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Metabolic Degradation of the Nitrofurans

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Spectrophotometric studies of nitrofurazone† solutions incubated with mammalian tissue slices[1] or with milk xanthine oxidase[2] have shown that a different absorption maximum, ascribed to 'reduced' nitrofurazone, appears in the 330 mμ region during incubation. Similar observations have been reported by Beckett and Robinson[3–5] and by Asnis[6] with bacteria and by Austin[7] after Raney-nickel reduction of nitrofurazone. Austin[7] also reported the isolation of glyoxylopropionitrile semicarbazone, an open chain isomer believed to be formed following ring cleavage of 5-amino-2-furaldehyde semicarbazone in aged reduction mixtures of nitrofurazone.

The present study is concerned with the purification and characterization of the end-products appearing in the urine of nitrofurazone-fed animals and the degradation pattern by liver tissues of nitrofurazone and certain related nitrofurans. Nitrofurazone (Furacin), whose antibacterial properties were first described by Dodd and Stillman,[8] is used as a topical antibacterial agent.

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† The following are trademarks of The Norwich Pharmacal Company for certain nitrofurans: Furacin, brand of nitrofurazone (5-nitro-2-furaldehyde semicarbazone); Furadroxyl, brand of nidroxyzone [5-nitro-2-furaldehyde 2-(2-hydroxyethyl)semicarbazone]; Furamazone, brand of nifuraldezone (5-nitro-2-furaldehyde semioxamazone); Furadantin, brand of nitrofurantoin [*N*-(5-nitro-2-furfurylidene)-1-aminohydantoin]; Furoxone, brand of furazolidone [*N*-(5-nitro-2-furfurylidene)-3-amino-2-oxazolidone]; Altafur, brand of furaltadone [*N*-(5-nitro-2-furfurylidene)-3-amino-5-(*N'*-morpholinylmethyl)-2-oxazolidone]; Micofur, brand of nifuroxime (*anti*-5-nitro-2-furaldoxime). Nihydrazone is the generic name of 5-nitro-2-furaldehyde acetylhydrazone.

Methods

Metabolic End-Products

The presence of nitrofurans and end-products in incubation media, in urine, or in chromatographic fractions of urine was determined spectrophotometrically as described in earlier publications[1, 2, 9] using the Beckman model DU quartz spectrophotometer and appropriate correction blanks. The absorption maxima and $E^{1\%}_{1cm}$* in aqueous solutions of certain of the nitrofurans studied are: nitrofurazone (NF–7)—375 mμ, 790; nidroxyzone (NF–67)—385 mμ, 689; nifuraldezone (NF–84)—362·5 mμ, 830; nitrofurantoin (NF–153)—367·5 mμ, 753; furazolidone (NF–180)—367 mμ, 747; and furaltadone (NF–260)—366 mμ, 543.

Urine samples from nitrofuran-fed laboratory animals or human subjects were diluted with 4 parts of 95 per cent ethanol and introduced into chromatographic columns of $Mg(OH)_2$†–Celite‡ or Superfiltrol§–Celite. A flowing chromatogram was used with 76–80 per cent ethanol as the eluent. The Superfiltrol–Celite columns were most useful for separation and detection of unchanged nitrofurazone, and material having an absorption maximum near 270 mμ. The $Mg(OH)_2$–Celite column was most useful for the separation of the yellow fraction from animal urine having a maximum absorption in the 420 mμ range. The two columns were about equally useful for separating the material having an absorption maximum near 335 mμ. The nitrofurazone fraction passed readily through the magnesia column. In some cases the urines were pretreated by adjusting to pH 4, adsorbing on charcoal (Darco type G–60; about 1/100 the volume of the urine), centrifuging and washing three times with slightly acidified distilled water (HCl to pH 4–5), and eluting with two successive portions of 76 per cent ethanol made slightly alkaline (pH 10) with 2N NaOH. This treatment left many of the natural constituents of urine on the charcoal. The eluate was then further purified on $Mg(OH)_2$–Celite or Superfiltrol–Celite columns. In

* The absorbance of a 1 cm layer of a 1 per cent solution is designated as $E^{1\%}_{1cm}$.

† Magnesium hydroxide, J. T. Baker medicinal grade.

‡ Johns-Manville Company, Celite No. 545 or No. 501.

§ Johns-Manville Company.

some cases, successive fractions were collected from the magnesia columns and further purified by passing them through the Superfiltrol columns.

Tissue Degradation Studies

Slices 0·5–1 mm thick of liver tissue from healthy adult albino rats were used. The medium was M/15 phosphate buffer pH 7·4 with added glucose (100 mg per 100 ml). Sufficient nitrofuran was added to give a final concentration of approximately 0·5 millimolar in the medium. One-gram samples of the tissue slices were introduced into 50-ml small-mouthed, round-bottom centrifuge tubes, followed by 4 ml of the buffer–glucose medium. The tubes were stoppered, placed in a water-bath at 37·5° and agitated mechanically throughout the incubation period. Tubes containing the tissue and medium without added drug were prepared concurrently to serve as controls. At specified intervals, 0·5 ml aliquots were removed to small centrifuge tubes containing 2 ml of 95 per cent ethanol. After mixing and centrifuging, 1 ml of the clear supernatant liquid was removed and diluted with 70 per cent ethanol as required for spectrophotometric determinations.

