IN VITRO STUDIES ON THE EFFECT OF STEROID HORMONES
UPON HORMONE BIOSYNTHESIS OF BEEF THYROID GLAND
1. EFFECTS OF ESTRADIOL AND PROGESTERONE

KINZOO HASEGAWA

*Department of Physiology, Institute of Endocrinology,
Gunma University, Maebashi*

It has been widely accepted that there exists an intimate relationship between thyroid function and female gonad. For instance, enlargement of the thyroid is frequently observed in puberty, menstruation and pregnancy (Garnier, 1921; Marine, 1935; Neumann, 1937). Furthermore, the increase of PBI value in pregnant woman has been pointed out by many authors (Heinemann *et al.*, 1948; Man, 1951; Yamazaki and Kumaoka, 1954). These facts have shown the stimulating action of gonad on both the size and function of the thyroid. To analyse the relationship of these endocrine glands, many attempts have been made, mainly observing thyroid activity in various functional states of female gonad *in vivo*.

In order to obtain further information about the direct influence of these hormones, the author attempted to see the effects of estradiol and progesterone on the hormone synthesis in thyroid slices *in vitro*, aiming at excluding the influence of other endocrine glands such as the anterior pituitary.

MATERIALS AND METHODS

Fresh thyroid glands were obtained mainly from oxen sacrificed at a local slaughterhouse and brought back in a box packed with cracked ice. Thyroids were freed of surrounding and connective tissues and the central part made into a rectangular block. Slices of 0.4–0.6 mm thicknesses were prepared by hand with a sharp razor blade. Slices used in any single experiment were made from one gland only.

Iodine metabolism Measurement was made after the method reported by Suzuki (1956). Outline of the method was as follows: Thyroid slices were divided into groups consisting of similar number of them and after blotting on filter paper, weighed on a torsion balance. Slices of 200 mg were put into a Warburg flask containing 2.8 ml of Krebs-Ringer phosphate solution (pH 7.4) as medium, with 0.2 ml of 2 N NaOH and filter paper in the center well and incubated at 37°C for 2 hrs. with air as gas phase. Oscillation frequency was 100 times per minute. After 10 mins. temperature equilibration, oxygen consumption was measured at 15 mins. intervals. The Krebs-Ringer solution used in each flask contained 8 μ c I¹³¹ as carrier free NaI¹³¹.

Estradiol and progesterone were dissolved in 99% ethanol and 0.1 ml of each of these solutions was added to Krebs-Ringer phosphate solution. In the control flask, 0.1 ml of ethanol was used instead of hormone solutions. After incubation, the slices were quickly removed from the flasks, blotted on filter paper and put into physiological saline solution of approximately 30 ml to wash the radioactive iodide attached to the surfaces. After washing 3 times, it was found that the final saline solution did not show any appreciable amount of radioactivity.

Immediately after washing, slices were transferred to Potter-Elvehjem whole glass homogenizers with 1 ml of 0.1 *M* carbonate buffer pH 8.5 and heated in a 100°C water bath for 5 mins. Boiled slices were homogenized with protease solution (trypsin and pancreatin, each 2 % in the buffer mentioned above) to which a drop of toluene was added.

Digestion was made in a 37°C water bath for 72 hrs. with a further addition of 1 ml protease solution 48 hrs. later. After the completion of digestion, 1 ml of hydrolysate was taken out of each homogenizer, put into a steel dish, alkalized with two drops of 10 % NaOH and desiccated.

Paper chromatography The hydrolysate was analysed paper-chromatographically to detect iodine fractions of inorganic iodine, monoiodotyrosine and diiodotyrosine. Toyo-Roshi Co. paper No. 50 (2×40 cm) was employed for one dimensional ascending chromatography. On the paper were applied 0.1 ml of hydrolysate and 0.05 ml of carrier solution (0.1 *N* KI solution containing 15% NH₄OH, 0.005% thyroxine and 0.0025% diiodotyrosine) and developed with the use of a solvent, consisting of *n*-butanol, acetic acid and water (78:5:17). After development and drying, the paper was placed under a 1 cm thick lead plate with a window (2×1 cm) on which Geiger-Mueller tube was fixed and the radioactivity of each 1 cm of paper was measured. After the determination of radioactivity, color development was carried out by spraying ninhydrin in order to detect amino acid and 0.1 *M* of CuSO₄ for iodide to identify peaks of radioactivity with the spots of corresponding carriers. Total c.p.m. which corresponded to the color spot developed by the treatment, was designated as an iodine fraction newly formed. Figure 1 shows the radiochromatograms as the results of effect of estradiol (10⁻⁴ *M*) on iodine fractions in thyroid slices. A detailed explanation of this figure will be given in the following account.

