THE BIOLOGICAL ASSAY OF THYROACTIVE MATERIALS BY THE GOITRE PREVENTION TECHNIQUE

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The goitre prevention technique was employed to measure the biological activity of synthetic and natural thyroproteins as well as L-thyroxine and L-triiodothyronine. These studies indicated that L-thyroxine could not be used as a reference standard for natural thyroid preparations since the log dose response lines were not parallel. However, L-thyroxine was a suitable reference standard for the biological assay of the synthetic iodinated proteins.

The British Pharmacopoeia (1958) defines the potency of desiccated thyroid in terms of thyroxine iodine, while the United States Pharmacopeia XVI employs total organic iodine for measuring thyroid activity. However Stasilli and Kroc (1956) have reported recently that the total iodine content of desiccated thyroid and thyroglobulin is not necessarily correlated with biological activity. It was found that pork thyroid preparations were consistently more active in the goitre prevention assay than beef desiccated thyroid. In addition to the dried thyroid, some consideration must be given to synthetic thyroproteins such as iodinated casein (Protamone). The total organic iodine content of these compounds provides very little information about their biological activity because only a small percentage of the iodine is present as thyroxine (Reinecke, 1946).

In view of these apparent differences between biological effectiveness of thyroactive substances and their iodine content, a study was undertaken to examine the possibility of using L-thyroxine as the reference compound for the standardisation of thyroid preparations. The prevention of goitre in thiouracil-treated rats was employed as the criterion of response in the bioassay of the thyroactive substances. In addition to desiccated thyroid, iodinated proteins and L-triiodothyronine were included in the investigation. A comparison was also made of oral and parenteral potencies of the thyroid preparations.

Experimental

Oral Route of Administration

Adult female rats of an inbred Wistar strain, weighing 150-180 g. were used as the test animals. The goitre-prevention assay procedure employed was similar to that described by Stasilli and Kroc (1956). After random distribution into dose groups of 10 animals each, two rats were placed in each cage and fed a diet of ground Master Fox cubes containing 0·3 per cent thiouracil. Graded amounts of the thyroactive substances were mixed into the appropriate diets to provide two doses levels each of the standard and unknown preparations. The actual doses used were based on the total organic iodine concentration and expressed as μg. thyroid iodine per 100 g. diet. The rats were fed the test diets *ad libitum*

for 14 days. On the fifteenth day, the animals were killed and the thyroid glands removed and weighed. The response was expressed as the relative thyroid weight, i.e., mg. thyroid/100 g. of final body weight. For each assay two control groups were included, one providing the goitrogenic

TABLE I

IODINE CONTENT OF MATERIALS STUDIED

Preparation	Iodine per cent
Thyroxine pentahydrate (Sodium Salt) ..	57·3
Triiodothyronine (Sodium Salt)	58·9
Desiccated pork thyroid[1] (house standard) ..	0·202
Desiccated pork thyroid[2]	0·79
Thyroglobulin[3]	0·86
Iodinated protein[4]	6·90
Iodinated protein[5]	7·60

1. Wilson Laboratories. 2. Armour Pharmaceutical Co. 3. Warner-Chilcott Laboratories. 4. Protamone-AgriTech Ltd. 5. Lechavit-Pure. H.F. Cordes and Co. Hamburg, Germany.

effect of thiouracil alone on the thyroid gland, and the other the normal untreated thyroid weight.

Parenteral Administration

When the thyroactive substances were administered by intraperitoneal injection, they were dissolved or suspended in a 1 per cent (w/v) solution of sodium bicarbonate. A Potter-Elvehjem glass homogeniser was employed to make the suspensions when the material did not dissolve completely. The rats were distributed at random into dose groups and

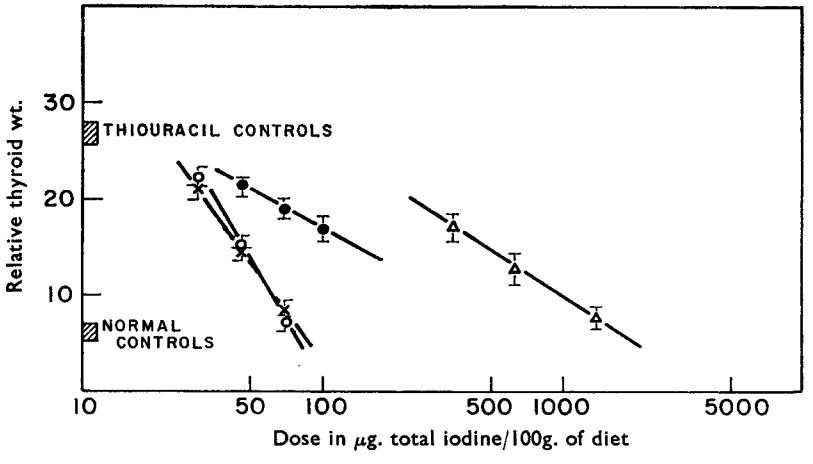

FIG. 1. Log. dose-response curves for thyroactive materials based on relative thyroid weights in thiouracilized rats. Each point represents the mean of 10 animals. Route of administration—oral.

