THE PREPARATION OF BOVINE 19S THYROGLOBULIN BY AGAROSE-GEL FILTRATION*

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Several techniques have been used to prepare mature 19S thyroglobulin and to purify it from serum proteins and other thyroidal iodoproteins. Until about 1960 most preparations of thyroglobulin were obtained by salt fractionations[1] or fractionation with cold ethanol and zinc.[2] Of these, even the most carefully constituted preparations seldom exceeded 95% purity by ultracentrifugal criteria.[3]

Electrophoretic procedures also have their disadvantages. Thus, boundary electrophoresis may yield a homogeneous fraction, yet, when analysed by starch-gel or polyacrylamide disc-gel electrophoresis, the same sample may be heterogeneous.[2,4] Gel electrophoresis is useful for analytical procedures but less appropriate for preparative purposes of large quantities of pure thyroglobulin. Nevertheless, it has been suggested as one of 3 steps for the purification of human thyroglobulin.[5]

Highly purified thyroglobulin (i.e. $> 98\%$) of rather poor yield (i.e. $< 18\%$) has been obtained by column chromatography on DEAE-cellulose with step-wise elution procedures.[6] Beef thyroglobulin is, however, fractionated by this method according to its iodine content.[7,8] The iodine content of thyroglobulin may affect its sedimentation value during moving boundary ultracentrifugation.[9,10]

Of the centrifugation techniques, sucrose gradient centrifugation[11] can be used for analytical as well as preparative purposes,[12] but is mainly useful for the analysis or preparation of small quantities of thyroidal iodoproteins. Thus ultracentrifugation in a linear density gradient of sucrose has been used for the isolation of pure 27S thyroidal iodoprotein (MW $1{\cdot}2 \times 10^6$),[13] which is considered to be a polymer of 19S subunits, and for the isolation of a 12S iodoprotein (MW 331,000)[14] which is thought to be a subunit of thyroglobulin.

Edelhoch[15] made use of the differential ultracentrifugal behaviour of proteins for the preparation of essentially pure thyroglobulin, yet still not completely devoid of heavier iodoproteins. With this technique a relatively larger preparation of thyroid material can be processed in one batch but multiple preparative runs are required to concentrate and purify the sample, while a considerable loss of thyroglobulin may occur during the process of boundary washing.

Filtration through granulated agar-gels (5 and 7%), which separate thyroidal iodoproteins on the basis of size and shape, have been used by Salvatoré *et al.*[12] to prepare pure thyroglobulin. Since then, Edelhoch has used agar-gel filtration to remove all traces of the 27S component from his boundary-washed thyroglobulin preparations as a final process. It was shown subsequently that Sephadex G-200 can also be used for obtaining a pure 19S component from the descending slope of the thyroglobulin peak,[4,16,17] similar to the observations of Salvatoré *et al.*[12] when 5% agar-gel was used. Nevertheless, no packing material for column chromatography has yet been found which will completely fractionate a mixture of normally occurring iodoproteins (12S, 19S, 27S and 32S) into separate peaks in a single step.

Of the methods discussed above, the salting out[1] and boundary washing[15] techniques are the most useful for obtaining large quantities of relatively pure and concentrated 19S thyroglobulin as starting material which can be used for further purification by gel-chromatography. For the final purification step, we have used a 3% agarose column successfully. At the same time fractions can be pooled which are very rich in heavier iodoproteins (27S and 32S). One component with a sedimentation coefficient greater than 32S has been observed.

Since starch-gel electrophoresis and velocity ultracentrifugation seem to be two of the most analytical techniques, they were used to determine the degree of purity of the pooled fractions and were found to be in agreement with respect to the number of components. The slowest migrating band in starch-gel electrophoresis corresponded to the fastest sedimenting material in the ultracentrifuge.

MATERIALS AND METHODS

Fresh beef and pig thyroid glands were obtained from the abattoirs, packed on ice and removed for processing in our laboratory. Apart from these unlabelled glands, thyroids from 5 dogs, 3 prepubertal baboons and one cow were analysed after they had received ^{131}I or ^{125}I. The dogs and baboons were each injected with 500 μc ^{131}I intravenously. They were exsanguinated 48 hours afterwards, just before thyroidectomy. The thyroid of one baboon was perfused with cold normal saline before ablation of the gland. The cow received 400 μc ^{125}I two days before the thyroid was removed.

All glands were frozen and sliced with a Stadie-Riggs tissue-slicer. The slices were suspended in 0·1M KCl - 0·2M PO_4 buffer (KP-buffer), pH 7·4, at a ratio of 2 ml. buffer per gram of tissue and extracted for 6 hours at approximately 4°C. The extracts were centrifuged in a Sorvall at 31,500 × g for 30 minutes. The supernatant was cleared subsequently in a Spinco Model L ultracentrifuge for ½ hour at 105,000 × g. The soluble proteins were concentrated by vacuum dialysis at 4°C in cellophane bags and dialysed against fresh KP-buffer while the bags were tied down to prevent excessive dilution during dialysis. Thyroidal protein concentrations were determined in a Beckman DU spectrophotometer at 280 mμ and haemoglobin at 414 mμ by accepting $E^{1\%}_{1cm.}$ values of 10·5 for thyroglobulin and 18·7 for haemoglobin.

