# EFFECTS OF CHRONIC INGESTION OF ANTHRANILIC ACID ON MAMMARY GLAND GROWTH IN SHN MICE

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## Summary

Effects of anthranilic acid on normal mammary gland growth were examined in SHN/Mei female virgin mice. Anthranilic acid was given to the experimental groups as drinking water at the concentrations of 0.01, 0.02 or 0.04% for 21 days beginning 2-3 months of age. The control group received tap water only. RNA content and RNA/DNA ratio in mammary glands were significantly higher in mice given 0.04% anthranilic acid than in the control, while not mammary DNA content. The results indicate that chronic ingestion of anthranilic acid can induce an enhancement of proliferation and differentiation of mammary cells.
(Key Words: Anthranilic Acid, DNA, Mammary Growth, Mice)

## Introduction

Nakahara and his colleagues found in mice (Nakahara et al., 1939a) and rats (Nakahara and Inukai 1933; Nakahara et al., 1935) that the feeding of synthetic diet (polished rice powder: fish protein: butter: McCollum's salt mixture: brewer's yeast = 15:2:2:1:1) resulted in a complete failure of lactation despite normal body growth, pregnancy and parturition and that this failure of lactation was ameliorated completely by the supplement with liver extract, which they named vitamin $L_1$ (Nakahara et al.,1935, 1938a, 1939a,b, c). Furthermore, Nakahara et al.,(1945) demonstrated that anthranilic acid showed the same effect on lactation as vitamin $L_1$. However, the mechanism(s) of this process is(are) obscure at present.

As a possible step to evaluate the role of vitamin $L_1$ on lactation, the effects of anthranilic acid on normal growth of mammary glands of virgin female mice were studied in this paper, since a prior mammary gland growth is an essential factor for a subsequent lactation.

## Materials and Methods

### Animals and treatments

Virgin female SHN/Mei mice maintained in our laboratory were used. At 2-3 months of age, mice were divided into 4 groups. Group A received tap water only and served as the control. Groups B, C and D were given tap water containing anthranilic acid, which was extracted from citrus (*Citrus unshiu* MARC) as illustrated in figure 1, at the concentrations of 0.01, 0.02 and 0.04% for 21 days, respectively. Water was provided *ad libitum*.

Throughout the experiments, animals were kept in plastic cages (18 x 30 x 15 cm) with wood shavings, 5 each, maintained in a windowless animal room, which was air-conditioned (22-24°C and 55-75% relative humidity) and artificially illuminated (14 hours of light from 5:00 AM to 7:00 PM). They had free access to a commercial diet (Lab MR Breeder; Nihon Nosan Kogyo KK, Yokohama, Japan).

### Mammary nucleic acid contents

Mice were killed by decapitation under the light

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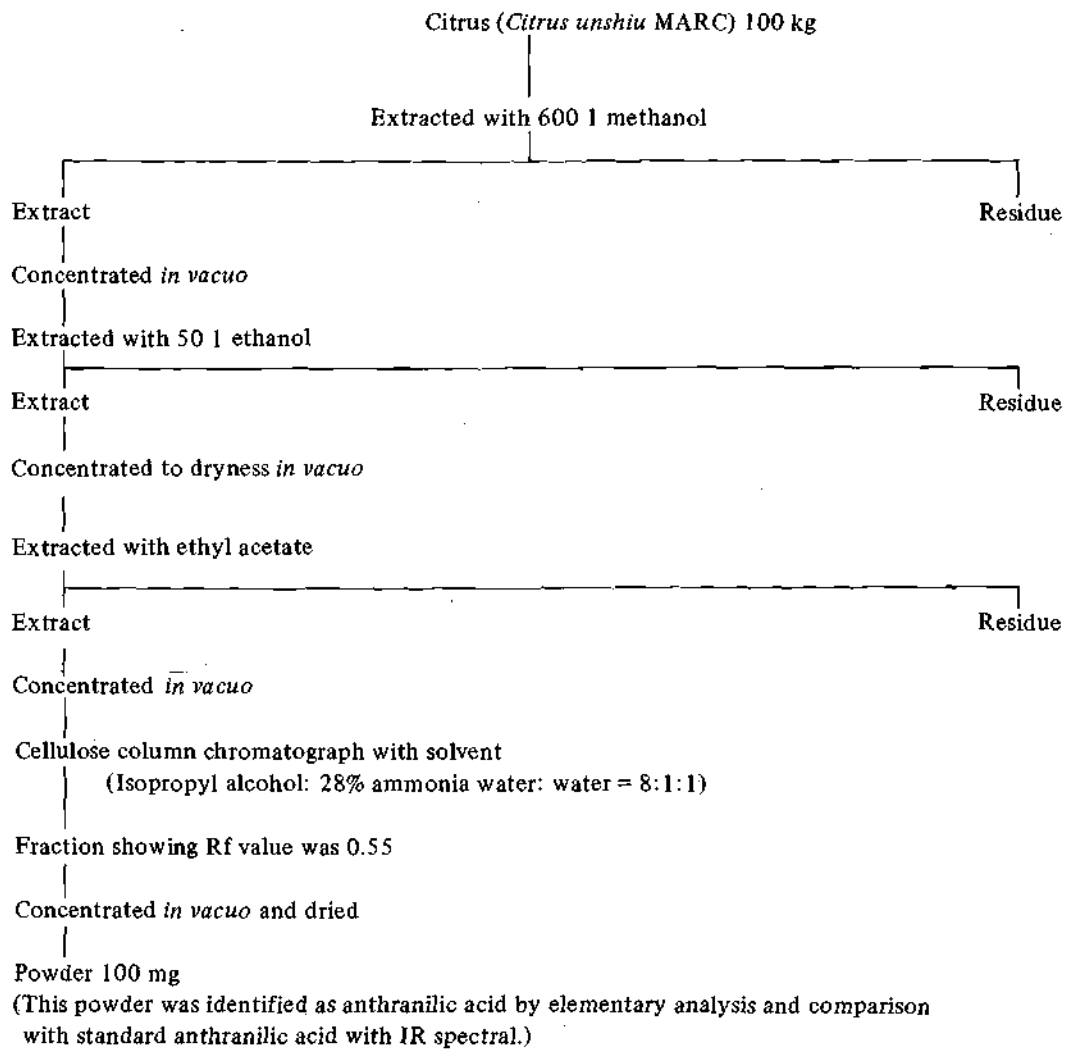


