Sequential Changes in Rat Liver Nuclear Tri-iodothyronine Receptors and Mitochondrial α -Glycerophosphate Dehydrogenase Activity after Administration of Tri-iodothyronine

By Hirotoshi NAKAMURA,* Satoshi HAMADA†‡ and Hiroo IMURA*

*Department of Internal Medicine II, Kyoto University School of Medicine, Kyoto 606, Japan,
and †Department of Endocrinology and Nuclear Medicine, Tenri General Hospital,
Tenri 632, Japan

(Received 12 October 1978)

The dynamics of the induction of nuclear tri-iodothyronine receptors and mitochondrial α -glycerophosphate dehydrogenase were studied in rat liver after a single injection of tri-iodothyronine. The maximal binding capacity ($C_{\max}$) and association constant (K_a) of the nuclear receptors were determined by Scatchard analyses with and without correction for the endogenous tri-iodothyronine measured by radioimmunoassay. The administration of tri-iodothyronine induced sequential increases in the concentration of nuclear receptors and α -glycerophosphate dehydrogenase activity in the liver. The nuclear-receptor concentration was increased to 2.5 times that in the hypothyroid rat 1 day after the administration of hormone, and then decreased, with a half-life of about 2 days. α -Glycerophosphate dehydrogenase activity changed in parallel with the nuclear-receptor concentration, showing a delayed response. The total amount of non-histone protein in the liver was significantly increased 3 days after the administration. It seems likely therefore that the tri-iodothyronine-induced increase in nuclear-receptor concentration is responsible, at least in part, for the induction of this enzyme. The possibility is also suggested that nuclear receptors may be one of the non-histone proteins selectively synthesized at an early stage of the hormonal stimulation. Throughout the time course, the K_a values of the nuclear receptors for tri-iodothyronine remained unchanged, when corrected for endogenous tri-iodothyronine bound to the non-histone proteins, although they were apparently changed when the correction was not made. The results obtained provide further evidence for hormonal modulation of the nuclear receptors which is closely linked with the hormonal effect.

Since the discovery of nuclear binding sites specific for tri-iodothyronine (Oppenheimer *et al.*, 1972) a number of studies have been performed to elucidate the nature and properties of nuclear sites (Samuels *et al.*, 1974; Torresani & DeGroot, 1975; Oppenheimer *et al.*, 1976; Latham *et al.*, 1976). The results so far obtained strongly suggest that the nuclear tri-iodothyronine-binding protein, a non-histone protein, may be a thyroid-hormone receptor responsible for the hormonal effect, because the binding affinities for thyroid-hormone analogues bear a close correlation with the thyromimetic potency (Oppenheimer *et al.*, 1973; Koerner *et al.*, 1975), and the maximal binding capacity ($C_{\max}$) for hormone is greater in tissues responsive to thyroid hormones than in non-responsive tissues (Oppenheimer *et al.*, 1974).

It is particularly important to know whether the hormone binding by the nuclear receptors is affected by various thyroid conditions, and whether changes

in hormone-binding, if any, are associated with the hormonal effect. Although no hormonal effect on the nuclear receptors in rat liver has so far been reported, this failure may well be ascribed to either the small amounts of tri-iodothyronine tested (DeGroot *et al.*, 1976) or the indirect method of estimation based on an assumption that the plasma/nuclear ratios of injected [125 I]tri-iodothyronine are equal to [intracellular free tri-iodothyronine]/ $C_{\max}$ ratios (Oppenheimer *et al.*, 1975). Furthermore, no previous studies took account of the endogenous hormone that occupies substantial portions of the binding sites.

Using Scatchard (1949) analyses corrected for the endogenous hormone, we previously demonstrated that $C_{\max}$ of the liver nuclear receptors and the mitochondrial α -glycerophosphate dehydrogenase activity were significantly increased in hyperthyroid rats given 40 μ g of tri-iodothyronine for 3 days, and that the association constant (K_a) for tri-iodothyronine remained constant, irrespective of the thyroid state (Hamada *et al.*, 1979). Oppenheimer *et al.*

‡ To whom reprint requests should be addressed.