Salting out was performed according to Derrien *et al.*,[1] and differential ultracentrifugation with boundary washing according to Edelhoch and Lippoldt.[18]

DEAE-Sephadex A50 was treated according to Griffin *et al.*,[19] packed in a 1·7 × 40 cm. column with a bed volume of 62 cm. and equilibrated overnight with 0·01M PO_4 buffer, pH 6·7. A 9-ml. sample of thyroid extract containing 288 mg. protein was applied and after 215 ml. of 0·01M PO_4 buffer had passed through the column a gradient

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was started with 200 ml. 0·01M PO_4, pH 6·7 in the mixing chamber against 0·05M PO_4 containing 0·5M NaCl of the same pH in the reservoir. The flow-rate was controlled at 13 ml./hr. After all proteins were eluted with this mixture, the gradient was continued with 0·1M PO_4 in 1·0M NaCl without any further recovery of proteins. Fractions of 0·5 ml. were counted in a Packard Auto-gamma counter. They were then diluted to 2·5 ml. and optical density readings taken in a Beckman spectrophotometer at 280 and 414 millimicrons.

Essentially the method of Hjerten[20] was used for the preparation of agarose gels of different concentrations, as described earlier,[21] with some modifications. Emulphor EL* was used as stabilizer instead of polyoxyethylene sorbitan monostearate (Tween 61) as suggested by Hjerten and in the same ratio as described for Tween 61. All agarose concentrations were liquefied by stirring the flask containing the agarose while it was immersed in a beaker containing a boiled saturated saline solution. After the gel was formed, the beaker containing the boiling saline solution was replaced by a beaker containing ice water.

When the suspension reached room temperature, it was centrifuged in 250-ml. cups at 2,000 r.p.m. for 5 minutes and the supernatant CCl_4 and toluene were decanted. The beads were subsequently washed with methanol. Methanol washings were continued until the supernatant gave no milkiness upon treatment with water in a test-tube.

Only successful preparations were used which resulted in almost perfectly spherical-shaped beads and in which not less than 60% of the beads had a diameter of between 50 and 100μ. No attempt was made to obtain greater uniformity in the diameter of the grains by wet-sieving.

The ability of various agarose concentrations to resolve thyroidal iodoproteins was tested out with columns provided with jackets through which water circulated at 17°C. All subsequent analyses were performed in a cold room at approximately 4°C.

Granulated agar† gels with granule size of 70 - 150 μ were prepared according to Andrews[22] and Salvatoré *et al.*[12] The agar column (2·1 × 75 cm., ratio 1:40) was packed under 40 - 50 cm. pressure.

Analytical ultracentrifugation by the moving boundary technique was performed in a Model E Spinco ultracentrifuge. Iodine analyses were performed according to a method previously described[23] and nitrogen analyses according to the method of Zwarenstein and Van der Schyff.[24] Vertical starch-gel electrophoresis was done according to Smithies.[25] The gel buffer was 0·025M Na-borate, pH 8·8, and the electrode buffer 0·3M NA-borate, pH 8·3.

RESULTS

Comparison of Salting-out and Boundary Washing Procedures

Fig. 1 (top) demonstrates a typical pattern obtained by the boundary washing technique which is about the same as when the salting-out technique was used. In both techniques the S19 thyroglobulin was the major component, with S27 and S32 iodoproteins recognizable. The percentage distribution and yield varied according to the thyroid sample used and the technique employed. Thus, if the contents of a tube were transferred to a different tube after each boundary washing procedure, some heavier iodoproteins would be lost in the gelatinous pellet. If the sediments were collected and suspended in KP-buffer and analysed, a mixture of the same iodoproteins was still obtained but the concentration of the heavier iodoproteins was greater and a component with greater sedimentation than that of S32 was observed (Fig. 1 bottom).

