RESEARCH PAPERS

A COMPARISON OF THE THYROXINE: TRI-IODOTHYRONINE CONTENT AND BIOLOGICAL ACTIVITY OF THYROID FROM VARIOUS SPECIES

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Samples of ox and sheep thyroid were less potent than pig thyroid in biological assays when the doses were administered either on the basis of B.P. "Thyroxine-iodine" or U.S.P. total combined iodine. Chemical analyisis for iodothyronines revealed that the molar ratio of thyroxine to tri-iodothyronine approximated 2:1 in pig thyroid but approached 3:1 in ox and sheep preparations. Thus the ox and sheep samples which were less potent in the biological assay than pig thyroid also contained less tri-iodothyronine. By using tri-iodothyronine as the dosage basis, there was very little if any difference in physiological efficacy in goitre prevention assay.

It has been established that the present official methods for the chemical assay of desiccated thyroid may not indicate the biological activity. Stasilli and Kroc (1956) have reported that on the basis of either the U.S.P. total combined iodine or the "Blau thyroxine-iodine", ox thyroid is much less active than pig thyroid in both the goitre-prevention and calorigenic assays in the adult rat. Similarly Johnson and Smith (1961) and Webb (1961) noted a lack of agreement between B.P. "thyroxine-iodine" values and physiological activity in preparations of pig, ox and sheep thyroid.

The present study was undertaken to determine whether the variation in biological potency reported for desiccated thyroid from different species could be explained on the basis of changes in content of thyroxine and tri-iodothyronine (liothyronine).

Experimental

Samples

An examination was made of representative beef and pork thyroid samples from an American source and sheep, ox and pig thyroid from a British supply. A highly purified pork thyroglobulin served as a primary reference standard. A sample of desiccated pork thyroid was used as a house standard and all assays were made against this preparation. Relevant details of the source and composition of the samples are given in Table I.

The biological activity of the thyroid products was determined orally by a goitre prevention assay (Wiberg and Stephenson, 1961) and subcutaneously by the mouse anoxia test (Burn, Finney and Goodwin, 1950).

The doses were administered on the basis of the "total combined iodine" (United States Pharmacopoeia XVI, p. 759) and were also computed in terms of "thyroxine-iodine" (British Pharmacopoeia, 1958, p. 678, Addendum, 1960, p. 60). The results of the assays were calculated by standard statistical procedures (Bliss, 1952; Emmens, 1948, Finney, 1952).

TABLE I

"THYROXINE IODINE" AND TOTAL IODINE CONTENT OF PREPARATIONS STUDIED

Preparation	B.P. "Thyroxine iodine" per cent	U.S.P. total combined iodine per cent	Ratio "Thyroxine iodine" / total iodine
Primary standard purified pig thyroglobulin[1]	0·24	0·86	0·279
House standard pig thyroid[2]	0·06	0·20	0·300
American samples—			
Pig[3]	0·26	0·79	0·329
Ox[4]	0·17	0·54	0·315
British samples—			
Pig[5]	0·20	0·57	0·351
Ox[5]	0·11	0·35	0·314
Sheep[5]	0·18	0·48	0·375

1. Warner Chilcott Laboratories. 2. Wilson Laboratories—this sample meets requirements of U.S.P XVI. 3. Armour Pharmaceutical Co.—a composited sample prepared from 7984 lb. of pig thyroids. 4. Armour Pharmaceutical Co.—a composited sample prepared from 3874 lb. of ox glands. 5. Burroughs Wellcome and Co.

In the chemical determination of the iodothyronines, the thyroid samples were hydrolysed by incubation with trypsin and erepsin, followed by chromatographic separation of a butanol extract of the hydrolysates (Devlin and Stephenson, 1962).

RESULTS

The combined results of three independent homogeneous assays are presented in Table II. In general, the total combined iodine and "thyroxine iodine" produced similar estimates of biological potency. The principal exception was the British sheep thyroid in which the assays based on the total iodine content were consistently higher than those employing the "thyroxine-iodine". This was observed with both the goitre prevention and mouse anoxia procedures. Reference to Table I, shows that the "thyroxine-iodine": total iodine ratio is highest in sheep thyroid which could account for these results.

A comparison of the relative potencies obtained from the two methods of biological assay also show good agreement. The British ox preparation indicated a higher level of activity in the goitre prevention test whereas the sheep thyroid was more potent when assayed by the mouse anoxia response. However, these particular results do not appear to be statistically significant.

Sheep and ox gland preparations, at equal concentrations of either total combined iodine or B.P. "thyroxine-iodine" were less potent physiologically than pig thyroid, with the one exception noted in Table II (sheep thyroid in the mouse anoxia assay based on total iodine content). This confirms the observations of Stasilli and Kroc (1956), Johnson and Smith (1961) and Webb (1961) in that the species of origin does affect the relative biological activity of desiccated thyroid.