(1976, 1978) have reported kinetic studies on the relationship between a single injection of a large amount of tri-iodothyronine and the induction of α -glycerophosphate dehydrogenase that can be blocked by α -amanitin. In the present studies therefore the single-injection method with tri-iodothyronine was used to elucidate whether such a single injection can result in an increase in nuclear-receptor concentration or whether any relationship exists between the changes in the receptor concentration and the induction of α -glycerophosphate dehydrogenase.

Materials and Methods

Preparation of nuclear extract

Wistar-strain male rats weighing about 140g were obtained from Shimizu Laboratory Animal Co. (Kyoto, Japan), fed on a laboratory rat diet (Oriental Yeast Industrial Co., Chiba, Japan) and housed throughout the studies in a constant-environment apparatus kept at 25°C and a humidity of 55%. After several days of these conditions, all the rats were thyroidectomized and given 0.5% CaCl₂ solution as drinking water. The animals were weighed daily for more than 2 weeks and used for experiments only when the body weights were nearly stabilized.

Thyroidectomized rats were injected through the femoral vein with a single dose of 1250 μ g of tri-iodothyronine (Sigma Chemical Co., St. Louis, MO, U.S.A.)/100g body wt. under light ether anaesthesia. The thyroidectomized rats were divided into four groups: group A, no injection; group B, killed 1 day after injection; group C, killed 3 days after injection; group D, killed 5 days after injection. The animals were killed by exsanguination through the abdominal aorta under ether anaesthesia, and the livers were perfused *in situ* with cold 0.9% NaCl until they had been blanched completely. All further procedures were carried out at below 4°C.

The excised livers were minced into small pieces with scissors, and washed repeatedly with large amounts of cold 0.9% NaCl and finally with 0.25M-sucrose/3mM-MgCl₂ solution. Small pieces of the liver were saved for determination of DNA and mitochondrial α -glycerophosphate dehydrogenase activity. The remaining minced liver was homogenized in 4vol. of 0.25M-sucrose/3mM-MgCl₂ with a Teflon/pestle homogenizer, followed by successive centrifugations to isolate the nuclei. The methods used are described in detail in the preceding paper (Hamada *et al.*, 1979). A portion of the purified nuclei was saved for determination of DNA and protein concentration. From the purified nuclei non-histone proteins were extracted with 0.4M-KCl/5mM-MgCl₂/0.05M-Tris/glycine buffer, pH8.5. The yield of proteins in the extract averaged 32.3 $\pm$ 2.8% for group A, 32.2 $\pm$ 4.6% for group B, 37.4 $\pm$ 5.6% for

group C and 37.0 $\pm$ 5.0% for group D, and the difference between these values was statistically insignificant. The KCl extract was enriched with 1mM-dithiothreitol and stored at -20°C. The experiments were performed within 1 week of preparation, although the binding activity of nuclear proteins for tri-iodothyronine remained unchanged for about 3 weeks.

Determination of K_a and C_{max} .

Binding properties of the nuclear receptors were estimated by Scatchard analyses with and without correction for the amount of tri-iodothyronine endogenously bound to the receptors. The methods used were as in the preceding paper (Hamada *et al.*, 1979), although in the present studies the amount of endogenous hormone was determined by a radioimmunoassay described below. In the analyses all the determinations were performed in duplicate, and the K_a and C_{max} values for tri-iodothyronine were estimated by a regression line using at least four points close to the ordinate. The values for K_a and C_{max} were the means of at least two determinations.

