*Europ. J. Cancer*, Vol. 16, pp. 473–480

0014-2964/80/0401-0473 $02.00/0

# Inhibitory Effects of Potassium Thiocyanate on Normal and Neoplastic Mammary Development in Female Mice*

HIROSHI NAGASAWA,† REIKO YANAI,† YUKO NAKAJIMA,† HIDEO NAMIKI,‡ SAKAE KIKUYAMA‡ and KOHEI SHIOTA‡

†*Pharmacology Division, National Cancer Center Research Institute, Tsukiji* 5-1-1, *Chuo-ku, Tokyo* 104, ‡*Department of Biology, School of Education, Waseda University, Nishiwaseda* 16-1, *Shinjuku-ku, Tokyo* 160 *and* §*Department of Anatomy, Kyoto University School of Medicine, Yoshida-Konoecho, Sakyo-ku, Kyoto* 606, *Japan*

**Abstract**—*Effects of potassium thiocyanate (KSCN) on normal and neoplastic mammary development in female mice were studied. Administration of KSCN as drinking water* (0.1 *or* 0.3%) *for* 12 weeks *to* 8-week-*old SHN female mice resulted in the dose-related inhibition of development of normal mammary lobulo-alveolar system, hyperplastic alveolar nodules and spontaneous mammary tumors associated with marked decrease in plasma levels of triiodothyronine and thyroxine. On the other hand, the treatment affected little estrous cycles, ovarian structures, plasma prolactin levels and weights of body, anterior pituitary and adrenals.*

*The incidence and number of pregnancy-dependent mammary tumors in GR/A female mice were also significantly suppressed by the similar treatment with KSCN with no influence on reproductivity as well as pituitary levels of prolactin and growth hormone.*

*These results suggest that KSCN does not affect the secretion of pituitary and ovarian mammotropic hormones and that thyroid hormones are principally involved in normal and neoplastic development of mammary glands.*

## INTRODUCTION

IN ORDER to evaluate the role of thyroid hormones in mammary tumorigenesis, several works have been done on the effects of altered thyroid function on carcinogen-induced mammary tumors in rats, although the results are still controversial; oral administration of propylthiouracil [1–4], methimazole [2] and 2-mercaptobenzimidazole [2], injection of $^{131}I$ [5], thyroidectomy [6–8] or injection of thyroxine [6, 8] resulted in enhancement [1], suppression [2–6] or no effects [7, 8]. It was also reported that mammary tumor enhancement in $^{131}I$-induced hypothyroidism was attributable to a consequence of radiation injury to mammary tissue rather than to hypothyroidism itself [9]. Furthermore, Vazquez-Lopez [10] reported that feeding of thiourea induced no alteration of the incidence of spontaneous mammary tumors in R3 female mice, but it decreased mammary tumor incidence in C3H female mice. Delayed appearance of mammary tumors and considerable atrophy of normal glands in C3H mice were also observed in another laboratory [11].

According to Anderson [12], potassium thiocyanate (KSCN) acts on thyroid glands to block the uptake of iodine and Pyska [13] recently found that chronic administration of KSCN as drinking water resulted in the marked inhibition of mammary gland growth and function in female rats associated with the lowered plasma protein-bound iodine level.

In this paper, the effects of KSCN on normal and neoplastic development of mammary glands in SHN and GR/A strains of female mice were studied as a possible step to evaluate the role of thyroid gland in mammary tumorigenesis. Estrous cycles, body weight change, reproductivity or plasma levels of triiodothyronine (T3), thyroxine (T4) and prolactin in these animals were also examined.

Accepted 21 August 1979.

*This work was supported in part by the grant in-aid for Cancer Research from the Ministry of Education, Science and Culture, Japan (No. 301082).

