Manganese Ion as a Goitrogen in the Female Mouse

JUN KAWADA, MIKIO NISHIDA, YOSHIYUKI YOSHIMURA
AND KIKUJI YAMASHITA

*Department of Biochemistry, Faculty of Pharmaceutical Sciences
The University of Tokushima, 77 Shomachi 1, Tokushima 770*

Abstract

Effect of excessive ingestion of manganese (Mn) on the mouse thyroid was assessed under the conditions of normal intake of iodide. Female mouse thyroids were enlarged after 7 weeks of administration of 200 mg/l $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ in drinking water; 2.74 ± 0.25 mg for control (N=56), and 3.31 ± 0.28 mg for Mn-treated group (N=85) ($p < 0.001$). In contrast, male mouse thyroids never became goitrous following this treatment. Manganese was goitrogenic to the castrated male mouse, but it had no effect on the testosterone-treated castrated male mouse, indicating the involvement of androgen in goiter formation. Oral administration of Mn did not severely affect blocked T/S of ^{125}I or iodine metabolism in the thyroid. A morphological study, however, revealed that the epithelial cell in the Mn-treated mouse thyroid became flatter than that of the control. The lumens were filled with colloid in Mn-treated female mouse thyroid. The serum levels of thyroxine (T_4), but not triiodothyronine (T_3), were slightly reduced by Mn.

These informations suggest that Mn can be a mild goitrogen for the female mouse and that the etiology of goiter formation can be interpreted by retention of colloid in the lumen.

According to Mănescu *et al.* (1960), manganese ion (Mn^{2+}) could be goitrogenic when iodide intake was insufficient. Manganese contained in various foods was also suspected as a causative factor in endemic goiter (Savina, 1969). Since Mn acutely injected into rats blocks uptake of iodide by the thyroid (Buthieu and Autissier, 1977), Mn can be one of the factors to cause goiter. However, a low iodine state itself is sufficient to create goiter in the thyroid (Studer and Greer, 1965, Riesco,

et al., 1977). If excessive Mn is present with insufficient iodide, it is unclear whether the metal ion itself is goitrogenic or can be only co-goitrogenic. Thus, the etiology of Mn dependent goiter remains to be clarified.

The present study was designed to assess whether or not large amounts of Mn per os were goitrogenic in the mouse maintained on normal iodide intake and to clarify the mechanism of the Mn effect on thyroid iodine metabolism.

Materials and Methods

ddY male and female mice (4 weeks old) were fed on a regular chow which was obtained from a commercial source (Type MF, Oriental Yeast Co., Ltd, Tokyo) and given tap water *ad libitum*. Manganese was added in the drinking water for 7 weeks unless otherwise stated. In some experiments, ip injection was applied for comparison with oral administration. Amounts of Mn were expressed in parts per million (ppm, equivalent to mg/l) as $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$. As a comparison, NaSCN (200 ppm) was also given in the drinking water for 7 weeks. The animals were killed by bleeding from the carotid artery. The blood was collected for serum T_3 and T_4 measurements by radioimmunoassay (RIA). The thyroid was immediately excised and weighed on a semi-micro torsion balance (Metronex, Warsaw).

Castration was carried out immediately after the animals were purchased. Testosterone propionate pellets (10–20 mg each) were prepared by a micro press and inserted subcutaneously in the back.

Organ distribution of Mn was examined after ^{54}Mn administration by stomach tubing (1 μCi /animal/day) for consecutive 4 weeks. The animals were anesthetized with ether. Blood was washed away by circulating saline solution containing 10 U/ml of heparin. This was done with a cannula inserted into the arcuate aorta through the left ventricle and the pressure was released by an incision at the right atrium. Radioactivity localized in each tissue was measured with an Aloka gamma scintillation counter.

The uptake of serum iodide into the thyroid (T/S) was examined by the method of Halmi *et al.* (1953).

