METABOLISM OF THE NITROFURANS
I. ULTRAVIOLET ABSORPTION STUDIES OF URINARY END-PRODUCTS AFTER ORAL ADMINISTRATION

By H. E. PAUL, F. L. AUSTIN, M. F. PAUL, AND V. R. ELLS
(From the Eaton Laboratories, Inc., Norwich, New York)
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The general antibacterial action in vitro of a variety of furan derivatives substituted in the 5 position with a nitro group has been reported by Dodd and Stillman (1, 2). That the presence of the nitro group is essential was established by comparison of the activities with those of the corresponding non-nitrated derivatives. All the nitrofurans except those limited by insolubility exhibited a marked antibacterial action with the exception of nitrofuroic acid. The latter compound, in contrast, was but weakly active.

With the in vitro activity of these derivatives firmly established, attention was directed to their possible use in the treatment of systemic infections. A report by Dodd (3) gave in detail the treatment of animal infections with one of the compounds, 5-nitro-2-furaldehyde semicarbazone (Furacin). In marked contrast to their behavior in vitro, not all of the nitrofurans gave a chemotherapeutic response in animal infections. An evaluation of more than sixty compounds has shown that the presence of a semicarbazone or closely allied grouping as substituent in position 2 of the furan ring is almost uniquely favorable to pronounced response in this respect.

\[
\begin{align*}
&\text{(I)} & \text{(II)} \\
&\text{Class A} & \text{Class B} \\
&\text{O}_2N-C=O-R & \text{O}_2N-C=O-C=N-R''
\end{align*}
\]

From a chemical stand-point the nitrofurans prepared to date can be grouped broadly into two general classes. Class A comprises the simpler nitrofurans represented by Formula I where \( R \) may be an alkyl, acyl, hydroxyalkyl, or carboxyl group together with the esters or certain other derivatives. Thus Class A would include nitrofuraldehyde and its diace-
tate, methyl nitrofuryl ketone, nitrosilvan (5-nitro-2-methylfuran), nitro-
furfuryl alcohol and its esters, nitrofuroic acid and its amide and esters, and similar compounds.

Class B (Formula II) comprises those derivatives of nitrofuraldehyde (R' = H) or of the nitrofuryl ketones (R' = alkyl or substituted alkyl group) in which condensation has taken place between the carbonyl group and a nitrogen-containing molecule with the elimination of water. Thus in Class B are included the ordinary carbonyl derivatives such as the semicarbazone and oxime as well as more complex analogues which have been prepared in these laboratories.

Although compounds in both Class A and Class B have marked activity in vitro, appreciable activity in vivo has been exhibited by certain members only of Class B. Chemotherapeutic activity depends not only on antibacterial activity of the drug but also on other factors such as its ability to reach the site of infection. Presumably, changes in the compound produced by metabolic processes would explain in part differences between in vitro and in vivo activity. It was, therefore, of prime importance to submit the nitrofurans to biochemical studies to determine the effect of variations in structure on their metabolism.

As a first step in such a study it was considered desirable to determine the urinary end-products after ingestion of various typical nitrofurans of Class A and Class B. For purposes of comparison it was also considered desirable to feed certain non-nitrated furans.

**EXPERIMENTAL**

Solvent extraction methods proved of little value in this laboratory in isolating excretory products from the urine after nitrofuran dosage. Chemical methods for the identification and determination of the nitrofurans and their excretory products were not available. Procedures for purifying urine by precipitation were of little help. However, since the nitrofurans have characteristic absorption spectra (4), it was believed that the location and identification of excretory products might be aided by submitting the fresh untreated urine to spectral absorption analysis. This technique proved of great value, and because of its potential usefulness in related problems of metabolism it is described in some detail here. Although such a method is not suitable for absolute identification of all compounds, it has been extremely useful in indicating the presence or absence of many nitrofurans. In many cases it is reliable for both identification and quantitative analysis of known compounds. After preliminary trials the following technique was evolved.

Albino rats of 300 to 400 gm. of body weight were used. The animals were fasted overnight to reduce as far as possible interference from metabolic end-products arising directly from foods. Water was supplied ad
libitum. Doses of 20 mg. of the drug suspended in simple syrup (sucrose solution) were administered by stomach tube. The animals were caged individually on raised screens over trays covered by waxed paper. Urinary collections by manipulation of the animals were made at 2 hour intervals for a 6 hour period after administration of the drug. Preliminary experiments indicated that appreciable excretion of the drug or its end-products had occurred by this time. If urination occurred before the regular collection, the urine was removed from the waxed paper by medicine dropper. Collections were made in 15 ml. graduated centrifuge tubes and the volume of the urine recorded.