Faecal suspensions were prepared by making a paste of fresh rat faeces with a small amount of 0·9 per cent NaCl solution. Nitrofuran solutions (about 100 mg/l.) were prepared in 0·9 per cent saline. One-gram samples of the faecal paste were added to 50-ml quantities of the nitrofuran solutions in 125-ml Erlenmeyer flasks. The flasks were shaken thoroughly and incubated at 37°. Samples were removed at appropriate intervals, diluted with 4 parts of 95 per cent ethanol and further diluted to the proper concentration with 70 per cent ethanol for spectrophotometric observations. Appropriate faecal suspension controls were used for correction.

Results

Hydrolysis of Nitrofurazone by Dilute HCl Solutions

Incubation of nitrofurazone (10 mg/l.) in an aqueous HCl solution (pH 1·3) at 37° resulted in degradation of the nitrofurazone as measured by the 375 mμ absorption maximum. In Fig. 1 are

shown the absorption curves of the nitrofurazone solution at the beginning and 1 h after incubation. A rapid loss of absorption at 375 mμ with increasing absorption in the 310 mμ region is apparent. A similar experiment was conducted in which the incubation was carried out for 20 h to allow equilibrium to be reached. In this case the loss of nitrofurazone was 53 per cent. Since the expected end-product of such a hydrolysis would be

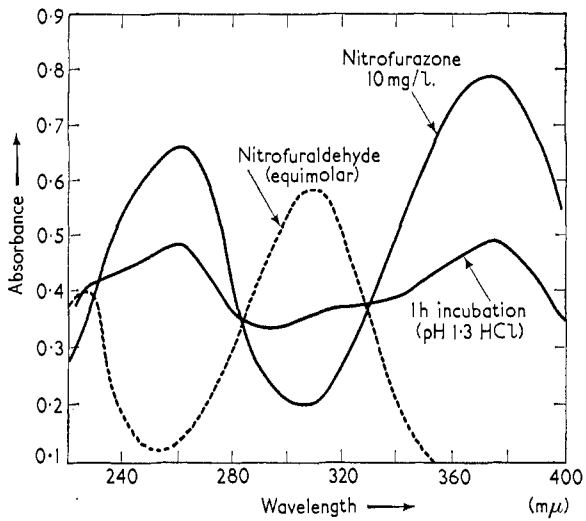


Fig. 1. Acid hydrolysis of nitrofurazone solution. Nitrofurazone (10 mg/l.) was incubated at 37° for 1 h in HCl solution (pH 1·3). The spectrophotometric curves of the solution at the start and at the end of 1 h incubation are shown as well as the spectral absorption curve of a solution of nitrofuraldehyde equimolar to the nitrofurazone at the start.

nitrofuraldehyde with an absorption maximum at 310 mμ, absorption curves for 47 per cent nitrofurazone (4·7 mg/l.) and for that amount of nitrofuraldehyde corresponding to 53 per cent of the nitrofurazone, were calculated and plotted. The actual curve obtained from the incubation mixture and the calculated curve are shown in Fig. 2. The marked similarity of the two curves is at once apparent and strongly indicates the expected hydrolysis of nitrofurazone to nitrofuraldehyde in dilute aqueous HCl.

As further proof of hydrolysis to nitrofuraldehyde, a similar nitrofurazone solution was incubated in dilute aqueous HCl to which an excess of semicarbazide hydrochloride had been added at the start. Under these conditions a typical nitrofurazone curve was maintained up to 20 h after incubation with only a minimal (7 per cent) loss of absorption at the nitrofurazone maximum (375 mμ). A colorimetric method[10] for the quantitative determination of nitrofurazone, furazolidone, nitrofurantoin,

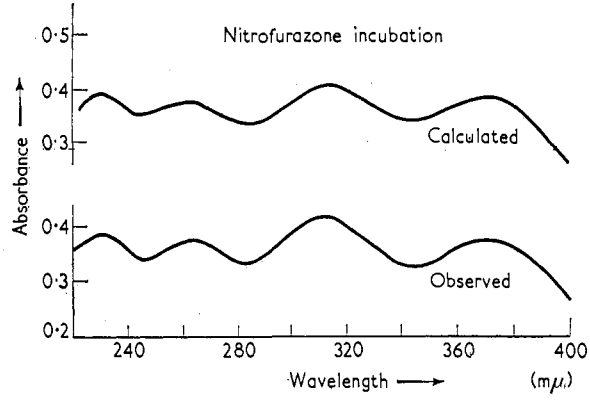


Fig. 2. Spectral absorption curves of a mixture of nitrofuraldehyde and nitrofurazone. The lower curve was that observed when a solution of nitrofurazone (10 mg/l.) in HCl (pH 1·3) was incubated at 37° for 24 h. Fifty-three per cent loss of nitrofurazone was calculated from the decrease in absorption at 375 mμ. The upper curve is a curve calculated for a mixture containing 4·7 mg/l. of nitrofurazone and 3·8 mg/l. nitrofuraldehyde (molar equivalent of 5·3 mg/l. of nitrofurazone). The absorption maximum of nitrofuraldehyde is 310 mμ and $E^{1\%}_{1\,cm}$ is 810.

and other nitrofurans is based upon acid (HCl) hydrolysis of the compound to nitrofuraldehyde in the presence of phenylhydrazine, and the formation of the coloured phenylhydrazone.

