for oxidant consumption are expected, since the galactopyranose ring contains a susceptible *cis*-hydroxyl pair. Clancy & Whelan (1959) noted that the methyl galactopyranosides were attacked somewhat faster than the methyl glucopyranosides. The consumption of 5 mol.prop. of periodate, with little or no oxidation of the furanose ring, is consistent with the postulated structure (II) for the galactobi-itol.

The difference between the molecular rotation of methyl β-galactopyranoside (0°) and methyl β-galactofuranoside (−21 700°) (calculated from the data of Augestad & Berner, 1954) is very close to that between the molecular rotation of 6-*O*-β-D-galactopyranosyl-D-galactose (+11 600°, calculated from data of Elsner, 1935) and the galactobiose from *M. mycoides* (−9600°). We conclude that the latter is 6-*O*-β-D-galactofuranosyl-D-galactose (I).

SUMMARY

1. A galactobiose was isolated from a partial acid hydrolysate of *Mycoplasma mycoides* galactan, and degraded via the corresponding galactobi-itol.

2. The results are consistent with the structure: 6-*O*-β-D-galactofuranosyl-D-galactose.

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Localization of Protein-Bound Radioactive Iodine in Rat Thyroid Glands Labelled with ^{125}I or ^{131}I

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The site of iodination of thyroglobulin has been the subject of a number of radioautographic studies in the past 15 years. Leblond & Gross (1948) found that, in the thyroids of rats on a moderately low-iodine diet, most of the organic ^{131}I was localized in the colloid, even when the animals were killed 2 min. after injection of ^{131}I. Rats whose diet was supplemented with 22 mg. of iodine/day had less active glands, and the presence of organic ^{131}I was demonstrated in the thyroid epithelium of one animal, 1 hr. after injection of the dose. In hypophysectomized rats, the thyroidal radioiodine uptake was low, and organic ^{131}I remained in the epithelial cells up to 24 hr. after injection of the dose.

Doniach & Pelc (1949) also found that organic ^{131}I was detectable only in the follicular lumen of the thyroids of normal rats, within 10 min. of injection of the dose, and that prolonged pre-treatment with thyroxine (to suppress secretion of thyrotrophin; Yamada, Iino & Greer, 1961) restricted organic ^{131}I to the epithelial cells. Nadler & Leblond (1955) confirmed the rapid appearance of organic ^{131}I in the colloid of normal rats; Wollman & Wodinsky (1955) were unable to demonstrate radioactivity in mouse thyroid cells even within a few seconds of injection of ^{131}I.

Mayer, Dimick & Kelly (1956) studied the incorporation of ^{131}I by fresh bovine thyroid slices and by slices from glands that had been stored at −16° for 2 weeks; they found that, whereas the the fresh slices formed organic ^{131}I in the colloid,

the stored tissue formed only cellular organic ^{131}I. This group (Mayer, Kelly & Morton, 1956) also showed that the frozen tissue was unable to effect thyroid hormone biosynthesis in thyroglobulin beyond the stage of monoiodotyrosine.

For these reasons it has been suggested that, in the normal thyroid gland, iodination of thyroglobulin occurs within the follicular lumen. Nevertheless, other evidence indicates that thyroid cells do incorporate radioiodine into organic combination. Levenson (1960) labelled rat thyroids with ^{131}I *in vivo* for 1 hr. and removed the colloid from frozen sections of the glands by treatment with ethanol and water; the remaining rings of epithelial cells were then exposed to radioautographic emulsion for a longer time than would be possible with entire sections without getting fogging of the picture. Levenson was thus able to show that cellular organic ^{131}I was present in normal rat thyroids. Schmidt, Henning & Witte (1963), using radioautographic techniques, have demonstrated the presence of intracellular organic ^{131}I in needle-biopsy material from human thyroids in various functional states, at different times after the injection of ^{131}I. The radioautographs of rat thyroid sections labelled with ^{131}I for 15 min. *in vivo*, obtained by Nandi, Poddar & Pyne (1956), also indicate some cellular organic radioiodine. Kayes, Maunsbach & Ullberg (1962), in electron-microscopic radioautography of ^{125}I in rat thyroid, calculated that in the gland labelled for 1 hr. *in vivo* the ratio of silver grains over the colloid to those over comparable areas of cells was 25:1.

