materials and methods. Adult male hamsters were fed either a stock diet or an iodine-deficient ration previously described(2). After varying periods on the latter diet (Table II) these animals, as well as normal controls, were sacrificed, each having received intraperitoneally 24 hours before 20-25 μc I^{131} without carrier. The thyroids of all animals were perfused with .9% sodium chloride through the ascending aorta after clamping the descending portion. Glands were dissected under low magnification, cleaned of fat and muscle and weighed. Since the entire gland of a normal animal weighs only 4 mg, two to three were pooled. The glands from iodine deficient animals ranged from 7 to 73 mg, depending on the duration of the deficiency. Small portions of the iodine deficient glands were taken for microscopic ex-

amination. Tissue was homogenized in small amounts of .9% sodium chloride with a glass tube-Teflon pestle homogenizer. The homogenate was centrifuged at 20,000 rpm; the supernate was used for electrophoretic studies.

Appropriate amounts of the supernate were electrophoresed on polyacrylamide gel in 5 mm tubes using TRIS-glycine buffer, pH 8.4 (3). Gel cylinders were removed from the tubes, fixed and stained with amido black in acetic acid and destained in an electric field. Gels were sucked into glass tubes of 6 mm diameter, photographed, and slowly extruded so that the stained bands could be cut out with a razor blade. Radioactivity of each of the stained and unstained portions of the entire cylinder was determined in a well scintillation detector. Other gel cylinders were placed on heavy filter paper (Whatman 3 mm), dried for 24 hours in an incubator, and placed in contact with x-ray film for varying periods of time. After developing the film, active areas of the gel were identified, cut out, and counted in a well crystal detector.

The supernate was also subjected to electrophoresis on cellulose acetate paper with barbital buffer, pH 8.6(4).

Results. (a) Normal group (Fig. 1; A, B): In the stained acrylamide gel 2 well-defined bands can be demonstrated, a slow and a more rapidly moving component. These have been designated zones 2 and 4. The latter has the approximate mobility of serum albumin. The radioiodine activity of the slow band is always appreciably higher than that of the faster moving material. Zone 2 undoubtedly represents thyroglobulin. In Table I the quantitative values for the entire gel cylinder are presented. It will be noted that the radioactivity of zone 2, the slow component, averages 37.5 times that of the faster band, zone 4. Appreciable radioactivity is found in the first zone, which, however, can-

TABLE I. Normal Hamsters. Percentage activity acrylamide gel zones after fixation and staining.*

No.	1	2	3	4	5	2/4†
5	10.5	80.8	3.5	4.8	.4	13.4
12	17.1	76.5	3.7	2.2	.5	34.4
14	13.7	80.7	2.9	2.7	—	28.9
15	28.6	62.2	5.2	4.0	—	14.3
16	19.0	77.1	1.7	1.6	.6	48.5
17	24.8	72.0	1.9	1.0	.3	64.8
20	15.6	79.4	2.9	1.5	.6	54.2
21	12.7	82.8	2.8	1.8	.9	42.2
					Mean	37.6

* See Fig. 1 and text for explanation.
† Ratio of zone 2 to zone 4.

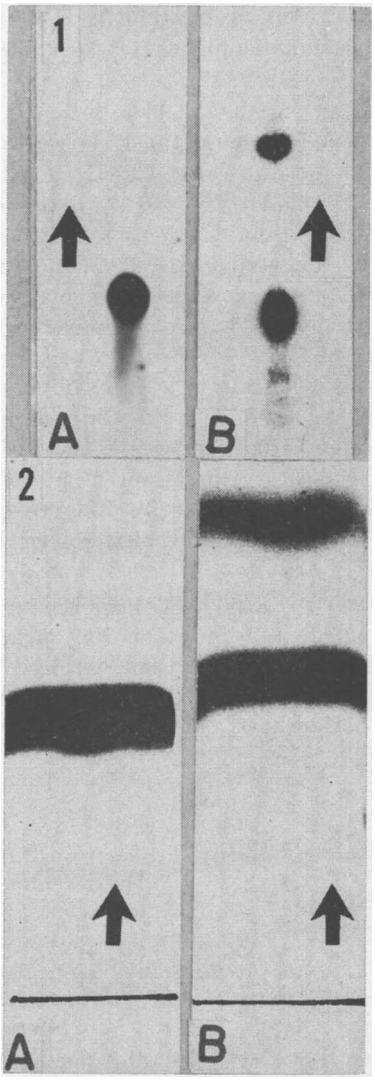

FIG. 1. *A.* Electrophoretic pattern in acrylamide gel of supernate of thyroid homogenate from 3 normal hamsters which had received I^{131} 24 hr before. Note localized area of radioactivity moving relatively slowly. This corresponds to zone 2 in Table I. *B.* Similar pattern from hamster on iodine-deficient diet. Note intense activity of faster moving zone (which corresponds to zone 4 in Table II).