## MATERIALS AND METHODS

*Experiment* I

*Animals.* A high mammary tumor strain of SHN female mice [14] was used. They were given 0.1 or 0.3% KSCN (Kokusan Chemical Works Ltd., Tokyo, Japan) as drinking water beginning 8 weeks of age. The control mice received tap water. Vaginal smears were checked every morning in all mice. They were kept six per cage in Teflon cages (15 × 30 × 12 cm) with wood shavings, maintained in an animal room that was air-conditioned (24 ±0.5°C and 65–70% r.h.) and artificially illuminated (14 hr of light from 5.00 a.m. to 7.00 p.m.) and provided with a commercial diet (CA-1: CLEA Japan Inc., Tokyo, Japan) *ad libitum.*

*Normal and neoplastic mammary development.* At 20 weeks of age, mice were checked for palpable mammary tumors, bled from vena cava under light ether anesthesia and killed by decapitation. Plasma was kept at −20°C for assay of T3, T4 and prolactin. Bilateral thoracic mammary glands were prepared for wholemount evaluation. Normal mammary end-bud or lobulo-alveolar growth was rated from one to seven in increments of one as presented in Fig. 1. The number and size of preneoplastic mammary hyperplastic alveolar nodules (HAN) expressed in terms of mathematical mean of the major two diameters were examined at 10 × magnification.

*Organ weights and ovarian histology.* The pituitary and adrenals were removed and weighed. Ovaries were fixed in Bouin's solution, embedded in paraffin, serially sectioned at 6μm and stained with hematoxylin and eosin.

*Plasma levels of T3, T4 and prolactin.* Plasma levels of T3, T4 and prolactin were measured by radioimmunoassay using the commercial kits, T-3 RIAKIT II and T-4 RIAKIT II (Dinabott RI laboratory, Tokyo, Japan) for T3 and T4, respectively and the kit supplied by Dr. VanderLaan, La Jolla, CA, U.S.A., for prolactin.

*Experiment* II

*Animals.* Highly inbred GR/A strain of female mice [15] was used. Beginning 5 weeks of age, experimental groups received 0.1 or 0.3% KSCN as drinking water throughout the experiment except for 3 days of mating. Control mice were given tap water. At 10 weeks of age, each group of mice was placed with males for 3 nights during which all groups were given tap water. The feeding conditions were the same as in experiment I except that pregnant and lactating mice were kept singly in a cage.

*Development of pregnancy-dependent mammary tumors.* At parturition, a number of pregnancy-dependent mammary tumors (PDMT) was checked by palpation and the major two diameters of each PDMT were measured by a caliper and the size was expressed in terms of the mathematical mean of the diameters.

*Reproductivity.* Litter size was counted and the weight was measured at parturition (on day 0 of lactation). All pups were then mixed, returned to mothers randomly 6 (3 males and 3 females) each in order to equalize the litter condition, weighed again and nursed normally until day 4 of lactation. The per cent litter weight gains during 4 days were calculated as an index of lactational performance of each mother.

*Statistics.* The significance of differences was evaluated by $\chi^2$-test for mammary tumor incidence and by Student's *t*-test for other parameters.

## RESULTS

*Experiment* I (*SHN mice*)

*Estrous cycle, body weight change, organ weights and ovarian histology.* Both experimental and control mice did not cycle regularly and no difference in the pattern of estrous cycles was observed between groups.

As presented in Table 1, the initial and the final body weights and the per cent changes in weight were little different between groups. Body weight increased significantly in all groups.

No difference was observed between groups in the weights of pituitary and adrenals and ovarian histology. The ovaries in all groups were normal and contained both follicles and corpora lutea at various phases of development.

*Normal, preneoplastic and neoplastic mammary development.* The results are illustrated in Fig. 2. Mammary rating as an index of development of end-bud or lobulo-alveolar system was significantly lower in 0.3% KSCN group than in the other two groups between which little difference was observed.

Both number of HAN and mammary tumor incidence decreased linearly with the increasing doses of KSCN; the differences in these parameters between the control and 0.3% KSCN group were significant, while the dif-