The rate of hydrolysis of nitrofurazone at various HCl concentrations was determined. The results appear in Fig. 3 and show that no detectable hydrolysis of nitrofurazone occurs at pH 4 or above and that the rate of hydrolysis below pH 4 varies with the pH. Since the pH value of gastric contents usually lies in the 1·4

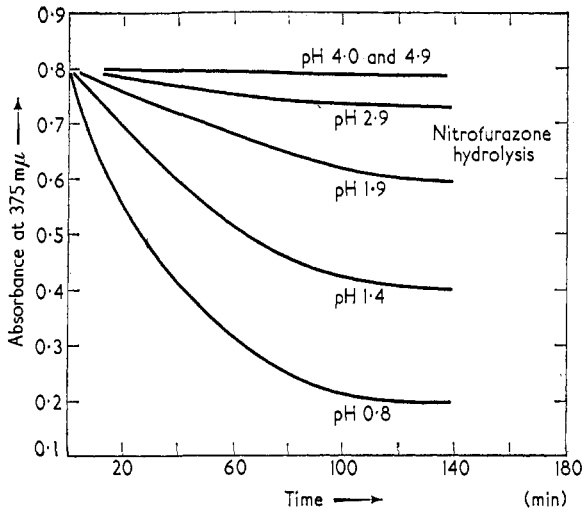


Fig. 3. Rate of hydrolysis of nitrofurazone as affected by pH. Nitrofurazone solutions (10 mg/l.) were incubated in different concentrations of HCl and the rate of hydrolysis followed by the decrease in absorption at the nitrofurazone maximum (375 mμ) with time.

to 3 range, it is assumed that a small amount of nitrofuraldehyde may be formed from nitrofurazone in the stomach, the amount varying with the pH of the contents and with the length of time the drug remains in the stomach.

Hydrolysis of Nitrofurans Related to Nitrofurazone

Selecting a single set of conditions for hydrolysis (pH 1·6, 37°) the stability for a 4-h period of a number of nitrofurans related to nitrofurazone was studied. The results appear in Table I. The stability of the —CH=N— linkage to acid hydrolysis varies with the structure of the side chain. Compounds with a substituent in the 2-position of the side chain appear to be more stable to acid hydrolysis than are the other nitrofurans studied. Therefore, the relative amounts of nitrofuraldehyde formed during metabolism would be dependent on the structure of the nitrofuran.

End-Products in Urine

Indications of absorption maxima in the 270, 320 and 420 mμ regions in the urine of nitrofurazone-fed animals have been reported.[9] The results of chromatographic treatment of the urine of a nitrofurazone-fed rat on a $Mg(OH)_2$–Celite column with 80

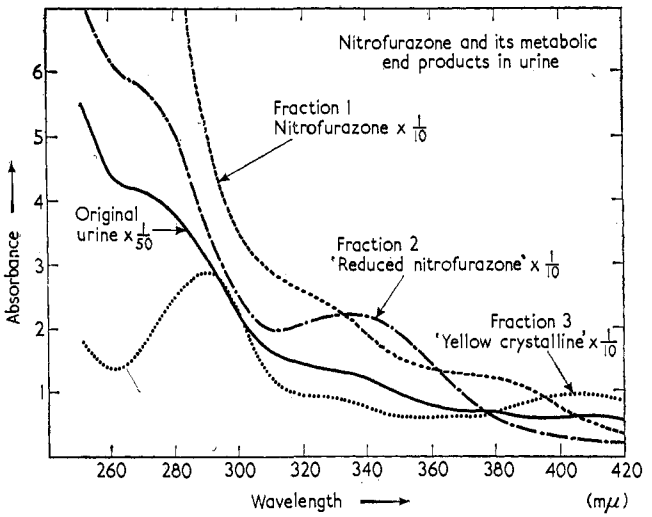
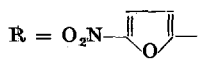


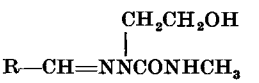
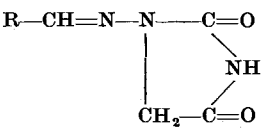
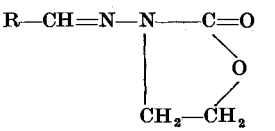
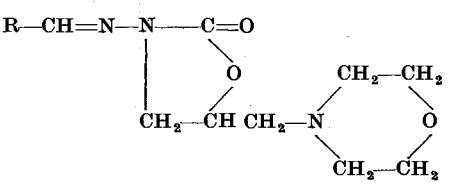
Fig. 4. Fractionation of urine of nitrofurazone-fed rat on $Mg(OH)_2$–Celite column; the spectral absorption curve of a 1 : 50 dilution of the untreated urine. The urine was then diluted 1·2 → 5·5 ml with C_2H_5OH and introduced into a $Mg(OH)_2$–Celite column, followed with 80 per cent C_2H_5OH for a flowing chromatogram. Successive 5 ml samples were collected. Fraction 1 (– – – – –) was shown to contain unchanged nitrofurazone (1 : 10 dilution); fraction 2 (— — — — — — —) contained 'reduced' nitrofurazone (1 : 10 dilution); fraction 3 (.....) contained a yellow crystalline material with maximum absorption at 412·5 mμ (1 : 10 dilution).

per cent ethanol as the eluent are shown in Fig. 4. The ultraviolet absorption characteristics of successive 5-ml fractions reveal that several components are separable. That the first fraction contained unchanged nitrofurazone was suspected from the inflection of the curve in the 375 mμ region (nitrofurazone absorption