FIG. 2. *A* and *B.* Comparison of cellulose acetate paper electrophoretic pattern from normal *(A)* and iodine-deficient *(B).*

TABLE II. Iodine Deficient Hamsters. Percentage activity acrylamide gel zones after fixation and staining.*

No.	Days on diet	1	2	3	4	5	2/4†
18	82	28.3	62.9	3.6	4.4	.8	14.3
19	82	18.3	72.2	3.1	5.5	.9	12.8
24	87	17.4	64.6	3.4	6.5	8.1	9.9
25	87	8.5	84.9	2.2	2.4	2.0	35.5
22	167	20.8	50.4	5.9	21.4	1.5	2.36
23	167	20.0	62.1	5.0	11.4	1.5	5.5
30	174	7.8	50.4	7.7	33.0	1.1	1.5
31	174	13.8	57.9	15.1	9.8	3.4	5.8
3	208	6.1	71.4	2.3	20.2	—	3.4
6	221	6.7	57.6	6.9	25.1	3.6	2.05
9	228	3.1	29.7	4.2	62.3	.7	.43
11	229	4.2	21.1	4.7	69.7	.3	.28
13	230	4.5	50.1	2.1	41.6	.7	1.16
32	256	14.8	29.8	11.7	42.2	1.5	.71
33	256	7.4	49.2	3.3	40.0	1	1.2

* See Fig. 1 and text for explanation.
† Ratio of zone 2 to zone 4.

not be localized as a discrete band by staining. Autoradiographs of the dried gel confirm the distribution of the activity, which may be further verified by counting these segments. Electrophoresis on cellulose acetate strips reveals only a single band which appears to contain all of the radioiodine activity when autoradiographed (Fig. 2, A).

(b). Iodine-deficient group (Fig. 1; C, D): The pattern in acrylamide gel is similar to the normal with respect to the presence of two principal bands, yet the degree of radioiodine concentration is far different. In Table II are shown the proportions of slow to fast moving fractions. As the period of iodine deficiency increases, the amount of activity of the fast component may fall to below unity in those animals which have been on the iodine-deficient ration for prolonged periods. In cellulose acetate strips a prominent zone is found which moves at a more rapid rate than that of the zone which conforms to the normal, which presumably is thyroglobulin (Fig. 2, B).

Discussion. Studies of soluble iodine containing proteins derived from normal and diseased thyroid glands of humans and other species have revealed components other than classical thyroglobulin(5). The most consistently appearing soluble fraction has been designated S-1. This has a lower molecular weight as determined by the ultracentrifuge, is precipitated by 1.92 to 2.98 M PO_4 buffer, and with paper electrophoresis in barbital buffer pH 8.6 migrates ahead of thyroglobulin. Normal human thyroids contain an average of 3.5% of their total radioiodine content in the form of S-1 protein(6). Sheep thyroids contain 1.7%(6), while the glands of rats contain only negligible amounts(7). A human follicular adenoma contained as much as 19%(6). Similar material has been found in a congenital goiter(8). One of the complicating features in certain studies is the resemblance of S-1 protein to serum albumin, even immunologically(8). The influence of blood constituents in nonperfused glands must of course be considered. It is of interest that electrophoretic studies of certain human thyroid tumors indicated that the eluate from a "parenchymatous microfollicular and trabecular adenoma" contained only albumin(9); this may well have been S-1 protein. Soluble proteins other than thyroglobulin and S-1 protein have not yet been well characterized.

The data presented herein indicate that when hamsters are subjected to increasing periods of iodine deficiency the iodinated protein patterns of their glands are so altered that the electrophoretically faster moving iodinated component, which may be similar to the S-1 protein described in other species, increases to exceed thyroglobulin.

Obviously much remains to be done to characterize more precisely by chemical and immunological studies the nature of the 2 components which have been observed in the thyroid glands of these iodine deficient hamsters. In addition, studies of the patterns which may accompany the transformation of hyperplastic to colloid goiters may aid in understanding the process under investigation, particularly when these are correlated with the anatomical alterations.

Summary. The patterns of soluble iodinated proteins from the thyroid glands of iodine deficient hamsters differ from the normal in that a component other than thyroglobulin is found in increasing amounts as the deficient state develops.

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The Persistence of Isotopically Labeled Cells in Corneal Grafts.* (27422)

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It is essential to learn the fate of cellular constituents of homograft tissue if we are to decide whether donor cells can become incorporated in the host without an immunological reaction to them. It is possible that grafts of some tissues serve simply as an inert scaffold which is invaded by host cells which repopulate the structure after the death of the donor cells. Current interpretation of the rejection phenomena in skin and other organ grafts implies that donor cells remain. The frequent persistence of clear corneal homotransplants is an important variant from the usual experience with grafts of other tissues. Corneal grafts, because they consist largely of regularly arranged collagen with relatively few connective tissue cells, could well act as a scaffold which, following death of the donor cells, would be populated by host corneal corpuscles invading it. Certainly the covering epithelium of such grafts is regularly replaced by host epithelium. The endothelium covering the posterior surface is also known to regenerate rapidly and could behave as does the epithelium. However, ordinary histological study of such grafts have not been able to solve the problem of whether there is a replacement of the endothelial or the stromal cells because of the impossibility of distinguishing those of the host from those of the donor.

Basu, Miller and Ormsby(1) investigated the fate of corneal stroma cells by utilizing the sex chromatin as the identifying characteristic of the donor cells, and reported that cells in transplants to hosts of the opposite sex persisted by at least 3½ months. Using the same technic Espiritu *et al.*(2), reported that grafted endothelial cells survived for 4 months and then were replaced. Another possible means of investigating this problem depends on distinguishing host from donor cells by long lived isotopically labeled constituents incorporated in stable compounds in the cell structure. Tritiated thymidine is incorporated in DNA at the time of its synthesis and presumably undergoes no degradation or turnover. In cells with a low mitotic rate such a label would be extremely stable and not diluted by cell division. Mitosis is not found in the normal adult corneal endothelial or connective tissue cells so they form an ideal material for such a study. DNA synthesis occurs both during development and regeneration of these cells. Experiments were carried out in which tritiated thymidine was incorporated in the corneal endothelium and keratoblasts in adult rabbit corneas during regeneration of these cells after those in the central portion of the cornea had been killed by freezing. Labeled thymidine injected into the anterior chamber during regeneration, following mechanical injury in the endothelium

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