FURTHER STUDIES ON THE 27S AND 32S OPTICAL DENSITY PEAKS OF THE RAT THYROID

By
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ABSTRACT

In this paper evidence is presented that a 27S Optical Density (O.D.) peak is absent and a 32S O.D. peak is regularly present on analysis of the soluble thyroid proteins of the goitrous rat. This occurs irrespective of the goitrogenic stimulus and a normal O.D. pattern can be restored by reversal of the goitrous process by thyroxine. In rats rendered goitrous by a low iodine diet or KClO₄ treatment, iodine supplementation also removed the 32S O.D. peak and resulted in reformation of the 27S peak. In rats treated by antithyroid drugs of the thiocarbamide group loss of the 32S O.D. peak was not associated with recovery of a 27S peak. This may be related to differences in potency of the various antithyroid drugs. \(^{125}\)I and \(^{3}H\)-leucine were not incorporated into the 32S O.D. peak material of the rat thyroid in vivo or into the 32S O.D. peak material of sheep thyroid slices in vitro. The view that this 32S O.D. peak was not iodoprotein nature was confirmed by showing that this peak had a maximal absorbance at 260 nm favouring the hypothesis that it was composed of nucleic acid.

The ultracentrifugal pattern of the thyroid proteins from normal vertebrate thyroids shows three main optical density (O.D.) peaks namely a predominant peak in the thyroglobulin (19S) region, a smaller peak in the 27S region and a broad lightweight peak in the 3-8S region (Salvatore et al. 1965). In a previous publication alterations in the pattern of the thyroid proteins in goitrous rats have been described (Thomson & Goldberg 1968). These changes consist of a relative loss of the 19S protein, a striking increase in the 3-8S protein...
and loss of the 27S peak with the appearance of a new O. D. peak in the 32S region.

An O. D. peak in the 32S region has been previously described by others in proteins from sheep and rat thyroid glands (Nunez et al. 1965; Robbins et al. 1966). In the experience of our laboratory a small 32S O. D. peak is usually present in addition to the 19S, 27S and 3-8S peaks in the sheep and occasionally in the ‘normal’ human thyroid (Thomson & Bissett 1969a). Shulman et al. (1967) have also described a 34S peak in the normal human thyroid. In Hashimoto’s thyroiditis a large 32S O. D. peak was present in 2 of 5 glands studied; a 27S peak was not seen from any thyroid gland affected by this pathological process (Thomson & Bissett 1969a).

In this present paper the situations under which this 32S material is found in the rat thyroid are explored and experiments to determine the nature of the material are described.

MATERIALS AND METHODS

Male Sprague-Dawley rats of approximately 150 g were obtained from A. J. Tuck & Son Limited, Rayleigh, Essex, England.

Propylthiouracil (PTU) was obtained from L. Light & Company Limited; potassium perchlorate KClO₄ from British Drug Houses Limited; carbimazole was a gift from Nicholas Laboratories Limited; thyroxine (T₄) from Koch-Light Laboratories Limited; [4,5-³H]L-leucine (S. A. 250–1000 Ci/mole) and carrier-free ¹²³I was obtained from the Radiochemical Centre, Amersham, England.

The dietary regimes used were similar to those previously described (Thomson & Goldberg 1968) and are detailed in Table 1.

The routine methods of preparation and ultracentrifugal analysis of the soluble thyroid proteins used in this study have been detailed in previous publications (Thomson & Goldberg 1968; Thomson & Bissett 1969b). The amount of protein applied

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<th>Table 1.</th>
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<td>Rat diets.</td>
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<td>A. Basic diet – Low iodine test diet (Nutritional Biochemical Corporation).</td>
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<td>B. PTU group – Basic diet + 5 mg KI + 200 mg PTU/kg diet.</td>
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<td>C. KClO₄ group – Basic diet + 20 g KClO₄/kg diet.</td>
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<td>D. Carbimazole group – Basic diet + 5 mg KI + 1500 mg carbimazole/kg diet.</td>
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<td>E. Iodine supplements – Low iodine group and KClO₄ group received distilled water and the remainder tap water. In certain experiments iodine supplements in the form of potassium iodide (KI) 0.05 °/o in the drinking water was given for the last 7 days of the dietary regime.</td>
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<td>F. Thyroxine supplements – 2 mg T₄/kg diet for last 7 days of the dietary regime. Low iodine diet alone given for 12 weeks; all other diets given for 3 weeks.</td>
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to the gradient was that extracted from the thyroid glands of two rats; no specific estimation was made of the amount of protein applied. Soluble thyroid proteins were also prepared by a) the method of Derrien et al. (1948) and b) by centrifugation of a thyroid homogenate at 105 000 g for 1 h to obtain a cell sap preparation and by precipitating the soluble proteins by 50 % saturation with ammonium sulphate.

