**Brief Communication: Null Effect of Concurrent Feeding of Sodium Nitrite and Amino Acids to MRC Rats**

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**SUMMARY**—The secondary L-amino acids, proline, hydroxyproline, and arginine, were fed together with sodium nitrite (NaNO₂) to MRC rats of both sexes for their lifetime. Controls were rats given the amino acid or NaNO₂ alone, nitrosopyrrolidine (positive control), or no treatment. No tumorigenic effect was observed in animals receiving the amino acids and NaNO₂ combined treatment. The null effect is probably due to the noncarcinogenicity of the nitrosoamino acids formed in the stomach and the lack of decarboxylation of nitrosoproline and nitrosohydroxyproline to the corresponding nitrosamine.—J Natl Cancer In 50:799–802, 1973.

THREE naturally occurring amino acids, proline, hydroxyproline, and arginine, are secondary amino compounds. We considered it probable that these compounds, like other secondary amines (1–3), tertiary amines (4), and alkylureas (5), when given together with sodium nitrite (NaNO₂), would undergo nitrosation in the stomach and produce the corresponding nitrosoamino acids or nitrosamines. Chemical studies (6) showed that proline and hydroxyproline undergo such reactions under physiologic conditions. The carcinogenicity of these compounds or their products was not known then.

We have completed an experiment on 280 MRC rats, studied for their lifetimes, in which they were fed these 3 amino acids and NaNO₂ in their drinking water. Controls were rats given the amino acid or NaNO₂ alone, nitrosopyrrolidine (positive control), or no treatment.

**MATERIALS AND METHODS**

Chemicals were prepared as described in (7).

Thirty MRC (Wistar-derived) randombred rats (15 males, 15 females) comprised each experimental group. They were 6–8 weeks old at the start of the experiment; males weighed an average of 268 g, and females 165 g. All animals had been pooled by sex at weaning and allocated to experimental groups at random. They were housed 5 animals per 2000 cubic inch of polycarbonate cage, bedded on ground cornsobs, and fed commercial rat and mouse diet and tap water ad libitum. Forty animals served as untreated controls.

All solutions were prepared in distilled water weekly and given as drinking water in dark-brown bottles at night. Solutions were consumed by morning and ordinary tap water was offered. Each rat drank 20 ml of the treatment solution containing 250 mg/liter of L-amino acid, 500 mg/liter NaNO₂, or 200 mg/liter nitrosopyrrolidine 5 days each week for 67 weeks. The total dose was 1.67 g amino acid, 3.35 g NaNO₂, or 1.34 g nitrosopyrrolidine per rat (table 1).

Animals were observed daily, weighed every 2 weeks, and killed when moribund. All survivors were killed at 122 weeks. A complete autopsy (including the brain) was performed and all viscera were examined histologically.

**RESULTS**

**Survival and Weight**

There was no significant difference in survival curves, mean age at death, or weight curves in animals receiving the combined treatment as compared with animals given the amino acids or NaNO₂ alone. Nitrosopyrrolidine-treated animals

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male rats given arginine, either alone or in combination with NaNO₂. For example, 9/13 of the tumors in male rats receiving arginine were endocrine and 3 animals had ≥3 endocrine tumors each. The combination of interstitial adenoma of the testis, islet cell adenoma of the pancreas, and an adrenal tumor (either cortical or medullary) was the most common pathologic triad. Similarly, in male rats given arginine and NaNO₂, 6/13 tumors were endocrine, and 2 animals had 2 tumors each. The remaining tumors, some of which were unusual histologically, were distributed equally in all groups without significant clustering in organ involvement or histologic type that would suggest a relationship to treatment.

**DISCUSSION**

The amino acids proline, hydroxyproline, and arginine are secondary amino compounds that occur in their free form in certain foodstuffs (11) or are liberated by hydrolysis of protein during food processing. The chemical considerations leading to this study have been discussed in (6, 7).

The absence of carcinogenic effect in this study and in our previous similar experiment in mice is probably due to lack of decarboxylation of the nitrosamino acids formed in the stomach. The nitrosamino acids themselves seem to be noncarcinogenic [(7) and Garcia and Lijinsky—unpublished observations]. Although decarboxylase activity is widely distributed in bacterial and animal tissues (12–14), often with a broad substrate specificity, the activity may not extend to nitroso-substituted cyclic L-amino acids.

We must assume that the formation of nitrosamines by decarboxylation of nitrosamino acids in vivo is insufficient to give rise to tumors within...
Table 2.—Organ location and histologic type of tumors in MRC rats fed amino acids and nitrite

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of rats*</th>
<th>Total (all tumors)</th>
<th>Total malignant</th>
<th>Pitu.</th>
<th>Mam.</th>
<th>Liver</th>
<th>Pan.</th>
<th>Test.</th>
<th>Ovary</th>
<th>Adren. cortex</th>
<th>Adren. medulla</th>
<th>Uterus</th>
<th>Soft tissues</th>
<th>Other</th>
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<td>Arginine</td>
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<td>3</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>A§</td>
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<tr>
<td>Arginine + NaNO₂</td>
<td>15</td>
<td>22</td>
<td>14</td>
<td>7</td>
<td>6</td>
<td>7</td>
<td>0</td>
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<td>0</td>
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*Number of autopsied rats surviving 35 weeks or longer, the time of earliest tumor appearance.
†Adenoma of islets of Langerhans.
‡Interstitial cell adenoma.
§A: carcinoma of duodenum, thyroid adenoma; B: squamous carcinoma of auditory canal, malignant lymphoma, adenocarcinoma of pylorus, acanthotic ameloblastoma of mandible; C: transitional cell papilloma grade 3 of urinary bladder, cystadenoma of epididymis, malignant lymphoma, squamous papilloma of forestomach, cervical node lymphangiosis, parathyroid adenoma; D: squamous carcinoma of forestomach; E: squamous papilloma of forestomach; F: ependymoma of 4th ventricle, thyroid adenoma; G: transitional cell papilloma grade 1 of renal pelvis, adenocarcinoma of thymus, adenocarcinoma of parathyroid gland, adenocarcinoma of bronchus, acute granulocytic leukemia; H: salivary gland adenoma, malignant lymphoma; I: malignant thymoma; J: cerebral astrocytoma grade 3, malignant lymphoma; K: malignant lymphoma; L: renal tubular adenoma, salivary gland adenoma, malignant lymphoma; M: epidermal carcinoma of thymus, adenocarcinoma of parathyroid gland, adenocarcinoma of bronchus, acute granulocytic leukemia; N: papilloma of choroid plexus; O: acute granulocytic leukemia; P: squamous papilloma of forestomach, papillary mesothelioma of tunica vaginalis testis (3), cavernous hemangioma of spermatic cord, tubular adenoma of kidney. (All lesions are single isolated tumors unless noted.)
the lifetime of a rat; perhaps it does not occur at all. However, the formation of nitrosoprine from proline and nitrite is not without significance, since nitrosoprine is almost certainly the source of the nitrosopyrrolidine present in fried bacon but not in uncooked bacon (15).

REFERENCES

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