The urines were combined for each drug for the 6 hour period, diluted appropriately, centrifuged, and the optical densities from 450 to 250 μ (occasionally to 220 μ) determined, usually at 10 μ intervals, with the use of a Beckman model D quartz spectrophotometer. Since the dilution required varied with the intensity of the absorbing material excreted and since the volume of urine varied from time to time, it was necessary to correct for volume and dilution. This was done as follows: (1) The optical density at a given wave-length was multiplied by the dilution. This gave the density of the original urine excreted. (2) This value was then multiplied by the volume of the urine excreted. We termed this resultant value “density units.” For example, an animal excreted 2.5 ml. of urine in a 6 hour period. The urine was diluted 1:500. The optical density at the desired wave-length was 0.63. Multiplying 0.63 (the optical density) by 500 (the dilution), we obtain 315, the density of the original urine. This value is multiplied by the volume of the urine (315 × 2.5 = 787.5). There were excreted 787.5 “density units” at this chosen wave-length. The total number of “density units” over a period of time is a measure of the total amount of absorbing substance excreted.

By the above method correction values for normal urine have also been determined. Correction for absorption at corresponding wave-lengths due to normal urinary constituents was then made by subtracting the “density unit” values for normal urine from the “density unit” values of the urine of the experimental animals. Urine curves have been plotted with “density units” as ordinates and wave-length in μ as abscissas.

In the case of identifiable compounds appearing in the urine the method could become quantitative by selecting the wave-length of a major absorption peak and using the following formula,

\[
\frac{\text{Density units} \times 10}{E_{\text{1% cm.}}} = \text{mg. of compound}
\]

(1)

when \(E_{\text{1% cm.}}\) is the extinction of a 1 per cent solution of the compound in a 1 cm. cell at the specified wave-length. Solutions of pure furans and
nitrofurans which have been investigated have been found to obey Beer's law up to saturation concentrations.

The average correction curve for a 6 hour period for a representative fasted animal appears in Fig. 1. To show the desirability of fasting the animal before drug dosage, a similar curve for a non-fasted animal is included. In either case absorption becomes great at the lower wave-lengths and care must be exercised in drawing conclusions at wave-lengths at which the correction becomes a large part of the total absorption.

In order to check the reliability of this spectral method, a furan compound whose metabolism had been studied by earlier workers was fed.

Schempp (5) fed the sodium salt of furoic acid and was able to account for 70 per cent of the compound as furoylglycine in the urine. In Fig. 2 our results from feeding furoic acid (20 mg. per animal to four rats) are to be seen. The curves for the furoic acid fed and the corrected urine obtained for a 6 hour period are shown. It is apparent that the absorption peak of the material excreted is not that of the furoic acid fed (245 m\(\mu\)) but that it is at 255 m\(\mu\). The spectral curve of a solution of furoylglycine was determined and is included in Fig. 2. The peak for furoylglycine is at 255 m\(\mu\) and the \(E_{1\%}^{1\%}\) is 864. The shape and peak of the corrected urine curve indicate that the furoic acid ingested is excreted largely as furoyl-
glycine. In order to make the data quantitative, urine collections were made on these rats until the urine curve returned to normal, i.e. until little or no furoylglycine was shown spectrophotometrically. The average total "density units" at 255 mp over this period (10 hours) calculated and corrected for normal urine for the same period were 1693. Therefore, by the use of Formula 1, we obtain \( \frac{1693 \times 10}{864} = 19.6 \) mg. of furoylglycine. This is equivalent to 13 mg. of furoic acid or 65 per cent of the 20 mg. dose fed, an excellent agreement with the 70 per cent excretion found by Schempp (5).

Several furan compounds were then studied and spectrophotometric evidence (Table I) has also been obtained to indicate furoylglycine as the major end-product in the urine of rats after feeding furfural, furfural diacetate, furfural hydrazone, furfuryl alcohol, furfuryl propionate, furylacrylic acid, and methylfuryl acrylate. Jaffé and Cohn (6) in 1887 had shown that furoylglycine was an end-product of furaldehyde metabolism. Friedmann (7) identified furoylglycine in rabbit urine after injection of furylacrylic acid and Sasaki (8) found furoylglycine in the urine after feeding furylpropionic acid to dogs. Other end-products were also identified by the above workers after chemical treatment of the urine. It is of

**Fig. 2.** Ultraviolet absorption characteristics of urine of rat fed 20 mg. of furoic acid compared to absorption curves of furoic acid and furoyl glycine. The urine curve represents average "density units" per rat (after correction for normal urine) for a 6 hour period. Solid line, urine of rat fed furoic acid; dashed line, furoic acid 0.00005 M solution; dotted line, furoylglycine 0.00005 M solution.
interest to note (Table I) that the urinary end-product after feeding furaldehyde semicarbazone is not furoylglycine.