Table I. Acid hydrolysis of nitrofurans at pH 1·6 and 37°

R = O_2N–  –

Compound no.	Generic name	Formula	% Hydrolysis after ½ h	% Hydrolysis after 4 h
NF–6 *anti*	nifuroxime	R—CH=NOH	2	24
NF–7	nitrofurazone	R—CH=NNHCONH$_2$	17	45
NF–9	–	R—CH=NNH$_2$	85	100[a]
NF–61	–	R—C(CH$_3$)=NNHCONH$_2$	2	6
NF–62	–	R—CH=NNHCONHCH$_3$	15	48
NF–64	nihydrazone	R—CH=NNHCOCH$_3$	68	79
NF–67	nidroxyzone	R—C(CH$_2$CH$_2$OH)=NNHCONH$_2$	4	13
NF–84	nifuraldezone	R—CH=NNHCOCONH$_2$	53	67

NF–89	–	R—CH=NNHCOCONHCH$_2$CH$_2$OH	57	69
NF–144	–		1	7
NF–145	–	R—CH=NNHCONHCH$_2$CH$_2$OH	13	44
NF–153	nitrofurantoin		3	14
NF–180	furazolidone		4	16
NF–260	furaltadone		4	16

[a] All except NF–9 seemed to reach an equilibrium point characteristic of each nitrofuran.

maximum) and the demonstration of antibacterial activity of this fraction.

The isolation and identification of nitrofurazone from urine was accomplished as follows. Urine (28 ml) collected from nitrofurazone-fed rats (100 mg/kg *per os*) was 'freeze-dried' to near dryness and taken up in 1 ml of H_2O, with 95 per cent C_2H_5OH added to a total volume of 7 ml, and centrifuged. The clear supernatant was put on a $Mg(OH)_2$–Celite column followed by 80 per cent C_2H_5OH, and the yellow band which passed immediately through the column was collected (7 ml). This fraction was then put on a Superfiltrol–Celite column (previously adjusted to pH 7·5 with NH_4OH in 95 per cent C_2H_5OH wash) followed by 80 per cent C_2H_5OH and three 25-ml fractions were collected. These fractions had similar spectrophotometric characteristics (those of nitrofurazone) and were composited, 'freeze-dried' to 3 ml, and stored in the refrigerator for two days. Slight crystallization occurred. Further slight evaporation was accomplished by freeze-drying and the material was returned to the refrigerator. The next day the crystals were dissolved in 10 ml of water and put back into the refrigerator for recrystallization. After a second recrystallization from water, the ultraviolet absorption characteristics of the crystals were determined. The resulting spectral curve closely resembled that of nitrofurazone (Fig. 1) in all respects (max. 375, min. 310, max. 260 mμ). The $E^{1\%}_{1cm}$ of the crystals at the point of maximum absorption (375 mμ) was found to be 740, somewhat lower than that of nitrofurazone (790), indicating slight contamination. The ratio of the absorption at the minimum (310 mμ) to that at the maximum (375 mμ) for the crystals was $170/740 = 0 \cdot 23$, while the value for pure nitrofurazone is $180/790 = 0 \cdot 23$. The crystals were examined on a hot stage under the microscope and compared with an authentic specimen of nitrofurazone crystals. Both exhibited the same behaviour, softening and charring at 225–228°. A solution of the crystals to which sodium or potassium hydroxide had been added gave the red colour of nitrofurazone under similar conditions.

Fraction 2 exhibited an absorption maximum at 335 mμ and a marked inflection in the 270 mμ region (Fig. 4). In studying a number of such fractions from urine it was observed that the intensity of absorption at 335 mμ and that in the 270 mμ region

bore no constant relationship to each other. In some cases the 270 mμ maximum was much higher than that of the 335 mμ (Fig. 4, fraction 2) and in some cases much lower. It was also observed that on standing at room temperature the absorption at 335 mμ decreased with an accompanying increase in the 270 mμ absorption indicating that the 270 mμ material might represent further degradation of the 335 mμ material. This has been reported for solutions of chemically reduced nitrofurazone[7] and for solutions of nitrofurazone degraded by bacteria.[3] Studies with solutions containing a mixture of nitrofurazone and chemically reduced nitrofurazone* on chromatographic columns revealed behaviour remarkably similar to that of urine fractions 1 and 2. The lability of the chemically reduced material having maximum absorption at 335 mμ[7] or of the material in urine, has so far precluded its isolation and characterization. Tentatively, the material in urine purified by chromatography and exhibiting maximum absorption at 335 mμ is assumed to be either hydroxylamino- or aminofuraldehyde semicarbazone, or both. Fraction 2 as well as chemically reduced nitrofurazone was found to be antibacterially inactive in cup plate tests; the instability of the latter may have led to this result rather than the inactivity of the compound *per se*.

In the 270 mμ region (Fig. 4), the inflection of the spectrophotometric curve of the urine and the increase in absorption when fraction 2 is allowed to age are suggestive of the presence of glyoxylopropionitrile semicarbazone (272 mμ maximum) isolated by Austin[7] after the chemical reduction of nitrofurazone. A fraction with a better defined maximum in the 270 mμ region could be obtained using a Superfiltrol–Celite column. Acid treatment of the urine suggested also that the absorption characteristics of this end-product may be pH sensitive. The large number of normal metabolites with absorption maxima in the 240–300 mμ range, the inability to separate the nitrofuran metabolites by liquid–liquid extraction, the lack of suitable chemical methods for identification of nitrofuran end-products as well as the lability of certain end-products have so far prevented final isolation and identification of the '270 mμ' material. It may possibly be the glyoxylopropionitrile semicarbazone, as isolated by Austin[7] from chemical reduction of nitrofurazone.