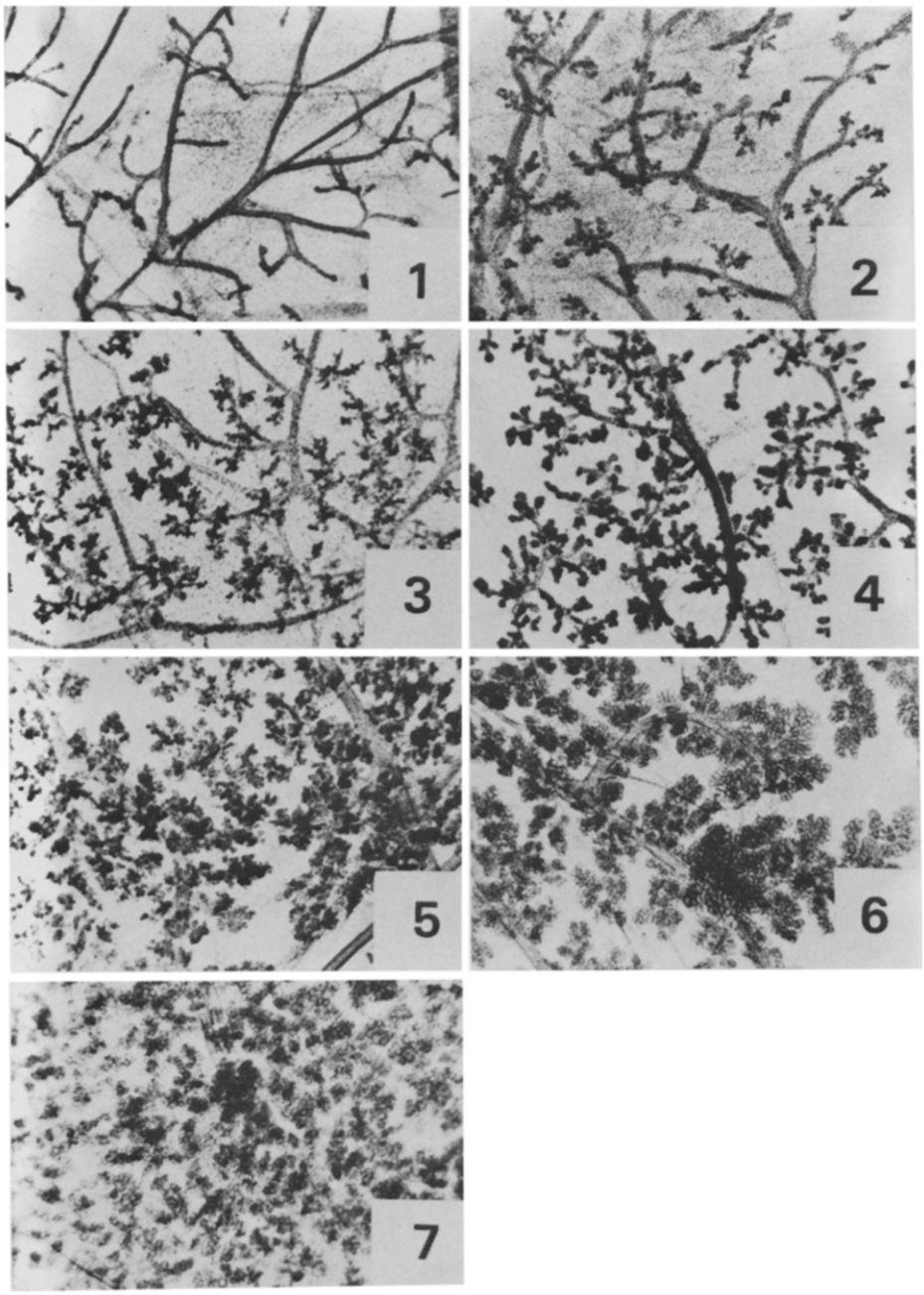


*Fig. 1. Mammary rating as the index of the growth of end-bud or lobulo-alveolar system of the gland. Numbers in each photograph is the rating.*

*Table 1. Effects of potassium thiocyanate (KSCN) on the weights of body, anterior pituitary and adrenals in SHN strain of female mice*

| Group and treatment* | No. of mice | Body weight | | | Organ weight (mg/100 g body weight) | |
|---|---|---|---|---|---|---|
| | | Initial (g) | Final (g) | Change‡ (%) | Anterior pituitary | Adrenals |
| Control | 18 | 27.2±0.5† | 29.1±0.6 | 7.4±1.0 | 8.4±0.5 | 35.3±0.8 |
| 0.1% KSCN | 18 | 26.7±0.5 | 28.7±0.4 | 7.4±1.1 | 7.5±0.2 | 37.2±1.0 |
| 0.3% KSCN | 18 | 27.2±0.3 | 28.5±0.3 | 4.9±1.0 | 7.6±0.3 | 36.2±0.9 |

*Each dose of KSCN was given to the experimental mice as drinking water *ad libitum* for 12 weeks beginning 8 weeks of age. The control received tap water.
†Mean±S.E.M.
‡All differ from zero at $P<0.01$.