-X-	desiccated pork thyroid	b = − 39·6 ± 1·04	λ = 0·065
-○-	pork thyroglobulin	b = − 40·0 ± 0·97	λ = 0·093
-●-	thyroxine	b = − 14·2 ± 1·27	λ = 0·161
-△-	iodinated casein (*Protamone*)	b = − 18·7 ± 1·24	λ = 0·161

fed a diet containing 0·3 per cent thiouracil *ad libitum* during the assay period. Each animal received a daily intraperitoneal injection (0·2 ml.) of the material under test for a period of 14 consecutive days. The doses

TABLE II

BIOLOGICAL ASSAY OF A SYNTHETIC IODINATED PROTEIN AGAINST SODIUM L-THYROXINE

Preparation	No. of animals	Dose µg. total iodine / 100 g. diet	Relative thyroid weight ± S.E.M.*
Standard			
Na L-thyroxine ..	10	66·6	19·0 ± 0·8
	10	100	15·6 ● 0·5
	10	150	10·3 ± 0·7
Unknown			
Protamone ..	10	345	20·3 ± 0·7
	10	690	12·6 ● 1·5
	10	1380	7·2 ± 0·6
	Thiouracil Normal	Controls Controls	28·8 ± 2·2 5·5 ± 0·6

s = 2·64 b = −22·8 λ = 0·115
Relative potency 17·0 per cent
Confidence limits (P = 0·95) 15·0 — 19·4 per cent

* mg. thyroid/100 g. final body weight ± standard error of the mean.

were not administered on the basis of body weight because of the narrow range (150–160 g.) of the initial body weights. The control groups of rats were fed either the untreated diet or that containing 0·3 per cent thiouracil, and were given intraperitoneal injections of a 1 per cent solution of sodium bicarbonate for the period of the assay. On the fifteenth day of the test,

TABLE III

BIOLOGICAL ASSAY OF A DESICCATED PORK THYROID PREPARATION AGAINST A PURIFIED PORK THYROGLOBULIN

Preparation	No. of animals	Dose µg. total iodine / 100 g. diet	Relative thyroid weight ± S.E.M.*
Standard			
Thyroglobulin ..	10	45·0	15·2 ± 1·1
	10	67·5	6·3 ± 1·1
Unknown			
Desiccated thyroid	10	45·0	14·7 ± 1·7
	10	67·5	8·2 ± 0·1
	Thiouracil controls		26·8 ± 1·4
	Normal controls		6·5 ± 0·4

s = 3·72 b = −43·49 λ = 0·085
Relative potency 96·4 per cent
Confidence limits (P = 0·95) 85·0 — 109·2 per cent

* mg. thyroid/100 g. final body weight ± standard error of the mean.

the animals were killed, and the thyroid glands removed and weighed. The results were expressed as the relative thyroid weight.

The total iodine content of the compounds tested in this study are shown in Table I.

RESULTS

Oral Route of Administration

Fig. 1 shows typical log dose-response lines obtained for a number of thyroactive substances by plotting the relative thyroid weight against the

log of the dose expressed as μg. of total iodine/100 g. of diet. The slopes for thyroxine and iodinated casein (Protamone) were significantly smaller than those for desiccated pork thyroid and pork thyroglobulin. This significant difference in slopes clearly indicates that thyroxine cannot be used as a reference standard for the bioassay of dried thyroid by the goitre-prevention technique, at least by the method of parallel lines.

However this method can be used for estimating the biologically available thyroxine content of iodinated proteins, and the results of such an assay are given in Table II. On the basis of the total organic iodine, the potency of Protamone is approximately 17 per cent relative to that of thyroxine. Since 17 per cent of the total iodine is assumed to be thyroxine, it is estimated that Protamone contains approximately 1·2 per cent biologically available thyroxine. The thyroxine content of Protamone was

TABLE IV

BIOLOGICAL ASSAY OF DESICCATED PORK THYROID (AGAINST ANOTHER DESICCATED PORK THYROID PREPARATION)

Preparation	No. of animals	Dose μg. total iodine / 100 g. diet	Relative thyroid weight ± S.E.M.*
Standard			
Wilson	10	40	20·5 ± 0·7
Desiccated thyroid	10	60	12·7 ± 0·9
Unknown			
Armour desiccated	10	40	19·3 ± 0·4
Thyroid	10	80	8·0 ± 0·7
Thiouracil controls			24·3 ± 1·0
Normal controls			6·8 ± 0·8

s = 1·83 b = −39·3 λ = 0·047
Relative potency 103 per cent
Confidence limits (P = 0·95) 95–112 per cent

* mg. thyroid/100 g. body weight ± standard error of the mean.

measured by the method described in the British Pharmacopoeia 1958 and it was found to be 1·2 per cent, showing excellent agreement with the value obtained by the bioassay procedure.