Following sucrose density gradient ultracentrifugation various O.D. peaks were collected and their maximal absorbance determined by scanning in a Unicam SP 800 spectrophotometer.

In certain experiments sheep thyroid slices were incubated in vitro with either 125I or 3H-leucine as previously described (Thomson & Bissett 1969a).

RESULTS

1) Effect of different techniques of preparation of the soluble thyroid proteins on the presence of the 32S O.D. peak

The fact that the 32S O.D. peak was not an artefact was demonstrated by showing it to be present following 3 different techniques of preparation of the soluble thyroid proteins, namely the routine method used in this work which consists essentially of precipitation of the soluble thyroid proteins with 50 % saturation with ammonium sulphate following an initial centrifugation step at 20 000 g for 10 min to spin down cellular debris; by preparation of the soluble thyroid proteins by the method of Derrien et al. (1948) which consists essentially of a series of fractionation steps using ammonium sulphate; and by demonstrating that the 32S O.D. material was present in a 50 % ammonium sulphate precipitate of a thyroid cell sap preparation.

2) Conditions under which alteration of the O.D. peaks > 19S are shown

The middle part of Fig. 1 shows the pattern of the thyroid proteins from a goitrous rat with the protein pattern of control sheep thyroid proteins shown above. In the particular rats shown the goitrogenic stimulus was PTU but the same patterns were obtained from all the goitrogenic regimens used (PTU, methylthiouracil, carbimazole, KClO4) and from a low iodine diet.

In the lower part of Fig. 1 is shown the thyroid protein pattern from PTU treated rats supplemented with iodine. The 32S O.D. peak is virtually abolished, the 19S peak is increased and the 3-8S peak is relatively diminished. It should be noted that in neither the PTU treated nor the PTU + iodide treated rat is a 27S peak formed. A similar result was obtained in rats made goitrous by carbimazole with and without iodide supplements.

In Fig. 2 are shown the results from rats rendered goitrous with KClO4 and supplemented with iodide. The O.D. pattern of the thyroid proteins from a KClO4 treated animals is similar to that from the PTU treated rat. Iodine supplementation of the KClO4 treatment resulted as expected in increased 19S

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Ultracentrifugal pattern of thyroid proteins from (a) upper normal sheep, (b) middle PTU treated rat and (c) lower PTU + iodine treated rat. 
S. W. 41 rotor at 28 000 r. p. m. for 16 h.

protein and relative diminution of the 3-8S protein but on this occasion a 27S peak was reformed.

A similar pattern of results to the KClO₄ treated rats was found in rats made goitrous by a low iodine diet and then exposed to iodine or thyroxine dietary supplements (Fig. 3). A similar effect of thyroxine in increasing the relative amount of thyroglobulin and diminishing the 3-8S peak with reconstitution of the 27S peak was seen in rats treated by other goitrous regimes such as PTU, carbimazole and KClO₄.

3) Evidence of the nature of the 32S O. D. peak

As can be seen from Fig. 4, ³H-leucine was not incorporated into the O. D. peak > 19S at either early (15 min) or late time intervals (4 injections daily for 4 days, the last being 24 h before sacrifice) after subcutaneous injection of the isotope.
Ultrasound pattern of thyroid proteins from (a) upper normal sheep, (b) middle KC104 treated rat, (c) lower KC104 + iodine treated rat. S. W. 41 rotor at 28000 r. p. m. for 16 h.

Similarly 125I and 3H-leucine were not incorporated in vitro into the 32S O. D. peak of the soluble thyroid proteins from sheep thyroid slices at 4 and 24 h after incubation although the labels were incorporated at both times into the 19S and 27S O. D. peaks.