* Prepared by Dr. F. L. Austin.[7]

Other known furans with absorption maxima in the 270 mμ range have been considered as possible end-products of nitrofuran metabolism. Furaldehyde has an absorption maximum at 276 mμ but studies in this laboratory[9] have confirmed the results of earlier workers[11] that furaldehyde is excreted as furoyl glycine with an absorption maximum at 255 mμ. Feeding of furaldehyde semi-carbazone to rats[9] was also found to result in an end-product in urine having an absorption maximum in the 270 mμ range. Isolation and identification of this product have not yet been accomplished.

Fraction 3 (the third 5-ml portion) exhibited an absorption maximum in the 410–420 mμ region and was inactive against bacteria. A yellow crystalline material with an absorption maximum at 412·5 mμ and a secondary maximum at 320 mμ was isolated from rabbit urine by the following procedure.

Eleven rabbits were each given 300 mg nitrofurazone crystals *per os* and urine was collected by catheterization three times during the day. The urine samples were pooled (780 ml), adjusted to pH 3 and allowed to stand overnight in the refrigerator. They were centrifuged, the precipitate was discarded and the clear supernatant adjusted with HCl to pH 5·2. Darco G–60 charcoal was added (10 g) and stirred for an additional 10 min. The material was centrifuged and the supernatant discarded. The charcoal was washed with one 200-ml and two 100-ml portions of acidified water (pH 5). Elution was carried out using 16 ml of water with 64 ml of 95 per cent C_2H_5OH adjusted to pH 10·4 with NaOH (7 ml of 2N NaOH). Elution was repeated using 7 ml of water with 28 ml of 95 per cent C_2H_5OH and 0·3 ml of 2N NaOH. A third elution was carried out with 7 ml of water and 28 ml of 95 per cent C_2H_5OH. The three eluates were combined, filtered through Celite and the pH adjusted to 8·9. The total volume of eluate was 149 ml. To this were added four volumes of 95 per cent alcohol and the entire sample was put through a $Mg(OH)_2$–Celite column using 80 per cent C_2H_5OH for a flowing chromatogram. The material passing through as a yellow band was collected. This material was put through a second $Mg(OH)_2$–Celite column by the same procedure and the material passing through as a yellow band again collected. No improvement in purity, as judged by the spectral characteristics, was obtained by the use of

a third column. The solution was adjusted to pH 5 and 'freeze-dried'. The dry material was taken up in 95 per cent alcohol and recrystallized three times. The spectrophotometric absorption curve obtained from an aqueous solution of the crystals (third recrystallization) is shown in Fig. 5. The $E^{1\%}_{1\,cm}$ value of the crystals as obtained was 820 at 412·5 mμ. Carbon, hydrogen and

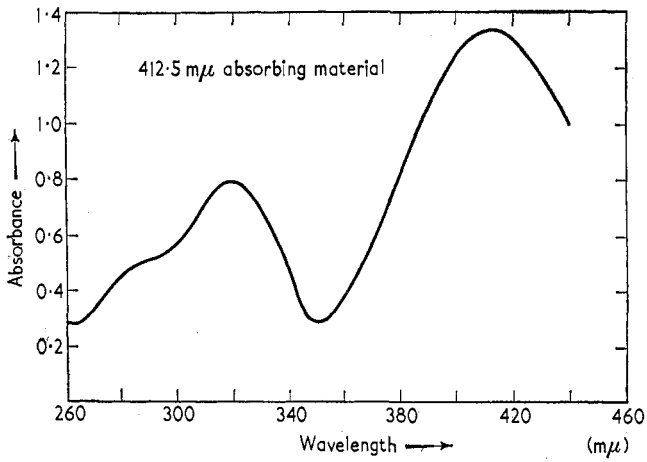


Fig. 5. Spectral absorption curve of yellow crystalline material isolated from the urine of nitrofurazone-fed rabbits 0·8 mg to 50 ml. Absorption maximum is 412·5 mμ, $E^{1\%}_{1\,cm}$ = 820.

nitrogen analyses were: C, 27·78 per cent; H, 1·50 per cent; and N, 21·25 per cent; accounting for about half of the material. Some contamination from the column was present since emission spectrographic studies showed the presence of magnesium. Further recrystallization from alcohol was not attempted since the $E^{1\%}_{1cm}$ values for the second and third recrystallizations were not appreciably different (805, 2nd; 820, 3rd), indicating that the material was quite pure. The material has not been further identified but is completely inactive toward bacteria. This yellow material has been found in the urine of all nitrofurazone-fed animals studied to date (rat, guinea-pig, rabbit, dog, calf) but was totally absent from the urine of human beings who had received nitrofurazone. This yellow crystalline material disappears on heating in solution at 95° at pH values below 10 with

a new maximum forming at 277·5 mμ. The new maximum becomes more than ten times the height of the original 412·5 mμ maximum and then eventually decreases.

In addition to the above intermediates the urines of nitrofurazone-fed animals and humans contained brown polymeric material which tended to remain in chromatographic columns but could be eluted with 1 per cent ammonia. A similar material also appears after the chemical reduction of nitrofurazone and after the reduction of nitrofurazone by susceptible strains of *Escherichia coli* and *Staphylococcus aureus* or by cell-free extracts of nitrofurazone susceptible *E. coli*.[6] This material has not been characterized other than descriptively as 'brown-coloured resins'.[1,2] The brown materials from the urine of nitrofurazone-fed animals and from the chemical reduction of nitrofurazone were found to behave almost identically when subjected to acid or alkaline hydrolysis or heat followed by chromatographic treatment. The range of spread throughout a chromatographic column indicates the presence of polymers of varying chain lengths.