Pastan (1961) and Tong, Kerkof & Chaikoff (1962) have shown that isolated thyroid cells obtained by trypsinization of thyroid tissues can form ^{131}I-labelled iodotyrosines and indeed iodothyronines (Tong *et al.* 1962). Tissue cultures of thyroid cells have also been shown to form ^{131}I-labelled iodotyrosines and, in one instance, iodothyronines (Pulvertaft, Davies, Weiss & Wilkinson, 1959).

It is possible therefore that, in the normal thyroid gland, iodination of thyroglobulin may occur in the cell, but the iodinated protein is secreted so rapidly into the lumen of the follicle that it is difficult to detect in the epithelium except in glands whose activity has been damped down by treatment with iodide.

The purpose of the present work was to resolve, if possible, the contradictory findings discussed above, and to ascertain whether organic radioiodine could be located in cells of the thyroid follicle at short times after the injection of radioiodide. The isotope ^{125}I was used as well as ^{131}I because of the higher resolution that can be obtained with ^{125}I (Kayes *et al.* 1962; Appelgren, Söremark & Ullberg, 1963).

MATERIALS AND METHODS

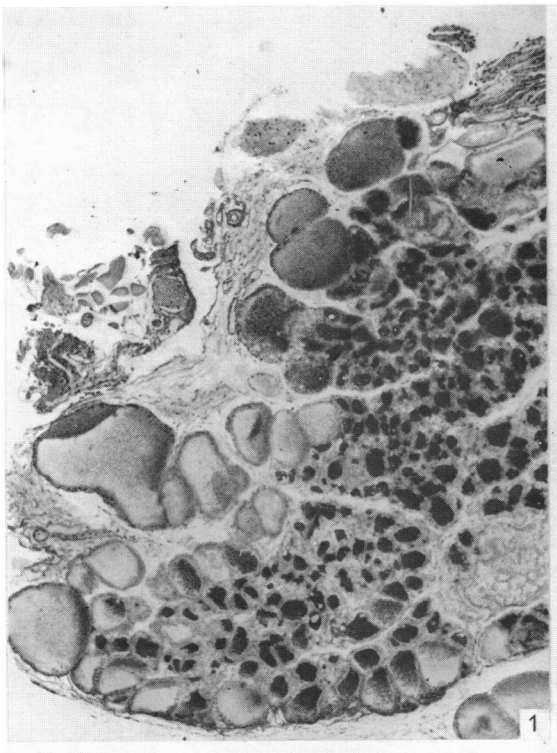
Male hooded rats weighing about 220 g. were given diet 41 B (Bruce, 1958) and tap water *ad lib.* Duplicate iodine analyses of the diet by Dr B. I. Sacks of University College Hospital Medical School gave 0·21 and 0·22 μg. of I/g. In a second group of animals, the drinking water was supplemented with 2 μg. of I (as KI)/ml.; this gave an additional intake of about 20 μg. of I. This treatment was continued for 20 days and was stopped 36 hr. before injection of radioiodine. The animals were injected intraperitoneally with a carrier-free solution of ^{125}I in 0·02 N-NaOH or with carrier-free ^{131}I, both supplied by The Radiochemical Centre, Amersham, Bucks., and diluted with aq. 0·9% NaCl. The animals were anaesthetized with chloroform and killed by exsanguination at intervals from 10 min. to 2 hr. after the injection. The thyroids were quickly removed and fixed in neutral formalin, Bouin's solution or Carnoy's fluid. Fixation with Carnoy's fluid gave in general the most satisfactory material. Serial paraffin sections 4 μ thick and mounted on gelatin-coated slides were taken down to water and covered with Kodak A.R. 10 radioautographic stripping emulsion in the conventional way. Some sections were coated with Kodak experimental stripping films nos. 305 A and 305 B; in these emulsions the thickness of the gelatin undercoat was 5 and 7·5 μ instead of the usual 10 μ. Although these films required more careful handling, they gave better results than those obtained with standard film when double-staining procedures were used. In sections counterstained with eosin and indigo carmine, the 'background' dye was strongest with the standard emulsion and weakest with 305 A.

