Effects of Pharmacological Doses of Iodide on the Hyperplastic Rat Thyroid Gland. Roles of Intrathyroidal Iodide, Thyrotropin and Thyroglobulin in the Wolff-Chaikoff Phenomenon [1]

H. BÜRGI, A. RADVILA, H. KOHLER, AND H. STUDER

Medizinische Universitätsklinik, Inselspital, CH 3010 Berne, Switzerland

ABSTRACT. The block of organic iodine formation by excess iodide was reinvestigated, paying particular attention to the intrathyroidal iodide concentration, to the role of TSH and to changes in physicochemical properties of thyroglobulin. Maximal TSH stimulation was obtained by pretreating rats for 4 weeks with propylthiouracil followed by 2 days of a low-iodine diet. Three mg iodide was then injected every 12 hr and the thyroid glands were analyzed at daily intervals. The initially very high intrathyroidal iodide concentration decreased rapidly during the first 4 days of excess iodide, irrespective of whether TSH was high or suppressed by thyroxine injections. Thus TSH played only a minor part in the adaptation of the active iodide transport mechanism. Organic iodine formation was blocked during the first 4 days of iodide treatment. As soon as the intrathyroidal iodide fell below 0.1 μg per mg tissue, organic iodination resumed in TSH-stimulated glands. In animals with low TSH the escape from the block was delayed for another day until the intrathyroidal iodide fell below 0.05 μg/mg. Thus TSH diminished the blocking effect of a given intrathyroidal iodide concentration.

Under high doses of iodide the glands reaccumulated thyroglobulin. Its iodine content was initially low, but rose to a normal value of 0.6% after 6 days of iodide treatment. Despite this normal iodine content the distribution of iodoamino acids was abnormal, more monoiodotyrosine and less diiodotyrosine and thyroxine being present than in normal rat thyroglobulin of identical iodine content. Moreover sedimentation in sucrose gradient ultracentrifugation was slightly slower than that of comparable normal rat thyroglobulin. (*Endocrinology* **95:** 388, 1974)

IN addition to the well-known phenomena of iodide goiter and iodide myxedema (4) several conditions have recently been described where the human thyroid gland is unusually susceptile to pharmacological doses of iodide (5,6). This has led to renewed interest in the blocking effect of iodide on thyroid hormone biosynthesis as originally described by Morton, Chaikoff and Rosenfeld (7) and by Wolff and Chaikoff (8).[2] Many features of the action of iodide have been well studied and the results have been exhaustively reviewed by Wolff (4,9).

Although it has been reported recently that 1 to 3 mM iodide inhibited thyroglobulin iodination catalyzed *in vitro* by a purified thyroid peroxidase (10,11), it was doubted whether the necessary high iodide concentration could ever be reached *in vivo* (10), and thus the mechanism of the Wolff-Chaikoff effect remained obscure. Estimates of the inhibitory intrathyroidal iodide concentrations have been made *in vivo* (12,13,14), but exact measurements have not been reported. We have therefore reinvestigated the problem with the particular aim to measure as exactly as possible the inhibitory intrathyroidal iodide concentration *in vivo*. In addition we were interested in the effect of TSH on the block of iodine organification, and finally we wanted to study the causes of the high MIT/DIT ratio found during the escape period of the block (15–19).

The results show that iodine organification is blocked at intrathyroidal iodide

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[1] Part of this work has been published in preliminary and abstract form (1,2,3).

[2] We shall use the term Wolff-Chaikoff effect to describe the inhibition by iodide of organic iodination. Other inhibitory effects of iodide, such as the block of hormone release from stored thyroglobulin, are not included in this definition.

concentrations which are of the same order of magnitude as those which block thyroid peroxidase *in vitro*. Moreover, we found that, contrary to general belief, TSH diminished the blocking effect of a given intrathyroidal iodide concentration. Thirdly, we observed that the thyroglobulin synthesized in the presence of high amounts of iodide during the escape period has an abnormal iodoamino acid distribution and sedimentation behavior, which both cannot be explained by a low-iodine content of the protein.

Materials and Methods

Experimental protocol

Wistar rats of 180 to 250 g were pretreated for 4 weeks with a diet containing 0.15% propylthiouracil (PTU). Two days before the experiment started the food was changed to a low iodine diet without PTU (less than 0.1 μg iodine per g food) in most cases. In some experiments the change to the PTU-free low-iodine food was made only with the beginning of the iodide injections. This pretreatment produced intensely stimulated hyperplastic goiters that were depleted of thyroglobulin and iodine. It allowed a convenient assessment of iodine content by isotope equilibration as described below. 3 mg iodide (as KI or NaI) labeled with 40 μCi of ^{125}I were then injected ip every 12 hr during periods specified in the results. TSH secretion was suppressed in some experiments by the injection of 9 μg L-thyroxine every 12 hr. That this dose of thyroxine was adequate was established by a McKenzie type TSH bioassay (20) in pooled sera, which had been dialyzed to remove the excess iodide. Unmeasurably low TSH levels were found in the thyroxine-treated rats, while in the untreated rats TSH remained very high (400% to 500% stimulation over baseline) throughout 7 days of excess iodide administration. In all experiments rats were killed 6 hr after the last iodide injection.

