THE METABOLIC EFFECTS OF HUMAN THYROGLOBULIN AND ITS PROTEOLYTIC CLEAVAGE PRODUCTS

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In previous communications, (1, 2, 3, 4, 5) it has been pointed out that two important questions with regard to the nature of thyroid colloid must be answered because they bear upon the normal and pathological physiology of the gland. The first of these is: Does the colloid of "toxic" glands show significant differences from normal or "non-toxic" colloid? The second question is: Does the activity of the thyroid secretion depend solely upon its thyroxin content? This paper includes both chemical analyses and biological assays so correlated as to bring out possible causal relationships to the metabolic effects produced. As explained elsewhere, (1) a routine standard procedure was adopted for administering thyroid material to patients with myxedema. The magnitude of the metabolic response could then be used as a gauge of the potency of the preparation or drug administered.

The thyroglobulins were prepared by extraction of freshly iced, surgically excised glands with 0.02 normal sodium hydroxide solution. The protein was subsequently precipitated isoelectrically; and then redissolved and reprecipitated repeatedly. The preparation of the di-iodotyrosine peptone and thyroxin peptone digests was accomplished as described by Harington and Salter (6). Undigested protein was eliminated by heat coagulation and by the acetone treatment described by these authors. Total iodine was estimated according to the procedure of Kendall (7). The thyroxin moiety was estimated by the procedure of Harington and Randall (8) and checked by the methods of Leland and Foster (9) or of Blau (10).

The response of patients to test fractions of thyroid material was judged by comparison with the standard curve of reference obtained by administering natural thyroxin polypeptide (1). When this substance was fed in solution in daily doses containing 0.5 mgm. iodine, the daily rise

in basal metabolic rate was found to average about 2.5 points (1). By this method the response of myxedematous patients could be calibrated when test preparations were administered. The reliability of this procedure has been discussed in a previous communication (5). A patient was never used for more than one assay, unless the first result was completely negative.

Effect of thyroglobulins upon basal metabolic rate

Colloid goiter. Two adult patients with typical spontaneous myxedema were treated with thyroglobulin prepared from human multiple colloid adenomatous goiters, excised surgically. The daily dose, by mouth, was 0.5 mgm. in terms of total iodine. The responses produced (recorded in Figure 1, Curve *A* and *B*) corresponded to the standard response obtained from an equivalent amount of thyroxin polypeptide, based upon the total iodine content. One cretin, aged twenty-one, previously untreated, was given 0.5 mgm. of iodine in the form of this same thyroglobulin. The result (shown in Figure 1, Curve *C*) was likewise of the same order of magnitude as the standard response. The average daily rise in basal metabolic rate was 2.8 points per day, as against the standard rise (1) of 2.5 points per day.

Chemical analysis by the method of Harington and Randall (8) showed 30 per cent of the iodine in this preparation to exist apparently as thyroxin. Nevertheless, the response indicates that 100 per cent of the iodine is active. This confirms work previously published to the effect that the activity of thyroid hormone is determined not by its thyroxin iodine, but by its total organic iodine.

Exophthalmic goiter. Three other patients were treated with thyroglobulin prepared from glands surgically excised from patients with hyperthyroidism, showing hyperplastic glands. Chemical analysis by the method of Harington and

FIG. 1.

Curves *A* and *B* represent the calorigenic responses of two patients with spontaneous myxedema who received daily oral doses of thyroglobulin (containing 0.5 mgm. of total iodine) derived from human, multiple colloid adenomatous goiters. Curve *C* represents the calorigenic response of a cretin to this same form of thyroglobulin. Curve *D* represents the calorigenic response of one patient with spontaneous myxedema to the daily oral administration of 0.5 mgm. of iodine in the form of the thyroglobulin obtained from a colloid gland removed in an endemic goiter region. The heavy solid line, present in all the charts, is the standard response to daily doses of 1.0 mgm. of natural thyroxin polypeptide (0.5 mgm. total iodine).

Randall (8) showed 25 per cent of the iodine in this preparation to exist apparently as thyroxin. By the method of Leland and Foster (9) the value was 20 per cent. Two of the patients had spontaneous myxedema; the third had cachexia strumipriva, complicated by rheumatic heart disease (without myocardial failure). The responses of the three patients insofar as average slope is concerned were altogether similar to those of patients receiving non-toxic colloid thyroglobulin, the average daily rise in metabolism being in the case of "toxic" thyroglobulin 2.9 points per day, and in that of "non-toxic" thyroglobulin 2.8 points per day. It is true that inspection of the three curves for "toxic" thyroglobulin shown in Figure 2 reveals a hump in each about the fifth to sixth day. We have observed similar humps

in isolated instances in the past and have not discovered that they have any significance.

In the interpretation of these results, it must be remembered that the source of thyroglobulin was tissue excised from patients who had received iodine therapy preliminary to operation.