Fig. 1. Radiochromatograms of beef thyroid hydrolysate. Open circle: control, closed circle: estradiol (10⁻⁴ *M*). I⁻: inorganic iodide, MIT: monoiodotyrosine, DIT: diiodotyrosine.

In the tables and figures, "organic iodine fraction" represented the total counts of monoiodotyrosine and diiodotyrosine fractions. In this study, duplicated experiments were carried out in each case and the data obtained were represented as average values.

Phosphorus metabolism The incorporation of P³² (orthophosphate) into acid-soluble organic phosphate fraction was determined after the method of Katsura *et al.* (1958), employing similar

experimental conditions as used in "iodine metabolism" mentioned above. In this case, the concentration of phosphate buffer solution was reduced to half in order to obtain the higher specific activity of radioactivity of phosphate incorporated in thyroid slices. Radioactivity (c. p. m.) of P^{32} newly incorporated in the acid-soluble organic phosphate fraction, divided by μ l of oxygen consumed was found to be the relative index of "oxidative phosphorylation" when it was compared with control value. This expression is the most significant index for energy metabolism of tissue slices (Katsura *et al.*, 1958).

RESULTS

Effects of estradiol

1) *On oxygen consumption*

As is seen in Table 1 and Figure 2, estradiol suppressed the oxygen consumption of thyroid slices at the higher concentration of the hormone (10^{-4} M), while, with the dilution of the hormone, this suppressing effect disappeared: the average value obtained by repeated trials at a concentration of 10^{-6} M were almost identical with the control value.

Table 1. Effect of estradiol on iodine metabolism of beef thyroid slices

No. exp.	Conc. of estradiol	O_2 uptake (μ l)	Radioactivity (c.p.m.)					
			Hydrolysate (I^{131} uptake)	Inorg. I	Fraction in thyroid slices			
					Inorg. I		Organic I (MIT+DIT)	
	0	71.2	9,286	503			816	
1	1×10^{-4} M	50.9	4,503	48.5%	391	77.6%	244	29.2%
	0	115.9	9,005		847		586	
2	1×10^{-5} M	112.2	8,927	99.1%	791	93.3%	701	119.6%
	0	99.7	19,681		1,469		1,162	
3	1×10^{-5} M	98.0	17,829	90.6%	1,421	96.7%	1,023	88.0%
	0	71.4	6,503		1,134		672	
4	5×10^{-6} M	59.5	6,543	100.3%	1,027	90.4%	626	93.2%
	0	74.3	12,300		1,069		506	
5	2×10^{-6} M	75.2	14,626	118.9%	1,245	116.3%	615	121.3%
	0	68.9	9,357		813		587	
6	1×10^{-6} M	63.4	11,239	120.1%	969	119.0%	754	128.3%
	0	186.6	13,308		2,215		1,731	
7	1×10^{-6} M	189.4	13,671	102.6%	2,028	91.8%	1,624	94.8%
	0	57.3	6,017		902		944	
8	1×10^{-6} M	61.0	6,196	102.9%	988	109.5%	1,216	128.8%
	0	95.6	12,118		1,907		2,574	
9	5×10^{-7} M	93.6	13,512	111.0%	1,994	104.6%	2,687	104.5%
	0	128.1	7,769		1,879		2,140	
10	1×10^{-7} M	112.5	6,176	79.5%	1,771	94.3%	1,815	84.8%

2) *On inorganic iodide collection*

At the higher concentration of the hormone (10^{-4} M), the iodide uptake of thyroid slices was more profoundly inhibited, compared with oxygen consumption (Table 1 and Fig. 3). While at the lower concentrations, 2×10^{-6} to 5×10^{-7} M, the hormone showed rather an augmenting effect on the iodide uptake of thyroid slices.