A third of the sections from each experiment were set aside for coating with Saran F-120 resin before covering with stripping film. This protective layer ensures results which are free from pseudophotographic reduction resembling radioactivity and served as controls with which the uncoated sections were compared. Coated sections cannot be stained satisfactorily and therefore after exposure and development all preparations were examined by phase-contrast microscopy. Uncoated sections showing artifacts were discarded and the remainder stained with either haematoxylin and eosin or with neutral fast red and indigo carmine.

The method of coating employed was similar to that described by Hill (1959), except that a concentration of 1·5% (w/v) of Saran F-120 resin was found simpler to work with and gave satisfactory results. Saran films floated on water were transferred to sections by passing the slide underneath the film and carefully raising the slide out of the water, picking up the film at a slight angle, draining off excess of water and drying thoroughly before applying the radioautographic emulsion. All radioautographs were developed in Kodak D 19 b developer at 20° in parallel for

EXPLANATION OF PLATE 1

Radioautographs of thyroid sections from rat no. 192. Thyroid gland removed 2 hr. after 50 μc of ^{125}I. Sections were coated with Kodak A.R. 16 experimental emulsion 305 B and stained with neutral fast red and indigo carmine. Magnification: 1, ×65; 2 and 3, ×265; 4, ×1320.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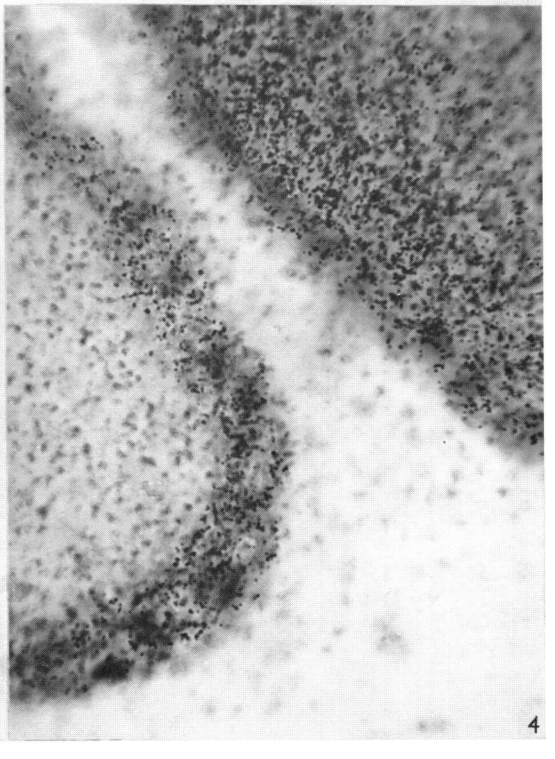
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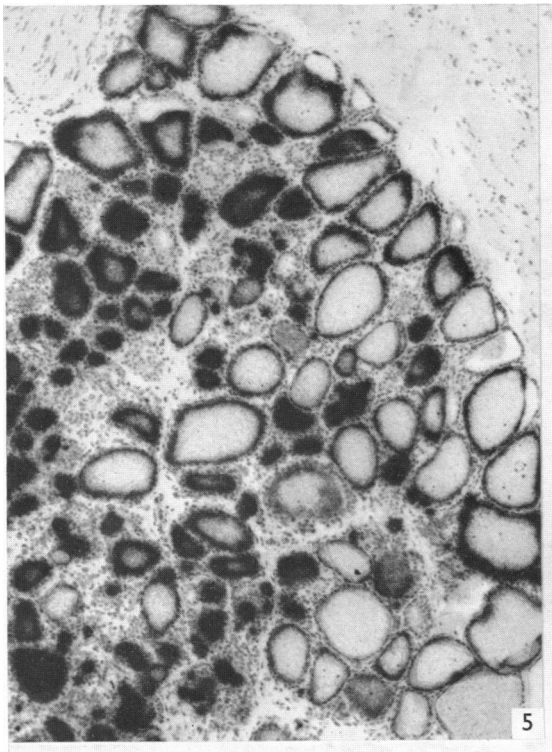
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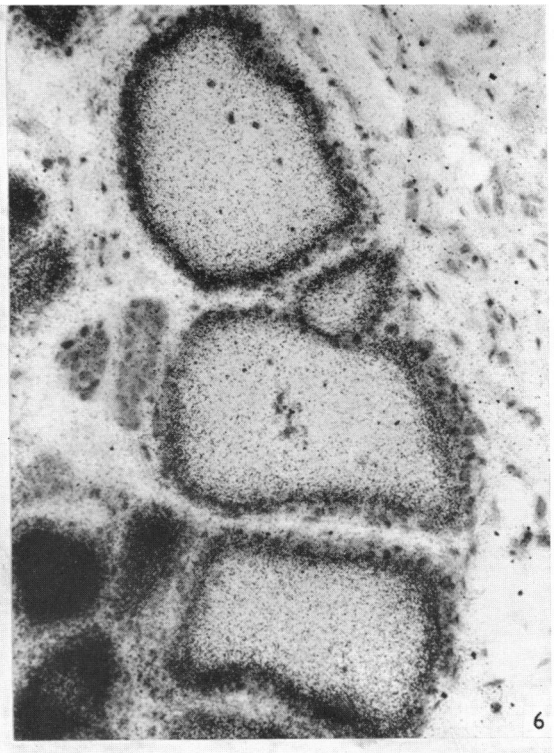
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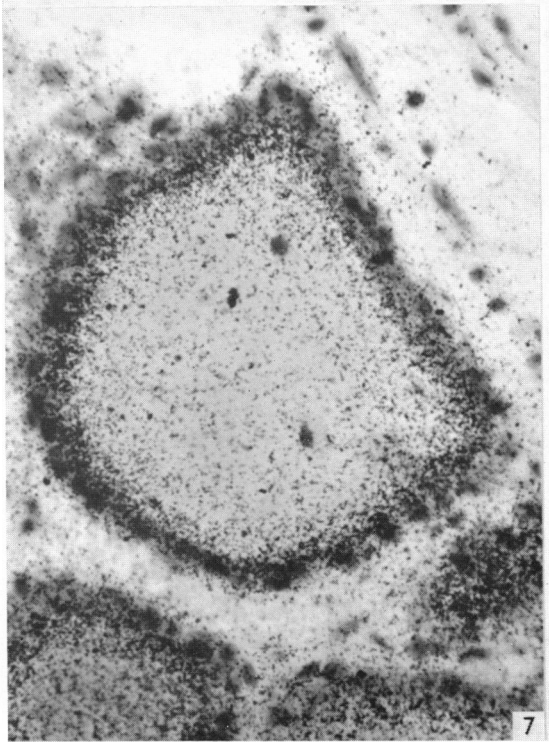
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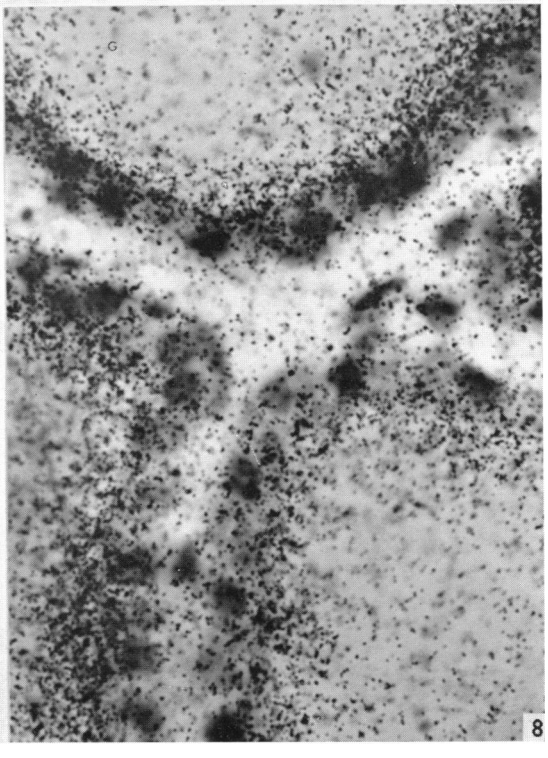
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3 or 5 min. The shorter development time showed a good registration of silver grains over the cells, whereas the standard time (5 min.) gave a heavy concentration of silver grains which obscured the cells, and prevented their being made visible.