Iodine metabolism in the thyroid was examined by ip injection with ^{125}I (2 μCi /animal). After 24 h the thyroid was excised and then subjected to hydrolysis with Pronase in a sealed test tube under N_2 gas flush. The iodinated compounds were analyzed by paper chromatography described elsewhere (Kawada *et al.*, 1980).

Morphological observation was carried out by ordinary dehydration and fixation procedures. The sections were stained with hematoxylin and periodate. The height of the epithelia was measured on photographs.

The amounts of iodide and Mn in the chow were determined as follows; after pulverizing of

the pellets, followed by ashing on a low temperature plasma asher (Yanagimoto Ltd, type LTA-4sn), extraction was carried out with re-distilled water and sequentially with acid solution (0.7 N $\text{H}_2\text{SO}_4 + 0.2$ N HCl). The extracts were appropriately diluted for analysis. Iodide was measured by the method of Barker *et al.* (1951). A linear relation was obtained up to 5.0 ppm for iodide. The recovery of added authentic iodide for ashing and extraction procedures was essentially 100% below 10 ppm, but gradually reduced when the added amounts were increased to more than 10 ppm. Manganese was determined with a flame atomic absorption spectrometer (AAS, Japan Jarrell-Ash Co., Ltd). The standard curve became straight up to 8.0 ppm. The recovery was 70–90% for ashing and 80–100% for extraction. Analytical conditions were as follows; C_2H_2 0.5–0.8 kg/cm², oxidant, 1.5 kg/cm², and wavelength, 279.5 nm.

Reagents of AAS were of special grade for metal analysis obtained from Wako Chemical Co. (Osaka). Other reagents were of the highest grade available from commercial sources. RIA kits were purchased from Mallincrodt Co. through Daiichi Isotope Institute (Tokyo). Radioactive manganese (^{54}Mn , 36.0 mCi/mg) and iodide (^{125}I , 17.4 Ci/mg) were purchased from New England Nuclear (Massachusetts, USA).

Results

The iodide and Mn contents in the chow were 1.04 ± 0.15 ppm (N=14) and 64.76 ± 7.34 ppm (N=7) as the element respectively. Daily consumption of chow per mouse was 4.87 g/day (an average of 6 identical experiments consisting of 6 animals every time for 3 consecutive days). Therefore, daily intake of iodide was approximate 5 μg per mouse. Since the daily requirement is reported to be in the range of a few $\mu\text{g/kg}$ (Greenspan and Forsham, 1983), the detected amount of iodide was sufficient to maintain normal functions in the thyroid. The commercially available regular chow for growth and breeding was fortified with 50 ppm of Mn (Oriental Yeast, 1981). The detected amounts of Mn in the diet were assumed

to be the basal level for normal maintenance and approximate 300 μg of Mn/day was consumed by a mouse. The addition of 200 ppm of $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ (approximate 50 ppm as the metal ion) in the drinking water was enough to induce colloid-rich goiter in female mice.

^{54}Mn was orally given for 4 weeks to male and female mice and its radioactivity distribution was examined. The pituitary, thyroid, pancreas, adrenal and kidney showed a high accumulation of the radioactivity in both sexes (Tab. 1). The general trend of Mn retention in mice was similar to the patterns reported in rat (Buthieu and Autissier, 1983) and monkey (Suzuki *et al.*, 1975). Further prolonged administration of ^{54}Mn , however resulted in less distinct distribution of isotope in tissues (data not shown).