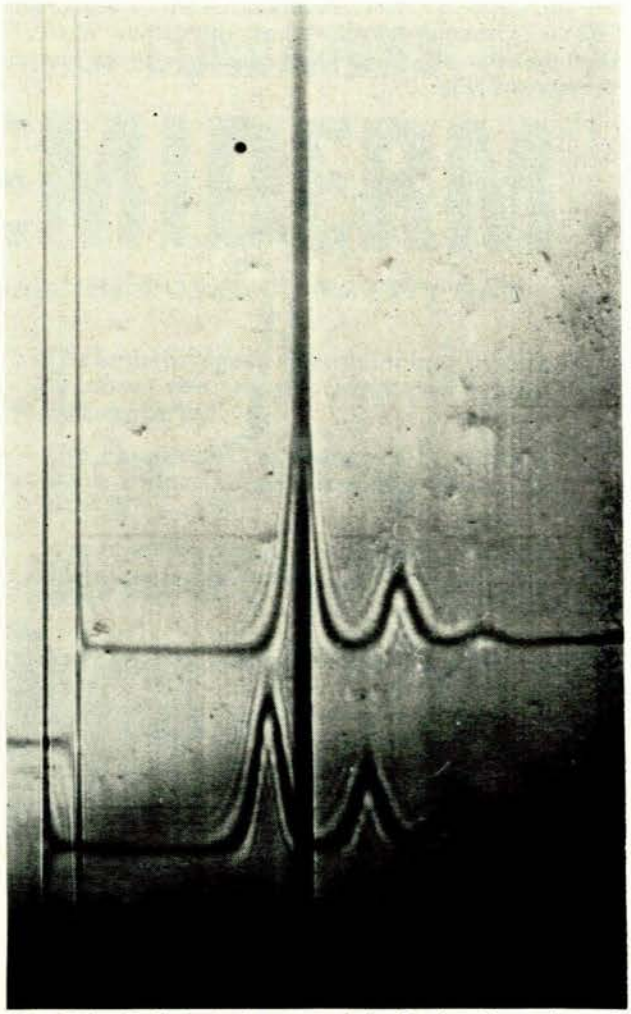

Fig. 1. Thyroidal iodoprotein pattern obtained by boundary washing of a beef thyroid extract showing S19, S27 and S32 components (top). The sediment was suspended in buffer and, when analysed, it showed an additional protein with sedimentation larger than S32 (bottom). Speed 52,000 r.p.m., bar angle 65°; protein concentration 0·75%. The photograph was taken 52 minutes after 52,000 r.p.m. had been achieved.

The yield is somewhat greater with the salting-out procedure than that which is obtained by boundary washing. Thus, the mean yield from 4 large-scale preparations of beef thyroids was 18·6 mg. thyroglobulin/G of minced thyroids for the salting-out technique as compared with 13·4 mg./G obtained by boundary washing. These results, however, are empirical since in the differential ultracentrifugation procedure the boundary washings may be continued indefinitely, resulting in concentrating and purifying the S19 thyroglobulin every time to a greater extent since lighter proteins, such as serum proteins, haemoglobin and

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†Difco Bacto-Agar, Difco Laboratories, Detroit, Michigan, USA.

12S iodoproteins, are sucked off while the heavier iodoproteins (S27 and S32) are partially lost in the pellet.

DEAE-Sephadex Salt-gradient Separation of Bovine Thyroid Extract

A bovine thyroid was labelled by injecting 400 μc ^{125}I intravenously 2 days before thyroidectomy. Of the thyroid extract a 9·0-ml. sample in 0·01M PO_4, pH 6·7, containing 288 mg. of soluble thyroidal proteins, was placed on a 1·7 × 40 cm. DEAE-Sephadex A-50 column with a bed volume of 62 cm. The column was washed with 215 ml. of 0·01M PO_4 of the same pH. Under these conditions all the proteins were retained (Fig. 2).

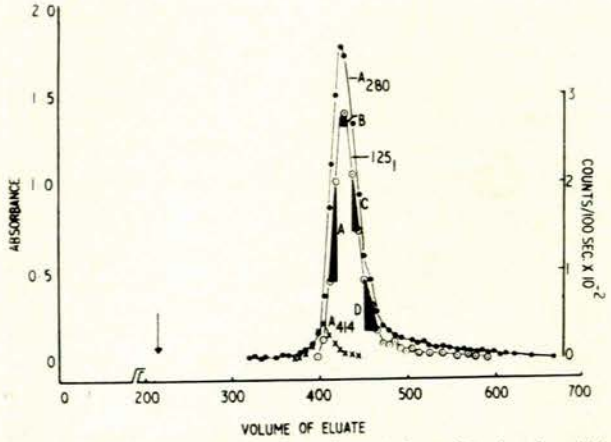

Fig. 2. DEAE-Sephadex A-50 salt-gradient elution of bovine thyroidal proteins. The sample volume was 9·0 ml. containing 188 mg. protein. Column dimensions were 1·7 × 40 cm. with a bed volume of approximately 62 ml. The flow-rate was 13 ml./hour.

Subsequently, a gradient was started with 200 ml. 0·01M PO_4, pH 6·7, in the mixing chamber with 0·05M PO_4 of the same pH and containing 0·5M NaCl in the reservoir. The flow-rate was controlled at 13 ml./hr. Fractions of 0·5 ml. were counted in a Packard Auto-gamma counter. These fractions were subsequently diluted to 2·5 ml., and OD_{280} and OD_{414} readings were taken in a Beckman DU spectrophotometer.

The small amount of haemoglobin contained in the extract was eluted in the first part of the ascending limb. The rest of the graph was symmetrical, except for a slight shoulder in the last part of the descending limb. The pattern showed a good correlation between OD_{280} readings and ^{125}I counts.

Samples were pooled as indicated by the blackened portions in Fig. 2, and marked A, B, C and D respectively. Fractions A, C and D were concentrated by vacuum dialysis and diluted afterwards to a 1% concentration while fraction B, which had a concentration of 0·83%, was retained as such.

In Fig. 3(a) the sedimentation pattern of fraction A of Fig. 2 appears on top and of the peak (fraction B, Fig. 2) at the bottom. In fraction A, no appreciable heavier iodoproteins than 19S were observed, while the lighter serum proteins did not move from the meniscus. Since the serum proteins can be separated from thyroidal iodoproteins by Sephadex G-100,[26] Sephadex G-200,[4] 3% agarose[21] or by the differential centrifugation and boundary washing technique of Edelhoch and Lippoldt,[18] the DEAE-Sephadex A-50 method could be used for the collection of ultracentrifugally-pure thyroglobulin from the ascending limb of the DEAE-Sephadex separation.

At the peak of the DEAE-Sephadex A-50 gradient elution pattern (fraction B, Fig. 2), the 27S iodoprotein starts to appear (Fig. 3(a), bottom) when examined in the ultracentrifuge.