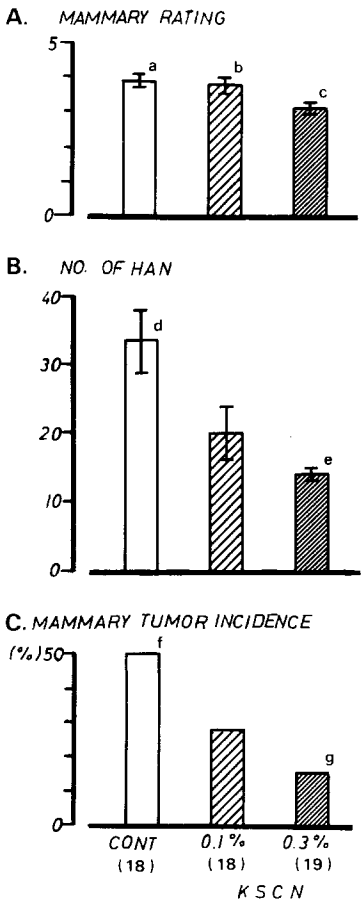


*Fig. 2. Effects of potassium thiocyanate (KSCN) on normal and neoplastic mammary development in SHN strain of female mice. Values given in A and B are means±S.E.M. See Table 1 for details of each treatment. Number in parentheses is number of mice examined. Significance of difference: a, b/c; d/e; $P<0.01$; f/g: $P<0.02$.*

ferences between the control and 0.1% KSCN group were statistically marginal at 5% level.

There were little differences between groups in the size of HAN.

*Plasma levels of T3 and T4.* As seen in Fig. 3, plasma levels of T3 and T4 were declined

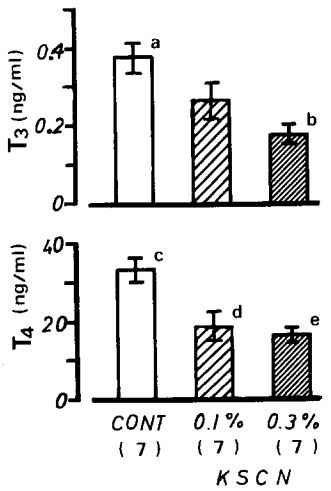


*Fig. 3. Effects of potassium thiocyanate (KSCN) on plasma levels of triiodothyronine (T3) and thyroxine (T4) in SHN strain of female mice. Values given are means±S.E.M. See Table 1 for details of each treatment. Number in parentheses is number of samples. Significance of difference: a/b; c/d, e: $P<0.01$.*

significantly by treatment with KSCN when compared to the control except for T3 level in 0.1% KSCN group.

*Plasma prolactin levels.* Plasma prolactin levels were 182±84, 188±48 and 101±16 ng/ml (means±S.E.M.) in the control, 0.1% and 0.3% KSCN groups, respectively, the differences between groups being statistically not significant.

*Experiment II (GR/A mice)*

*Incidence, number and size of PDMT.* The results are shown in Fig. 4. The incidence of PDMT was less in both of the experimental groups by more than 50% than in the control, the differences being statistically significant. The number of PDMT per mouse with PDMT was not different between groups. On the other hand, mammary tumor size was