Radioimmunoassay of tri-iodothyronine in serum and nuclear extract

Radioimmunoassay for serum tri-iodothyronine was carried out as follows; a 0.1 ml serum sample was added with 0.1 ml (20pg) of [¹²⁵I]tri-iodothyronine (sp. radioactivity 500–600 μ Ci/ μ g; Dainabott Laboratories, Tokyo, Japan), 0.5 ml of 1,8-anilino-naphthalenesulphonate (500 μ g/ml) and 0.1 ml of (1:4000) diluted antiserum, followed by incubation at 37°C for 1h and then 4°C overnight. Hormone-free rat serum, adsorbed on 2 $\times$ 25mg of Dowex 1 anion-exchange resin (Dow Chemical Co., Midland, MI, U.S.A.) per ml, was used for preparation of the standard curves. The mixture was added to 0.5 ml of 2.5% charcoal/0.25% Dextran T-40/0.075M-barbital/HCl, pH8.6, to separate unbound hormone. After centrifugation at 3000rev./min for 20min the supernatant and pellet were counted for radioactivity. The lowest detectable concentration of tri-iodothyronine was 20pg/0.1 ml of serum.

Radioimmunoassay of endogenous tri-iodothyronine in the nuclear extract was performed by a modified method of Surks *et al.* (1972). A mixture of the nuclear extract ranging from 0.1 to 0.5 ml or increasing amounts of standard tri-iodothyronine (0.1 ml), [¹²⁵I]tri-iodothyronine (0.1 ml), 0.1M-NaOH (0.3 ml), 0.03% bovine serum albumin/0.01M-NaOH (0.1 ml) and the extraction buffer to give a total volume of 1.0 ml was prepared in duplicate, and ¹²⁵I radioactivity (T_0) was counted for recovery correction. It was applied to a small column (1.5 ml volume) of Sephadex G-25 (Pharmacia Fine Chemicals, Uppsala, Sweden) equilibrated with 0.1M-NaOH. The column was washed successively with

2×0.2 ml of 0.1 M-NaOH, 3×1 ml of 0.075 M-barbital/HCl, pH 8.6, and 0.7 ml of 0.4% bovine serum albumin in the barbital buffer. Subsequently tri-iodothyronine was eluted with 2.2 ml of 0.4% bovine serum albumin in the barbital buffer. After gentle mixing, 2 ml of the eluate was pipetted into a disposable plastic tube, and counted for ^{125}I (T); 0.1 ml of the diluted antiserum was added, and the mixture was incubated at room temperature overnight and then at 4°C for 12 h. Separation of the unbound from the bound hormone was performed by the dextran-coated charcoal method described above. The amount of tri-iodothyronine estimated on the standard curve was corrected further by using the recovery of [^{125}I]tri-iodothyronine (T_0/T) in each determination. The recovery ranged from 80 to 90%, and the lowest detectable concentration of tri-iodothyronine was 2 pg/0.1 ml of nuclear extract. The value for the endogenous hormone was the mean of four determinations.

Other assays

Mitochondrial α -glycerophosphate dehydrogenase activity was measured by the method of Lee & Lardy (1965). Protein concentration was determined by the Lowry *et al.* (1951) method, with bovine serum albumin as standard. DNA was extracted by the method of Schmidt-Thannhauser-Schneider (Schneider, 1946) and measured by Burton's (1956) method.

Results

Concentrations of tri-iodothyronine in serum and non-histone protein, concentrations of nuclear proteins, non-histone proteins and DNA, and α -glycerophosphate dehydrogenase activity after injection of tri-iodothyronine

The results obtained are summarized in Table 1. Tri-iodothyronine was not detectable (less than

20 ng/100 ml) in the sera of the control group of thyroidectomized rats. After a single intravenous injection of tri-iodothyronine (1250 μg /100 g body wt.), the hormone concentrations were as high as 18300 ± 3400 ng/100 ml (mean $\pm$ s.d.) on the first day, and rapidly diminished thereafter. The concentration of tri-iodothyronine in non-histone protein was also elevated on the first day, and then decreased, but more gradually than in serum.