Fig. 2. Effects of estradiol and progesterone on oxygen consumption of beef thyroid slices. Open circle : estradiol, closed circle : progesterone

Fig. 3. Effects of estradiol and progesterone on inorganic iodide collection of beef thyroid slices. Open circle : estradiol, closed circle : progesterone

3) On organic binding of iodide

The highest concentration of estradiol tested in this study retarded the organic binding of iodide in thyroid slices to a higher degree than the oxygen consumption and iodide uptake. On the contrary, estradiol at the concentrations ranging

from 2×10^{-6} to 5×10^{-7} *M* accelerated binding as is illustrated in Figure 4. Figure 1 shows the radiochromatograms of hydrolysate of thyroid slices with solvent (open circle) and with estradiol (closed circle). In this figure, it is clearly shown that organic iodine fractions are especially suppressed by the hormone. At the solvent front, a radioactive spot with *R_f* value close to that of thyroxine was found. However, it was previously identified as iodinated lipid fraction by Suzuki (1956).

Fig. 4. Effects of estradiol and progesterone on organic binding of iodide of beef thyroid slices. Open circle: estradiol, closed circle: progesterone

4) On phosphorus metabolism

Table 2 shows incorporation of radioactive inorganic phosphate from surrounding media into inorganic and organic phosphate fractions in thyroid slices. Oxidative phosphorylation of the thyroid slice, expressed as c. p. m. $P/\mu l O_2$, was not affected appreciably. With the addition of estradiol in both concentrations (10^{-4} and 10^{-6} *M*) the values remained almost equivalent to those of control, as

Table 2. Effect of estradiol on phosphorus metabolism of thyroid slices

Conc. of estradiol	O_2 uptake (μl)	Radioactivity			
		Organic phosphate fraction		c. p. m./ O_2	
		c. p. m.	%		
0	85.8	814		9.48	
10^{-4} <i>M</i>	68.8	597	73.3%	8.67	
10^{-6} <i>M</i>	70.0	822	101.0%	11.73	

shown in Table 2, considering the limitation of this method. It might be tentatively concluded that the estradiol has no uncoupling effect on the oxidative phosphorylation of thyroid slices.

Effect of progesterone

1) *On oxygen consumption*

Progesterone at the concentration of 10^{-3} M, inhibited oxygen consumption of thyroid slices, while at the lower concentrations the inhibiting effect seemed to disappear, as seen in Table 3 and Figure 2. However, the effect of the hormone was not so remarkable as that of estradiol at the same molar concentration basis. At the lowest concentration examined in this experiment, progesterone does not influence oxygen consumption of the slices.

Table 3. Effect of progesterone on iodine metabolism of beef thyroid slices

No. of exp.	Conc. of progesterone	O ₂ uptake (μ l)	Radioactivity (c.p.m.)					
			Fraction in thyroid slices					
			Hydrolysate (131 uptake)		Inorg. I			
	0	97.6		3,385		2,296		4,900
11	1×10^{-3} M	45.3	45.9%	1,898	56.0%	2,004	87.2%	2,785
	0	85.4		22,123		6,699		5,323
12	1×10^{-4} M	78.4	91.8%	19,063	86.2%	4,766	71.3%	3,362
	0	130.5		5,842		3,221		832
13	1×10^{-5} M	143.2	109.9%	5,227	89.5%	2,665	82.8%	930
	0	117.5		35,836		942		2,919
14	1×10^{-6} M	113.0	96.2%	38,526	107.3%	1,055	111.9%	3,015
	0	91.8		11,950		1,743		3,032
15	1×10^{-6} M	84.5	92.0%	11,636	97.4%	1,770	101.5%	2,581
	0	114.8		2,721		1,685		2,093
16	1×10^{-6} M	109.0	95.1%	2,407	88.5%	1,300	77.1%	1,422
	0	132.4		12,617		2,404		2,658
17	1×10^{-6} M	145.5	109.9%	15,599	123.7%	2,757	114.3%	3,611

2) *On inorganic iodide collection*

At the higher concentration of the hormone (10^{-4} M), inorganic iodide collection of thyroid slices was suppressed to similar degree to the case of oxygen consumption (Table 3 and Fig. 3). As is seen in the figure, this suppressing effect of the hormone disappeared as the amount of hormone in the solution was decreased.

Table 4. Effect of progesterone on phosphorous metabolism of thyroid slices

Conc. of progesterone	O ₂ uptake (μ l)	Radioactivity		
		Organic phosphate fraction		
		c. p. m.	%	c. p. m/O ₂
0	84.3	779		9.24
10^{-4} M	73.7	591	75.8%	8.02
10^{-6} M	83.4	729	93.5%	8.74

3) *On organic binding of iodide*

Results are shown in Table 3 and Figure 4. A similar result to that observed in the effect on oxygen consumption (Fig. 2) was seen with progesterone.

