Urinary nonvolatile phenols (NVP) were determined by the method of Valterra (18), results being reported as mg. phenol.

RESULTS AND DISCUSSION

Nutritional studies in our laboratories have indicated that 0.025 mg riboflavin/day/kg of body weight is an adequate intake of this vitamin for adult dogs. Animals fed this amount of vitamin in the presence of aminofluorene, however, do not conjugate a maximum amount of diazotizable amine. The data in Table 1 illustrate the increase in conjugation as the dietary riboflavin is raised from 0.025 to 0.25 to 2.5 mg/day/kg of body weight. These data were obtained by feeding the test level of vitamin for 7 days prior to administration of the carcinogen. Continued feeding of 2.5 mg. of riboflavin for 16 days increased the percentage of conjugation (expressed as conjugated aminofluorene) still further to an average of 51.

Riggs and Hegsted (10) found that the conjugation of p-aminobenzoic acid was significantly lower in riboflavin-deficient rats than in the controls. On the other hand, Shils et al. (11) did not find riboflavin to be a factor in the acetylation of sul-

MATERIALS AND METHODS

Adult beagle dogs (2—8 years of age, 8-10 kg.) were fed the synthetic diet previously reported (1). The carcinogen and the additional vitamins were administered orally in gelatin capsules. The carcinogen was administered after the animals had been on the test level of riboflavin or pantothenic acid for 7 days. Urine was collected for 8 days following administration of the carcinogen.

Aminofluorene and diazotizable amino groups were determined by diazotisation followed by coupling with the o-toluidide of 3-hydroxy-4-naphthoic acid (naphthol ASD). The resultant stable red-orange dye was compared to a standard solution treated in the same manner. Diazotisation and coupling after hydrolysis with 5 N HCl permitted an estimation of free and conjugated amino groups excreted in the urine. The details of the analysis are as follows: 1 ml. of 5 N hydrochloric acid and 1 ml. of 0.05 N sodium nitrite were added to 1 or 2 ml. of urine (containing at least 5 μg/m1 of the carcinogen). After mixing and waiting 1 minute, 2 ml. of freshly prepared 0.01 N naphthol ASD in 2.5 N NaOH were added, slowly, but with rapid agitation of the mixture. The volume was brought to 25 ml. with water and the reaction allowed to proceed for 30 minutes. The optical density was read at 530 μm, the peak of the absorption curve (Chart 1). Blank determinations were made routinely in normal urine. The color was always negligible. The quantity of diazotizable amine was computed from a standard curve prepared with known amounts of 2-aminofluorene. A linear relationship was observed between concentration and optical density. The conjugated amine was determined by adding 2 ml. of the 5 N HCl to 2 ml. of the unknown and heating for 1 hour at 100° C. The base so liberated was determined as above. This is a modification of a method previously reported by Westfall and Morris (15).

* These studies were made possible by a grant from the Committee on Growth of the American Cancer Society.

1 The sample of naphthol ASD was generously supplied by R. Erdmann, Sr., of the Sinclair Valentine Company, N.Y.

Received for publication February 4, 1952.
fanilamide. The latter authors point out that conjugation may be related to liver size, a factor which can be effected by riboflavin intake. These papers, however, emphasize the importance of pantothenic acid in conjugation reactions.

This increased conjugation of 2-aminofluorene was established in the presence of a constant amount of pantothenic acid in the diet (0.2 mg/day/kg body weight), a vitamin which has an effect upon conjugation of amines (9, 10, 12). The experiments were repeated, therefore, varying the pantothenate as well as the riboflavin content of the diet. The data in Table 2 illustrate again the systems, forces the equilibrium in the direction of increased conjugation, a reaction which is associated with pantothenic acid. It is also possible that increased synthesis of liver protein, associated with adequate riboflavin (1), is a factor in developing maximum function.