The thyroid of young adult female mice was clearly enlarged after 4 week oral administration of Mn. The effect was dependent on the given doses and periods

of Mn treatment. The administration of 200 ppm $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ for 7 weeks caused a high incidence of goiter formation. One of the typical results on Mn effect is shown in Table 2. The potency of the goitrogenic action of Mn was comparable to that of SCN in female, while no change was observed in male mouse thyroids with Mn or even with SCN. To check the above observations on the Mn effect, the same experiments were repeated. In Table 3, the average weight

Table 1. Distribution of ^{54}Mn administered orally in the mouse

Organs	Relative Distribution	
	Male	Female
Liver	1.00	1.00
Thyroid	22.7	18.7
Pituitary	25.7	19.6
Brain	0.67	0.39
Submaxillary gland	4.95	2.74
Lung	0.58	0.34
Pancreas	4.78	1.96
Spleen	0.32	0.21
Testis	1.54	—
Adrenal	3.79	3.56
Kidney	6.26	2.14
Seminal vesicle	1.70	—
Ovary	—	0.93

The dose schedule and the method of sample preparation are described in the text. ^{54}Mn activity was expressed as the ratio of cpm/mg of each organ to that of the liver. Each value represents the mean of two determinations for each sex.

Table 2. Change in thyroid weight of mice treated with manganese and/or thiocyanate

Sex	Treatment	Thyroid weight	
Female		mg	
	Control	2.65 $\pm$ 0.24	
	Mn	3.37 $\pm$ 0.23	p<0.02
	SCN	3.38 $\pm$ 0.31	p<0.02
Male	Mn+SCN	3.83 $\pm$ 0.52	p<0.01
	Control	2.95 $\pm$ 0.30	
	Mn	2.71 $\pm$ 0.49	NS
	SCN	2.82 $\pm$ 0.12	NS
	Mn+SCN	3.20 $\pm$ 0.24	NS

Each group of females consisted of 5 animals and that of males consisted of 6. Mn (200 ppm as $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$) and thiocyanate (200 ppm as NaSCN) were given in the drinking water for 7 weeks. Thyroid weight is expressed as mean $\pm$ SD. Statistical significance was assessed by Student's *t*-test. NS: Not significant

Table 3. Goitrogenic effect of manganese chloride administered orally to mice

Sex	Group	No. of animal	Thyroid weight	
Female			mg	
	Control	56	2.74 $\pm$ 0.25	
	Mn	85	3.31 $\pm$ 0.28	p<0.001
Male	Control	42	3.15 $\pm$ 0.50	
	Mn	49	3.02 $\pm$ 0.55	NS

The experimental conditions were same as those described in Table 2. The values represent the mean $\pm$ SD. NS: Not significant

was 3.31 mg in the treated group (N=85), compared with 2.74 mg in the control group (N=56). The thyroid enlargement due to Mn was clear only in female mice. Even when male mice were treated with 200 ppm of $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ for 7 weeks, the thyroid weight never exceeded that of the control animals.

Castration, followed by the Mn treatment

Fig. 1. Manganese effect on the thyroid weight of castrated male mice with and without testosterone supplement

C; Control, Mn; Given 200 ppm $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ in drinking water for 7 weeks, TP; Testosterone propionate pellet was inserted on the back of the animals. The number of animals is shown in parenthesis.

resulted in enlargement of the thyroid, while castrated animals which received a supplement of testosterone propionate did not show any measurable thyroid weight gain (Fig. 1).

To study the role of Mn in goiter formation, the blocked iodide uptake (T/S) was measured after a 7 week administration of Mn. The ratio was not greatly changed in the two groups: 76.25 ± 18.88 (N=7) in

Fig. 2. Manganese and thiocyanate effects on serum T_4 and T_3 levels.

Each group consisted of 8 mice. C; Control, Mn; Given 200 ppm $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ in drinking water for 7 weeks. SCN; Given 200 ppm NaSCN in drinking water for 7 weeks. Vertical bar represents standard deviation. Statistical difference was: for T_4 , Mn versus C, $p < 0.01$; SCN versus C, $P < 0.001$; for T_3 , Mn versus C, not significant; SCN versus C, $p < 0.01$.