4) *On phosphorus metabolism*

Influence of progesterone on oxidative phosphorylation were examined as that of estradiol. Results are listed in Table 4. Oxidative phosphorylation of thyroid was not affected by progesterone.

Table 5. Effect of cholesterol on iodine metabolism of beef thyroid slices

No. exp.	Cont. of cholesterol	O ₂ uptake (μ l)	Radioactivity (c. p. m.)						
			Fraction in thyroid slices						
			Hydrolysate (¹³¹ I uptake)						
	0	94.2	10,281		Inorg. I		Organic I (MIT+DIT)		
18	1×10^{-4} M	97.8	103.8%	10,931	106.3%	1,535	1,987	2,056	103.5%
						1,476	96.2%		

Effect of cholesterol on iodine metabolism

As a control substance of steroid compound, the effect of cholesterol on iodine metabolism of thyroid slices was tested. Table 5 shows the results. Cholesterol at the concentration of 10^{-4} M neither increased nor decreased oxygen consumption, iodide collection and formation of organic iodine compounds.

DISCUSSION

There are several indices indicating the thyroid function, such as weight and histological findings of thyroid gland, rate of ¹³¹I uptake by thyroid, protein bound iodine (PBI), cholesterol in serum, and measurement of basal metabolic rate. However, these indices are not satisfactory in representing the thyroid function completely. In this study, the author has directly measured the hormone synthesis of thyroid *in vitro* through observations on iodine metabolism in the slices; a method which should be most suitable for examining the direct action of sex hormones on thyroid function.

There are several reports concerning the suppressing effects of estrogen upon the thyroid function *in vivo* (Bialet-Laprida, 1933; Gardner, 1949; Wolterink *et al.*, 1950; Mercier-Parot and Tuchmann-Duplessis, 1951; Mercier-Parot, 1952). However, results obtained in these experiments appears to depend upon the quantities of the hormone administered. Inhibition of the thyroid function by estrogen was observed with rather a larger amount of the hormone, while, with a smaller amount estrogen, an enhancing effect was noted as pointed out by some of the authors cited above. In recent studies, many authors have reported the stimulating effect of the hormone. Hurst and Turner (1948) have found the increased secretion of thyroid hormone in mice after the administration of a minute amount of dianyl-hexene. With the use of rat as an experimental animal, Money *et al.* (1950, 1951), Reineke and Soliman (1953), Ogawa *et al.* (1954), Soliman and Reineke (1955) and Feldman (1956 a, b, c) have observed

that estrogen enhanced I^{131} uptake by the thyroid (1-200 μg /per rat, as estradiol benzoate). Ogawa *et al.* (1954) had noted the increase of the thyroid weight at the same time. Furthermore, Takaori (1954) has reported the hyperfunctional states of rat thyroid from his histological findings, *i. e.*, proliferation of thyroid epithelial cells and decrease of colloid contents, as the results of estrogen administration. Finally, activation of human thyroid function by administration of estradiol was found (Engstrom *et al.*, 1952; Engstrom and Markardt, 1954; Lederer, 1953).

In the present *in vitro* experiments, it was shown that estradiol shows a dual effect on the hormone biosynthesis of thyroid, namely inhibition at the higher concentration (10^{-4} M) and enhancement at the lower concentrations (2×10^{-6} - 5×10^{-7} M ; 554-136 $\mu\text{g}/\text{ml}$). Such effects of the hormone in the present study appear to confirm the phenomena found in *in vivo* experiments.

In regard to the mechanism of estrogen action on the thyroid function *in vivo*, it has been postulated that anterior pituitary participates in facilitating the thyroid function through TSH secretion promoted by estrogen (Soliman and Reineke, 1955; Noach 1955, Takaori, 1954), while, the augmentative effects of estrogen on thyroid function are demonstrated by Feldman (1956 a, b), using hypophysectomized rats. These inconsistent results appear to show the difficulty of deciding whether anterior pituitary mediates the estrogen effect on the thyroid function through *in vivo* experiments. However, the results presented in this paper might give a solution to this problem, because of the observation of direct influences of the hormone on thyroid slices *in vitro*.