TABLE II

A COMPARISON OF THE BIOLOGICAL ACTIVITIES OF DESICCATED THYROID PREPARATIONS OBTAINED FROM DIFFERENT SPECIES

Preparation	Relative potency			
	Goitre prevention assay		Mouse anoxia assay	
	B.P. "Thyroxine iodine" per cent	U.S.P. total iodine [3] per cent	B.P. "Thyroxine iodine" per cent	US.P. total iodine per cent
Primary standard pork thyroglobulin	100	100	—	—
House standard desiccated pork thyroid	99·3 (94·0–107·4)	100·4 (95·3–105·9)	100	100
American samples—				
Pig	97·2 (93·7–100·6)	101·0 (96·0–108·0)	104·8 (80·1–137·2)	104·5 (79·9–136·7)
Ox	63·0 (56·3–70·3)	69·0 (61·6–77·2)	56·7 (45·2–71·3)	58·0 (46·2–72·5)
British samples—				
Pig	103·3 (95·9–111·4)	97·5 (91·0–104·3)	96·3 (80·5–115·3)	105·2 (88·2–126·3)
Ox	81·2 (74·7–88·7)	81·7 (75·2–88·8)	66·6 (49·2–90·2)	70·3 (52·1–95·5)
Sheep	73·8 (67·9–80·2)	86·6 (79·7–94·2)	88·1 (74·4–104·3)	104·1 (87·9–113·9)

Each value shown represents the weighted mean of three assays together with the range of potency in brackets.

The goitre prevention assay was superior to the mouse anoxia technique in precision, reproducibility and sensitivity. The Index of Precision (s/b) for the individual goitre prevention assay was always less than 0·2 and frequently below 0·1, whereas the mouse anoxia test produced Indices of Precision which lay between 0·35–0·45. The high residual error term found in the mouse anoxia assay was the major reason for the reduced precision. Transformation of the response parameter to either the reciprocal or to the log of the survival time not only reduced the error variance but also decreased the slope, and consequently there was no appreciable gain in the precision.

The thyroxine and liothyronine content of enzymic hydrolysates of the various thyroid samples is given in Table III. The liothyronine levels are lower in the ox and sheep samples than in preparations of pig thyroid. Thus it would seem that the molar ratio of thyroxine: liothyronine is about 2: 1 in pork thyroid but approaches 3: 1 in sheep and beef samples.

TABLE III

THYROXINE AND LIOTHYRONINE CONTENT OF DESICCATED THYROID SAMPLES

Preparation	Thyroxine		Liothyronine		
	mg./100 g.	micromoles/ 100 g.	mg./100 g.	micromoles/ 100 g.	Molar ratio[1] T_4/T_3
Primary standard pork thyroglobulin	230	296	108	166	1·78
House standard pork thyroid	47	60	19	29	2·06
American samples—					
Pig	212	273	73	112	2·43
Ox	116	149	35	53	2·80
British samples—					
Pig	146	188	75	115	1·64
Ox	119	154	38	58	2·65
Sheep	135	174	43	66	2·64

Each value shown is the mean of four determinations.
1. T_4 = Thyroxine. T_3 = Liothyronine.

The molar ratio (T_4/T_3) appears to be inversely related to the biological effectiveness of the dried thyroid samples. This observation suggested that liothyronine and not thyroxine might be responsible for the greater amount of the activity in the biological assays. Accordingly log dose response lines were plotted for the various goitre prevention assays on the basis of the liothyronine content of the samples. The results from one of these assays is presented in Fig. 1. For the sake of comparison,

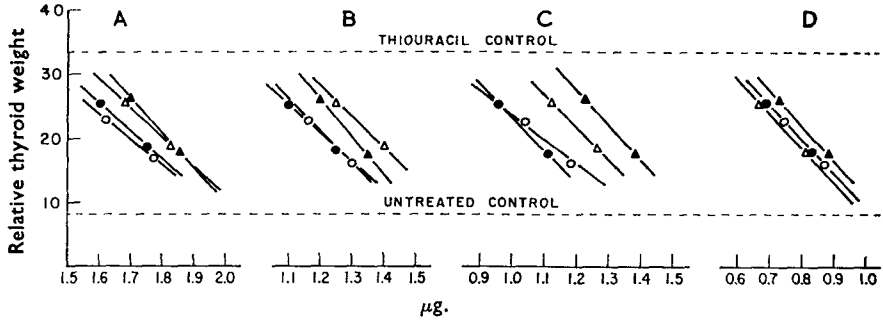

FIG. 1. Log. dose response lines showing the relation between the various parameters of thyroid dosage and response in the goitre prevention assay with adult female rats. Dosages are expressed as per 100 g. diet. Each point represents the mean of 10 animals. Samples assayed were: House standard ●, British pig ○, British ox △, and British sheep ▲.

A, U.S.P. total combined iodine. B, B.P. "thyroxine iodine." C, thyroxine. D, liothyronine.

the log dose response lines for the other iodine-measurements are also shown, namely the B.P. "thyroxine iodine", the U.S.P. total iodine and the thyroxine value as obtained by chromatographic resolution. Visual inspection of the graph indicates that only liothyronine removes this species difference in terms of the biological potency of the dried thyroid preparation. All the other assays produced similar results to those shown in Fig. 1. Hence it seemed of interest to recalculate these assays using the liothyronine content as the basis of dosage. These results are given in Table IV and it is evident that the various preparations now possess equivalent potency, in the goitre prevention test, regardless of species of origin. The obvious conclusion is therefore that liothyronine appears to provide the greater part of the biological activity when thyroid preparations are administered orally.