The ultracentrifuge patterns of fractions C and D which form the descending limb of the DEAE-Sephadex elution pattern in Fig. 2, are shown in Fig. 3(b), in which it is clear that in fraction C (top) and fraction D (bottom) the heavier iodoprotein component increased in this order.

Since at a 1% concentration there was a clear difference in the sedimentation velocity between the 19S thyroglobulin peaks of fractions C and D, it was decided to determine the sedimentation coefficients at different concentrations, and to extrapolate to zero concentrations in order to see whether DEAE-Sephadex A-50 can resolve thyroglobulin molecules of different sedimentation values. These results are shown in Fig. 4, from which it is clear that the $S_{20,w}$ values of the S19 components at zero concentration did not differ by any appreciable extent in these 2 fractions. The difference between an $S_{20,w}$ value of 18·7 for the S19 component in fraction C and 18·9 for fraction D is moreover

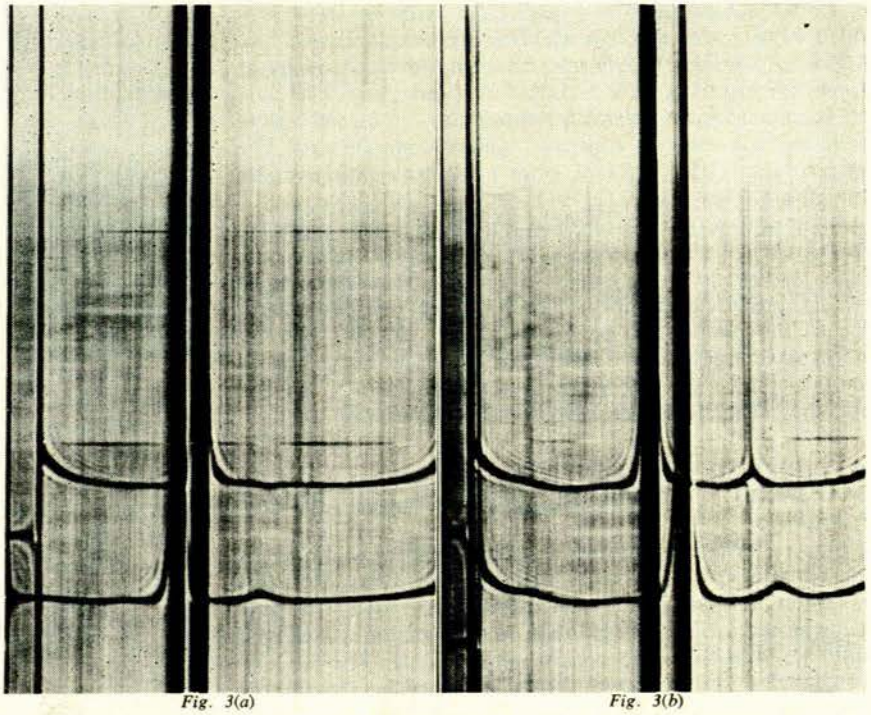

Fig. 3(a) *Fig. 3(b)*

Fig. 3. Ultracentrifugation analyses of a bovine thyroid extract of the pooled fractions A, B, C and D obtained by DEAE-Sephadex fractionation as illustrated in Fig. 2. *3(a)*: Pattern of sample A (top) and of sample B (bottom). The protein concentration was 1% for sample A and 0·83% for sample B. Bar angle was 70° and the photo taken 24 minutes after maximum speed of 52,000 r.p.m. was achieved. *3(b)*: Pattern of sample C (top) and of sample D (bottom). The protein concentrations of samples C and D were both 1%. Bar angle was 70° and the photo was taken 50 minutes after maximum speed of 48,000 r.p.m. was obtained.

in accordance with a greater Johnston-Ogston effect in the sample where the S27 component is present in greater concentration.

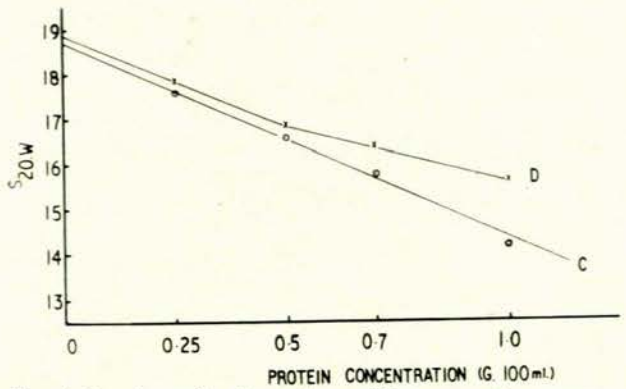

Fig. 4. Dependence of sedimentation coefficient on protein concentration for bovine 19S thyroglobulin proteins obtained in fractions C and D by salt-gradient elution from DEAE-Sephadex A-50 column.

Because it is known that DEAE-cellulose fractionates pure thyroglobulin according to its iodine content—by which the inhomogeneity of S19 thyroglobulin species was demonstrated[7,8]—it was of value to compare the iodine/protein ratio of these fractions. For fractions A, B, C and D the relative ratios were 8·6, 14·9, 9·7 and 10·2 respectively. Because of the haemoglobin content of fraction A it was expected to yield the lowest iodine/protein ratio since, according to our experience, the I/N ratio of haemoglobin is the lowest of all the soluble components fractionated from thyroid material, and is lower than the I/N ratio of plasma proteins (see below).