Concentrations of total nuclear proteins and non-histone proteins in the isolated nuclei were increased slightly but significantly ($P < 0.05$) on the third day, and returned to control values on the fifth day. However, DNA content per g of liver remained the same throughout the time course of the studies.

Mitochondrial α -glycerophosphate dehydrogenase activity was 0.030 absorbance unit/min per mg of protein in the control group, the value corresponding to that in hypothyroid rats. After injection of tri-iodothyronine, α -glycerophosphate dehydrogenase activity began to rise on the first day, and reached a maximum on the third day. The activity gradually decreased thereafter, but was still elevated above the control on the fifth day.

K_a and C_{max} values with and without correction for endogenous tri-iodothyronine

The results obtained for K_a and C_{max} of the nuclear receptors are shown in Table 2. When Scatchard analyses were performed without correction for endogenous tri-iodothyronine bound to non-histone protein, the apparent K_a was markedly decreased on the first day, and then gradually returned to the control value. The apparent C_{max} was increased to 1.5 times the control value on the first day, and decreased subsequently.

The correction for endogenous hormone had marked effects on the Scatchard analyses. As shown

Table 1. Concentrations of serum tri-iodothyronine (T_3), endogenous tri-iodothyronine bound to non-histone protein, DNA, nuclear proteins and non-histone proteins, and α -glycerophosphate dehydrogenase activity after the injection of tri-iodothyronine. All the data given are means $\pm$ s.d. Significance of difference from the corresponding values of group A, * $P < 0.05$, † $P < 0.005$.

Group	Number	Serum T_3 (ng/100 ml)	Endogenous T_3 bound to non- histone protein (ng/100 ml)	DNA (mg/g of liver)	Nuclear protein (mg/g of liver)	Non-histone protein (mg/g of liver)	$10^3 \times \alpha$ -Gly- cerophosphate dehydrogenase (ΔA /min per mg of protein)
A (before injection)	5	<20	8.0 ± 3.8	2.67 ± 0.26	4.40 ± 0.65	1.48 ± 0.22	30.0 ± 9.4
B (1 day after injection)	6	18300 ± 3400	140.1 ± 18.9	2.67 ± 0.47	5.36 ± 0.40	1.78 ± 0.29	83.4 ± 24.4
C (3 days after injection)	6	148 ± 37	64.3 ± 14.6	2.83 ± 0.85	$5.49^* \pm 0.40$	$2.10^* \pm 0.28$	243.0 ± 56.0
D (5 days after injection)	5	24 ± 9.5	20.2 ± 5.7	2.35 ± 0.48	4.59 ± 0.33	1.78 ± 0.13	166.5 ± 68.0

Table 2. Association constant (K_a) and maximal binding capacity ($C_{max.}$) of the nuclear tri-iodothyronine receptor with and without correction for endogenous hormone

Results are means $\pm$ s.d. The value in parenthesis was a mean of four preparations, because in the remaining two preparations no correction was possible for the reason described in the text. Significance of difference from the corresponding values of group A, * $P < 0.05$, † $P < 0.005$.

Group	Number	Uncorrected		Corrected	
		$10^9 \times K_a$ (M^{-1})	$C_{max.}$ (pg of T_3 /g of liver)	$10^9 \times K_a$ (M^{-1})	$C_{max.}$ (pg of T_3 /g of liver)
A (before injection)	5	11.4 ± 2.1	256.8 ± 86.6	14.5 ± 3.5	276.0 ± 90.1
B (1 day after injection)	6	$4.1 \dagger \pm 1.3$	$393.5^* \pm 88.5$	—	$697.9 \dagger \pm 163.7$
C (3 days after injection)	6	7.5 ± 3.2	321.4 ± 105.0	(14.9 ± 1.7)	$473.6^* \pm 123.9$
D (5 days after injection)	5	10.1 ± 2.9	246.2 ± 85.7	16.0 ± 5.2	293.9 ± 95.9

in Table 2, the corrected values for K_a remained virtually the same in all the preparations, except for those obtained soon after the tri-iodothyronine injection, i.e. group B, and two out of six preparations of group C. In the latter preparations the corrected values could not be obtained, because the points in the Scatchard plot converged near the intercept of the abscissa, which corresponded to the $C_{max.}$. These findings were quite similar to those obtained in the experiments *in vitro* (shown below) in which the preparations were preincubated with unlabelled hormone at a concentration equivalent to that used for group B.