Since thyroxine could not be used as the reference standard for determining the physiological activity of dried thyroid, it was necessary to use a thyroid preparation as a reference standard. Tables III and IV show the results of two bioassays in which a pork thyroglobulin and a desiccated pork thyroid were employed as the reference standards. All the samples were derived from pork and displayed similar biological activity on the basis of total organic iodine in the goitre-prevention assay.

Intraperitoneal Route of Administration

Fig. 2 shows that the slopes of the log dose-response lines for desiccated thyroid, administered by intraperitoneal injections is significantly steeper than those obtained under similar conditions for triiodothyronine, thyroxine, and the two iodinated proteins, Protamone and Lechavit. These findings agree with those observed when the thyroactive substances were given orally in the diet.

As the slopes for triiodothyronine, Lechavit and Protamone did not differ significantly from thyroxine, it was possible to get an approximate indication of the physiological activity by measuring the horizontal difference between the log dose-response lines. By using this method thyroxine had 19 per cent of the biological activity of triiodothyronine, Protamone had 16 per cent of the potency of thyroxine, while Lechavit

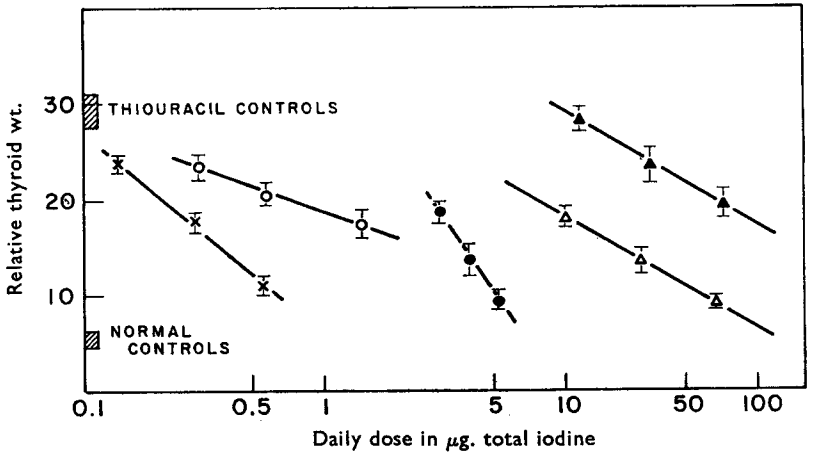

FIG. 2. Log. dose-response curves based on relative thyroid weights of thiouracilized rats receiving thyroactive substances by intraperitoneal injection. Each point represents the mean of 10 animals.

–X–	triiodothyronine	b = – 20·79 ± 1·42	λ = 0·099
–○–	thyroxine	b = – 15·07 ± 2·82	λ = 0·176
–●–	desiccated pork thyroid	b = – 42·01 ± 1·91	λ = 0·072
–△–	iodinated casein (*Protamone*)	b = – 14·67 ± 0·30	λ = 0·162
–▲–	iodinated protein (*Lechavit*)	b = – 15·19 ± 0·90	λ = 0·265

was only 2 per cent as potent as thyroxine and 19 per cent as active biologically as Protamone. These estimates are all in good agreement with data obtained by oral assay.

DISCUSSION

Numerous workers have commented on the existence of greater biological activity in dried thyroid preparations than their thyroxine content would indicate. The extensive earlier literature on this subject has been summarised by Frieden and Winzler (1948). Since the majority of the studies antedate the discovery of triiodothyronine by Gross and Pitt-Rivers (1953), the presence of this hormone might explain the enhanced physiological response of the natural dried thyroid products.

Nevertheless the results presented here suggest that one characteristic of the biological response elicited by thyroxine and triiodothyronine differs from that found with desiccated thyroid, namely, the slope of the log dose-response line. Although few investigators have published actual estimates of the slopes of the linear relationship between log of the dose and the relative thyroid weight, examination of their data provides an

approximate assessment of the probable values. In general, the results of Cortell (1949), Dempsey and Astwood (1943), Gross and Pitt-Rivers (1953), Hemming and Holtcamp (1953), Kroc, Phillips, Stasilli and Malament (1954), and Mussett and Pitt-Rivers (1957) yielded log dose-response lines with slopes which were of the same order of magnitude as those shown in Figs. 1 and 2. Cortell (1949) found that the slope of the log dose-response line was much steeper for thyroglobulin than it was for thyroxine, whereas the results of Kroc and others (1954) indicate that the log dose-response lines for these two materials were essentially parallel. In a later study, however, Stasilli and Kroc (1956) reported that the slopes of log dose-response lines in a large series of bioassays of desiccated thyroid preparations, ranged from −23·8 to −58·3 with a mean of −40·5 for 15 assays. These values do not differ significantly from those obtained in the present study. Ferguson and Warson (1953) found that the log dose-response lines for thyroxine, triiodothyronine and desiccated thyroid varied from −6 to −36 with an average value of −16.

The results obtained in the goitre-prevention assay suggest that thyroid preparations must be assayed against a standard derived from glandular material in order to obtain a meaningful estimate of biological potency. Chemical methods which determine total iodine or thyroxine content may not be indicative of physiological activity.

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