Experience with 2-aminofluorene in dogs indicates that the conjugation is initially greater with aminofluorene than with the acetylated derivative. Six dogs, for example, were fed single 1-gm. doses of 2-acetylaminofluorene, and the amounts of the free and conjugated material excreted were measured. The percentage of conjugated carcino
gen increased conjugation of diazotizable amines associated with a rise in riboflavin intake. These data demonstrate also that increasing the pantothenate content of the diet raised the level of conjugation in the urine, an effect which was minimal in the presence of relatively large amounts of riboflavin. Thus, conjugation of the carcinogen is associated with the dietary level of both pantothenic acid and riboflavin. These data emphasize the variation in vitamin requirements of animals according to the stress placed upon them. The significance of these findings in terms of activity of enzyme systems is under investigation. It is possible that the dietary riboflavin, as a component of particular enzyme
first few hours to values obtained from animals fed the acetyl derivative. Deacetylation of 2-acetylaminofluorene has been demonstrated by Morris, Weisberger, and Weisberger (6). Thus, a dynamic equilibrium between deacetylation and conjugation could be established in the animal. It will be necessary to determine the chemical nature of the compounds excreted in the urine and labeled diazotizable amine before the significance of this dynamic equilibrium can be explored further. Data have also been obtained to demonstrate conjugation of β-naphthylamine in the dog (2), a conjugation which is a function of the pantothenic acid content of the diet.

2-Aminofluorene is carcinogenic in the rat (7) and is more toxic than the acetylated derivative in the dog. Dogs fed large amounts of the 2-acetylaminofluorene showed no clinical abnormalities; but those fed the amine exhibited marked cyanosis, impaired respiration, and methemoglobin formation, effects which began to disappear 3 hours after the administration of 1 gm. of the hydrochloride. It is possible, as suggested by Ray and Argus (8), that conjugation is a step in detoxification reactions.

The data in Table 3 illustrate the larger than normal excretion of nonvolatile phenols in the presence of the carcinogen and a high riboflavin intake. It is possible, therefore, that excess riboflavin has the general effect of increasing the metabolism of 2-aminofluorene resulting not only in an increased conjugation but also in hydroxylation. Beilschowsky (8) has demonstrated that a hydroxylated compound is the principal metabolite of aminofluorene in the rat. The protective effects of riboflavin could be the result of increased detoxification of the carcinogen through various metabolic pathways.

### TABLE 3

<table>
<thead>
<tr>
<th>Dog no.</th>
<th>2-Aminofluorene (mg/day)</th>
<th>Riboflavin intake (mg/day/kg body weight)</th>
<th>N.V.P. excreted (mg/day/kg body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>147</td>
<td>none</td>
<td>2.5</td>
<td>2.7</td>
</tr>
<tr>
<td>158</td>
<td>none</td>
<td>2.5</td>
<td>2.3</td>
</tr>
<tr>
<td>159</td>
<td>none</td>
<td>2.5</td>
<td>2.3</td>
</tr>
<tr>
<td>87</td>
<td>316 mg.</td>
<td>0.025</td>
<td>5.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.25</td>
<td>5.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.50</td>
<td>6.5</td>
</tr>
<tr>
<td>106</td>
<td>516 mg.</td>
<td>0.025</td>
<td>4.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.25</td>
<td>5.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.50</td>
<td>7.2</td>
</tr>
<tr>
<td>141</td>
<td>516 mg.</td>
<td>0.025</td>
<td>4.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.25</td>
<td>5.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.50</td>
<td>6.0</td>
</tr>
</tbody>
</table>

### REFERENCES


### SUMMARY

A modified method of analysis for 2-aminofluorene is described. This method involves diazotization followed by coupling with the o-toluidide of 2-hydroxy-4-naphthoic acid to form a stable dye. Conjugation was determined by liberating amine with acid hydrolysis.

A study was made of the effects of dietary pantothenic acid and riboflavin on the conjugation of diazotizable amine in the urine of dogs fed 2-aminofluorene. It was found that raising the level of dietary riboflavin increases the excretion of the conjugated amine. Conjugation is also a function of the pantothenic acid intake, being reduced by a pantothenic acid deficiency. The data demonstrate that dogs fed the acetyl derivative of 2-aminofluorene excrete a constant percentage of the conjugated form, whereas those fed the hydrochloride excrete a high percentage initially, a percentage which decreases rapidly to values obtained in animals fed the acetyl derivative.

The free amine is more toxic than the acetylated carcinogen, suggesting that conjugation is a form of detoxification—a reaction associated with the maintenance of adequate protein stores as well as with specific enzyme systems. Increasing dietary riboflavin increases the excretion of nonvolatile phenols in the dogs fed this carcinogen.


The Effects of Dietary Riboflavin and Pantothenic Acid on the Metabolism of 2-Aminofluorene

James B. Allison and Arthur W. Wase


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/12/9/647

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.