since either glucose or glycerol as carbon source give in *C. utilis* high concentrations of pyruvate kinase. Then the inducer could well be FDP, the metabolite that activates pyruvate kinase in other yeasts.

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Biochem. J. (1967) **102**, 25c

Biosynthesis of Thyroglobulin and Sub-units in the Rat Thyroid Gland *in vivo*

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Seed & Goldberg (1963, 1965a), in studies of protein biosynthesis in thyroid tissue slices, have shown that labelled amino acids are incorporated into proteins sedimenting more slowly than thyroglobulin during the initial minutes of incubation, before any label could be detected in thyroglobulin itself. Because of the limitations of experiments with thyroid slices *in vitro*, specifically in view of the possibility of artifacts (Seed & Goldberg, 1965b; Nunez, Mauchamp, Macchia & Roche, 1965), we chose to study the biosynthesis of thyroid proteins in intact animals. The present communication describes the sequence of labelling of soluble proteins of the thyroid glands of rats injected with labelled amino acid.

Methods. Male Sprague-Dawley rats (weighing 150–200g.) were fed on a low-iodine diet (General Biochemicals, Chagrin Falls, Ohio, U.S.A.) for 10–14 days. L-[U-¹⁴C]Leucine (specific activity greater than 200mc/m-mole) was injected intraperitoneally in a dose of 15µc/animal. At intervals between 30min. and 48hr. after injection groups of two or three rats were killed by exsanguination under ether anaesthesia.

The thyroid glands were chilled to 4° within 1 min. after removal and homogenized in an all-glass homogenizer in cold phosphate-buffered saline (0.15M-NaCl–0.01M-potassium phosphate buffer, pH 6.8). Care was taken to avoid freezing the glands and homogenates.

Previous work in this Laboratory has shown that the concentration of diffusible ¹⁴C-labelled material in the blood of rats reaches a maximum within 10min. and falls to less than 15% of the maximum within 60min. after a single intraperitoneal injection of [¹⁴C]leucine. Therefore the present studies may be considered pulse-labelling experiments.

Results and discussion. The sequence of labelling of soluble proteins extracted from the thyroid glands is illustrated in Fig. 1. The sedimentation pattern of extracts dialysed at pH 6.8 (top panels) showed a progressive shift in the major radioactive peak from 12s at 1hr. to 16s at 4hr. and 18s at 20hr. The relative specific radioactivity, defined as the ¹⁴C/extinction ratio, of the 12s protein exceeded that of all other soluble proteins both at 30min. (not shown in Fig. 1) and at 1hr. after the injection

Fig. 1. Sedimentation of extracts of rat thyroid glands labelled *in vivo*. Rats were injected with L-[U- ^{14}C]leucine and killed at 1 hr. (a), 4 hr. (b) and 20 hr. (c) after injection. At each time-interval the glands from two rats were homogenized in 1.0 ml. of cold phosphate-buffered saline and centrifuged at 9000g for 10 min. One-half of the supernatant was dialysed against phosphate-buffered saline, and the remainder was dialysed against 0.01N-NH₃-0.15M-NaCl, pH 11.0, for 16 hr. at 4°. After dialysis, 0.40 ml. of each preparation was applied to a gradient of sucrose (5–20%, w/v) in phosphate-buffered saline and sedimented immediately at 30000 rev./min. for 18 hr. in a Spinco model L centrifuge (SW-39 rotor). From 25 to 35 fractions were collected from each tube. The extinction was measured at 280 m μ (—), and the total content of ^{14}C (●....●) in each fraction was determined by liquid-scintillation counting. The error of counting was less than 10%. The sedimentation coefficients of the radioactive peaks were estimated by reference to the extinction peaks at 4 s and at 19 s. At the top of the Figure are shown the sedimentation patterns obtained in extracts dialysed at pH 6.8, and at the bottom are the patterns in extracts dialysed at pH 11.

of labelled amino acid. The results of a 12 hr. experiment showed a pattern intermediate between that obtained at 4 hr. and that found at 20 hr. The position of the major radioactive peak in extracts of glands removed at 48 hr. after injection corresponded exactly with the extinction peak at 19 s.

These results confirm the observations *in vitro* made by Seed & Goldberg (1963, 1965a) and add support to the hypothesis that thyroglobulin biosynthesis proceeds by way of a 12 s sub-unit.