The corrected values for $C_{max.}$ were even greater than the uncorrected ones, especially in preparations containing substantial amounts of endogenous tri-iodothyronine (Table 2). The $C_{max.}$ value was augmented to 2.5 times the control value on the first day, and thereafter decreased, with a half-life of 2 days. A significant increment in $C_{max.}$ was still observed on the third day, but not on the fifth day.

Relationship between $C_{max.}$ and α -glycerophosphate dehydrogenase activity

It is noteworthy that the change in $C_{max.}$ after injection of tri-iodothyronine appeared to be followed by a concomitant, but delayed, increase in mitochondrial α -glycerophosphate dehydrogenase activity (Fig. 1). The ratio of maximum to basal values was even greater for the enzyme activity (8.1 times) than for $C_{max.}$ (2.5 times). However, both of them decreased at similar rates after they had reached the maximum values. On the other hand, the hormone concentrations in serum and non-histone protein rapidly decreased (Table 1).

Effect on Scatchard plots of preincubation with unlabelled tri-iodothyronine in experiments *in vitro*

As mentioned above, Scatchard analyses corrected for endogenous tri-iodothyronine could not be performed on preparations obtained 1 day after the

injection of hormone. It is most likely that this failure was due to the large amounts of endogenous tri-iodothyronine contained in the non-histone protein in excess of $C_{max.}$. To verify this possibility, non-histone proteins obtained from hypothyroid rats were preincubated with two graded doses of unlabelled tri-iodothyronine, and Scatchard analyses with and without correction for added hormone were carried out. Without the correction, the enrichment with 20 or 100 pg of tri-iodothyronine/0.1 ml of nuclear extracts resulted in substantial decreases in K_a and $C_{max.}$, especially with the large dose of added hormone (Figs. 2a and 2b). With the correction, a Scatchard plot of the preparation enriched with 20 pg of tri-iodothyronine was exactly the same as that without added hormone (Fig. 2a). For enrichment with the hormone at a concentration nearly equivalent to that used for group B, however, the corrected points (Δ) converged near the intersection of the abscissa with the line of the Scatchard plot that was obtained for the original preparation without added hormone ($\circ$) (Fig. 2b). As a rule, only the $C_{max.}$ value that corresponded to the intercept of the abscissa was available in the experiments using large amounts of added tri-iodothyronine. These results indicate that correction for unlabelled hormone, either added in advance or bound endogenously, is essential for accurate determination of K_a and $C_{max.}$.

Discussion

The single injection of tri-iodothyronine at the dose employed by Oppenheimer *et al.* (1976, 1977) was found to induce a 250% increase in the $C_{max.}$ of the nuclear receptors without any accompanying changes in K_a for the hormone. This is probably a true increase in the nuclear-receptor concentration, because the DNA content per g of liver remained unchanged, as observed in the preceding paper (Hamada *et al.*, 1979). The present results are consistent with those obtained in the preceding paper

Fig. 1. Sequential changes in maximal binding capacity ($C_{\max}$) of the nuclear tri-iodothyronine receptors and mitochondrial α -glycerophosphate dehydrogenase activity after administration of tri-iodothyronine

Thyroidectomized rats were given a single intravenous injection of tri-iodothyronine (1250 μ g/100 g body wt.), and the nuclear non-histone proteins and mitochondrial fractions were prepared from the liver at the time intervals indicated. $C_{\max}$ of the tri-iodothyronine receptors in the non-histone proteins (○) was determined by Scatchard analysis with correction for endogenous hormone. Mitochondrial α -glycerophosphate dehydrogenase activity (●) was measured by the method of Lee & Lardy (1965). Each point represents the mean $\pm$ S.D.

and provide further evidence for induction of nuclear receptors by tri-iodothyronine.