Although at present it is not clear whether the iodine content of thyroglobulin alone is responsible for differences in sedimentation values,[27] these results indicate that DEAE-Sephadex A-50, under the existing experimental conditions, did not separate bovine S19 thyroglobulin molecules according to sedimentation values or according to their iodine content, since no gradual increase in the iodine concentration of the late eluted fractions were observed.

Separation of Proteins in Crude Thyroid Extracts by Different Agarose Concentrations

In exclusion chromatography, using gels, the gel concentration determines the pore size and the degree of hardness. If the gel concentration is too low, the gel may be too soft, with a resultant deformation when packed in the column, so that the resolving power of the gel is decreased. Agarose spheres have the advantage over agar granules that the hardness of agarose gels allows preparation of very low concentrations with satisfactory flow-rates. Such low concentrations should theoretically differentiate to a greater extent between relatively large protein molecules in the order of thyroidal iodoproteins. It was therefore decided to prepare and to test out 3%, 5% and 8% agarose gel concentrations for their ability to resolve the soluble proteins of thyroid extracts (Fig. 5). This experiment was done on thyroid extracts prepared from 3 dogs, 2 of which received 500 μc ^{131}I 48 hours before thyroidectomy. Fig. 5(a) shows the pattern obtained by an 8% agarose gel concentration which indicates that the gel is capable of separating the thyroidal iodoproteins from the serum proteins. In the region of thyroidal iodoproteins the OD_{280} and ^{131}I peaks coincided and were both of a symmetrical nature. A difference between the OD_{414} and OD_{280} patterns in the serum protein region indicates partial differentiation by 8% agarose.

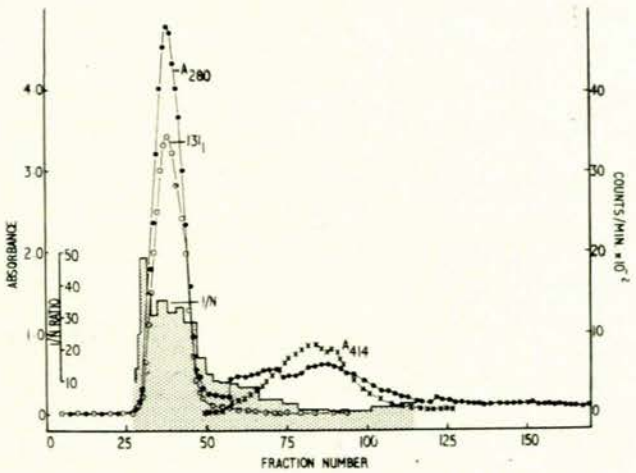

Fig. 5(a)

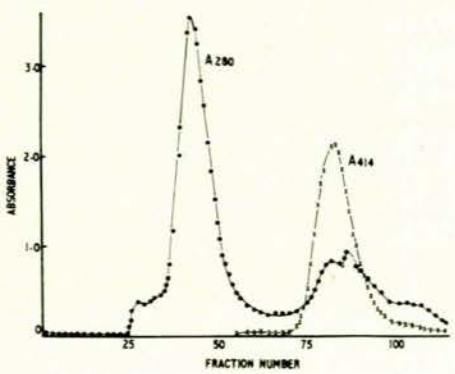

Fig. 5(b)

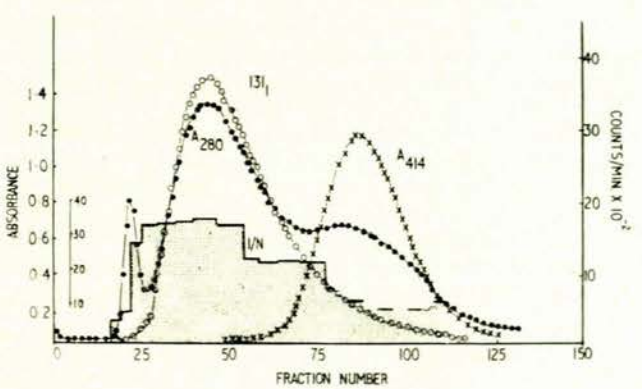

Fig. 5(c)

Fig. 5. Fractionation of the soluble proteins in dog thyroid extracts by columns of 8% agarose (*a*); 5% agarose (*b*); and 3% agarose (*c*). In the case of (*a*) and (*c*) the dogs received 400μc ^{131}I intravenously 48 hours before thyroidectomy. A280 and A414 refer to absorption of thyroidal proteins and haemoglobin respectively. The hatched areas indicate the I/N ratio, i.e. stable iodine (μg.) per nitrogen (mg.) of protein.

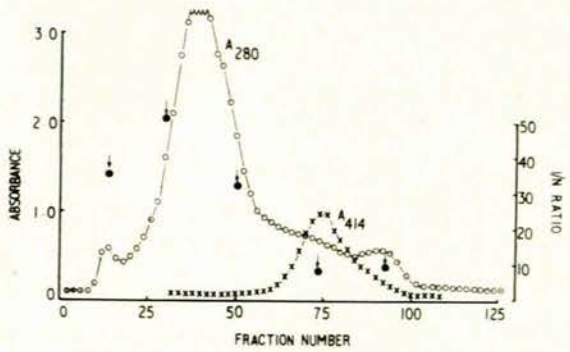

Fig. 6(a). 3% agarose fractionation of a pig thyroid extract. The arrows point to stable iodine/protein ratio values of five fractions analysed.