Control rats were similarly pretreated but instead of injections of pharmacological doses received a normal amount of iodine by feeding the low-iodine diet and by adding NaI to the drinking water (3 μg ^{127}I and 0.3 μCi ^{125}I per ml). The average water consumption was 8 ml per day, corresponding to 24 μg of iodine. The iodine consumed in the food, approximately 0.8 μg per day, was neglected in the calculations.

Thyroid homogenates and serum iodide

At the conclusion of the experiment the rats were anesthetized with ether and exsanguinated by aortic puncture. The thyroid gland was removed, weighed, and one lobe was homogenized in 1 ml 0.06M phosphate buffer, pH 8.2, containing 0.01M thiouracil, with an all glass homogenizer. The other lobe was used for isolation of thyroglobulin or sometimes for histological examination to rule out iodide-induced thyroiditis. 1 mg pancreatin and a drop of toluol were added and the homogenate was digested for 18 hr at 37 C. 20 to 100 μl of the digest was applied on Whatman No 1 paper with appropriate carrier substances and chromatographed in butanol-acetic acid-water (300:60:240). The iodoamino acid spots were visualized in ultraviolet light and iodide was identified by spraying with palladium chloride. The spots were cut out and counted in a well type gamma counter, and the distribution of iodoamino acids and iodide were calculated. Since it had been reported that in normal thyroid glands the iodide concentration determined by paper chromatography was spuriously high (21), this method was checked by paper electrophoresis of the undigested homogenate and also by precipitation of 0.05 ml homogenate in 0.5 ml 10% trichloroacetic acid. In the glands treated with high doses of iodide all three methods gave measurements within 10% of each other and paper chromatography was therefore considered satisfactory. Serum iodide was determined by adding 0.2 ml of serum to 1 ml of 20% trichloroacetic acid and counting the radioactivity in the supernatant.

Sucrose gradient ultracentrifugation

For the isolation of thyroglobulin 5 to 15 thyroid lobes were homogenized with an all glass homogenizer in 1 or 1.5 ml of 0.1M KCl, 0.01M phosphate buffer pH 7.4. A trace amount of a thyroid homogenate from a normal rat injected 2 days previously with ^{131}I was added as a marker. The homogenate was centrifuged at 100,000 $\times$ g for 1 hr and the supernatant was dialyzed for 3 hr against the above buffer. 0.3 ml were applied on top of an 8 ml gradient of 5 to 20% sucrose in the same buffer and spun at 4 C for 15 to 17 hr at 30,000 rpm in a swinging

bucket rotor (model B-60, rotor SB 283, International Equipment Co). Fractions of either 3 or 5 drops were collected and the radioactivity and the optical density at 280 nm were measured. Sedimentation coefficients were estimated as suggested by Inoue and Taurog (22), assigning a value of 19.0 S to the ^{131}I-labeled normal marker thyroglobulin and assuming linear migration (23). The fractions containing the 19 S thyroglobulin peak were pooled and the radioactivity and the optical density in the pool were measured. The thyroglobulin pools were dialyzed 3 hr in 0.008M phosphate buffer pH 8.2 and then lyophilized, 0.2 mg sucrose being added as an adhesive to prevent losses of protein. The dried material was dissolved in 0.2 ml water containing 2 mmoles thiouracil and 1 mg pronase (Calbiochem Co). Chromatography was done as for the digests of the homogenates, using an additional solvent system with butanol-ethanol-2N ammonia (5:1:2) for separation of T_3 and T_4.

Protein determination

The extinction coefficient $E_{1\,cm}^{1\%}$ at 280 nm of 10.4 was obtained in a sample of rat thyroglobulin purified on Sephadex G-200 (24) and rendered salt-free by dialysis. Assuming a nitrogen content of 14.8% for thyroglobulin the protein content of lyophilized aliquots was calculated from the measurement of nitrogen and water contents.

Iodine determinations

^{127}I concentrations were calculated from the ^{125}I measurements, using the specific activity of the injected or ingested tracer. It was assumed that after the PTU pretreatment the ^{127}I content of the rat was negligible compared to the amounts injected or fed in the drinking water. In 5 excess iodide and in 2 control thyroglobulins the ^{127}I was also checked by chemical measurement according to Stolc and Knopp[3] (25). The chemical method gave values which were on the average 20% lower than the isotope method, which was thought to be within the error limits of the methods.