Degradation by Tissue Slices

Degradation studies with liver slices have been carried out on a number of nitrofurans. For comparative purposes a study similar to that reported by Bender and Paul[1] with nitrofurazone was repeated. The corrected spectrophotometric curves of the nitrofurazone solution incubated with liver slices (Fig. 6) reveal the gradual decrease with time of the 375 mμ maximum of nitrofurazone with a new maximum appearing in the 335 mμ region. The appearance of the 335 mμ maximum is in agreement with the observations of Bender and Paul with several mammalian tissues;[1] of Asnis, Cohen and Gots,[1,2] and of Beckett and Robinson with bacteria;[3,5] of Taylor, Paul and Paul with xanthine oxidase;[2] and of Austin with Raney-nickel reduction of nitrofurazone.[7] This type of degradation (disappearance of the major absorption maximum of the compound accompanied by the appearance of a different maximum of the 'reduced nitrofuran' in the 335 mμ region) was also found to be characteristic of certain related semicarbazones of 5-nitro-2-furaldehyde with substituents in the 4-position of the side chain, *viz.* NF–62, 5-nitro-2-furaldehyde

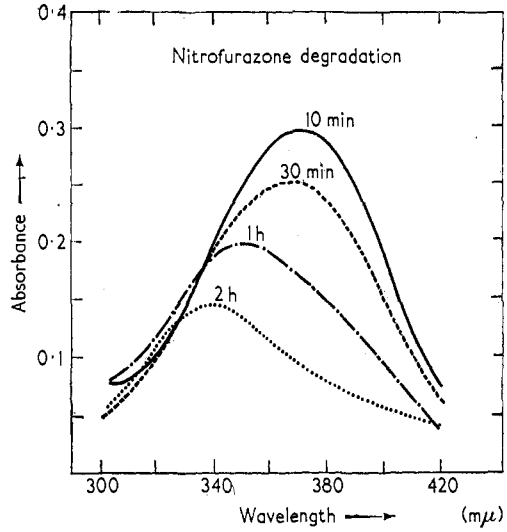


Fig. 6. Spectral absorption characteristics of solutions of nitrofurazone after incubation at 37° for various time intervals in the presence of liver slices under aerobic conditions. The curves represent the corrected absorbances of 1 : 20 dilutions of the incubation mixture.

4-methyl semicarbazone; NF–145, 5-nitro-2-furaldehyde 4-(2-hydroxyethyl) semicarbazone. Certain 5-nitro-2-furyl ketone semicarbazones also exhibit the same spectrophotometric pattern during breakdown, for example, NF–57, methyl 5-nitro-2-furyl ketone semicarbazone.

Other nitrofurans were degraded without the formation of a different maximum in the 335 mμ region. Fig. 7 shows the spectrophotometric data obtained upon incubation of nidroxyzone [5-nitro-2-furaldehyde 2-(2-hydroxyethyl)semicarbazone] (NF–67) with liver slices. Nitrofurantoin [*N*-(5-nitro-2-furfurylidene)-1-aminohydantoin] (NF–153), 5-nitro-2-furaldehyde 2-methylsemicarbazone (NF–61), furazolidone [*N*-(5-nitro-2-furfurylidene)-3-amino-2-oxazolidone] (NF–180), 5-nitro-2-furaldehyde 2-(2-dimethylaminoethyl) semicarbazone (NF–189), furaltadone [*N*-(5-nitro-2-furfurylidene)-3-amino-5-(*N'*-morpholinylmethyl)-2-oxazolidone] (NF–260) and other related nitrofurans in which the 2'-NH group bears an alkyl or hydroxyalkyl substituent or is blocked by ring formation, have been found to be degraded with-

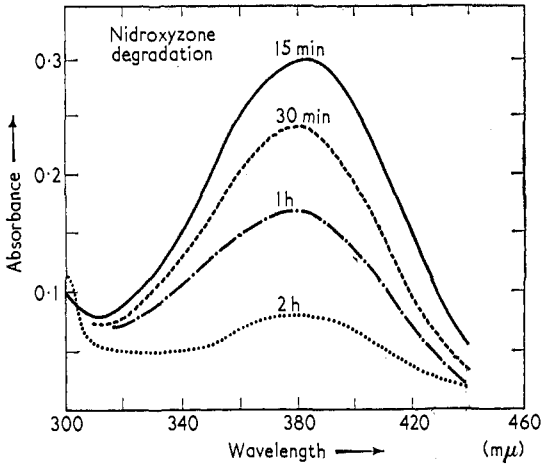


Fig. 7. Spectral absorption characteristics of solutions of nidroxyzone after incubation at 37° for various time intervals in the presence of liver slices under aerobic conditions. The curves represent the corrected absorbance of 1 : 20 dilutions of the incubation mixture.

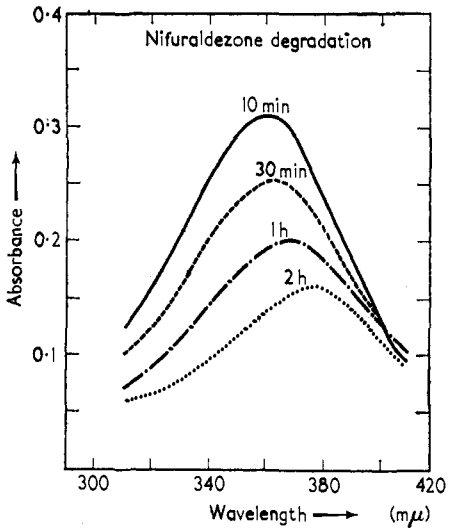


Fig. 8. Spectral absorption characteristics of solutions of furamazone after incubation at 37° with liver slices for various time intervals under aerobic conditions. The curves represent the corrected absorbance of 1 : 20 dilutions of the incubation mixture.

out the concomitant formation of material with maximum absorption in the 335 mμ range. This is in agreement with Beckett's[4, 13] observation that 'Bacterial reductions of nitrofurans . . . in which the 2′-NH-group is blocked by an alkyl group or by formation of a ring involving the 2′-NH- and the 4′-NH_2-groups, involves reduction of the nitro group accompanied by furan ring cleavage'.