**Table I**

_Ultraviolet Absorption Characteristics of Urine from Rats Fed 20 Mg. Doses of Various Furans_

<table>
<thead>
<tr>
<th>Compound</th>
<th>Absorption maximum</th>
<th>E1 cm</th>
<th>Absorption characteristics of urine</th>
<th>Compound indicated in urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>R—COOH</td>
<td>245</td>
<td>1000</td>
<td>High peak at 255 μm</td>
<td>Furoylglycine</td>
</tr>
<tr>
<td>R—CHO</td>
<td>276</td>
<td>1438</td>
<td>High peak at 255 μm</td>
<td>&quot;</td>
</tr>
<tr>
<td>R—CH(OCOCH₃)₂</td>
<td>278</td>
<td>393</td>
<td>High peak at 255 μm</td>
<td>&quot;</td>
</tr>
<tr>
<td>R—CH=N—NH₂</td>
<td>335</td>
<td>1709</td>
<td>High peak at 255 μm</td>
<td>&quot;</td>
</tr>
<tr>
<td>R—CH₂OH</td>
<td>Below 220</td>
<td></td>
<td>High peak at 255 μm</td>
<td>&quot;</td>
</tr>
<tr>
<td>R—CH₂OCOC₂H₄</td>
<td>&quot; 220</td>
<td></td>
<td>High peak at 255 μm</td>
<td>&quot;</td>
</tr>
<tr>
<td>R—CH=CH—COOH</td>
<td>295</td>
<td>1483</td>
<td>High peak at 255 μm, low shelf in 295 μm region</td>
<td>also small amount of furylacrylic acid</td>
</tr>
<tr>
<td>R—CH=CH—COOCH₃</td>
<td>307</td>
<td>1487</td>
<td>High peak at 255 μm, low shelf in 290-310 μm region</td>
<td>Furoylglycine; also small amount of methylfuryl acrylate</td>
</tr>
<tr>
<td>R—CONHCH₂COOH</td>
<td>255</td>
<td>864</td>
<td>High peak at 255 μm</td>
<td>Furoylglycine</td>
</tr>
<tr>
<td>R—CH=N—NHCONH₂</td>
<td>293</td>
<td>1617</td>
<td>High peak at 270 μm</td>
<td>Not identified</td>
</tr>
</tbody>
</table>

With this background on furan compounds, investigation of the urinary end-products of nitrofuran metabolism was then actively pursued by means of the spectral method for identification or indication of compounds.

**Nitrofurans of Class A**

The results from feeding various simple nitrofurans of Class A are shown in Table II. The urinary curves obtained from certain typical compounds
appear in Figs. 3 and 4. In Fig. 3 are shown the results from the feeding of nitrofuraldehyde. The shape of the urinary curve and the position of the peak (315 m\(\mu\)) indicate that this compound is excreted as nitrofuroic acid. The height of the peak (550 "density units") indicates that about 35 per cent of the nitrofuraldehyde fed appears in the urine in 6 hours as nitrofuroic acid.

Since several of the nitrofurans have spectral absorption peaks in the 310 to 320 m\(\mu\) region, isolation and identification of the material causing the 315 m\(\mu\) peak in the urine of nitrofuraldehyde-fed rats was attempted. Nitrofuraldehyde was administered in 30 mg. doses to six albino rats, and the urine was collected over a 6 hour period and then treated in a manner similar to that described by Sherwin and Hynes (9) for the isolation of o-nitrobenzoic acid. The urine was adjusted to pH 3.0 to 4.0 with sulfuric acid, reduced to a low volume by lyophilization, and extracted several times with ether. The ether extracts were evaporated to dryness, and the residue taken up in a small amount of hot water and placed in the refrigerator for several days. A few large crystals precipitated and were removed from the mother liquor. Spectral analysis of the mother liquor indicated that it was saturated with nitrofuroic acid.

The crystals were purified by sublimation. The sublimed crystals melted at 185–186°. Pure nitrofuroic acid melts at 185.5°. The melting point of the crystals mixed with pure nitrofuroic acid remained unchanged.

The purified crystals were dissolved in water and the ultraviolet absorption characteristics determined on the Beckman spectrophotometer. The peak was at 315 m\(\mu\), the \(E_{1\%}\) was 731, and the ratio of the minimum at 260 m\(\mu\) to the maximum at 315 m\(\mu\) was 0.191. Pure nitrofuroic acid has a peak at 315 m\(\mu\), an \(E_{1\%}\) of 725, and a ratio of the minimum at 260 m\(\mu\) to the maximum at 315 m\(\mu\) of 0.188. The melting point and absorption data thus confirm the spectral data obtained on the untreated urine and show that nitrofuroic acid is an end-product of nitrofuraldehyde metabolism.