Results

The thyroid glands were highly stimulated by the pretreatment with PTU and the low-iodine diet. Six hr after the first injection of iodide they still had a tissue to serum (T/S) ratio for iodide of 50. After 7 days of iodide injections it had fallen to 2.2 in the rats with high TSH and to 0.9 in the rats with suppressed TSH (not shown). The high initial T/S ratio was reflected in the extremely high intrathyroidal iodide concentration of 0.75 μg per mg thyroid tissue (Fig. 1). During the first days the intrathyroidal iodide decreased in a very similar way in both the group with low and the group with high circulating TSH. From day 4 onward, however, the iodide was slightly but significantly lower in the rats with suppressed TSH (0.018 ± 0.002 *vs* 0.040 ± 0.003 μg/mg at day 7).

Organic iodine formation, low or absent at high intrathyroidal iodide, started at a rapid rate when the intrathyroidal iodide fell below 0.1 μg/mg (0.8 mmole/kg) in the case of TSH-stimulated glands (Fig. 1, bottom). In the absence of TSH organic iodination was delayed for another 1 or 2 days until the iodide concentration had reached about 0.05 μg/mg (0.4 mmole/kg). The rate of organic iodine accumulation remained much lower in the absence of TSH. The two critical iodide levels (0.1 and 0.05 μg/mg) where organic iodination resumed were consistently observed in a whole series of experiments.

Figure 2 gives the rates of organic iodine formation as a function of the intrathyroidal iodide concentration calculated from the experiments in Fig. 1. This display again shows that organic iodination is very low at an iodide concentration of 1 mmole per kg tissue and that it resumes at an intrathyroidal iodide of about 0.8 mmole/kg (0.1 μg/mg).

After the 4 weeks of PTU pretreatment the thyroglobulin content of the glands had fallen to 2.2 ± 0.2 μg/mg tissue, which is less than one-tenth of the content of normal rat thyroid glands. Eight days after stopping the PTU and 6 days after the start of the normal or excess iodide feeding the thyroglobulin had again risen to 17 μg/mg and 13 μg/mg respectively. Figure 3 (top)

[3] These measurements were kindly performed by Dr. J. Knopp, Bratislava.

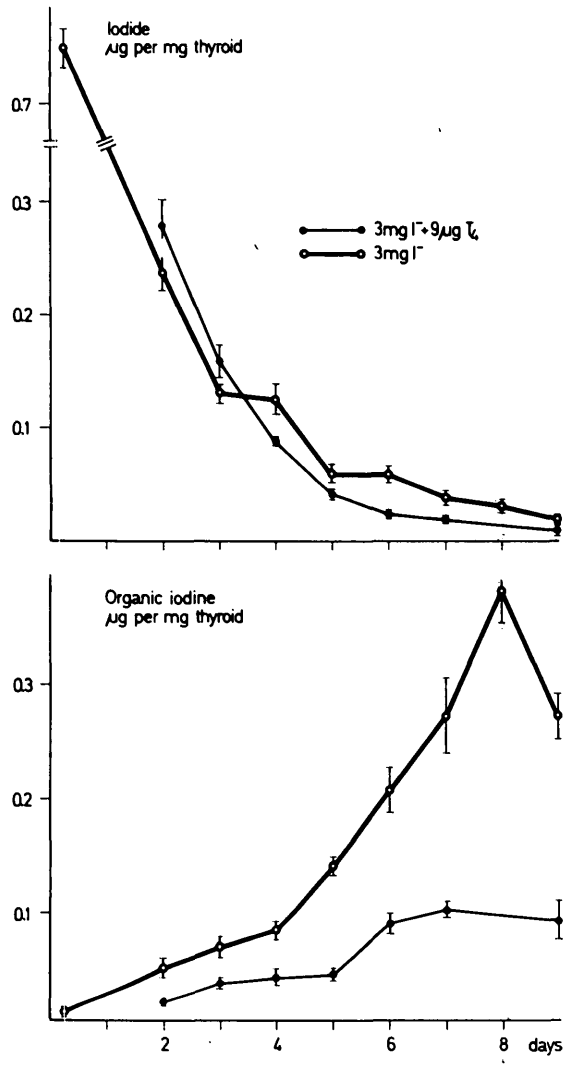

FIG. 1. Iodide (upper panel) and organic iodine (lower panel) content of thyroid glands. Animals were pretreated with PTU for 4 weeks and switched to a low-iodine diet without PTU 2 days before the iodide injections were started. 3 mg iodide without (o — o) or with (• — •) 9 µg L-thyroxine were injected ip every 12 hr, starting on day 1. The figure represents a composite graph of 5 identical experiments in which only the duration of treatment was varied. Each point represents the mean and standard error of at least 10 animals, except at 6 hours and at days 2, 7 and 9 (5 animals each). The difference of the intrathyroidal iodide between the two groups was significant from day 4 onward ($p < 0.025$ or better in t test).