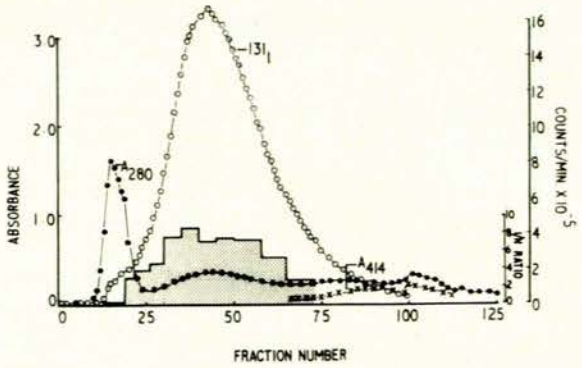

Fig. 6(b). 3% agarose fractionation of a baboon thyroid extract. The hatched area indicates stable iodine/nitrogen ratio values performed on pooled fractions.

When 5% agarose was used (Fig. 5(b)), the major OD_{280} peak showed a shoulder in the region of the exclusion volume indicating a partially fractionated 'prethyroglobulin'. Judging from the haemoglobin (A 414) peak, the indications are that a 5% gel concentration separated the serum proteins somewhat better from the thyroidal iodoproteins than the 8% agarose.

Using a 3% agarose concentration, the 'prethyroglobulin' separation was nearly complete, while there was no differentiation between different serum proteins (Fig. 5(c)). Thus, a low concentration of agarose seemed more useful to differentiate between relatively large proteins, while a high concentration of agarose may be employed for the fractionation of smaller proteins.

The ability of a 3% agarose concentration to fractionate thyroglobulin from the unknown 'prethyroglobulin' component was further substantiated in baboon, beef and pig thyroid extracts. In every instance a 'prethyroglobulin-peak' was observed when the crude extract was applied to the column. The smallest concentration of this component was observed in pig thyroids (Fig. 6(a)), while the greatest concentration was found in the baboon (*Papio ursinus*, Fig. 6(b)). Approximately 50% of the total OD_{280} absorption occurred in this region.[25] When the UV-spectrum of baboon thyroglobulin was compared with that of the 'prethyroglobulin' component, the main difference was that thyroglobulin showed a more prominent peak at OD_{280}.

Of interest in the pattern obtained from baboon thyroids with 3% agarose sieving, is the small and flattened thyroglobulin OD_{280} peak, yet it contained almost all the radioactivity (Fig. 6(b)). Three baboon thyroids chromatographed on 3% agarose columns all showed identical peaks. These, however, were all very young prepubertal baboons, between 6 months and 1 year old. Whether age plays a role in the distribution of thyroidal iodoproteins remains to be determined.

It was first thought that the 'prethyroglobulin' peak seen in a 3% agarose pattern consists of heavier iodoproteins such as S27 and S32 components. The material, however, was extremely labile and sparingly soluble in 0·02M PO_4 - 0·1M KCl, and behaved like a protein aggregate or a denatured protein.

Further observations on iodoprotein fractionation by different agarose concentrations relate to the iodine content of the proteins with respect to iodination with radioactive or with stable iodine.

In every case where the 'prethyroglobulin' component was isolated from radioactively labelled proteins, very little radioactivity occurred in the former (Figs. 5(a), 5(c) and 6(b)). It is therefore unlikely that the aggregate formed from labelled thyroglobulin, since in such a case the radioactive peak of the 'prethyroglobulin' component was expected to be higher. The two most unstable compounds in a mixture of thyroidal iodoproteins, collectively designated as thyroglobulin, are the poorly iodinated species (14 - 15S)[27] and the heavier iodoproteins, particularly the 27S component.[13] If the 'prethyroglobulin' peak were formed from the latter, radioactive iodine would be poorly incorporated into it because of its originally high stable iodine content.[13] It was therefore of interest to determine the stable iodine/nitrogen (μg. I/mg. N) values of these peaks.

Although the I/N values differed in different thyroids, the following general conclusions were drawn (Figs. 5(a), 5(c), 6(a) and 6(b)).

(*a*) The I/N ratio of 'prethyroglobulin' is always lower than that of thyroglobulin.

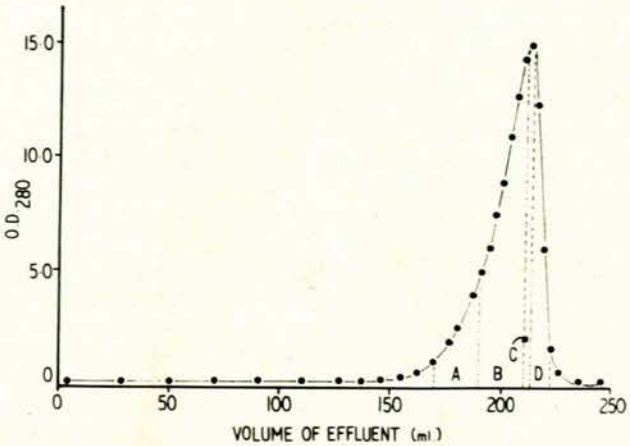

Fig. 7. 5% agar separation of partially purified bovine thyroglobulin. Fractions A, B, C and D were pooled and analysed by starch-gel electrophoresis and analytical ultracentrifugation.