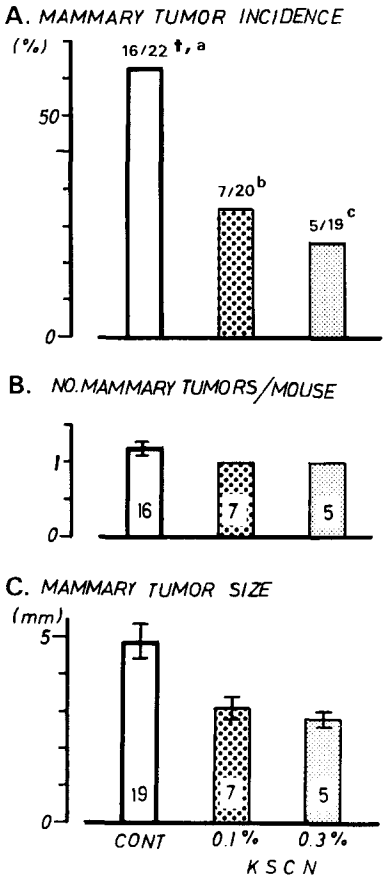


*Fig. 4. Effects of potassium thiocyanate (KSCN) on pregnancy-dependent mammary tumorigenesis in GR/A strain of female mice. Values given in B and C are means ± S.E.M. and number in each column is number of mice (B) or tumors (C) examined. See Table 2 for details of each treatment. †Number of mice with tumors/total number of mice examined. Significance of difference: a/b: $P<0.02$; a/c: $P<0.01$.*

apparently reduced by KSCN, while the statistical significance of differences was marginal at 5% level owing to the small number of estimates in the experimental groups.

*Reproductivity.* There were little differences between groups in any item examined as the index of reproductivity (Table 2).

*Pituitary levels of prolactin and growth hormone.* Pituitary levels of prolactin and growth hormone (GH) on day 4 of lactation assayed by disc electrophoresis were not altered by KSCN treatment.

## DISCUSSION

In the present experiments, the chronic treatment with KSCN resulted in the inhibition of normal, preneoplastic and neoplastic mammary gland development in mice associated with the apparent decrease in plasma levels of T3 and T4. On the other hand, estrous cycles, ovarian structure and pituitary and/or plasma levels of prolactin and GH were affected little by the treatment. These results suggest that the secretion of pituitary and ovarian mammotropic hormones was not impaired by KSCN.

It has been reported that inhibition of mammary tumorigenesis induced by hypothyroidism is often attributable to the decreased food intake and consequent inanition in the animals [2, 5, 6]. In the present study, body weight increased during the KSCN treatment and the weights of pituitary and adrenals were not different between groups. Moreover, reproductivity was quite normal in both experimental groups. It is, therefore, unlikely that the inhibition of normal and neoplastic mammary development in the experimental mice is due to the inanition and the consequent disturbance of normal physiological function.

Thus, all findings obtained in the present study suggest that the inhibited development

*Table 2. Effects of potassium thiocyanate (KSCN) on reproductivity in GR/A female mice*

| Group and treatment* | No. of mice | No. and % of pregnancy | Mother weight at parturition (g) | Litter size | Rate of still-born pups (%) | Av. pup's weight (g) | Litter growth rate on day 4 of lactation (%) |
|---|---|---|---|---|---|---|---|
| Control | 32 | 22 (68.8) | 25.8±0.3† | 6.7±0.3 | 0.7 (1/150)‡ | 1.41±0.02 | 66.6±4.3 (12)§ |
| 0.1% KSCN | 32 | 20 (62.5) | 26.3±0.2 | 7.0±0.3 | 1.4 (2/141) | 1.41±0.02 | 75.4±3.0 (11) |
| 0.3% KSCN | 29 | 19 (65.5) | 25.2±0.2 | 6.4±0.5 | 4.6 (5/108) | 1.35±0.02 | 63.5±4.5 (11) |

*Each dose of KSCN was given to the experimental mice as drinking water *ad libitum* for 9 weeks beginning 5 weeks of age. The control received tap water.
†Means ± S.E.M.
‡Number of still-born pups/total number of pups.
§Number of litters examined.

of normal and neoplastic mammary glands may principally be involved in the decreased thyroid hormones in the circulation by KSCN.

The precise mechanism of the action of thyroid hormones on mammary glands is little understood. Vonderhaar [16] reported that thyroid hormones increase *in vitro* mammary responsiveness to prolactin, a primary hormone for normal and neoplastic mammary development [17], and she suggested that thyroid hormones might enhance prolactin binding.

No alteration of estrous cycles and ovarian histology was observed in this study. Vazquez-Lopez [10] found that the estrous cycles remained unaffected by thiourea feeding in R3 female mice, but C3H mice receiving the same treatment became diestrus soon after the beginning of treatment. Less regular, infrequent or absent estrous cycles [11] or decreased large ovarian follicles and increased degenerating ova [18] were also found in C3H mice fed thiourea. These discrepancies may primarily be due to the difference in the degree of impaired thyroid function and partially due to the strain-dependent difference as pointed out by Vazquez-Lopez [10].

