THE CHEMICAL ASSAY OF BIOLOGICALLY ACTIVE IODINATED PROTEINS: ISOLATION OF THYROXINE

by

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INTRODUCTION

According to HARINGTON AND RANDALL[1], the thyroid gland contains only two iodinated compounds; thyroxine and diiodotyrosine. Thyroxine, which is the active principle of the gland, can be separated from diiodotyrosine by virtue of its insolubility in acid solution. This is the basis of the chemical method of thyroid assay (HARINGTON AND RANDALL[2]) in which thyroid substance is hydrolysed with alkali, the thyroxine fraction of the hydrolysate is precipitated with acid and the iodine of the precipitate determined either directly or indirectly (total iodine minus acid-soluble iodine gives acid-insoluble or thyroxine iodine). The technique of the method has been criticized by several workers mainly on the grounds that hydrolysis is inadequate and that diiodotyrosine iodine is therefore included in the acid-insoluble fraction. Nor is the method always accepted on theoretical grounds: the relationship between biological activity and acid-insoluble iodine was questioned by GADDUM AND HETHERINGTON[3]; these authors believed that the activity of the gland was related to the total rather than the thyroxine iodine. However, TAUROG AND CHAIKOFF'S[4] recent evidence in favour of thyroxine being the circulating thyroid hormone indicates that the thyroxine content of any preparation is likely to be the best index of its activity.

HARINGTON AND RANDALL'S[2] method of chemical assay cannot be used with biologically active iodinated proteins, first prepared by LUDWIG AND VON MUTZENBECHER[5] in 1939 and since then extensively studied by other workers. During iodination, acid-insoluble iodine-containing products other than thyroxine are formed, so that the acid-insoluble iodine content is no longer a guide to the thyroxine content of the proteins. This was amply demonstrated when a number of iodinated protein preparations were made from casein, "Ardein" (ground-nut protein), and ox-plasma by PITT-RIVERS AND RANDALL[6] for experiments on the milk yield response in cows (BLAXTER[7]). Preliminary chemical assays by the method of HARINGTON AND RANDALL[2] were done in the expectation that products with high acid-insoluble iodine contents would show high physiological activity. The chemical assay however, was no guide to activity, and in later experiments on the effect of iodinated proteins on the metamorphosis of *Xenopus* tadpoles, DEANESLY AND PARKES[8] confirmed these findings, that the acid-insoluble iodine content of many proteins tested (prepared by different workers) could not be correlated with their activity; these authors however found that biological response as measured on tadpoles was quantitatively related to the response in cows. Later PITT-

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RIVERS[9] showed that two iodinated proteins with high acid-insoluble iodine values and low activity yielded only minute amounts of thyroxine after alkaline hydrolysis.

Evidence is brought forward from the experiments described below that the activity of artificially iodinated proteins is proportional to their thyroxine content. A simple method for thyroxine isolation is described.

EXPERIMENTAL

For these experiments, six iodinated proteins were chosen with different biological activities. One of them (casein C4 + 5) was according to DEANESLY AND PARKES[8] slightly more active than thyroid powder when administered in an equal dose; another (casein NCB 3/62) had an activity very similar to that of thyroid powder. The iodinated proteins used include casein and "Ardein" preparations and one plasma preparation.

Hydrolysis and isolation

The iodinated proteins (30–35 g) were boiled under reflux in an electric heating mantle for 20 hours with 200 ml water and 100 g hydrated baryta. (These quantities were all doubled for the hydrolysis of Plasma N4 as the expected amount of thyroxine was small). The precipitated barium salts were separated and decomposed with 1% NaOH and Na_2SO_4 according to the method of HARINGTON[10]. A small crop of acid-insoluble material was also obtained from the baryta filtrate and was combined with the product from the barium salt. After drying, this material was extracted twice with ether and dissolved in the minimum amount of boiling 0.1 N sodium carbonate solution and filtered. The sodium salt of thyroxine was allowed to separate in the ice-chest during 72 hours after which it was collected by centrifuging; the salt was dissolved in the same volume of boiling 0.1 N sodium carbonate solution and acidified with acetic acid (c.f. TAUROG AND CHAIKOFF[4]). The crystalline thyroxine obtained was collected, washed with water and dried *in vacuo*. Melting points of the samples of thyroxine obtained varied between 228° and 235° (decomp.). One of the iodinated proteins "Ardein" N4SF) was also hydrolysed with sodium hydroxide and the thyroxine isolated by n-butanol extraction according to LELAND AND FORSTER[11]. The results of these experiments are summarized in the last column of Table I; other data on the iodinated proteins investigated are taken from DEANESLY AND PARKES's[8] paper. The relationship between the biological activity of iodinated proteins and their thyroxine content as determined by isolation is shown in Fig. 1.