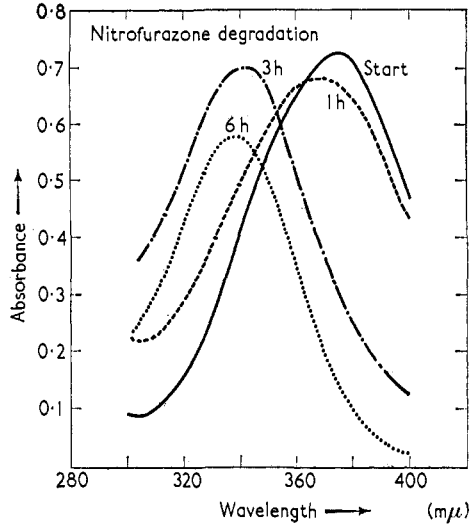


Fig. 9. Spectral absorption characteristics of solutions of nitrofurazone after incubation at 37° with rat faecal suspensions for various time intervals. The curves represent the corrected absorbance of 1 : 10 dilutions of the incubation mixture.

Whether furan ring cleavage necessarily accompanies the reduction of all of these 2′-N blocked compounds or whether it follows very rapidly in bacterial or mammalian systems can only be answered by a study of the proposed intermediates.

A third type of spectral change upon incubation of certain nitrofurans with mammalian liver tissue has been observed. In Fig. 8 appears the absorption curve obtained with nifuraldezone, 5-nitro-2-furaldehyde semioxamazone (NF–84). In this case, the formation of a new compound with an absorption maximum at a longer wavelength than that of the parent compound is indicated. This compound has not yet been isolated or characterized. To

date, the only other nitrofurans which have exhibited this degradation pattern are also semioxamazones, for example NF–89, 5-nitro-2-furaldehyde 5-(2-hydroxyethyl) semioxamazone (see Beckett and Robinson,[5] p. 141, for an explanation of this wavelength shift).

Studies have been made of the breakdown of nitrofurans when incubated with faecal suspensions. In every case where data are available the process of degradation appears similar for either tissue or faecal incubation. This breakdown by faecal suspensions is considered to be due partially to bacterial flora and may well explain why only a small amount of most nitrofurans escapes degradation to appear in the faeces. The breakdown of nitrofurans by faecal suspensions under certain *in vitro* conditions produces very clear-cut spectral absorption curves when carefully timed samples are assayed. The degradation of nitrofurazone is shown in Fig. 9 with the following apparent steps: a decrease in the 375 mμ absorption occurs together with a slight shift in maximum absorption (the spectral curve at 1 h is presumably the summation of the curves for nitrofurazone and 'reduced nitrofurazone'). At 3 h the curve suggests almost complete transformation to 'reduced nitrofurazone' and at 6 h the 'reduced nitrofurazone' has begun to disappear.

Discussion

The identity of the material with the absorption maximum at 335 mμ appearing as a metabolite in the urine, or after degradation by tissue slices, has not been thoroughly elucidated. Austin[7], and Beckett *et al.*[4,5] concluded that the product obtained from nitrofurazone by chemical reduction or bacterial metabolism with an absorption maximum of 335 mμ is 5-amino-2-furaldehyde semicarbazone. In studies with purified xanthine oxidase,[2] calculations of the stoichiometric relationship between the nitrofurazone disappearance and the uric acid formed from xanthine or hypoxanthine favoured 5-hydroxylamino-2-furaldehyde semicarbazone as the product of nitrofurazone reduction. The compound was not isolated. It seems possible that under the conditions of the xanthine oxidase study, reduction may have progressed only to the hydroxylamino compound whereas in the bacterial

and chemical studies more rigorous conditions may have carried the reduction to the amino compound. In *in vitro* studies with another nitro compound, Bueding and Jolliffe[14] detected hydroxylamino dinitrotoluene following the action of xanthine oxidase on trinitrotoluene (TNT), isolated 4-amino 2,5-dinitrotoluene after incubation of TNT with pig liver, and speculated on the probable presence of nitroso dinitrotoluene after the incubation of Straub's flavoprotein and diphosphopyridine nucleotide with TNT. Channon, Mills and Williams[15] were able to isolate 2,6-dinitro-4-hydroxylaminotoluene; 2,6-dinitro-4- aminotoluene, and 2,4-dinitro-6-aminotoluene from the urine of rabbits which had received 2,4,6-trinitrotoluene. Smith and Worrel[16] isolated and identified at least 18 decomposition products from the action of bacteria on chloramphenicol. End-products were the result of oxidative, hydrolytic reductive and condensation processes. Glazko, Dill and Rebstock[17] found that aromatic nitro-compounds in the urine accounted for 75 to 90 per cent of the administered dose of chloramphenicol in humans. These were identified as unchanged chloramphenicol, a hydrolysis product of chloramphenicol and a conjugate of chloramphenicol with glucuronic acid.

From the present studies and from comparisons with studies which have been made on other nitro compounds, the following pathways of certain steps of nitrofuran metabolism are postulated (Fig. 10).