It has been known that progesterone at a certain concentration has a stimulating effect on thyroid function (Money *et al.*, 1950, 1951; Takaori, 1954; Reineke and Soliman, 1953), while, in other reports, or in employing other concentrations, opposite results have been obtained. Reineke and Soliman (1953), with the use of 0.4 mg progesterone, Ogawa (1954) with 0.2 mg and then Takaori (1954) with 5 mg have all found the inhibitory action of the hormone on thyroid function of rats. Concerning the influences of progesterone on human thyroid, Zingg and Perry (1953) have shown that I^{131} uptake rate and I^{131} clearance are decreased by the hormone administration (25 mg/day, 3 days).

In the present study, the author found that progesterone at the concentration of 10^{-3} - 10^{-4} M inhibited oxygen consumption, inorganic I^{131} uptake and organic binding of iodide of thyroid slices. While, with the hormone at the concentration of 10^{-5} M and 10^{-6} M , the inhibiting effect of progesterone on thyroid slices was abolished and increased I^{131} uptake and organic binding of iodide were seen in a few instances of 10^{-6} M (Table 3). However, these effects were not as obvious as in the case of estradiol (Table 1). Here, it is of interest that the blood concentrations of these two female gonadal hormones greatly differ from each other. The highest estrogen concentration in women's blood in normal menstrual cycle is about 5 $\mu\text{g}/\text{L}$ (approx. 2×10^{-8} M) expressed as estrone concentration, whereas progesterone concentration is 3 mg/L (approx. 1×10^{-5} M) (Smith, 1955). Accordingly, blood progesterone concentration in women in the non-pregnant state seems to reach almost the higher concentrations, which showed suppression of thyroid hormone biosynthesis in the present experiment, on the

contrary, estrogen concentrations *in vivo* are usually similar to the lower concentrations, which activated the thyroid function *in vitro*.

The author is of the opinion that both these female gonadal hormones have same mode of action on thyroid function, namely suppression at higher concentrations and enhancement at the lower. And *in vivo* conditions, estrogen acts in an activating manner on the thyroid function while progesterone acts in a suppressing way due to the concentration of these hormones in the blood.

It has been confirmed that the thyroid functions, especially marked potency of inorganic iodide collection by the gland from the blood stream, are closely related to the energy supply generated through oxidative phosphorylation (Slingerland, 1955; Freinkel and Ingbar, 1955; Suzuki, 1956). Progesterone at the concentration of around 10^{-4} *M* is known as an uncoupling reagent on oxidative phosphorylation of liver and brain mitochondria (Wade and Jone, 1956 a, b). In the present investigation, from the results of the tests of both hormones on oxidative phosphorylation in thyroid tissue *in vitro*, the mechanisms of inhibition of thyroid functions by the hormones should not be ascribed to the uncoupling effect of the hormones but to the primary action on oxygen consumption. As is seen in Tables 1, 3 and Figure 1, the organic binding of iodide in thyroid slices was more profoundly inhibited by estradiol (10^{-4} *M*) and progesterone (10^{-4} *M*), as compared with percent inhibition of oxygen consumption, I^{131} uptake and inorganic iodide fraction. However, these patterns of thyroid hydrolysates on the paper-chromatogram have often been seen when inhibitors such as cyanide, an inhibitor of oxygen consumption, and dinitrophenol, uncoupler of oxidative phosphorylation, suppressed iodide collecting function of thyroid (Suzuki, 1956). Accordingly, these hormones are not regarded as "goitrogen" which has marked potency of inhibition on organic binding of iodide in the thyroid gland.

SUMMARY

1. The oxygen consumption of beef thyroid slices in Krebs-Ringer phosphate solution containing carrier free NaI^{131} was measured. After incubation, thyroid slices were digested and iodine fractions of hydrolysate were determined by paper-chromatography.

2. Estradiol suppressed oxygen consumption, inorganic I^{131} uptake and organic binding of iodide of thyroid slices at the higher concentration, 10^{-4} *M*. While, at the lower concentration, around 10^{-6} *M*, the hormone slightly stimulated hormonogenesis.

3. Progesterone showed an inhibiting effect on hormonogenesis of thyroid slices at higher concentrations, 10^{-3} and 10^{-4} *M* but not so profoundly as estradiol, when both effects were compared in same molar concentration. At the lower concentration, 10^{-6} *M*, the inhibiting effect was absent.

4. Cholesterol at the concentration of 10^{-4} *M*, used as a control substance of steroid compound, had no effect on hormonogenesis of thyroid slices.

5. Estradiol and progesterone at concentrations of 10^{-4} and 10^{-6} *M* did not show significant effect on oxidative phosphorylation of thyroid slices.