shows that the iodine content of the thyroglobulin was very low at the end of the PTU pretreatment. It then rose, incidentally at almost the same rate in both the control

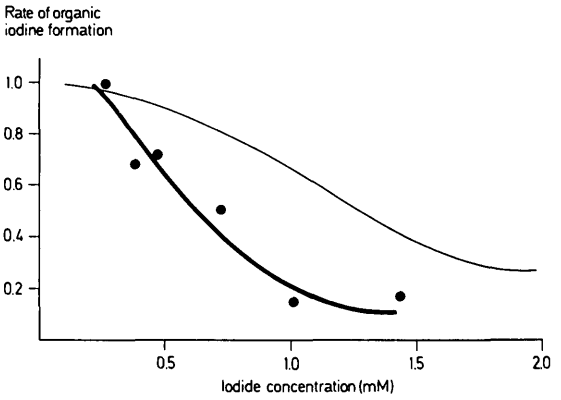

FIG. 2. Rate of organic iodine formation correlated with the intrathyroidal iodide concentration (abscissa). From the data of Fig. 1 the mean iodide concentration for each daily interval between days 2 and 8 and the corresponding rates of organic iodine formation were calculated and plotted as dots. The curve was fitted by hand. The thin line gives for comparison the inhibition of iodination catalyzed *in vitro* by purified thyroid peroxidase as reported by Pommier *et al.* (11). The units on the ordinate are arbitrary with the highest measurement being assigned a value of 1.

and excess iodide group, to reach a near normal level between 0.7% and 0.8% after 6 days.

In the rats receiving a normal amount of iodide the MIT/DIT ratio[4] of the thyroid homogenates had a normal value below unity when the iodine content of thyroglobulin was higher than 0.2%. In contrast the MIT/DIT ratio remained grossly elevated in the excess iodide rats, despite the fact that the degree of thyroglobulin iodination became normal after 3 days (Fig. 3, bottom).

The thyroglobulins of varying iodine contents produced during the escape period of the Wolff-Chaikoff effect (days 2 to 10) were compared to thyroglobulins of rats which were refed a normal amount of iodide. Figure 4 clearly shows that for any given iodine content the excess iodide thyroglobulins contained more MIT and less DIT and less T_4 than the control thyroglobulins, while the T_3 content was identical.

[4] This ratio refers to the amount of iodine found in MIT and DIT and not to the number of amino acyl residues.

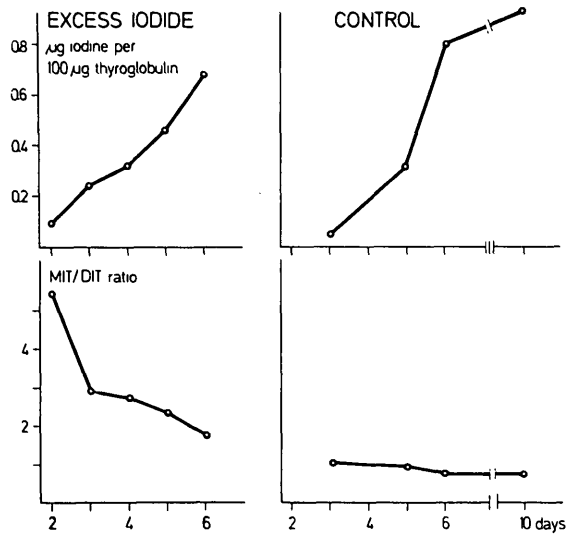

FIG. 3. Degree of thyroglobulin iodination (upper panels) and MIT/DIT ratios of thyroid homogenates (lower panels) from rats pretreated as in Fig. 1. Starting on day 1 the excess iodide rats received 3 mg iodide intraperitoneally every 12 hr (left), while the control rats were given 3 μg iodide per ml drinking water (right). Each point was obtained from a homogenate of a pool of 5 rat thyroid glands.

Figure 5 gives the results of the sucrose gradient ultracentrifugations. Thyroglobulin synthesized under the influence of excess iodide had a clear cut peak in the 19 S region. However, the peak of this thyroglobulin was displaced by 1 fraction toward the top of the gradient when compared to the normal marker thyroglobulin which was co-centrifuged in the same tube. By contrast, the thyroglobulin of identical iodine content synthesized under a normal iodide intake gave a peak which was superimposable to that of the marker.