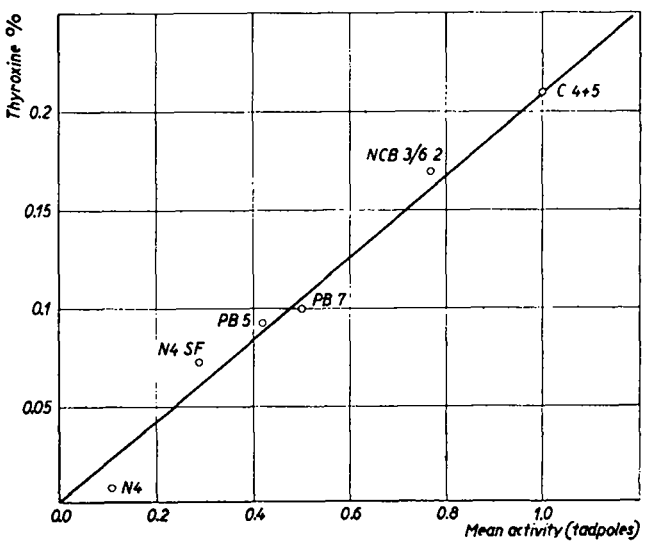

Fig. 1. Relationship between the thyroxine content by isolation and biological activity of iodinated proteins

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TABLE I

Iodinated Protein	Iodine %		Mean biological activity (tadpoles)	Thyroxine % by isolation
	Total	Acid-insoluble		
Casein C 4 + 5	8.1	1.6	1.0	0.21
Casein NCB 3/62	8.02	1.46	0.77	0.17
"Ardein" PB7(DT/S/824)	7.2	1.2	0.50	0.10
"Ardein" PB5(DT/S/822)	7.3	1.3	0.42	0.094
"Ardein" N4SF	3.61	0.50	0.29	0.074 0.071*
Plasma N_4	5.4	0.4	0.11	0.007

* Duplicate isolation by LELAND AND FOSTER's[11] method

DISCUSSION

The above findings indicate that the biological activity of iodinated proteins is directly proportional to their thyroxine content when they are compared with each other. Of greater interest is the consideration whether the activity of such iodinated proteins can be compared with the activity of thyroid powder, the comparison being based on the thyroxine content of equivalent doses. Now DEANESLY AND PARKES[8] emphasized that the most active iodinated protein tested by them with 1–2% acid-insoluble iodine had only a slightly greater activity than commercial thyroid powder with 0.1% acid-insoluble iodine. On an acid-insoluble iodine basis therefore the artificially iodinated proteins are 10–20 times less active than thyroid powder. If however one considers the amount of thyroxine administered in equal doses of iodinated protein and thyroid powder then the biological responses to these doses can be explained in terms of thyroxine content only. For instance, the iodinated protein investigated in the present work whose activity was most similar to that of thyroid powder was the casein preparation NCB 3/62, and was found to contain 0.17% of thyroxine by isolation; thyroid preparations prepared according to the British Pharmacopoeia are standardized to contain approximately 0.15% thyroxine (0.099 to 0.11% thyroxine iodine). The dose of active principle is of the same order in both cases. It is therefore suggested that the thyroxine content of an artificially iodinated protein determined by isolation will give the most reliable index of its biological activity.

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SUMMARY

1. Crystalline thyroxine has been isolated from six iodinated proteins of known biological activity.

2. The amount of thyroxine isolated was found to be proportional to the biological activity of the proteins.

3. On the basis of thyroxine content, the activity of the iodinated proteins may be compared with that of thyroid powder.

RÉSUMÉ

1. La thyroxine a été isolée de six protéines iodées dont l'activité biologique est connue.

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2. La quantité de thyroxine isolée est proportionnelle à l'activité biologique des protéines.
3. L'activité biologique de ces protéines peut être comparée à celle de la poudre de thyroïde à l'égard de leur teneur en thyroxine.

ZUSAMMENFASSUNG

1. Kristallisiertes Thyroxin wurde aus sechs Jodoproteinen bekannter biologischer Aktivität isoliert.
2. Die isolierte Thyroxinmenge ist der biologischen Aktivität der Proteine proportional.
3. Die Aktivität der Jodoproteine kann auf Grund des Thyroxingehaltes mit derjenigen von Thyroidpulver verglichen werden.

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