Table 4. Distribution of ^{125}I in the hydrolysate of female mouse thyroid treated with manganese chloride and/or thiocyanate

Treatment	DIT	MIT	I ⁻	T ₄	T ₃	DIT/MIT	T ₄ +T ₃ /DIT+MIT
	%	%	%	%	%		
Control	47.35	25.63	4.24	6.83	2.08	1.847	0.122
Mn	48.01	23.03	5.14	8.87	2.18	2.085	0.156
SCN	45.32	19.82	3.49	7.18	2.34	2.287	0.146
Mn+SCN	48.69	16.22	3.39	6.55	1.98	3.002	0.131

Each group consisted of 4 mice which were administered a 200 ppm solution of either $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ or NaSCN. One group was given a mixture of the two. The administration period was 7 weeks. Since individual thyroid was not sufficient, the excised thyroids were pooled in each group for hydrolysis by Pronase. Results were expressed as a percentage of the count due to ^{125}I -labelled metabolite to the total count on the paper chromatogram. The values represent the mean of duplicate determinations.

the control and 69.56 ± 17.86 in the Mn-treated group ($N=6$). When Mn was intraperitoneally injected for 2 days, a significant reduction (by 30% to control) in the iodide uptake was observed. Table 4 depicts that the 24 h radioiodide distribution in the thyroid remained unchanged following oral administration of Mn, although a minor increase in fractions of DIT/MIT and $T_3 + T_4$ /DIT+MIT was observed in the Mn treated thyroid. A similar tendency was also seen in the SCN treated group.

The change in the serum T_4 level with the Mn treatment was minor, but still statistically significant (Mn given group versus control, $p < 0.01$). Serum T_3 was unchanged by this treatment (Mn given group versus control, not significant) (Fig. 2).

A morphological study of the female thyroid treated with Mn per os, revealed moderately flattened epithelial cells with ample colloid in the lumen (Fig. 3). Equal numbers ($N=50$ each) of the epithelial cells were chosen at random from follicles in the middle portion of the control and the Mn treated groups. The estimated cell height was $7.43 \pm 0.82 \mu$ in control and $5.86 \pm 0.31 \mu$ in the Mn treated ($p < 0.001$).

Discussion

Endemic goiters are usually associated with iodide deficiency and/or interfered iodide metabolism in the thyroid (DeGroot

Fig. 3. The appearance of Mn treated female mouse thyroid

(a) Control $\times 150$ (b) Mn treated $\times 150$

and Stanbury, 1975), except the iodide dependent goiter which is caused by excessive iodide ingestion (Suzuki, *et al.*, 1965, Nagataki, 1976). When the goitrogenic nature of Mn is discussed, it should be differentiated whether the Mn dependent goiter is caused by Mn itself or enhanced by iodide deficiency with an excess of Mn. In our experiment, the iodide content in the food consumed by animals was strictly measured and was confirmed to be sufficient to maintain normal growth. Under such conditions, the oral administration of large amounts of Mn resulted in the thyroid enlargement of female mouse. The actual dietary requirement of Mn has rarely been estimated. Manganese intake in mammals is said to be somewhere between 5 and 40 ppm (Mena, 1981). The estimated 60 ppm in the diet should be in the normal range and less than the threshold level required to develop goiter in female mouse. Additional Mn administration may trigger goiter formation. Castrated male mice developed goiter with Mn like female animals, while the castrated and testosterone-treated animals became resistant to the Mn effect. These findings suggest that androgens could exert an antagonistic effect on the goitrogenic action of Mn.

Goiter was found in female mice given Mn only by the oral route but not by a parenteral route. Although the rate of gastro-intestinal absorption of Mn is rather low, a radioisotopic study by stomach intubation showed that ^{54}Mn was substantially accumulated in the pituitary and thyroid of both sexes. It seems likely that sub-maximal amounts of Mn supplied continuously through gastro-intestinal absorption function differently in tissues from the excess ion given intermittently by injection. The mechanism by which only orally administered Mn induces goiter is not known, but it is reasonable to consider that the presence of Mn transferrin was demonstrated in the plasma of certain species (Foradori *et al.*, 1967, Hancock *et al.*, 1973, Gibbons *et al.*,

1976), and a similar carrier protein may exist in the mouse and facilitate Mn absorption through the digestive tract. The transferrin-bound Mn remains in circulation for longer periods than the free ion (Foradori *et al.*, 1967, Gibbons *et al.*, 1976) and it may release a free form of Mn gradually. Thus, mild and continual stimulus of the thyroid by Mn must play an important part in causing goiter.