The yield of crystals from this isolation procedure was low. This was thought to be due to poor partition between the water and the ether and to destruction of nitrofuroic acid during the isolation process. However, the existence in urine in addition to nitrofuroic acid of a conjugate with an absorption peak in the same region is not ruled out. The preparation of such a conjugate (i.e. nitrofuroylglycine) has not yet met with success when conventional synthetic procedures are used. This work is not being carried further, since nitrofuroic acid would be the metabolic pathway whether the end-product was nitrofuroic acid or a conjugate.

From the data in Table II, it appears that nitrofuroic acid, nitrofuraldehyde, nitrofuraldehyde diacetate, nitrofurfuryl alcohol, methyl
**TABLE II**

*Ultraviolet Absorption Characteristics of Urine from Rats Fed 20 Mg. Doses of Various Nitrofurans of Class A*

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>Compound</th>
<th>Absorption maxima</th>
<th>$\varepsilon_{1%}$ cm$^{-1}$</th>
<th>Absorption characteristics of urine</th>
<th>Compound indicated in urine</th>
<th>Chemotherapeutic activity†</th>
</tr>
</thead>
<tbody>
<tr>
<td>NF-4</td>
<td>R COOH</td>
<td>315 726</td>
<td></td>
<td>High peak at 315 $\mu$m</td>
<td>5-Nitro-2-furoic acid</td>
<td>Little or none</td>
</tr>
<tr>
<td>NF-2</td>
<td>R-CHO</td>
<td>225 310 840</td>
<td></td>
<td></td>
<td></td>
<td>Not tested</td>
</tr>
<tr>
<td>NF-1</td>
<td>R-CH(OOCCH$_3$)$_2$</td>
<td>308 465</td>
<td>“ “ “ 315 “</td>
<td>5-Nitro-2-furoic acid</td>
<td>Probably 5-nitro-2-furoic acid</td>
<td>“ “ “</td>
</tr>
<tr>
<td>NF-15</td>
<td>R-CH$_2$OOCCH$_3$</td>
<td>224 317 563</td>
<td>“ “ “ 315-317 $\mu$m</td>
<td>Not identified; little or no 5-nitro-2-furoic acid</td>
<td>Not tested</td>
<td>“ “ “</td>
</tr>
<tr>
<td>NF-41</td>
<td>R-CH$_2$OOC$_2$H$_5$</td>
<td>232 317 529</td>
<td>“ “ “ 315-317 $\mu$m</td>
<td>Not identified; some 5-nitro-2-furoic acid may be present</td>
<td>Little or none</td>
<td>“ “ “</td>
</tr>
<tr>
<td>NF-21</td>
<td>R-COCH$_3$</td>
<td>308 817</td>
<td>Broad peak at 320 $\mu$m</td>
<td>5-Nitro-2-furoic acid</td>
<td></td>
<td>“ “ “</td>
</tr>
<tr>
<td>NF-13</td>
<td>R-CONH$_2$</td>
<td>309 880</td>
<td>No peaks, rising absorption below 360 $\mu$m</td>
<td>5-Nitro-2-furoic acid</td>
<td></td>
<td>“ “ “</td>
</tr>
<tr>
<td>NF-86</td>
<td>R-CN</td>
<td>297 830</td>
<td>No definite peaks, low absorption below 400 $\mu$m</td>
<td>5-Nitro-2-furoic acid</td>
<td></td>
<td>“ “ “</td>
</tr>
<tr>
<td>NF-112</td>
<td>R—CH═CHCHO</td>
<td>242</td>
<td>355</td>
<td>1012</td>
<td>Slowly rising absorption below 400 μm, low peak at 280 μm</td>
<td>Not identified; little or no 5-nitro-2-furoic acid</td>
</tr>
<tr>
<td>--------</td>
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<td>------</td>
<td>----------------------------------------------------------</td>
<td>--------------------------------------------------</td>
</tr>
<tr>
<td>NF-23</td>
<td>R—CH═CHCOOC₂H₅</td>
<td>240</td>
<td>357</td>
<td>645</td>
<td>Broad low band at 310–360 μm</td>
<td>Not identified; little spectral absorption due to metabolic end-products</td>
</tr>
<tr>
<td>NF-20</td>
<td>R—H</td>
<td>315</td>
<td>1640</td>
<td></td>
<td>Low absorption below 380 μm</td>
<td>&quot;        &quot;</td>
</tr>
<tr>
<td>NF-33</td>
<td>R—NO₂</td>
<td>307</td>
<td>645</td>
<td></td>
<td>Very little absorption</td>
<td>&quot;        &quot;</td>
</tr>
</tbody>
</table>

