Furacin* (5-nitro-2-furaldehyde semicarbazone) is an antibacterial agent effective against both Gram-negative and Gram-positive organisms (1–3). It has also been studied extensively, along with other nitrofurans, to determine its fate during metabolism in the body (4, 5). Certain of these nitrofurans having chemotherapeutic activity partially withstand metabolic breakdown in the body and are excreted in the urine (4). To extend further the metabolic studies on the nitrofurans, information was sought on the site and mechanism of breakdown of Furacin in the body. Preliminary incubation studies on Furacin with various body tissues are reported in this paper. The studies on intestinal tissue received emphasis because of their significance in connection with concurrent work on the Furacin-xanthine oxidase system (6).

EXPERIMENTAL

All tissues studied were from normal, adult, albino rats. The animals were fasted overnight but had free access to water. Previous to the fast they received the uniform colony diet. All tissues were removed as rapidly as possible after the animals were killed and kept chilled until added to the medium for incubation. The contents of the small intestines were milked out and pooled, aliquots being subsequently measured by volume. Aliquots from the other tissues, including intestinal mucosa, were weighed. These tissues were cut by scissors into thin sections after excess blood was removed.

For most tests 1 or 2 gm. each of the various tissues were transferred to 15 ml. centrifuge tubes. Furacin in physiological saline solution was added in the ratio of 1 ml. per gm. of tissue. Concentrations of Furacin ranged from 84 to 210 mg. per liter. Tissues in physiological saline alone served as controls. In certain experiments 1/30 phosphate buffer of pH 7.2 replaced the physiological saline solution. The tubes were warmed quickly in a water bath to 37° and kept at this temperature with vigorous shaking at 5 minute intervals for the period of incubation, which ranged from 20 minutes to 3 hours.

1 Brand of nitrofurazone, “New and nonofficial remedies.”
After the incubation period each sample was centrifuged and an aliquot of the supernatant liquid was added immediately to twice its volume of 95 per cent ethyl alcohol and recentrifuged. The concentration of Furacin in the resulting clear solutions was estimated spectrophotometrically by the following formula.

\[
\text{Mg. Furacin per ml.} = \frac{\text{optical density} \times \text{dilution} \times 10}{E_{1\%}^{1\text{cm.}}}
\]

The \(E_{1\%}^{1\text{cm.}}\) value for Furacin at its peak of 371 \(\text{m}\mu\) is 800 in 63 per cent ethyl alcohol. Uric acid was estimated spectrophotometrically in certain instances. Chemical determinations of uric acid were also made by the method of Folin (7).

**Results**

Results obtained when a Furacin solution was incubated for a prolonged period in the presence of an equal volume of intestinal contents are presented in Fig. 1. It will be noted that the Furacin absorption maximum disappeared during incubation and that a new absorption peak near 290 \(\text{m}\mu\) appeared. Since the contents of the small intestines of fasted rats were rather meager in amount, intestinal mucosa was tried and found to produce quite similar results. The effect of time upon the spectral characteristics of a solution of Furacin incubated with intestinal mucosa is illustrated in Fig. 2. Each curve represents the spectral absorption of Furacin or end-products obtained during incubation after correction for suitable controls. It is to be observed that with a loss of Furacin an increase of material with an absorption maximum at 335 \(\text{m}\mu\) and a marked increase of material with an absorption maximum at 292 \(\text{m}\mu\) occurred. That these two maxima were not due to a single substance is indicated by the later disappearance of the maximum at 335 \(\text{m}\mu\), accompanied by an increase in the peak at 292 \(\text{m}\mu\). We have tentatively called the material absorbing at 335 \(\text{m}\mu\) "reduced Furacin" since it appears as a result of many chemical reducing actions, such as that of sodium hydrosulfite or catalytic reduction by Raney's nickel.\(^2\) Since it is relatively unstable, we have not been able to isolate it for identification.

Although Austin\(^2\) had observed the formation of two substances by catalytic reduction having absorption maxima at 335 \(\text{m}\mu\) and 270 \(\text{m}\mu\), a substance with an absorption maximum at 292 \(\text{m}\mu\) had not been observed as an end-product of chemical reduction of Furacin. Since a considerable amount of the 292 \(\text{m}\mu\) material was indicated in our experiment, the question arose whether it was a breakdown end-product of Furacin or a

\(^2\) Austin, F. L., to be published.
normal tissue metabolite. The principal metabolite having an absorption maximum in this region is uric acid, with a peak at 292 μm.

Since the absorption maximum of the material observed here was iden-
tical with that of uric acid, and since Bueding and Jolliffe (8) have shown the effectiveness of the xanthine oxidase system in the reduction of other nitro compounds, studies were conducted which would confirm or eliminate the possibility of uric acid being the material here observed.