Figure 1. Scheme of preparation of anthranilic acid.

ether anesthesia on the morning (8:30-9:00 AM) of 21 days of treatments and the left inguinal mammary fat pads were removed and defatted with hot alcohol-ether. DNA and RNA in mammary glands were extracted by hot trichroloacetic acid and determined by diphenylamine and orcinol reactions, respectively. All procedures were essentially the same as detailed previously (Nagasawa and Yanai, 1974).

**Body weight change and endocrine organ weights**

All mice were weighed every seven days throughout the experiment. At autopsy, anterior pituitary, adrenals and ovaries were removed and weighed.

**Statistics**

Statistical significance of difference in parameters between the control and each experimental group was evaluated by Student's t-test.

## Results

**Mammary nucleic acid contents (figure 2)**

RNA content and RNA/DNA ratio in mammary glands were significantly higher in group D than in group A (control). Group C was also higher than group A in these parameters, however, the difference was not statistically significant. There was little difference in mammary DNA content between the control and all experimental groups.

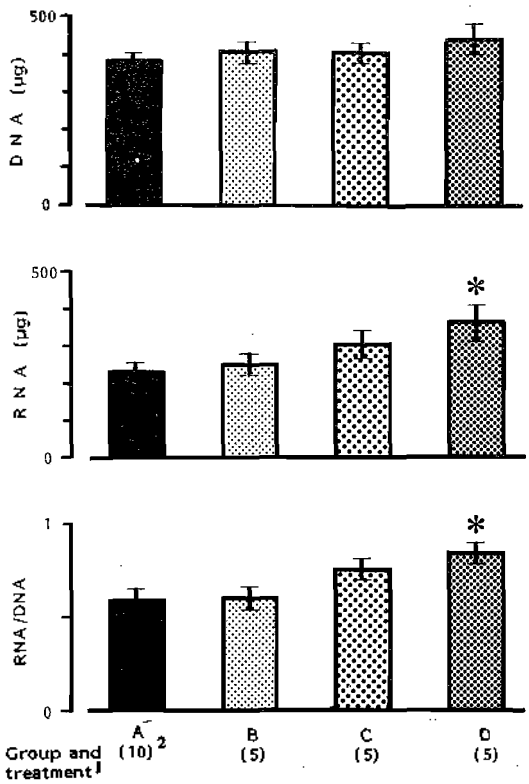


Figure 2. Mammary nucleic acid contents in each group (Mean ± SEM).
[1]Group A received tap water only and Groups B, C and D were given tap water containing anthranilic acid at the concentrations of 0.01, 0.02 and 0.04%, respectively, for 21 days beginning 2-3 months of age.
[2]Number of estimates.
*Significantly different from group A (control) at $p < 0.05$

**Body weight change and endocrine organ weights (table 1)**

Little differences were observed between the control and each experimental group in body weight change.

Endocrine organ weights were also affected little by anthranilic acid ingestion.

## Discussion

This study shows that chronic ingestion of anthranilic acid at the concentration of 0.04% resulted in a significant increase in RNA content and RNA/DNA ratio in mammary glands, while not mammary DNA content. The results indicate that anthranilic acid enhances differentiation and proliferation of mammary cells rather than cell division. Mammary glands of rats fed vitamin $L_1$ deficient diet were in the hypofunctional state and no visible evidence of milk secretion (Nakahara and Inukai, 1933; 1934) and Nakahara et al., (1938a, b) inferred that vitamin $L_1$ might contribute to mammary maturation. Furthermore, vitamin $L_1$ supplement was effective on lactation, if it was started earlier than 14 days before parturition, but not 2-5 days before parturition (Nakahara et al.,1939c).

These and our present observations strongly suggest that anthranilic acid or vitamin $L_1$ can render the failure of lactation by stimulating mammary gland growth, especially its maturation, before parturition.

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TABLE 1. BODY WEIGHT CHANGE AND ENDOCRINE ORGAN WEIGHTS IN EACH GROUP (MEAN ± SEM)

| Group and treatment[1] | No. of mice | Body weight | | | Endocrine organ weights (mg) | | |
|---|---|---|---|---|---|---|---|
| | | Initial (g) | Final (g) | % change | Anterior pituitary | Adrenals | Ovaries |
| A | 10 | 25.7± 0.5 | 28.3± 0.6 | 10.3± 1.5 | 2.3± 0.2 | 11.1 ±0.8 | 21.1± 0.9 |
| B | 5 | 25.1± 0.7 | 27.0± 0.5 | 7.8± 2.4 | 1.9± 0.4 | 10.8 ±0.6 | 20.1± 1.3 |
| C | 5 | 25.4± 0.7 | 27.6± 1.0 | 7.4± 1.7 | 2.0± 0.3 | 12.1 ±0.6 | 21.3± 0.6 |
| D | 5 | 26.0± 1.2 | 30.0± 0.7 | 8.0± 3.5 | 2.3± 0.5 | 11.2 ±0.5 | 21.7± 0.9 |

[1]See figure 2 for details of treatments.

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