As a cause of colloid-filled goiter formation, it has been postulated (Marine and Lenhart, 1909) that a recurrent cyclic change in hyperplasia with deprivation of iodide to a colloid-filled and involuted state with replenished iodide supply may take place in the thyroid. This early hyperplastic change should be caused by an increase in the amount of thyroid stimulating hormone (TSH) in response to the iodide deficiency. Subsequently when an adequate iodide supply is restored, the involuted follicles with colloid may appear, due to the lowered TSH (Greer *et al.*, 1967). In the present study, the Mn induced goiter in female mice had the appearance of colloid goiter. However, the amount of iodide fed to the mice should be sufficient throughout this study, as mentioned before. Furthermore only minor reduction in the T/S occurred following oral Mn ingestion. It is unlikely that orally administered Mn causes severe iodide deficiency due to blockage of the iodide pump or severe aberration in iodine metabolism.

The serum T_4 level was lowered slightly, but the T_3 level remained unchanged following oral Mn administration. As the major source of serum T_4 is the thyroid, the reduction in serum T_4 suggests a somewhat depressed hormone secretion from the thyroid, whereas the serum T_3 level is maintained by both the thyroid and peripheral tissues. In the latter organs, rapid conversion of T_4 to T_3 takes place. The generated T_3 may significantly contribute to the hormone level in the circulation (Oppenheimer

and Samuels, 1983). The unchanged serum T_3 level may indicate that peripheral deiodination in major organs is not affected by Mn treatment.

An early study (Ray and Deysach, 1942) reported that small doses of Mn induced a hyperfunctional state in the thyroid and the morphological examination (Mănescu *et al.*, 1960) revealed that Mn caused interstitial hyperplasia in the gland. These observations however, sharply contrasted with our observation in which the moderately flat parenchymatous cells with colloid in follicles and slightly lowered serum T_4 were observed. Upon these observations, we tentatively consider that endocytosis must be inhibited by the excess of Mn, presumably via lowered or at least a subnormal TSH level in blood. Unfortunately in this study, the mouse TSH level could not be correctly measured with a commercially available human TSH kit due to its incomplete immunoreactivity. Therefore, this theory remains to be proved. Buthieu and Autissier (1983) reported recently that the TSH level was lowered in the Mn injected rat, suggesting that Mn may depress the pituitary and thyroidal functions. If this evidence could be extended to mouse, the reduction in cell height with retained colloid due to the prolonged oral administration of Mn might be attributed to the reduced blood TSH level.

In addition to Mn, several other metals (Paley *et al.*, 1958, Suzuki *et al.*, 1966, Miloni *et al.*, 1983) have been reported as elements affecting the thyroid function. Among them, lithium (Miloni *et al.*, 1983) seems to be an interesting goitrogenic element. It could cause a thyroglobulin-rich goiter by inhibiting endocytosis in the presence of a minimal dose of methimazole, demonstrating the possibility of an alternative mechanism to Marine's hypothesis (Marine and Lenhart, 1909). The clear difference observed between conditions inducing goiter with lithium and goiter with Mn is that the former goiter was developed

in the presence of a classical goitrogen, methimazole to create an iodide-deficient state, but the latter was developed by Mn under a sufficient iodide supply. In this sense, the Mn-induced colloid goiter in female mice seems to be different from lithium-inducible goiter and an additional alternative to Marine's hypothesis. To prove this, the process in developing Mn-induced goiter should be investigated from the biochemical and histochemical viewpoints. Current studies at our laboratory are focussed on finding out whether the hyperplastic stage is involved in quite an early stage of Mn treatment and to determine iodide content in thyroid and TSH in blood.