6. From the data obtained in this study, the role of the hormones in the physiology of the thyroid gland is discussed in view of the results obtained in *in vivo* experiments.

ACKNOWLEDGEMENT

The author wishes to express his cordial thanks to Prof. K. Yamamoto for his kind encouragement and valuable discussion during the course of this work.

REFERENCES

- Bialet-Laprida, Z. (1933). *Buenos Aires Thesis* 4705.
- Engstrom, W. W., B. Markardt and A. Liebman (1952). *Proc. Soc. Exptl. Biol. Med.* **81**, 582.
- Engstrom, W. W. and B. Markardt (1954). *J. Clin. Endocrinol.* **14**, 215.
- Feldman, J. D. (1956 a). *Endocrinology* **58**, 327.
- Feldman, J. D. (1956 b). *Endocrinology* **59**, 289.
- Feldman, J. D. (1956 c). *Am. J. Physiol.* **184**, 369.
- Freinkel, N. and S. H. Ingbar (1955). *J. Clin. Endocrinol. and Metabolism* **15**, 598.
- Gardner, J. H. (1949). *Proc. Soc. Exptl. Biol. Med.* **72**, 305.
- Garnier, M. (1921). *J. Med. Franc.* **10**, 218.
- Heinemann, M., C. E. Johnson and E. B. Man (1948). *J. Clin. Invest.* **27**, 91.
- Hurst, V. and C. W. Turner (1948). *Missouri Agri. Exp. Sta. Bull.* 417, cited from *Iowa State Coll. J. Sci.* **28**, 67 (Reineke and Soliman).
- Katsura, H., R. Kanaya and K. Yamamoto (1958). *Endocrinol. Japon.* **5**, 103.
- Lederer, J. (1953). *Ann. endocrinol.* **14**, 916 (The Excerpta **8**, 362, 1954).
- Man, E. B., M. Heinemann, C. E. Johnson, D. C. Leary and J. P. Peters (1951). *J. Clin. Invest.* **30**, 137.
- Marine, D. Glandular Physiology and Therapy Amer. Med. Ass., Chicago p. 315. (1953).
- Mercier-Parot, L. and H. Tuchmann-Duplessis (1951). *Compt. rend. soc. biol.* **145**, 1883 (Ber. **158**, 251, 1953).
- Mercier-Parot, L. (1952). *Bull. microscop. appli.* **2**, 117 (Biol. Abst. **27**, 1818, 1952).
- Money, W. L., L. Kirschner, L. Krintz, P. Merrill and R. W. Rawson (1950). *J. Clin. Endocrinol.* **10**, 1282.
- Money, W. L., L. Kaintz, J. Fager, L. Kirschner and R. W. Rawson (1951). *Endocrinology* **48**, 682.
- Neumann, H. O. (1937). *Arch. Gynäk.* **163**: 406.
- Noach, E. L. (1955). *Acta Endocrinol.* **19**, 127.
- Ogawa, E., S. Itagaki and H. Nomura (1954). *Kitakanto Med. J.* **4**, 87.
- Ogawa, E., K. Arai and K. Shibata (1957). *Endocrinol. Japon.* **4**, 71.
- Reineke, E. P. and F. S. Soliman (1953). *Iowa State Coll. J. Sci.* **28**, 67.
- Slingerland, D. W. (1955). *J. Clin. Endocrinol. and Metabolism* **15**, 131.
- Smith, G. V. Text Book of Endocrinology. Saunders Co. Philadelphia p. 352, (1955).
- Soliman, F. A. and E. P. Reineke (1955). *Am. J. Physiol.* **183**, 63.
- Suzuki, M. (1956). *Endocrinol. Japon.* **3**, 291.
- Takaori, C. (1954). *Folia Endocrinol. Japon.* **30**, 543.
- Wade, R. and H. W. Jones (1956 a). *J. Biol. Chem.* **220**, 547.
- Wade, R. and H. W. Jones (1956 b). *J. Biol. Chem.* **220**, 553.
- Wolterink, L. F., C. C. Lee, K. Olsen and M. Murray (1950). *Federation Proc.* **9**, 138.
- Yamazaki, E. and S. Kumaoka (1954). *Clinical Endocrinology* **2**, 32. (In Japanese)
- Zingg, W. and W. F. Perry (1953). *J. Clin. Endocrinol.* **13**, 713.