Rat no. 192 (Plate 1) received no pretreatment and was injected with 50 μc of ^{125}I 2 hr. before killing. The thyroid was fixed in neutral formalin. The sections were exposed for periods of 1–8 days; the best results were obtained after 4–5 days' exposure.

Rats nos. 49 and 50 were pretreated with iodide; they were injected with 600 μc of ^{131}I and killed 10 and 15 min. later respectively. The thyroids were fixed in Carnoy's fluid. Sections were developed after 6, 24 and 48 hr. and good registration was obtained after 24 hr. exposure.

RESULTS

Radioautographs of stained sections of thyroid glands from a normal untreated rat (no. 192) and from two rats pretreated with iodide (nos. 49 and 50) are shown in Plates 1 and 2. In both Plates, the radioautographs at the lowest magnification show what has often already been demonstrated, that the distribution of organic radioiodine in rat thyroid is uneven from follicle to follicle, whether the gland has been labelled for a few minutes, or for as long as 10 days (Leblond & Gross, 1948; Doniach & Pelc, 1949; Clayton, 1953; Triantaphyllidis, 1958).

In Plate 1, photographs 1–3 show a number of follicles that contain very little radioactivity over the lumen compared with that registered over the cells. An even larger number of such follicles can be seen in Plate 2, photograph 5; this might be expected, since the gland had been labelled for a much shorter time, and its activity had been depressed by pretreatment of the animal with iodide.

As regards differences in resolution of the radioautographs with ^{125}I and ^{131}I, it is clear that equally good resolution can be obtained with the latter isotope, provided that the time of exposure to the radioautographic emulsion is short; with prolonged exposure of sections labelled with ^{131}I, considerable blurring is seen on the radioautographs. On the other hand, on prolonged exposure, radioautographs with ^{125}I showed more 'background' than did those with ^{131}I.

EXPLANATION OF PLATE 2

Radioautographs of rat-thyroid sections coated with Kodak A.R. 10 standard emulsion and stained with haematoxylin and eosin. 5, Rat no. 50, pretreated with iodide; thyroid removed 15 min. after 600 μc of ^{131}I; ×120. 6, 7 and 8, Rat no. 49, pretreated with iodide; thyroid removed 10 min. after 600 μc of ^{131}I (6, ×315; 7, ×700; 8, ×1320).

DISCUSSION

The present findings indicate that in the normal rat, as well as in the animal whose thyroid function has been depressed, a number of thyroid follicles contain much or most of the protein-bound radioiodine in the epithelial cells. The fact that cellular organic radioiodine can be seen in sections from an untreated animal suggests that it represents a normal process, and is not due to an abnormal physiological state of the animal.