Thiocyanate is known to be a goitrogen by inhibiting the iodide concentrating mechanism of the thyroid and was used as a reference compound in our experiments. When 200 ppm of NaSCN was given in drinking water for 7 weeks, it was indeed goitrogenic in female mouse, but not in male mouse. No observable aberration in intra-thyroidal iodine metabolism was caused by SCN in the conditions applied (Tab. 4). This result was rather unexpected, but in many studies (Wyngaarden *et al.*, 1953, Langer, 1966, Scranton *et al.*, 1967), a low iodine diet was given with SCN to demonstrate the drug effect. Other researches (Vanderlaan and Vanderlaan, 1947, Wolman, 1956), gave large amounts of the drug parenterally to cause blockage of the iodide pump in the thyroid. Therefore, the ineffectiveness of SCN on male mouse in the present study seems to be due to the difference in the experimental conditions; in fact, in another series of our studies showed that the administration of 300 ppm of SCN for 6 weeks caused a mean 28% weight gain in the thyroid of half the number of male mice. Whether there is a real sex-related difference in the effect of low level SCN on mouse thyroid is still not obvious. Research aimed at solving this problem is in progress.

The present study showed that Mn was a moderately mild goitrogen without iodide deficiency. The cause of goiter by Mn still remains putative, but it is speculated that excessive Mn administration may lead to suppression of the colloid pinocytosis and result in colloid retention in lumen. Flatter cells with ample luminal colloid support this assumption. Furthermore the sex hormone status should be profoundly involved as another etiological factor in goiter formation with Mn.

Acknowledgements

The present study was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Health and Welfare of Japan.

References

- Barker, S. B., M. J. Humphrey and M. H. Soley (1951). The clinical determination of protein-bound iodine. *J. Clin. Invest.* **30**, 55–62.
- Buthieau, A.-M. and N. Autissier (1977). Action des ions Mn^{2+} sur le métabolisme iodé thyroïdien du Rat. *Comp. rend. séan. Soc. Biol.* **171**, 1024–1028.
- Buthieau, A.-M. and N. Autissier (1983). Effects of manganese ions on thyroid function in rat. *Arch. Toxicol.* **54**, 243–246.
- DeGroot, L. J. and J. B. Stanbury (1975). *The Thyroid and Its Diseases* 4th ed. pp. 496–538. John Wiley and Sons, New York.
- Foradori, A. C., A. Bertinchamps, J. M. Gulibon and G. C. Cotzias (1967). The discrimination between magnesium and manganese by serum proteins. *J. Gen. Physiol.* **50**, 2255–2266.
- Gibbons, R. A., S. N. Dixon, K. Hallis, A. M. Russell, B. F. Sansom and H. W. Symonds (1976). Manganese metabolism in cows and goats. *Biochim. Biophys. Acta* **444**, 1–10.
- Greenspan, F. S. and P. H. Forsham (1983). In: *Basic and Clinical Endocrinology*. pp. 130–186, Lange Med. Pub. Los Altos.
- Greer, M. A., H. Studer and J. W. Kendall (1967). Studies on the pathogenesis of colloid goiter. *Endocrinology* **81**, 623–632.
- Halmi, N. S., B. N. Spirtos, E. M. Bogdanove and H. J. Lipner (1953). A study of various influences on the iodide concentrating mechanism of the rat thyroid. *Endocrinology* **52**, 19–32.
- Hancock, R. G. V., D. J. R. Evans and K. Fritze (1973). Manganese protein in blood plasma. *Biochim. Biophys. Acta* **320**, 486–493.
- Kawada, J., M. Nishida, Y. Yoshimura and K. Mitani (1980). Effects of organic and inorganic mercurials on thyroidal functions. *J. Pharm. Dyn.* **3**, 149–159.
- Langer, P. (1966). Antithyroid action in rats of small doses of some naturally occurring compounds. *Endocrinology* **79**, 1117–1122.
- Mănescu, S., I. Negoescu, A. Lupulescu and M.-L. Diaconescu (1960). Investigation on the goitrogenic action of manganese. *Rumanian Med. Rev.* **5**, 88–90.
- Marine, D. and C. H. Lenhart (1909). Colloid glands (goiter): their etiology and physiological significance. *Bull. Johns Hopkins Hosp.* **20**, 131–139.
- Mena, I. (1981). Manganese In: *Disorders of Mineral Metabolism* (Bronner, F. and Coburn, J. W. eds.) vol. 1, pp. 233–270, Academic Press, New York.
- Miloni, E., H. Bürgi, H. Studer, L. Siebenhüner and Th. Lemarchand-Béraud (1983). Thyroglobulin-rich colloid goiters: a result of the combined action of lithium and methimazole on the rat thyroid. *Acta Endocrinol.* **103**, 231–234.
- Nagataki, S. (1976). Iodine-induced goiter: effect of high iodine intake in thyroid function. *Pharmac. Ther. C.* **1**, 45–56.
- Oppenheimer, J. H. and H. H. Samuels (1983). In: *Molecular Basis of Thyroid hormone Action*. pp. 2–4. Academic Press, New York.
- Oriental Yeast Co. LTD, (1981). *Information in Nutritional Control* pp. 9, Tokyo.
- Paley, K. R., E. S. Sobel and R. S. Yalow (1958). Effect of oral and intravenous cobaltous chloride on thyroid function. *J. Clin. Endocrinol. Metab.* **18**, 850–859.
- Ray, T. W. and L. J. Deysach (1942). Storage of manganese by thyroid. Effect on oxygen consumption of the guinea pig. *Proc. Soc. Exp. Biol. Med.* **51**, 228–229.
- Riesco, G., A. Taurog, P. R. Larsen and L. Krulich (1977). Acute and chronic responses to iodide deficiency in rats. *Endocrinology* **100**, 303–313.