The present studies also confirm that correction for endogenous hormone is essential for accurate determination of K_a as well as of $C_{\max}$ of the receptors (Hamada *et al.*, 1979). If no correction is made, the increase in $C_{\max}$ is rather small and statistically insignificant on the third day. Furthermore, the K_a value seems to change from day to day after injection of the hormone. With the correction, however, K_a is shown to be constant and $C_{\max}$ substantially increases after the injection.

Mitochondrial α -glycerophosphate dehydrogenase activity increased to 8-fold the value found in hypothyroid rats 3 days after injection of hormone, and decreased thereafter. This finding is in good agreement with that reported previously (Oppenheimer *et al.*, 1977, 1978). It is of further interest that the enzyme activity changed in parallel with the $C_{\max}$ of the nuclear receptors after a time lag of at least 1 day. Up until now, this alteration in enzyme activity has been entirely ascribed to saturation with hormone of the nuclear receptors, which were assumed to be fixed in number (Oppenheimer *et al.*, 1975, 1977). However, the results obtained here strongly suggest that the increase in $C_{\max}$, as well as the hormone occupancy may be responsible for induction of the enzyme.

Furthermore it is likely that the augmented $C_{\max}$.

Fig. 2. Effects on Scatchard plots of two graded doses of unlabelled tri-iodothyronine added *in vitro*

Non-histone proteins obtained from thyroidectomized rats were preincubated *in vitro* with (a) 20 pg and (b) 100 pg of tri-iodothyronine/0.1 ml, and Scatchard plots were drawn with or without correction for the amount of hormone added in advance. ○, Scatchard plots of the original preparation which was not preincubated with unlabelled tri-iodothyronine. Δ, Scatchard plots without correction for the added hormone; ▲, Scatchard plot with the correction.

may participate in the so-called amplification of the enzymic response. Oppenheimer *et al.* (1978) have reported that α -glycerophosphate dehydrogenase activity was elevated by 9–19.5 times when the nuclear occupancy was increased from 47 to 100%, i.e. only twice the control. It followed that the enzymic response to the hormone occupancy must be amplified or non-linear. However, because the $C_{\max}$ is increased to 2.5 times the basal value, full saturation of the augmented receptor sites should indicate a 5-fold increase in the hormone occupancy. Therefore the increase in $C_{\max}$ appears to provide a partial, though not full, explanation for the amplification of enzyme induction.

The total amount of acidic non-histone proteins in the liver nuclei reached a maximum 3 days after administration of the hormone, as did the enzyme activity. Since the nuclear tri-iodothyronine receptors are acidic non-histone proteins (Oppenheimer *et al.*, 1976), the early increase in $C_{\max}$ of the receptors may indicate selective synthesis of the receptor proteins by the hormonal stimulation. It has been reported that many hormones, namely glucagon (Enea & Allfrey, 1973), oestrogen (Teng & Hamilton, 1970; Cohen & Hamilton, 1975), cortisol (Shelton & Allfrey, 1970) and tri-iodothyronine (Taningher *et al.*, 1977; Bernal *et al.*, 1978), induce selective synthesis or phosphorylation of non-histone proteins. It is not yet clear whether or not the increase in the concentration of nuclear tri-iodothyronine receptors is related to the synthesis *de novo* of nuclear proteins that occurs within 5h of the administration of thyroid hormone (Bernal *et al.*, 1978).