Simple colloid goiter. In addition, to one patient with spontaneous myxedema there was given 0.5 mgm. of iodine daily in the form of thyroglobulin obtained from a large colloid gland removed from a patient living in an endemic goiter region.¹ As shown in Figure 1, Curve *D*, there followed no significant metabolic response. This material was found on chemical analysis to contain only 0.006 per cent of total iodine, and of this only 5 per cent (relative percentage) was apparently in the form of thyroxin by the analytical method of Harington and Randall (8). The

FIG. 2. THE RESPONSE OF THREE PATIENTS WITH MYXEDEMA, TWO SPONTANEOUS AND ONE CACHEXIA STRUMIPRIVA, TO THE DAILY ORAL ADMINISTRATION OF THYROGLOBULIN DERIVED FROM HUMAN HYPERPLASTIC ("TOXIC") GLANDS, EACH DOSE CONTAINING 0.5 MGm. TOTAL IODINE (LIGHT SOLID LINES AND SOLID CIRCLES).

The heavy solid line is the standard curve, as in Figure 1.

¹ We are indebted to Professor Frederick A. Collier of the University of Michigan for this material.

check value obtained by Blau's method (10) was 3 per cent.

Action of peptones of human thyroglobulin

When thyroglobulin is digested with pepsin under appropriate conditions, a considerable portion of the organically-bound iodine remains insoluble in dilute acid at pH 5.0 (6). This insoluble fraction is variable in amount, but often amounts to one-third of the total iodine. It consists partly in (a) undigested protein and partly in (b) peptones containing thyroxine as the chief source of iodine. Harington and Salter (6) found that after such isoelectric precipitation of the digest, the iodine-containing peptones which remained in solution yielded no thyroxine. This second iodopeptone was shown by Harington and Randall (11) to yield di-iodotyrosine².

Thyroxine peptone. Three patients with spontaneous myxedema were treated with thyroxine peptone, administered by mouth in daily doses containing 0.5 mgm. iodine. One of these failed to respond (Curve A, Figure 3), and in this case the material was administered suspended in water, but undissolved. In the other two cases, the peptone was dissolved in dilute alkali before administration, and both these patients made significant responses (Curve B and C, Figure 3), although in one of them (Curve B) it was somewhat sub-standard. In the latter case, however, subsequent daily administrations of thyroglobulin (from colloid adenomatous glands) for six days failed to produce any additional rise. The inference, therefore, is that this patient had obtained a maximal response, even though sub-standard. Of these two effective peptone preparations, one (Curve B, Figure 3) was made from multiple colloid, non-toxic adenomatous goiter, and the other (Curve C, Figure 3) from toxic diffusely hyperplastic goiter.

Di-iodotyrosine peptone. Three patients with spontaneous myxedema were treated with di-iodotyrosine peptone in daily oral doses containing 0.5 mgm. of iodine. None of these patients made

² The thyroxine-containing peptone can be partially purified by re-precipitation and by treatment with acid acetone (6). The di-iodotyrosine peptone can be essentially freed from inorganic iodide by concentration of the acid solution under reduced pressure.

FIG. 3. THE RESPONSE OF THREE PATIENTS WITH SPONTANEOUS MYXEDEMA TO THE ADMINISTRATION OF THYROXIN PEPTONE IN DAILY ORAL DOSES CONTAINING 0.5 MGm. TOTAL IODINE.

The patient represented by Curve A received undissolved material. Curve B represents the response to thyroxine peptone derived from non-toxic colloid adenomatous goiter and Curve C to peptone derived from hyperplastic "toxic" goiter. The heavy solid line is the same as in Figure 1.

an unequivocal response. All three patients subsequently made a good response to an active thyroid preparation. The conclusion is to be drawn, therefore, that thyroxine peptone possesses standard activity, but that di-iodotyrosine peptone in standard doses is inert.³

Effect of synthetic (racemic) glycyl-thyroxine

Through the kindness of Professor C. R. Harington, we were able to test the activity of a synthetic dipeptide made in his laboratory and described by Ashley (12). This material was administered intravenously to two patients with spontaneous myxedema in daily doses corre-

³ It is necessary to note, however, that in assays in two other cases, in which three and six times the standard iodine dosage (0.5 mgm.) of di-iodotyrosine peptone was given, definite responses did occur. These data will be the subject of a later communication.

FIG. 4. THE RESPONSE OF TWO PATIENTS WITH MYXEDEMA (LIGHT SOLID LINES AND SOLID CIRCLES) TO THE ADMINISTRATION OF SYNTHETIC GLYCYL-THYROXIN IN DAILY ORAL DOSES CONTAINING 0.5 MG. IODINE.

The heavy solid line is the same as in Figure 1.

sponding to 0.5 mgm. iodine. The responses are shown in Figure 4. These curves follow closely the standard curve of reference. The average daily rise in basal metabolic rate was 2.5 points, as compared with the standard 2.5 points daily.

DISCUSSION

These metabolic assays in human myxedema indicate that the colloid in the common goiters of the eastern seaboard behaves like that derived from the normal gland. Our results show no definite deviation from the results obtained in previous studies (2) when standard U. S. P. desiccated thyroid was administered. On the other hand, the failure of the thyroglobulin, obtained from the endemic colloid goiter from the Great Lakes region to produce a metabolic response, is of great interest because of the extremely low iodine and thyroxin content of this protein.