Figure 6 shows that the sedimentation constant of excess iodide thyroglobulin increased with rising iodine content. However, only one single sampled reached a value of 19.0 S.

Discussion

During the first 4 days of excess iodide feeding the accumulation of organic iodine was slow (Fig. 1). It is unlikely that this was due to a continuing effect of PTU, since PTU was withheld two days before starting the iodide injections and since organic iodine formation is known to resume one day after PTU withdrawal (26). The fact that the little thyroglobulin present was iodinated initially to less than 0.1% (Fig. 3) rules out the possibility that iodination was blocked because thyroglobulin was present in rate-limiting amounts (27). We conclude that a real Wolff-Chaikoff effect was present during the first 4 days of iodide administration. Once the glands had "escaped," organic iodine accumulation reached values of 0.05 to 0.1 μg per mg thyroid weight per day, which compares well with maximal rates of 0.14 μg per mg per day found in normal rats on a high normal iodine intake (18).

The basic mechanism of the adaptation to excess iodide is a lowering of the intrathyroidal iodide concentration. Since this occurred when organic iodination was still virtually nil (Fig. 1), it must have been due to a decreased influx of iodide via the active transport mechanism, as previously suggested by others (14–19, 28).

Our results allow some important conclusions on the controversial role of TSH in this adaptation. While some (14,29) reported that TSH enhanced the block of iodination produced by excess iodide, others (19,30,31) thought that the reverse was true. Figure 1 shows that during the first 4 days the intrathyroidal iodide fell almost identically in both the rats with high and those with low circulating TSH. This is surprising since TSH is usually considered the major controlling factor of active iodide transport (32,33). It suggests that autoregulatory mechanisms (34) must have prevailed in the *early* phase of the adaptation. On the other hand the lower iodide concentration after the 4th day in the thyroxine-treated rats (Fig. 1) shows that TSH had some definite effect on iodide transport in the *late* phase of the adaptation, which is in keeping with Braverman and Ingbar (16). Organic iodine formation resumed clearly at a higher intrathyroidal iodide concentration in the TSH-stimulated glands (Fig. 1). Thus,

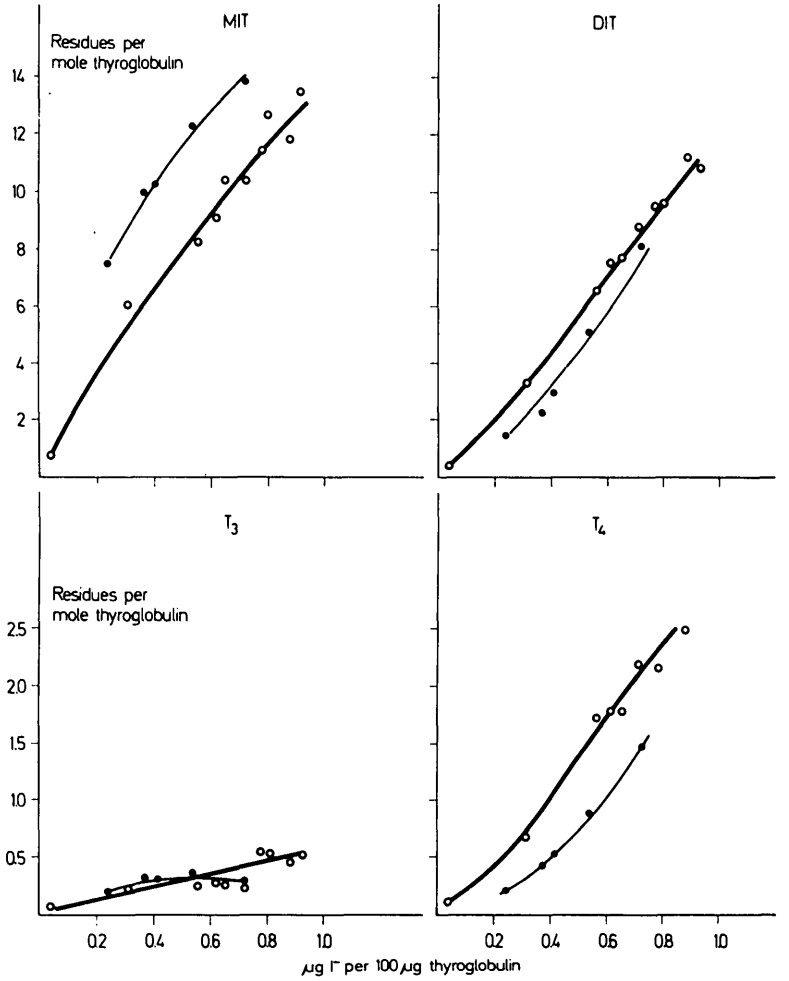

FIG. 4. Relation between iodine content and number of iodoamino acid residues of thyroglobulin synthetized under iodide excess (• — •) and under normal iodine intake (o — o). The thyroglobulins were obtained from animals treated similarly as in Fig. 3. The different iodine contents were achieved by varying the treatment period from 3 to 14 days.

though TSH did not influence much the adaptive decrease of the iodide pump, it clearly diminished the blocking effect of a given intrathyroidal iodide concentration.