- Savina, P. N. (1969). Importance of manganese in the formation of endemic goiter. *Tr. Tomsk. Nauch.-Issled. Vaksyn Syvocotok Tomsk. Med. Inst.* 20, 183-185.
- Scranton, J. R., W. M. Nissen and N. S. Halmi (1969). The kinetics of the inhibition of thyroidal iodide accumulation by thiocyanate: A reexamination. *Endocrinology* 85, 603-607.
- Studer, H. and M. A. Greer (1965). A study of the mechanisms involved in the production of iodine-deficiency goiter. *Acta Endocrinol.* 49, 610-628.
- Suzuki, H., T. Higuchi, K. Sawa, S. Ohtaki and Y. Horiuchi (1965). Endemic coast goiter in Hokkaido, Japan. *Acta Endocrinol.* 50, 161-176.
- Suzuki, T., T. Miyama and H. Katsunuma (1966). Affinity of mercury for the thyroid. *Ind. Health* 4, 69-75.
- Suzuki, Y., T. Mouri, Y. Suzuki, K. Nishiyama, N. Fujii and H. Yano (1975). Study of subacute toxicity of manganese dioxide in monkey. *Tokushima J. Exper. Med.* 22, 5-10.
- Vanderlaan, J. E. and W. P. Vanderlaan (1947). The iodide concentrating mechanism of the rat thyroid and its inhibition by thiocyanate. *Endocrinology* 40, 403-416.
- Wollman, S. H. (1956). Nature of the inhibition by thiocyanate of the iodide concentrating mechanism of the thyroid gland. *Amer. J. Physiol.* 186, 453-459.
- Wyngaarden, J. B., J. B. Stanbury and B. Rapp (1953). The effects of iodide, perchlorate, thiocyanate and nitrate administration upon the iodide concentrating mechanism of the rat thyroid. *Endocrinology* 52, 568-574.