* For longer wave-length.
† *Streptococcus hemolyticus* infections in mice (unpublished data).
‡ Also low peak at 277 μm.
Fig. 3. Ultraviolet absorption characteristics of urine of rat fed 20 mg. of 5-nitro-2-furaldehyde (NF-2). The urine curve represents average "density units" per rat (after correction for normal urine) for a 6 hour period. Solid line, urine of rat fed 5-nitro-2-furaldehyde; dashed line, 5-nitro-2-furaldehyde, 0.00005 \text{ M} \text{ solution}; dotted line, 5-nitro-2-furoic acid, 0.00005 \text{ M} \text{ solution}.

Fig. 4. Ultraviolet absorption characteristics of urine of rat fed 20 mg. of 5-nitro-2-furamide (NF-13). The urine curve represents average "density units" per rat (after correction for normal urine) for a 6 hour period. Solid line, urine of rat fed 5-nitro-2-furamide; dashed line, 5-nitro-2-furamide, 0.00005 \text{ M} \text{ solution}.
nitrofuratoate, and methyl nitrofuran are all excreted as nitrofuroic acid. Since the urinary absorption curves after the feeding of these compounds indicate little interference from end-products with spectral absorption other than nitrofuroic acid, the data have been treated quantitatively (Table III) as described earlier for furoylglycine. It is apparent that nitrofuroic acid is a major pathway of metabolism of these nitrofurans.

Nitrofurfuryl acetate and nitrofurfuryl propionate are probably also excreted as nitrofuroic acid, but the spectral curves of the three compounds are too similar to determine whether the urinary compound is nitrofuroic acid or the original nitrofuran. From our spectral data we cannot say whether the methyl and ethyl nitrofuryl ketones are excreted as nitrofuroic acid, for the urinary peak is at 320 rather than 315 mμ and is much broader than expected for a single compound. The data do not exclude the possibility of nitrofuroic acid as one of the end-products.

As a confirmatory procedure on these compounds of Class A the urine of albino rats fed 10 mg. doses of nitrofuraldehyde diacetate was submitted to bacterial assay by a standard cup plate method with Staphylococcus aureus as the test organism. If as much as 1 per cent of the drug were excreted, the urine (volume = 2 ml.) should be antibacterial, since in vitro activity for this compound is evident on Staphylococcus aureus at a concentration of 50 mg. per liter. The results were negative. This lack of antibacterial activity in the urine of animals receiving nitrofuraldehyde

### Table III

**Urinary Excretion of Nitrofuroic Acid in Urine in 6 Hours after 20 Mg. Doses of Various Nitrofurans to Albino Rats**

<table>
<thead>
<tr>
<th>Compound fed</th>
<th>Molecular weight</th>
<th>&quot;Density units&quot; in urine at 315 mμ</th>
<th>Nitrofuroic acid excreted* mg.</th>
<th>Theoretical nitrofuroic acid mg.</th>
<th>Conversion of compound to nitrofuroic acid (per cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R—COOH</td>
<td>157</td>
<td>1003</td>
<td>15.1</td>
<td>20</td>
<td>75</td>
</tr>
<tr>
<td>R—CHO</td>
<td>141</td>
<td>550</td>
<td>7.6</td>
<td>22</td>
<td>35</td>
</tr>
<tr>
<td>R—CH(OOCOCH₃)₂</td>
<td>243</td>
<td>400</td>
<td>5.5</td>
<td>12.9</td>
<td>43</td>
</tr>
<tr>
<td>R—CH₂OH</td>
<td>143</td>
<td>950</td>
<td>13.1</td>
<td>22</td>
<td>60</td>
</tr>
<tr>
<td>R—COOCH₂</td>
<td>171</td>
<td>770</td>
<td>10.6</td>
<td>18.4</td>
<td>58</td>
</tr>
<tr>
<td>R—CH₃</td>
<td>127</td>
<td>800</td>
<td>11.0</td>
<td>24.7</td>
<td>45</td>
</tr>
</tbody>
</table>

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* Calculated by formula, ("density units" × 10)/E₁% cm. = mg. of material. The E₁% cm. for nitrofuroic acid at 315 mμ = 726.
diacetate strengthens the spectrophotometric evidence which indicates excretion of over 40 per cent of the drug as the bacterially inactive nitrofuroic acid.