Our results also explain why it is virtually impossible to re-induce a block by raising the iodide dose, once the glands have "escaped" (16). The active iodide transport is so efficiently shut off, that the dose of iodide needed to reach a blocking intrathyroidal concentration would be enormous.

Wolff (4,9) has discussed a number of biochemical mechanisms which could explain the paradoxical iodide effect. Recently Pommier *et al.* (35) have postulated that the enzyme responsible for iodination, thyroid peroxidase, has two substrate binding sites, one for iodide and the other for the acceptor molecule tyrosine. At high iodide concentrations the latter binding site is occupied by iodide instead of tyrosine, and the iodination of tyrosine is inhibited. Figure 2 shows that in our experiments in vivo the Wolff-Chaikoff effect occurred at intrathyroidal iodide concentrations which were about two times lower than the concentrations which were inhibitory *in vitro* (11). The distribution of iodide in thyroid tissue is probably not uniform (36) and the concentration at the iodinating site may well be twice the mean tissue concentration. Under this reasonable assumption the inhibitory iodide concentration becomes identical *in vivo* and *in vitro*. Whatever the exact chemical mechanism, our results would thus be in good agreement with those of Pommier (35).

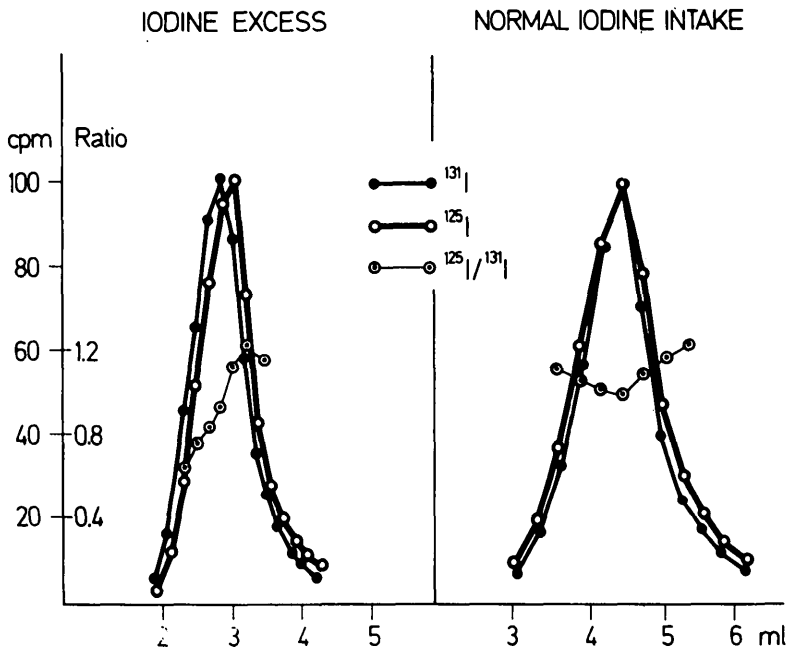

FIG. 5. Sucrose gradient ultracentrifugation of thyroglobulin synthetized under iodide excess (left) and under normal iodide intake (right). The thyroglobulins were obtained as outlined in Fig. 3. Both thyroglobulins were equilibrium labeled with ^{125}I. Their iodine content was 0.72 and 0.69% respectively. A tracer amount of normal ^{131}I-labeled rat thyroglobulin had been added to the homogenates before centrifugation. Sedimentation was from right to left and the abscissa gives the volume of the gradients eluted from bottom to top. The cpm for ^{131}I and ^{125}I were normalized, assigning a value of 100 to the highest fraction. The two thyroglobulins were centrifuged in different runs and the elution volumes are therefore not entirely comparable.