(*b*) The I/N ratio peak is not of a symmetrical nature; the highest ratios correlate with the ascending limb of the main thyroglobulin peak.

(*c*) The lowest I/N ratio of all serum and thyroidal proteins correlates with that of haemoglobin.

(*d*) In the young prepubertal baboon thyroid extract, in which a large percentage of total proteins is occupied by the 'prethyroglobulin' fraction, the thyroglobulin OD_{280} peak is broad and its I/N values relatively low.

These observations, together with analytical ultracentrifugation runs, indicated that the 'prethyroglobulin' peak consisted largely of non-iodinated protein aggregates and did not contain S27 and S32 iodoproteins. Nevertheless, since a 3% agarose concentration is capable of excluding non-iodinated protein aggregates from thyroidal iodoproteins on the one hand and of separating serum proteins from thyroidal iodoproteins on the other hand, it seemed useful for the preparation of thyroglobulin.

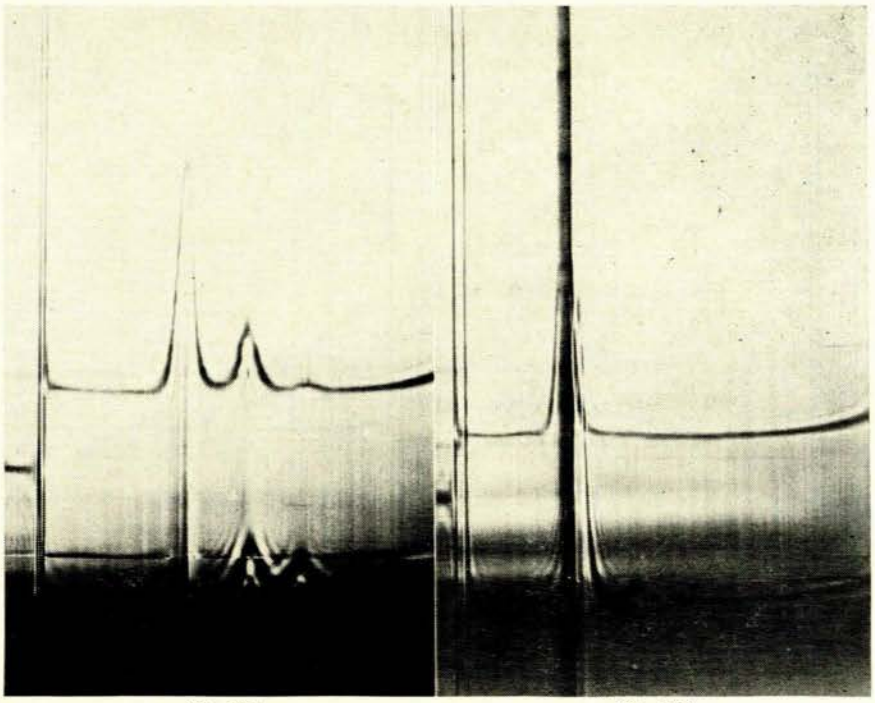

Fig. 9(a) *Fig. 9(b)*

Fig. 9. Ultracentrifugal analyses of fractions A, B, C and D obtained by 5% agar fractionation of partially purified bovine thyroglobulin. *9(a)*: Fraction A is shown at the bottom and fraction B at the top. Protein concentration in both fractions was 0·36%. The photograph was taken after 32 minutes at 48,000 r.p.m. in a Spinco Model E centrifuge at approximately 20°; bar angle 55°. *9(b)*: Fraction C appears at the bottom and fraction D at the top. Protein concentration for fraction C was 0·44% and for fraction D 0·47%. The photograph was taken after 40 minutes at 52,000 r.p.m.; bar angle 60°.

It was therefore important to compare the protein components obtained by molecular sieving of partially purified thyroglobulin through a 3% agarose column with those following 5% agar-gel chromatography, since the latter seems to be preferred to 7% agar for the preparation of pure 19S thyroglobulin.[22]

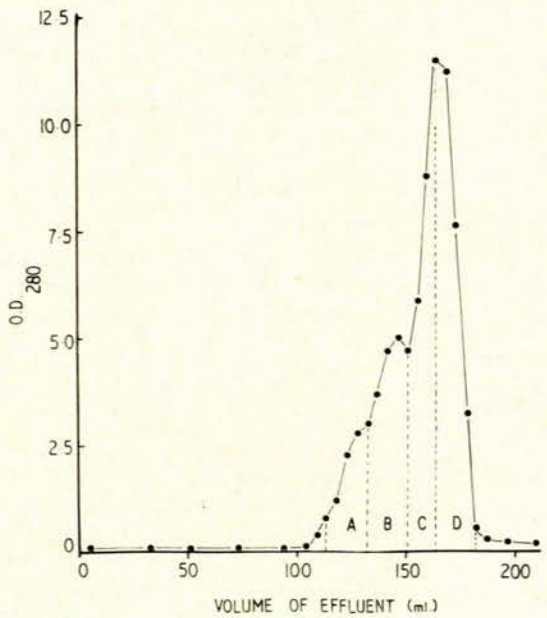

Fig. 8. 3% agarose fractionation of partially purified bovine thyroglobulin. Fractions A, B, C and D were pooled and analysed by starch-gel electrophoresis and by analytical ultracentrifugation.