There are a few compounds of Class A which appear to be metabolized by processes which do not lead to a predominance of nitrofuroic acid. In Fig. 4 the urine curve after the feeding of 5-nitro-2-furamid is shown. When this type of curve is obtained, we cannot draw conclusions as to the identity of the end-products but we can make the following deductions: (1) the compound fed is not excreted in the urine in appreciable quantities; (2) the spectral characteristics denote the absence of other known nitrofurans (e.g. nitrofuroic acid) with marked absorption peaks. Other compounds of Class A which are not metabolized predominantly to nitrofuroic acid are nitrofuronitrile, nitrofurylacrylic acid derivatives, nitrofuran, and dinitrofuran.

The conversion of the simpler nitrofurans to nitrofuroic acid in vivo finds an analogy with certain non-nitrated derivatives which have been shown to be excreted as furoylglycine, the simple conjugate of furoic acid. The chemical stability of nitrofuroic acid in contrast to that of a number of other nitrofurans permits its ready elimination from the body in appreciable amounts. By the spectral method (Table III) about 75 per cent of the nitrofuroic acid fed has been found in the urine. The low in vivo or chemotherapeutic activity (Table II) of certain compounds which exhibit good in vitro antibacterial activity may be readily explained for those compounds which are converted to nitrofuroic acid, since nitrofuroic acid has been shown to be only weakly active. From a metabolic standpoint the elimination of certain nitrofurans as nitrofuroic acid finds a parallel in the elimination of o-nitrobenzaldehyde as o-nitrobenzoic acid (9, 10).

**Nitrofurans of Class B**

The results from feeding various nitrofurans of Class B are shown in Table IV. The urinary curves obtained after feeding certain compounds of this class are shown in Figs. 5, 6, and 7. Some of the compounds undergo decomposition resulting in a complex series of end-products and some are partially excreted in the urine in unchanged form.

In Fig. 5 is shown the urine of an animal fed β-5-nitro-2-furaldehyde oxime. The major peak of the pure compound is at 346 mμ, but the peak of the material appearing in the urine is in the 335 mμ range.

It is of interest to note here that the α and β forms of the oxime (Table IV) are metabolized to quite different end-products. The breakdown is extensive in either case. Confirmation of the extensive (complete?) breakdown has been obtained by bacterial assay against Staphylococcus
 aureus of the urine of albino rats fed these drugs. The urine of rats fed 25 mg. doses of either the α- or β-nitrofuraldehyde oxime exhibited no antibacterial activity, whereas the in vitro activity of these drugs is evident at a concentration of 50 mg. per liter (1). These compounds exhibit no in vivo activity undoubtedly because they have been changed to inactive forms by metabolic processes. Similarly, it may be seen from Table IV that 5-nitro-2-furaldehyde hydrazone has undergone extensive change and shows no in vivo activity.

The chemotherapeutic properties of one of the members of Class B, 5-nitro-2-furaldehyde semicarbazone (Furacin), have been reported by Dodd (3). The urinary curve obtained after feeding this compound is shown in Fig. 6. It is apparent from this curve and from Table IV that extensive breakdown of the drug has occurred during its passage through the animal body. The apparently complete breakdown of this compound was puzzling in view of its in vivo activity. However, by refinement of methods and chromatographic treatment of urines it was demonstrated that a portion of the Furacin was in fact being excreted unchanged. This was not obvious from the spectral curve of the untreated urine, since the small amount of compound was masked by the presence of other metabolic end-products with spectral absorption in the same region. With adequate oral doses, unchanged Furacin at an antibacterial level is excreted in the urine. This has been confirmed by isolation of the compound by chromatography. Since other metabolic end-products of the drug isolated to date are without antibacterial activity, the activity of the urine must be due to the presence of the unchanged drug. It is believed that the fact that this nitrofuran escapes complete metabolic transformation within the animal body accounts for its chemotherapeutic activity. The details of the metabolism of Furacin in animals form the subject of a separate report.

A similar picture to that of Furacin (Table IV) is obtained with 5-nitro-2-furyl methyl ketone semicarbazone (NF-57) and with the 5-nitro-2-furyl hydroxymethyl ketone semicarbazone (NF-100). The urine curves indicate complete breakdown of the drug. Nevertheless, in the case of one of these (NF-106), the presence of some of the parent nitrofuran has been demonstrated by further purification of the urine by chromatography.