**Acknowledgements**—We thank Dr. W. P. VanderLaan, Division of Diabetes and Endocrinology, Scripps Clinic and Research Foundation, La Jolla, California, U. S. A., for the kit for radioimmunoassay of mouse prolactin.

Technical help by H. Taniguchi is also acknowledged.

## REFERENCES

1. B. A. Eskin, A. Murphey and R. Dunn, Induction of breast cancer in altered thyroid states. *Nature (Lond.)* **218,** 1162 (1968).
2. J. A. Kellen, Effects of hypothyroidism on induction of mammary tumors in rats by 7,12-dimethylbenz(a)anthracene. *J. nat. Cancer Inst.* **48,** 1901 (1972).
3. J. H. Helfenstein, S. Young and A. R. Currie, Effects of thiouracil on the development of mammary tumors in rats induced with 9,10-dimethyl-1,2-benzanthracene. *Nature (Lond.)* **196,** 1108 (1962).
4. W. C. Newman and R. C. Moon, Chemically induced mammary cancer in rats with altered thyroid function. *Cancer Res.* **28,** 864 (1968).
5. A. G. Jabara and J. S. Maritz, Effects of hypothyroidism and progesterone on mammary tumours induced by 7,12-dimethylbenz(a)anthracene in Sprague–Dawley rats. *Brit. J. Cancer* **28,** 161 (1973).
6. W. Jull and C. Huggins, Influence of hyperthyroidism and thyroidectomy in induced mammary cancer. *Nature (Lond.)* **188,** 73 (1960).
7. H. J. Chen, C. J. Bradley and J. Meites, Stimulation of growth of carcinogen-induced mammary cancers in rats by thyrotropin-releasing hormone. *Cancer Res.* **37,** 64 (1977).
8. M. Gruenstein, D. R. Meranze, M. Acuff and M. B. Shimkin, The role of the thyroid in hydrocarbon-induced mammary carcinogenesis in rats. *Cancer Res.* **28,** 471 (1968).
9. A. Davidson, J. Owen and C. G. Thomas, Jr., Further studies on the role of altered thyroid function on experimentally induced breast cancer in Sprague–Dawley rats. *Proc. Amer. Ass. Cancer Res.* **10,** 17 (1969).
10. E. Vazquez-Lopez, The effects of thiourea on the development of spontaneous tumours on mice. *Brit. J. Cancer* **3,** 401 (1949).
11. H. P. Morris, C. S. Dubnik and A. J. Dalton, Effect of prolonged injection of thiourea on mammary glands and the appearance of mammary tumors in adult C3H mice. *J. nat. Cancer Inst.* **7,** 159 (1946).
12. G. W. Anderson, Antithyroid compounds. *Med. Chem.* **1,** 1 (1951).
13. H. Pyska, Effect of thiocyanate on mammary gland growth in rats. *J. Dairy Res.* **44,** 427 (1977).
14. H. Nagasawa, R. Yanai, H. Taniguchi, R. Tokuzen and W. Nakahara, Two-way selection of a stock of Swiss albino mice for mammary tumorigenesis: establishment of two new strains (SHN and SLN). *J. nat. Cancer Inst.* **57,** 425 (1976).
15. R. Yanai and H. Nagasawa, Development and growth of pregnancy-dependent and -independent mammary tumors in GR/A strain of mice and their relationship. *Gann* **69,** 25 (1978).

16. B. K. VONDERHAAR, Studies on the mechanism by which thyroid hormones enhance α-lactoalbumin activity in explants from mouse mammary glands. *Endocrinology* **100,** 1423 (1977).
17. C. W. WELSCH and H. NAGASAWA, Prolactin and murine mammary tumorigenesis: a review. *Cancer Res.* **37,** 951 (1977).
18. A. J. DALTON, H. P. MORRIS and C. S. DUBNIK, Morphologic changes in the organs of female C3H mice after longterm ingestion of thiourea and thiouracil. *J. nat. Cancer Inst.* **8,** 201 (1947).