Preparation of Pure 19S Thyroglobulin

A 5% granulated agar-gel with granular size between 70 and 150 μ was prepared according to Andrews.[22] The agar column (2·1 × 75 cm., ratio 1:40, bed volume 250 ml.) was packed under 40 - 50 cm. pressure. A bovine thyroglobulin preparation of 350 mg., which, after partial purification by boundary washing, was composed of 70% 19S, 23·7% 27S, 4·3% 32S and 1·1% of heavier iodoproteins, was used as starting material. Fractions were pooled as indicated in Fig. 7 and marked A, B, C and D. A batch of the same sample was also filtered through a 3% agarose column (Fig. 8) and the fractions were pooled and marked in a similar way.

The pattern obtained in Fig. 7 with 5% agar was that of a single asymmetrical peak. Ultracentrifugal analyses of the pooled fraction A and B (Fig. 7) are shown in Fig. 9(a). Similar analyses of the fractions C and D (Fig. 7) are shown in Figure 9(b). The percentage distribution of the different iodoproteins is given in Table I.

TABLE I. DISTRIBUTION OF IODOPROTEINS IN FRACTIONS A, B, C AND D OBTAINED BY EXCLUSION CHROMATOGRAPHY ON 5% AGAR-GEL (FIG. 9) AND 3% AGAROSE GELS (FIG. 10)

	Fraction A		*Fraction B*		*Fraction C*		*Fraction D*		*Total*	
5% agar	*mg.*	*%*	*mg.*	*%*	*mg.*	*%*	*mg.*	*%*	*mg.*	*%*
Total protein	62·3	17·8	185·2	52·9	45·9	13·1	57·8	16·5	350·0	100·0
19S	3·7	6·0	140·8	76·0	42·7	93·0	57·8	100·0	245·0	70·0
27S	37·4	60·0	42·6	23·0	2·8	6·0	—	—	82·8	23·7
32S	13·1	21·0	1·9	1·0	—	—	—	—	15·0	4·3
>32S	3·7	6·0	—	—	—	—	—	—	3·7	1·1
3% agarose										
Total protein	46·4	13·9	78·5	23·6	11·0	33·0	98·0	29·4	333·0	100·0
19S	3·4	7·4	7·8	9·9	103·3	93·9	98·0	100·0	212·5	63·8
27S	8·6	18·5	60·1	76·6	6·7	6·1	—	—	75·4	22·6
32S	24·1	51·9	10·6	13·5	—	—	—	—	34·7	10·4
>32S	10·3	22·2	—	—	—	—	—	—	10·3	3·1

in fact pure 19S thyroglobulin as has been seen by analytical ultracentrifugation.

SUMMARY

Results with ammonium sulphate precipitation of thyroidal iodoproteins, as preparation for thyroglobulin from beef thyroids, were compared by the differential ultracentrifugal technique; the mean yields in 4 large-scale preparations of each were 18·6 mg. and 13·4 mg. thyroglobulin/G of minced thyroid respectively.

DEAE-Sephadex A-50 salt-gradient separation yielded a fraction which contained no iodoproteins appreciably heavier than 19S. This fraction which appeared in the ascending limb of the curve was, however, contaminated with serum proteins. This procedure could therefore be combined with Sephadex G-100, Sephadex G-200 or 3% agarose exclusion chromatography for pure 19S thyroglobulin preparations. DEAE-Sephadex A-50 did not separate bovine 19S thyroglobulin molecules according to sedimentation values or according to their iodine content.

Spheres of 3%, 5% and 8% agarose gel were tested for their ability to fractionate thyroidal iodoproteins and to separate them from non-thyroidal iodoproteins as a one-step procedure. With a 3% agarose concentration a 'prethyroglobulin' fraction was separated from thyroidal iodoproteins, while the latter were also separated from the serum proteins. Such a 'prethyroglobulin' peak was observed in baboon, beef, pig and dog thyroid extracts; the greatest concentration of this labile protein peak was observed in crude thyroid extracts of baboons. The 'prethyroglobulin' peak contained a low stable iodine protein ratio and a low specific activity after radioactive iodine treatment of the animals. Serial stable iodine/nitrogen ratios performed on samples obtained by 3% agarose column fractionation of thyroidal protein extracts indicate values for those fractions which contain heavier iodoproteins (i.e. S27 and S32 components) than the I/N ratio values of pure thyroglobulin (S19). The lowest I/N ratios were observed in the 'prethyroglobulin' fraction and in haemoglobin.

Three per cent agarose and 5% agar-gel columns were compared for their ability to resolve pure 19S thyroglobulin from partially purified thyroglobulin. In the case of agarose chromatography the main peak of the elution pattern could be included in the descending limb since both contained pure 19S thyroglobulin. In the case of 5% agar-gel filtration only the descending limb contained pure 19S thyroglobulin.

The number of iodoprotein components seen in starch-gel electrophoretograms correlated with their analytical ultracentrifugal patterns in so far as the slowest migrating band in starch-gel electrophoresis corresponded to the fastest sedimenting material in the ultracentrifuge.

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