DISCUSSION

Since preparations of desiccated thyroid are prescribed exclusively as oral medication, it would seem advisable to use the same route for its biological assay. The route of administration in the mouse anoxia test is subcutaneous and this method of dosing may not evaluate certain variables which could affect the oral potency of a sample of thyroid powder. Some of these variables are, the thyroxine and liothyronine content, the rate of release of these two substances plus completeness of

digestion in the gastrointestinal tract and, the relative absorption of the liberated hormones from the gut.

Although the content of the active ingredients, thyroxine and liothyronine, can be obtained by chemical analysis, the second and third factors listed above could produce marked changes in the physiological availability of these compounds. Little is known about the rate of release of these substances and completeness of digestion but there is some evidence that the thyroid hormones are only partially absorbed through the intestinal wall. Frieden, Tukich and Winzler (1949) reported that the ratio of oral to parenteral potency for (±)-thyroxine was 1 : 2, using the goitre prevention response in rats. Other workers have found also

TABLE IV

RELATIVE BIOLOGICAL POTENCY OF DESICCATED THYROID SAMPLES FROM VARIOUS SPECIES BASED ON THE LIOTHYRONINE CONTENT

Preparation	T_3[1]	Relative potency	Range of potency (P = 0·95)
House standard ..	19	100	—
American—			
Pig	73	96·1	92·6– 99·7
Ox	35	102·8	91·6–115·0
British—			
Pig	75	95·1	88·2–102·6
Ox	38	94·5	87·0–102·8
Sheep	43	105·3	96·9–114·2

Each value shown represents the weighted mean from three independent homogeneous assays employing the goitre prevention technique.
[1] Liothyronine concentration in mg./100 g. thyroid powder.

that (−)-thyroxine is less active by the oral route using a variety of assay techniques in different species. Kroc, Phillips, Stasilli and Malament (1954) noted oral dosing was only 34 per cent as effective in the antigoitrogenic assay and 26 per cent as effective in the calorigenic assay in rats compared to the subcutaneous route. Reineke, Travis and Kifer (1960) measured the suppression of the thyroid secretion rate in mink by (−)-thyroxine and concluded that only 34 per cent of the hormone was absorbed. In ruminants, the oral potency of thyroxine is even lower. Mixner and Lennon (1960) found 10–15 per cent of thyroxine is absorbed by lactating dairy cattle based on the protein bound iodine values while Turner and Reineke (1946) estimated that around 5 per cent of the oral dose is physiologically available in sheep based on a weight loss assay.

Desiccated thyroid samples do not show a comparable loss of oral potency in the goitre prevention assays as that recorded for (−)-thyroxine. Friedin and others (1949) and Kroc and others (1954) indicate desiccated thyroid is from 70–75 per cent as active by the oral route as that obtained by subcutaneous injection. A plausible explanation for this higher ratio of oral to parenteral potency for thyroid powder can be adduced from the results published by Gross and Pitt-Rivers (1953). These workers found that oral thyroxine had 39 per cent of the potency of the same parenteral dose whereas orally-administered liothyronine retained 86 per cent of the parenteral activity. Thus the greater extent of absorption of liothyronine coupled with its higher biological activity

could mean that the liothyronine content of desiccated thyroid is quantitatively much more important than the thyroxine level. This possibility has been discussed in detail by Levy and Knox (1961).

The feasibility of the hypothesis that liothyronine contributes the major portion of the biological activity of desiccated thyroid appears to be warranted on the basis of the results presented in Table IV. This does not exclude thyroxine as an active ingredient of dried thyroid but its role may be of minor importance. Since no firm values are available for the amounts of thyroxine and liothyronine released during digestion and then absorbed, it would be highly speculative to assign any definite fraction of the biological activity to the thyroxine content of the thyroid sample. In addition the species of origin would affect this value, since the relative liothyronine levels were lower in ox and sheep preparations. However, in consideration of the fact that liothyronine is from 3 to 8 times as potent as thyroxine (Gross and Pitt-Rivers, 1954; Tomich and Woollett, 1953; Stasilli, Kroc and Meltzer, 1959) the latter may contribute as little as 5 per cent and as much as 30 per cent of the total biological response.

These studies have been made with laboratory or domestic animals and it is quite possible that the digestion of desiccated thyroid by man and the subsequent absorption of the hormones follow a different pattern. Therefore the potency values reported in Table IV based on the liothyronine content of the thyroid samples may not be indicative of the therapeutic effectiveness in humans.

However, it is evident that the present chemical assays of the United States and British Pharmacopoeias do not always reflect the biological activity of desiccated thyroid. The goitre prevention assay involving the oral administration of the test preparations to rats seems to offer a more fruitful procedure at the present time for assessing physiological activity.

If the contributions of each of the active thyroid constituents to the overall biological response could be established with some degree of reliability, then it is quite possible that a chemical assay which measures thyroxine and liothyronine might obviate the present need for biological assay.

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