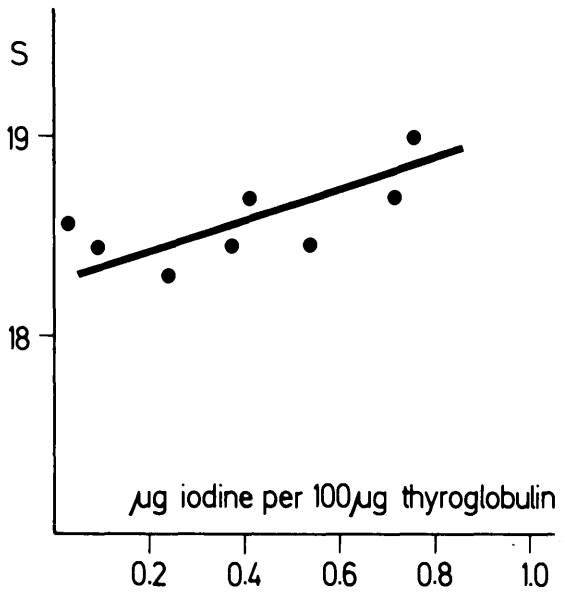

FIG. 6. Sedimentation coefficients in relation to iodine contents of thyroglobulins synthesized under iodide excess. The thyroglobulins were obtained in experiments as outlined in Fig. 3. The sedimentation coefficients were calculated from sucrose gradient ultracentrifugations, assigning a value of 19.0 S for normal marker thyroglobulin. The correlation coefficient was 0.80 ($p < 0.05$).

Pharmacological doses of iodide produce abnormally high MIT/DIT ratios, during the "escape" phase (15–19). Such an abnormal distribution of organic iodine could *a priori* be the result of a low-iodine content of thyroglobulin, since it is known that with increasing iodination of the protein the relative proportion of MIT decreases and that of DIT and T_4 increases (37–40). Indeed a low degree of iodination accounted entirely for the high MIT/DIT ratios found in thyroglobulin from euthyroid human goiters (39). It was therefore important to establish whether thyroglobulin synthetized under iodine excess also complied to this rule, *i.e.*, whether the high MIT/DIT ratios were entirely explained by a low-iodine content. Figure 4 shows clearly that the high MIT/DIT ratio is not the consequence of a low degree of iodination of thyroglobulin. Rather, the iodoamino acid distribution at each given iodine content is altered. Such a clear-cut deviation from the above mentioned rule has to our knowledge not been reported so

far in any other thyroid condition, except to some degree for the thyroglobulin from patients with drug-treated Graves' disease (41). Our results indicate that the formation of DIT is truly more inhibited than that of MIT by high doses of iodide, while the synthesis of T_4 from DIT does not appear to be particularly affected since the DIT/T_4 ratios are within the normal range. This result is in sharp contrast with a finding of Chevillard *et al.* (42), who observed that excess iodide in rats inhibited the biosynthesis of T_4, but not that of DIT. The discrepancy may be due to the fact that in their experiments rats received excess iodide *chronically* over 4 months.

On sucrose gradient centrifugation the sedimentation coefficient of Wolff-Chaikoff thyroglobulin increased with the degree of iodination. However, even at an iodine content of 0.7% the sedimentation was slightly slower than that of normal thyroglobulin (Figs. 5 and 6). The estimation of the sedimentation coefficient by the method used carries some error when the analyzed protein peak is displaced from the marker by no more than one or two fractions. Nonetheless we think that the differences are real, because the isotope ratio (^{125}I for the experimental and ^{131}I for the marker thyroglobulin) systematically rises from bottom to top of the gradient (Fig. 5) which rules out a chance displacement of the peak due to an erroneous ^{125}I measurement in a single fraction. Moreover, the marker was centrifuged in the same tube and the differences were reproducible in many experiments (Fig. 6). The abnormality in sedimentation behavior of the Wolff-Chaikoff thyroglobulins most probably reflects some conformational change leading to more molecular asymetry in the protein. Inoue and Taurog (22) found much lower sedimentation coefficients of around 15 S for poorly iodinated rat thyroglobulins. This low value compared to ours is probably due to the low ionic strength of their sucrose solution, a condition now recognized to produce conformational changes in thyroglobulin of low-iodine content (43).

Very similar changes in the iodoamino acid distribution and sedimentation behavior of thyroglobulin from humans exposed to dietary iodine excess have been observed.[5] These results in man lend further support to our findings in the rat.