Evidence that some nitrofurans at least partially escape degradation, conjugation, etc., and appear as the unchanged compound (I) in the urine has been presented in this paper, with the isolation of nitrofurazone from the urine. Although actual isolation after the feeding of other nitrofurans has not been carried out, evidence that the amount excreted remains unchanged in the urine varies considerably from compound to compound, as has been presented in other papers.[9, 18]

Evidence for nitrofuraldehyde (II) resulting from acid hydrolysis of nitrofurazone has been presented in the early part of this paper. Evidence for the metabolic transformation of nitrofuraldehyde to nitrofuroic acid (IV) was presented in an earlier paper[9] in which nitrofuroic acid was isolated as an end-product in the urine of animals fed with nitrofuraldehyde. Since only 30 per cent of the nitrofuraldehyde administered could be accounted for as nitrofuroic acid, transformation into polymers (III) is suspected.

Reduction of nitrofuraldehyde or nitrofuroic acid to the corresponding amine is not considered a likely pathway since neither of these compounds was readily reduced by xanthine oxidase.[2]

The material with maximum absorption at 412·5 mμ (V) was isolated from the urine of animals fed with nitrofurazone. The

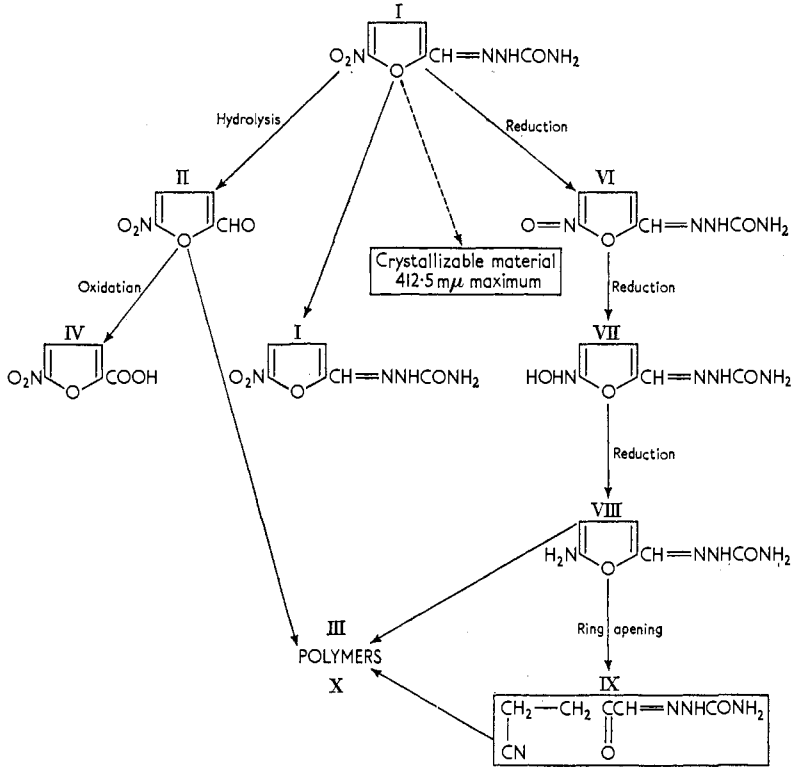


Fig. 10. Postulated pathways of physiological degradation of nitrofurans.

location of the absorption maximum would indicate that the nitro group might still be intact although the compound was devoid of antibacterial activity (infrared and polarographic studies are in progress). This compound was probably not formed via the nitrofuraldehyde or nitrofuroic acid pathway since it was not

observed in the urine of nitrofuraldehyde or nitrofuroic acid fed rats. This material was not found in human urine.

Evidence for the hydroxylamino compound (VII) as an intermediate is based on the stoichiometric relationship between nitrofuran reduction and uric acid formation from xanthine or hypoxanthine in the xanthine oxidase system observed by Taylor and co-workers.[2]

Evidence for the amine (VIII) as an end-product of bacterial metabolism has been presented by Beckett and Robinson.[4, 5] This, coupled with Bueding and Jolliffe's[14] studies on the metabolism of TNT by liver slices, suggests the formation of the amino compound as one of the metabolites of nitrofurans.

No direct evidence for formation of the nitroso compound (VI) is available from these studies. However, the stepwise reduction of the nitrofuran would be expected to include the nitroso compound, followed by the hydroxylamine and amine. Bueding and Jolliffe[14] were able to isolate a nitroso derivative of TNT using an isolated flavoprotein as an enzyme source.

Austin[7] has isolated glyoxylopropionitrile semicarbazone (IX) as a relatively stable end-product of the chemical reduction of nitrofurazone. The presence of a 270 mμ absorption maximum in the urine of nitrofurazone-fed animals suggests that this may also be an end-product of enzymatic reduction.

Polymers (X) might be expected to arise at the points indicated. The brownish materials in the urine of nitrofurazone-fed animals exhibited quite similar behaviour on chromatographic columns to the polymers arising from chemical reduction of the nitrofurans.

Some evidence has also been presented in this paper and elsewhere[2, 5, 13] that derivatives of nitrofuraldehyde other than the semicarbazone may be degraded by certain of these pathways. Each nitrofuran will require further individual study in order to establish similarities or differences in degradation pattern.

Summary. The degradation of a number of nitrofurans which are derivatives of nitrofuraldehyde has been studied under various physiological conditions. The molecule is subject to hydrolysis at the —CH=N— linkage with the formation of nitrofuraldehyde. This molecule also undergoes reduction of the nitro group when subjected to the action of various body tissues. None of the end products exhibited antibacterial activity. Pathways of metabolism have been postulated.

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