In the present studies a pulse injection of tri-iodothyronine was employed as described by Oppenheimer *et al.* (1977, 1978), and hence, the pharmacological dose was necessary to maintain full occupancy of the nuclear receptors for the induction of maximum enzymic responses. It seems, however, that it does not necessarily indicate a pharmacological effect of the hormone, because the $C_{\max}$ of the receptors together with the enzyme activity was increased in rats given 40 μ g of tri-iodothyronine for 3 days (Hamada *et al.*, 1979), and also in the animals given 7 μ g for 2 weeks (H. Nakamura, S. Hamada & H. Imura, unpublished work).

We thank Miss Keiko Okazaki for her secretarial work, and the Dainabbot Laboratories, Tokyo, for generously giving the radioactive material.

References

- Bernal, J., Coleoni, A. H. & DeGroot, L. J. (1978) *Endocrinology* **102**, 452–459
- Burton, K. (1956) *Biochem. J.* **62**, 315–323
- Cohen, M. E. & Hamilton, T. H. (1975) *Proc. Natl. Acad. Sci. U.S.A.* **72**, 4346–4350
- DeGroot, L. J., Torresani, J., Carrayon, P. & Tirard, A. (1976) *Acta Endocrinol. (Copenhagen)* **83**, 293–304
- Enea, V. & Allfrey, V. G. (1973) *Nature (London)* **242**, 265–267
- Hamada, S., Nakamura, H., Nanno, M. & Imura, H. (1979) *Biochem. J.* **182**, 371–375
- Koerner, D., Schwartz, H. L., Surks, M. I. & Oppenheimer, J. H. (1975) *J. Biol. Chem.* **250**, 6417–6423
- Latham, K. R., Ring, J. C. & Baxter, J. D. (1976) *J. Biol. Chem.* **251**, 7388–7397
- Lee, Y. P. & Lardy, H. A. (1965) *J. Biol. Chem.* **240**, 1427–1436
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Oppenheimer, J. H., Koerner, D., Schwartz, H. L. & Surks, M. I. (1972) *J. Clin. Endocrinol. Metab.* **35**, 330–333
- Oppenheimer, J. H., Schwartz, H. L., Dillman, W. & Surks, M. I. (1973) *Biochem. Biophys. Res. Commun.* **55**, 544–550
- Oppenheimer, J. H., Schwartz, H. L. & Surks, M. I. (1974) *Endocrinology* **95**, 897–903
- Oppenheimer, J. H., Schwartz, H. L. & Surks, M. I. (1975) *Endocr. Res. Commun.* **2**, 309–325
- Oppenheimer, J. H., Schwartz, H. L., Surks, M. I., Koerner, D. & Dillmann, W. H. (1976) *Recent Prog. Horm. Res.* **32**, 529–565
- Oppenheimer, J. H., Silva, E., Schwartz, H. L., Surks, M. I. (1977) *J. Clin. Invest.* **59**, 517–527
- Oppenheimer, J. H., Coulombe, P., Schwartz, H. L. & Gutfeld, N. W. (1978) *J. Clin. Invest.* **61**, 987–997
- Samuels, H. H., Tsai, J. S., Casanova, J. & Stanley, F. (1974) *J. Clin. Invest.* **54**, 853–865
- Scatchard, G. (1949) *Ann. N.Y. Acad. Sci.* **51**, 660–672
- Schneider, W. C. (1946) *J. Biol. Chem.* **164**, 747–751
- Shelton, K. R. & Allfrey, V. G. (1970) *Nature (London)* **228**, 132–134
- Surks, M. I., Schadow, A. R. & Oppenheimer, J. H. (1972) *J. Clin. Invest.* **51**, 3104–3113
- Taningher, M., Molinari, M. P. & Cesarone, C. F. (1977) *Endocrinology* **101**, 1221–1227
- Teng, C.-S. & Hamilton, T. H. (1970) *Biochem. Biophys. Res. Commun.* **40**, 1231–1238
- Torresani, J. & DeGroot, L. J. (1975) *Endocrinology* **96**, 1201–1209