In Fig. 7 is shown the urinary curve after the feeding of 5-nitro-2-furaldehyde 2-(2-hydroxyethyl) semicarbazone. Enough of this compound appears unchanged in the urine so that its presence is obvious from visual examination of the spectral absorption curve (i.e. the similarity of urine peaks to the peaks of the parent compound). Calculation of "density units" would indicate urinary excretion of about 20 per cent of the compound fed. It is thus easy to understand why this compound exhibits
TABLE IV

Ultraviolet Absorption Characteristics of Urine of Rats Fed 20 Mg. Doses of Various Nitrofurans of Class B

\[ R = O_2N-\]

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>Compound</th>
<th>Absorption maxima</th>
<th>Absorption characteristics of urine</th>
<th>Compound indicated in urine</th>
<th>Chemotherapeutic activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>NF-6α</td>
<td>R-CH=NOH</td>
<td>233 340 850</td>
<td>Peak at 435 μm, urine reddish</td>
<td>Not identified; extensive breakdown</td>
<td>None on α-, β mixture</td>
</tr>
<tr>
<td>NF-6β</td>
<td>R-CH=NOH</td>
<td>232 346 850</td>
<td>Peak at 335 μm</td>
<td></td>
<td>None on α-, β mixture</td>
</tr>
<tr>
<td>NF-9</td>
<td>R-CH=NNH₂</td>
<td>219 381 825</td>
<td>Low peak at 328 μm</td>
<td>Several end-products; mask presence of small amount of original compound</td>
<td>No indication</td>
</tr>
<tr>
<td>NF-7</td>
<td>R-CH=NNHCONH₂</td>
<td>260 375 786</td>
<td>&quot; or shelves in 270, 320, 490 μm regions</td>
<td>Several end-products; could mask original compound</td>
<td>Good</td>
</tr>
<tr>
<td>NF-57</td>
<td>R-C(CH₃)=NNHCONH₂</td>
<td>260 375 655</td>
<td>Low broad peaks at 270, 320, 400 μm</td>
<td>Some of original compound</td>
<td>Fair</td>
</tr>
<tr>
<td>NF-106</td>
<td>R-C(CH₂OH)=NNHCONH₂</td>
<td>257 375 540</td>
<td>Broad peak in 370 μm region, deflection in 260-270 μm region</td>
<td>Some of original compound</td>
<td>Good</td>
</tr>
<tr>
<td>NF-67</td>
<td>R-CH=NN(CH₂CH₂OH)CONH₂</td>
<td>268 384 670</td>
<td>Peaks at 384 μm and in 270 μm region</td>
<td>Original compound present</td>
<td>Slight</td>
</tr>
<tr>
<td>NF-62</td>
<td>R-CH=NNHCONHCH₃</td>
<td>266 380 734</td>
<td>Peak at 380 μm, shelf in 260-270 μm region</td>
<td>Some of original compound present</td>
<td></td>
</tr>
</tbody>
</table>

*μm, mμ, 1%α cm.†Metabolism of nitrofurans. ‡
<table>
<thead>
<tr>
<th>Compound</th>
<th>R—CH=NN(CH₂)CONH₂</th>
<th>267</th>
<th>384</th>
<th>760</th>
<th>Peaks in 410 and 285 μm regions</th>
<th>Other end-products; could mask original compound†</th>
<th>Slight</th>
</tr>
</thead>
<tbody>
<tr>
<td>NF-84</td>
<td>R—CH=NNHCOCONH₂</td>
<td>270</td>
<td>362</td>
<td>752</td>
<td>Shelves in 330-380 and 260-270 μm regions</td>
<td>Some of original compound‡</td>
<td>Good</td>
</tr>
<tr>
<td>NF-89</td>
<td>R—CH=NNHCOCONH₂CH₂CH₂OH</td>
<td>252</td>
<td>362</td>
<td>704</td>
<td>Shelves in 340-380 and 260-270 μm regions</td>
<td>Some of original compound?§</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

* For longer wave-length.
† *Streptococcus hemolyticus* infections in mice (Dodd, Cramer, and Ward and other unpublished data).
‡ Chromatography followed by spectrophotometric identification indicated the presence of a small amount of the original compound.
§ Spectral indication confirmed by chromatography and spectrophotometric identification of the original compound in appreciable amount.
‖ Could not be confirmed by chromatography, for column appeared to cause decomposition. See the text.
Fig. 5. Ultraviolet absorption characteristics of urine of rat fed 20 mg. of 5-nitro-2-furaldehyde β-oxime (NF-6). The urine curve represents average "density units" per rat (after correction for normal urine) for a 6 hour period. Solid line, urine of rat fed 5-nitro-2-furaldehyde β-oxime; dashed line, 5-nitro-2-furaldehyde β-oxime, 0.00005 M solution.

Fig. 6. Ultraviolet absorption characteristics of urine of rat fed 20 mg. of 5-nitro-2-furaldehyde semicarbazone (NF-7). The urine curve represents average "density units" per rat (after correction for normal urine) for a 6 hour period. Solid line, urine of rat fed 5-nitro-2-furaldehyde semicarbazone; dashed line, 5-nitro-2-furaldehyde semicarbazone (Furacin), 0.00005 M solution.
good antibacterial activity both in vitro and in vivo. Inspection of Table IV indicates that 5-nitro-2-furaldehyde 4-methyl semicarbazone (NF-62) is excreted in part unchanged, whereas 5-nitro-2-furaldehyde 2-methyl semicarbazone (NF-61), if excreted, is masked by the presence of other end-products; i.e. the peaks in the urine are different from those of the pure compound.