The end-products from Furacin incubation with intestinal mucosa were treated with uricase. The spectral absorption curves of the material before and after uricase treatment are shown in Fig. 3, in which the difference curve indicates that uric acid was removed by the specific action of uricase.

Estimations of the amount of uric acid present in these various incubations were made by calculation from the physical data. The $E_{1%^{\text{cm}}}$ value for uric acid at 290 m$\mu$ is 715. In the spectral assay for uric acid a certain amount of irrelevant absorption is encountered at these lower wavelengths. However, this may be corrected by subtraction of control values to permit the measurement by difference of increased uric acid production. These results, summarized in Table I, are compared with data obtained on the same samples by chemical analysis (7) before and after uricase treatment.

These results show that, while uric acid is formed by the incubation of intestinal mucosa without Furacin, the presence of Furacin results in increased uric acid formation. These data indicate that the material with the spectral absorption peak in the 290 m$\mu$ region is not a Furacin end-product but is uric acid.

Furacin was incubated with kidney, liver, lung, heart, spleen, brain, and skeletal muscle. As illustrated in Figs. 4 and 5, all such incubations led to a disappearance of Furacin. An absorption curve for Furacin itself has been included in Fig. 4 for comparison. Concurrent with loss
TABLE I

Uric Acid Removed from Mucosa-Furacin and Mucosa-Control Digestion Mixtures by Uricase in M/30 Phosphate Buffer at pH 7.2 after 1 Hour at 37°C

The values, in mg. per liter, represent the difference between the amount of uric acid in the sample before and after digestion with uricase.

<table>
<thead>
<tr>
<th>Mucosa-Furacin digestion mixture</th>
<th>Physical assay*</th>
<th>Chemical assay†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mucosa-control digestion mixture</td>
<td>430</td>
<td>271</td>
</tr>
<tr>
<td>Increased uric acid production arising from Furacin addition</td>
<td>367</td>
<td>193</td>
</tr>
</tbody>
</table>

* Calculated from spectrophotometric results by the method of Paul et al. (4).
† Obtained by the method of Folin (7).

Fig. 4. Spectral absorption curves of materials produced by incubation of Furacin with various tissues at 37°C for 2½ to 3 hours. 1 part of tissue plus 1 part of 0.9 percent NaCl containing 210 mg. of Furacin per liter was incubated, and an aliquot of the liquid was mixed with 9 volumes of alcohol. Each curve has been corrected for a control tissue digestion without Furacin. Curve 1, liver incubation; Curve 2, spleen incubation; Curve 3, kidney incubation; Curve 4, Furacin reference curve, one-half the starting concentration.

Fig. 5. Spectral absorption curves of materials produced by incubation of Furacin with various tissues. Conditions as for Fig. 4. Curve 1 muscle incubation; Curve 2, heart incubation; Curve 3, lung incubation; Curve 4, brain incubation.
of Furacin, the 335 m\(\mu\) material or "reduced Furacin" appeared in all the tissues studied.

In the case of liver, kidney, spleen (Fig. 4), and as has already been shown with intestinal mucosa, increased uric acid formation, as indicated by the appearance of an absorption maximum at 290 m\(\mu\), occurred in the presence of Furacin. In the case of muscle, lung, heart, and brain (Fig. 5) there was little or no increased uric acid formation.

**DISCUSSION**

The digestion of Furacin with certain mammalian tissues *in vitro* resulted in increased formation of uric acid. Taylor *et al.* (6) have concurrently shown that this nitrofuran can serve as a hydrogen acceptor for the anaerobic oxidation of xanthine to uric acid by xanthine oxidase. The conditions of our experiments, deep tube and periodic shaking, may have produced semianaerobic conditions. The relative order of activity of the tissues we studied in producing uric acid as a result of Furacin action correlates for the most part with the xanthine oxidase content of rat tissues as reported by Westerfeld and Richert (9), with the exception of lung tissue. These authors listed the tissues in the order of their activity as follows: liver (very high); small intestine, spleen, lung (high); kidney, stomach, skin (low); brain and muscle (zero).

**SUMMARY**

Loss of 5-nitro-2-furaldehyde semicarbazone (Furacin) occurred when it was incubated in solution with various mammalian tissues.

In all cases during the process of incubation of Furacin with tissues, a substance identified by a spectral absorption maximum at 335 m\(\mu\) was formed.

In the case of certain tissues (intestine, liver, spleen, and kidney) incubation with Furacin led to increased formation of a material with an absorption maximum at 290 m\(\mu\). Tests indicated this material to be uric acid.

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