Acknowledgments

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References

1. Bürgi H., A. Radvila, and H. Studer, *Rev Fr Endocrinol Clin* **11**: 419, 1970.
2. ———, ———, H. Kohler, and H. Studer, *Europ J Clin Invest* **1**: 365, 1971 (Abstract).
3. ———, ———, ———, and ———, Program 5th Annual Meeting European Thyroid Association, Jerusalem, 1973 (Abstract).
4. Wolff J., *Am J Med* **47**: 101, 1969.
5. Braverman L. E., K. A. Woeber, and S. H. Ingbar, *N Engl J Med* **281**: 816, 1969.
6. ———, S. H. Ingbar, A. G. Vagenakis, L. Adams, and F. Maloof, *J Clin Endocrinol Metab* **32**: 515, 1971.
7. Morton M. E., I. L. Chaikoff, and S. Rosenfeld, *J Biol Chem* **154**: 381, 1944.
8. Wolff J., and I. L. Chaikoff, *J Biol Chem* **172**: 855, 1948.
9. ———, *In* Decourt, J., and Gilbert-Dreyfus (eds.), Actualités Endocrinologiques, vol. 11, Expansion, Paris, 1970, p. 7.
10. Taurog A., *Arch Biochem Biophys* **139**: 212, 1970.
11. Pommier J., D. Deme, and J. Nunez, *Europ J Biochem* **37**: 406, 1973.
12. Wolff J., and I. L. Chaikoff, *J Biol Chem* **174**: 555, 1948.
13. ———, and ———, *Endocrinology* **42**: 468, 1948.
14. Raben M. S., *Endocrinology* **45**: 296, 1949.
15. Galton V., and R. Pitt-Rivers, *Endocrinology* **64**: 835, 1959.
16. Braverman L. E., and S. H. Ingbar, *J Clin Invest* **42**: 1216, 1963.

[5] Camus M., and A. M. Ermans, personal communication.

17. Nagataki S., and S. H. Ingbar, *Endocrinology* **74:** 731, 1964.
18. ———, K. Shizume, and K. Nakao, *Endocrinology* **79:** 667, 1966.
19. Greer M. A., and C. F. Allen, *Endocrinology* **90:** 915, 1972.
20. Iff H. W., A. Burger, H. Studer, and F. Wyss, *Am J Physiol* **213:** 250, 1967.
21. Nagataki S., and S. H. Ingbar, *Endocrinology* **72:** 480, 1963.
22. Inoue K., and A. Taurog, *Endocrinology* **83:** 816, 1968.
23. Martin R. G., and B. N. Ames, *J Biol Chem* **236:** 1372, 1961.
24. Salvatore G., M. Salvatore, H. J. Cahnmann and J. Robbins, *J Biol Chem* **239:** 3267, 1964.
25. Stolc V., and J. Knopp, *Mikrochim Acta* **5–6:** 941, 1963.
26. Studer H., and M. A. Greer, *Endocrinology* **80:** 52, 1967.
27. Nunez J., *In* Decourt, J., and Gilbert-Dreyfus (eds.), Actualités Endocrinologiques, vol. 11, Expansion, Paris 1970, p. 16.
28. Wolff J., I. L. Chaikoff, R. C. Goldberg, and J. R. Meier, *Endocrinology* **45:** 504, 1949.
29. Katakai S., T. Yamada, and K. Shichijo, *Metabolism* **15:** 271, 1966.
30. Studer H., and M. A. Greer, The Regulation of Thyroid Function in Iodine Deficiency, H. Huber, Berne and Stuttgart, 1968, p. 31.
31. Rosenfeld P. S., and I. N. Rosenberg, *Endocrinology* **78:** 621, 1966.
32. Halmi N. S., *Vitam Horm* **19:** 133, 1961.
33. Wolff J., *Physiol Rev* **44:** 45, 1964.
34. Studer H., H. Kohler, and H. Bürgi, *In* Solomon, D. H., and M. A. Greer (eds.), The Thyroid, Handbook Series of the American Physiological Society, Williams and Wilkins Co., Baltimore, 1974 (In press).
35. Pommier J., J. De Prailauné, and J. Nunez, *Biochimie* **54:** 483, 1972.
36. Andros G., and S. H. Wollman, *Am J Physiol* **213:** 198, 1967.
37. Edelhoch H., *J Biol Chem* **237:** 2778, 1962.
38. De Crombrugghe B., H. Edelhoch, C. Beckers, and M. DeVisscher, *J Biol Chem* **242:** 5681, 1967.
39. Ermans A. M., J. Kinthaert, and M. Camus, *J Clin Endocrinol Metab* **28:** 1307, 1968.
40. Cavalieri R. R., G. L. Searle, and L. L. Rosenberg, *Endocrinology* **86:** 10, 1970.
41. Rolland M., M.-F. Montfort, L. Valenta, and S. Lissitzky, *Clin Chim Acta* **39:** 95, 1972.
42. Chevillard L., J.-M. Gavaret, M.-F. Julien, and J. Nunez, *C R Acad Sci* [D] *(Paris)* **274:** 2782, 1972.
43. Valenta L., and S. Lissitzky, *Biochim Biophys Acta* **236:** 376, 1971.