The semioxamazones present a picture similar to that of the semicarbazones. In Table IV it is indicated that these compounds may be excreted in small amounts in the urine, although their presence is partially

![Ultraviolet absorption characteristics of urine of rat fed 20 mg. of 5-nitro-2-furaldehyde 2-(2-hydroxyethyl) semicarbazone (NF-67). The urine curve represents average "density units" per rat (after correction for normal urine) for a 6 hour period. Solid line, urine of rat fed 5-nitro-2-furaldehyde 2-(2-hydroxyethyl) semicarbazone; dashed line, 5-nitro-2-furaldehyde 2-(2-hydroxyethyl) semicarbazone, 0.00005 M solution.](http://www.jbc.org/)

masked by the presence of other end-products. In the case of 5-nitro-2-furaldehyde semioxamazone (NF-84) the presence of a small amount of the parent compound could be demonstrated by chromatography of the urine. This technique was not successful in the case of the 5-nitro-2-furaldehyde 5-(2-hydroxyethyl) semioxamazone (NF-89), for, although a band could first be seen, decomposition appeared to take place as the material went through the column. This nitrofuran is being investigated further.

A more complete study of the effect of various substituents on the stability of the nitrofurans of Class B is under way in these laboratories.
DISCUSSION

The use of spectrophotometric analysis of untreated urine has proved extremely valuable in the study of nitrofuran metabolism. The excretion of appreciable amounts of the compound fed is readily detected by this method (Fig. 7). The excretion of appreciable quantities of breakdown products with ultraviolet absorption characteristics is also readily detected (Figs. 2, 3, 5-7). In some cases a clue to the identity of the end-products is obtained by this method when the urine curves and peaks approximate those of known compounds (Figs. 2, 3). Further refinements of technique are necessary when breakdown products mask the presence of small amounts of compounds (Fig. 6). Other methods must be resorted to for end-products with no ultraviolet absorption characteristics.

From our present results we believe that certain conclusions may be drawn regarding the metabolic pathway of the nitrofurans. The simpler nitrofurans of Class A appear to be metabolized by oxidation to nitrofuroic acid and excreted as such. The Class A compounds such as nitrofuronitrile and nitrofuramide are apparently metabolized in a different manner with extensive breakdown. Further work will be necessary to determine the end-products of such compounds.

The compounds of Class B in some cases are excreted in varying amounts as the compound fed. In other cases they undergo extensive breakdown. In general, some end-products with ultraviolet absorption characteristics are formed. Nitrofuroic acid does not appear to be an end-product of the metabolism of compounds of Class B. The relation of the structure of various nitrofurans to their resistance to breakdown by metabolic processes is under investigation.

SUMMARY

A spectrophotometric method of examination of untreated urines of animals fed various furans and nitrofurans has been described.

By the use of this method furoylglycine has been found as the major urinary end-product after the feeding of furaldehyde, furoic acid, furfural diacetate, furfural hydrazone, furfuryl alcohol, furfuryl propionate, furyl-acrylic acid, and methylfurfuryl acrylate.

Nitrofuroic acid is believed to be the major urinary end-product after feeding nitrofuraldehyde, nitrofuroic acid, nitrofuraldehyde diacetate, nitrofurfuryl alcohol, nitrofurfuryl acetate, nitrofurfuryl propionate, methyl nitrofuroate, and methyl nitrofuran. The compounds tested which are metabolized to nitrofuroic acid show little or no \textit{in vivo} activity.

The oximes and hydrazone of nitrofuraldehyde are extensively broken down during metabolism. The end-products have not been identified,
but nitrofuroic acid is not indicated as a major metabolite. These compounds have shown little or no chemotherapeutic activity.

Certain semicarbazones and semioxamazones and their substitution products undergo extensive change in the body, while others are excreted in appreciable amounts as the compound fed. Nitrofuroic acid is not indicated as a metabolic end-product of these compounds. In general, these compounds which have shown chemotherapeutic activity have been shown to withstand metabolic breakdown partially and to be excreted in the urine.

The furans and nitrofurans used in this study were synthesized by the Divisions of Chemistry and Chemical Engineering and the chemotherapeutic tests were conducted by the Division of Pharmacology of the Eaton Laboratories, Inc. The authors wish to express their appreciation to John Gillespie and George Everett for their assistance in the numerous spectrophotometric determinations.

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Ells


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