In a number of the follicles in which the intensity of radioactive registration is high in both the cells and the colloid (Plate 1) there is no way of deciding whether the cellular radioiodine represents thyroglobulin synthesized in the cell, before being secreted into the lumen of the follicle, or on the way out; indeed, it is not known whether this cellular radioiodine represents thyroglobulin at all. It might be, totally or in part, the particulate iodoprotein, thyroid P-1 iodoprotein, found in normal and abnormal thyroid tissue (Robbins, Wolff & Rall, 1959*a*, *b*; Smith, Robbins & Rall, 1961), whose relationship to thyroglobulin, if any, is unknown.

Some of the follicles shown in both Plates exhibit a much higher concentration of organic radioiodine in the cells than in the colloid; if this cellular radioiodine represents, at least in part, thyroglobulin, one is tempted to suggest that this thyroglobulin was being made visible at the site of iodination, before being secreted into the follicular lumen.

It may seem somewhat surprising that the gland of a rat on a moderately low-iodine diet should manifest the same type of 'slow-motion' follicles as the glands of the animals whose diet had been supplemented with iodine. This may merely reflect an extreme case of the functional heterogeneity of the rat thyroid gland previously mentioned.

Maloof & Soodak (1963) have reviewed the evidence for and against the hypothesis that thyroglobulin is iodinated in the epithelial cell of the thyroid follicle, and have concluded in its favour. The present evidence supports this view. Thyroid cells can indeed form organically bound iodine even when the follicle has been disorganized. Whether the colloid contains enzymes that can iodinate thyroglobulin is not known; this should be susceptible to experimental proof.

SUMMARY

1. Radioautographic studies of rat thyroid glands have been made at different times after labelling with ^{125}I and ^{131}I.

2. Cellular organic radioiodine has been demonstrated in the thyroid of an untreated rat after 2 hr. labelling with ^{125}I.

3. Cellular organic radioiodine has been demonstrated in thyroids of rats pretreated with iodide, and labelled with ^{131}I for 10 and 15 min.

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The Kinetic Behaviour of Enzymes in Organized Systems

MITOCHONDRIAL SUCCINATE OXIDASE AND FUMARASE

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Effects of organization on the apparent kinetic parameters of enzymes give insight into the nature of the structural factors that may influence metabolic control. If two or more enzymes affected by a common substrate, inhibitor or activator are examined from this point of view, the relation between the composition of the external and internal medium of intact organized units, such as mitochondria, can also be elucidated. The present paper describes studies which illustrate two types of effects of organization on the kinetic behaviour of enzymes: control within closely linked reaction sequences and permeability barriers. These investigations are a continuation of those of Jones & Gutfreund (1963) on the control of succinate oxidation by the rate of electron transport and by competitive inhibition.

Previous studies (Chappell, 1962; Jones & Gutfreund, 1963) have shown that in several different types of mitochondria the rate of oxidation of succinate during closely coupled phosphorylation of ADP to ATP is limited by some step in the energy-conservation mechanism and not by the potential rate of the hydrogen and electron transfer from succinate to oxygen. Dinitrophenol or Ca^{2+} ions, which prevent the conservation of energy during electron transport, stimulate a considerable increase in the rate of oxidation of succinate. Studies with the pH-recording technique used by Gutfreund & Hammond (1963) to follow dinitrophenol-stimulated mitochondrial adenosine triphosphatase showed that stimulation by Ca^{2+} ions doubled the adenosinetriphosphatase activity compared with that with dinitrophenol (H. Gutfreund, unpublished work). The simplest kinetic scheme required for the analysis of our data can be represented as shown in Scheme 1, where E and E′ are the oxidized and reduced succinate dehydrogenase respectively, S is succinate and F is fumarate. The step characterized by k_0, the oxidation of reduced flavoprotein by the respiratory chain, involves the whole complex