Experiments were done to investigate the possibility that ^{14}C -labelled 12 s protein is an artifact produced by dissociation of an especially labile fraction of 19 s material during dialysis at pH 6.8. Homogenates of glands removed 30 or 60 min. after injection of ^{14}C -labelled amino acid were applied directly to gradients of sucrose. The quantity of ^{14}C -labelled 12 s material was equal to that found in the dialysed preparations, indicating that dialysis at pH 6.8 did not cause the formation of labelled 12 s protein as an artifact. Nevertheless, the

possibility of dissociation of an extremely labile protein during homogenization or ultracentrifugation cannot be excluded.

Edelhoch (1960) has shown that thyroglobulin can be dissociated easily into 12 s sub-units under various conditions, including alkaline pH. The results of dialysis of thyroid extracts at pH 11 are shown in the lower panels of Fig. 1. The labelled 16 s component that was present in the pH 6.8 extracts of glands removed at 4 hr. is almost completely dissociated into labelled 12 s protein as a result of exposure to pH 11. A comparison of the 4 hr. and 20 hr. experiments shows an increase in the proportion of radioactivity in alkali-stable thyroglobulin. At 48 hr. after injection (results not included in Fig. 1) labelled 12 s protein was detected only in the pH 11 extracts, and its relative specific activity was slightly lower than that of the 19 s protein.

An increase in stability of thyroglobulin toward alkali-induced dissociation with increasing time

after labelling has also been observed by Lissitzky, Roques, Torresani & Simon (1965). The maturation of newly synthesized thyroglobulin into a more-stable compact molecular configuration may be a function of iodine content. Nunez *et al.* (1965) have shown that chemical iodination of amino acid-labelled thyroglobulin derived from sheep-thyroid slices results in an increase in sedimentation coefficient from 17s to 19s. Seed & Goldberg (1965*b*) incubated rat-thyroid slices for periods as long as 5hr. During this time-interval labelled amino acids accumulated in 16s, 12s and smaller proteins, but labelled 19s thyroglobulin was not formed. These authors postulated a block in maturation peculiar to the system *in vitro*. An alternative explanation for the findings of Seed & Goldberg (1965*b*) may be that the periods of incubation were too short to obtain labelling in 19s thyroglobulin.

The question whether 3–8s proteins contain one or more precursors of 12s sub-units remains unanswered. The relative specific activity of proteins in the 3–8s fractions did not change within the time-interval of the present study. It is likely that the 3–8s proteins are heterogeneous with regard to turnover. A rapidly labelled precursor might be only one component of a group of proteins and thus escape detection by the present methods.

The possibility of contamination of thyroid extracts by plasma proteins labelled *in vivo* was examined. Sedimentation analyses of plasma

collected at intervals between 2 and 20hr. after injection of [^{14}C]leucine showed more than 96% of radioactive protein in the 3–8s fractions. From the content of ^{14}C in thyroids from rats given intravenous injections of ^{14}C -labelled plasma proteins it was estimated that contamination could account for 20–30% of the labelled 3–8s proteins and only 1% of the 12–19s proteins in the thyroid extracts.

In summary, the results of the present study are consistent with the following scheme of thyroglobulin biosynthesis in the rat: (a) formation of a 12s sub-unit, possibly from smaller polypeptide sub-units; (b) aggregation of two 12s sub-units into a 16s molecule; (c) maturation of 16s protein into 19s thyroglobulin.

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Biochem. J. (1967) **102**, 270

Purification and Physicochemical Properties of Human Placental Lactogen

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Several procedures have been reported for the preparation of HPL,* a placental hormone that possesses certain biological and immunological features in common with HGH. Typical of these preparative methods are those of Josimovich & MacLaren (1962), Cohen, Grumbach & Kaplan (1964) and Friesen (1965*a,b*). Recently Turtle, Beck & Daughaday (1966) have described a modification of the procedure of Cohen *et al.* (1964) to give a more purified preparation.

* Abbreviations: HPL, human placental lactogen; HGH, human growth hormone.

To enable structural studies of HPL to be performed, the purification of this protein from human placental tissue was carried out by a modification of the method of Friesen (1965*b*). The preparation of this material in considerable quantity was facilitated by the use of a side fraction obtained during the preparation of human serum albumin from term placentas by Commonwealth Serum Laboratories, Melbourne. Immunodiffusion studies of all fractions of this process against antiserum to HGH revealed that a considerable quantity of immunoreactive material was present in fraction