It must be remembered that the source of the "toxic" thyroglobulins used was thyroid tissue from patients who had received iodine prior to operation. Our failure to note any significant qualitative or quantitative difference from the

response to normal thyroid must be interpreted in the light of this fact. Assays with material from patients not treated with iodine would be desirable. Such material, however, is no longer obtainable, in this country, for no surgeon, who knows what he is about, would be willing to remove a toxic goiter without preoperative iodine administration.

The suggestion of Harington and Randall (11), that thyroxin in natural peptide combination should be unusually active, fails of confirmation in this work with thyroxin peptone. This finding harmonizes with results obtained previously (1) with natural thyroxin polypeptide. It is further substantiated by the responses to Harington's synthetic glycyL-thyroxin: the dipeptide, in terms of iodine, yielded metabolic responses essentially identical with those produced by thyroglobulin, by natural polypeptide (1), and indeed by thyroxin itself (in both racemic and optically active forms (1, 5)).

We have thus a series of thyroid derivatives ranging in molecular size from the relatively small thyroxin molecule to the relatively very large thyroglobulin molecule, with various intermediate forms such as dipeptide, polypeptide and peptone. The study of this series indicates that neither optical activity, nor peptide linkage, nor size of the molecule of which thyroxin is an integral component, influences, in a manner detectable by the method of assay used, the calorogenic activity of thyroxin in myxedematous human beings.

The role of di-iodotyrosine in calorogenic response merits special note. In pure form, as shown by various workers, it is inert. When it is a part of the thyroglobulin molecule, our work indicates that it possesses full calorogenic potency and, indeed, contributes the major part of the activity of natural human thyroglobulin. In its peptone form, it possesses no observable activity when given in our standard dosage.

SUMMARY AND CONCLUSIONS

Clinical assays of the metabolic potency of thyroglobulin and of the products resulting from the proteolytic cleavage thereof, showed that in terms of iodine, the following preparations were essentially equivalent within the error of the biological method used:

1. Thyroglobulin from (a) "colloid adenomatous" glands (assayed on three patients) and (b) iodinated "toxic" glands (assayed on three patients).
2. Thyroxin peptone from either type of gland (assayed on two patients).
3. Synthetic, racemic, glycyl-thyroxin, (assayed on two patients).

All of these preparations closely approximated the effect produced by natural thyroxin polypeptide, when appropriately assayed in cases of human myxedema.

Di-iodotyrosine peptone preparation (obtained from human thyroglobulin) failed, in three patients with myxedema, to produce a significant effect when administered in the standard daily dose containing 0.5 mgm. of iodine.

We are indebted for the human material used in our chemical preparation of thyroglobulin to the kind cooperation of Dr. Tracy B. Mallory, of the Massachusetts General Hospital, and Dr. Shields Warren, of the New England Deaconess Hospital, Boston, Massachusetts.

BIBLIOGRAPHY

1. Salter, W. T., Lerman, J., and Means, J. H., The calorogenic action of thyroxin polypeptide. *J. Clin. Invest.*, 1933, **12**, 327.
2. Means, J. H., Lerman, J., and Salter, W. T., The rôle of thyroxin iodine and total organic iodine in the calorogenic action of whole thyroid gland. *J. Clin. Invest.*, 1933, **12**, 683.
3. Lerman, J., and Salter, W. T., The calorogenic action of thyroid and some of its active constituents. *Endocrinology*, 1934, **18**, 317.
4. Lerman, J., and Salter, W. T., The maintenance requirements of myxedema patients: Clinical and chemical assay of commercial thyroid preparations. *J. Pharm. and Exper. Therap.*, 1934, **50**, 298.
5. Salter, W. T., Lerman, J., and Means, J. H., The calorogenic action of d- and l-thyroxin. *J. Clin. Invest.*, 1935, **14**, 37.
6. Harington, C. R., and Salter, W. T., The isolation of l-thyroxin from the thyroid gland by the action of proteolytic enzymes. *Biochem. J.*, 1930, **24**, 456.
7. Kendall, E. C., The determination of iodine in connection with studies of thyroid activity. *J. Biol. Chem.*, 1914, **19**, 251.
8. Harington, C. R., and Randall, S. S., The chemical assay of thyroid gland. *Quart. J. Pharm. and Pharmacol.*, 1929, **2**, 501.
9. Leland, J. P., and Foster, G. L., A method for the determination of thyroxine in the thyroid. *J. Biol. Chem.*, 1932, **95**, 165.
10. Blau, N. F., The determination of thyroxine in the thyroid gland. *J. Biol. Chem.*, 1933, **102**, 269.
11. Harington, C. R., and Randall, S. S., Observations on the iodine-containing compounds of the thyroid gland: Isolation of 1-3:5-diiodotyrosine. *Biochem. J.*, 1930, **23**, 373.
12. Ashley, J. H., and Harington, C. R., Some derivatives of thyroxine. *Biochem. J.*, 1928, **22**, 1436.