In Fig. 5 it can be seen that there is no significant incorporation of 125I into the 32S O. D. material after equilibrium labelling with the isotope (low iodine diet for 11 weeks followed by supplementing the drinking water for 5 weeks before sacrifice with a constant concentration of 125I). It should be noted that although no 27S O. D. peak is apparent that the 125I label is in fact incorporated into this area.

The fractions from the sucrose gradients which contained the 32S and 19S O. D. peaks were pooled and analysed in a scanning spectrophotometer. As can be seen from Fig. 6 the absorption spectrum of the 32S material is different.
Fig. 3.

Ultracentrifugal pattern of thyroid proteins from (a) upper rats on low iodine diet, (b) middle low iodine diet and iodine, (c) lower low iodine diet + thyroxine.

S. W. 41 rotor at 28 000 r. p. m. for 16 h.

from the 19S material in having a maximal absorbance in the region of 260 nm as opposed to 280 nm for the 19S O. D. peak material.

DISCUSSION

From the evidence presented in the results section it will be seen that a 32S O. D. peak is present on analysis of the soluble thyroid proteins of the goitrous rat irrespective of the goitrogenic stimulus.

Although not apparently regularly found by others this did not appear to be an artefact of one particular method of preparation of the soluble thyroid proteins.

Despite the similarity in the pattern of response of the thyroid proteins to a goitrogenic stimulus differences were apparent when the response of the thyroid glands from rats treated by goitrogenic drugs to iodine supplements was studied. In rats treated by a regime which simply stopped iodination of the thyroid protein by a low iodine diet or by KClO₄ treatment, a 27S O. D.
peak was readily reformed after iodine supplements. This was not, however, found after one of the thiocarbamide group of drugs despite similar alterations in the ratio of thyroglobulin and 3-8S protein being produced by the iodine supplements. This difference may simply reflect a difference in potency of blocking of thyroglobulin iodination by the different regimes but the possibility exists that it may indicate some more specific action of the thiocarbamide group of drugs on synthesis of thyroid proteins >19S. The fact that equal thyroxine supplements have a similar effect on the reformation of a 27S protein in all groups would favour the first hypothesis.

In the previous reports of a 31-34S peak on analysis of the thyroid proteins (Nunez et al. 1965; Robbins et al. 1966), evidence has been adduced to show its iodoprotein nature. In particular both groups who describe such a peak indicate that radioiodine was incorporated into this peak both in vivo and in vitro. This was not found in the present in vivo studies in the rat or in the in vitro studies using sheep thyroid slices; in all these experiments neither 126I nor 3H-leucine was incorporated into 32S O.D. peak. This is strong evidence
Fig. 5.
Pattern of in vivo equilibrium labelling with $^{125}$I of the thyroid proteins of iodine deficient rat (lower). Upper normal sheep thyroglobulin. S. W. 41 rotor at 28 000 r. p. m. for 16 h.

Fig. 6.
U. V. scan of the 19S and 32S fractions from PTU treated rat.
against its iodoprotein nature. It should be noted, however, that in the studies of Nunez et al. (1965) the protein studied was that obtained by solubilisation of the subcellular particles of the thyroid cells as opposed to the thyroid proteins obtained by ammonium sulphate precipitation of the soluble thyroid proteins as used in the present work. The theory that the 32S O.D. peak is not protein in nature is supported by its absorption spectrum which favours the view that the 32S O.D. peak is composed of nucleic acid. The possibility that the 32S material has some peptide fragments associated with nucleic acid cannot be excluded. Falconer (1969, personal communication) has also found that nucleic acid is precipitated by ammonium sulphate fractionation of the soluble thyroid proteins of goitreous sheep thyroid over a wide range of concentrations of ammonium sulphate.

Increased thyroidal nucleic acid in human thyrotoxic glands and in Hashimoto's thyroiditis has been previously described (Goldberg et al. 1968). A 32S O.D. peak in human thyroid glands affected by Hashimoto's thyroiditis has been found by us (Thomson & Bissett 1969a). This could be explained on the basis of the markedly increased cellularity of the thyroid — the greater part of which is of course due to infiltration with non thyroidal elements such as lymphocytes and plasma cells. It is, however, surprising that a 32S O.D. peak is not found in our laboratory in the extremely hyperplastic human thyrotoxic glands treated before operation with KClO₄. Further more detailed studies of the nature of the 32S material are indicated and are in progress.

REFERENCES


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