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COMPARING THE REDUCTION OF NITROIMIDAZOLES IN BACTERIA AND MAMMALIAN TISSUES AND RELATING IT TO BIOLOGICAL ACTIVITY

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The nitroimidazoles are compounds with a wide range of biological effects, some of which have particular value in human and animal therapeutics. Thus, such 5-nitroimidazoles as metronidazole and tinidazole have become drugs of choice for such human diseases as trichomoniasis, giardiasis and amebiasis; structurally similar drugs are used to treat turkey blackhead and swine dysentery. In addition, the 5-nitroimidazoles are potent antibiotics for anaerobic bacteria. They are also bacterial mutagens. Indeed, because they are bacterial mutagens and rodent tumorigens, some concern has been expressed about the safety of these valuable therapeutic agents [1].

A good deal of indirect evidence points to the importance of nitro group reduction in mediating the biological activity of the nitroimidazoles. Thus, anoxia is obligatory for their bactericidal and radiation sensitizing activities. Furthermore, the potency of nitroimidazoles as bacterial mutagens [2], radiation sensitizers [3], cytotoxic agents [4] and inhibitors of DNA synthesis [5] appears to relate rather closely to their one electron reduction potentials, in other words to the ease of adding the first electron in the process of nitro group reduction. The experimental demonstration of such correlations is made possible because the one electron potentials of various nitroimidazoles span a range of over a hundred millivolts, their potentials being determined in part by whether the nitro group is located at the 2-, 4- or 5-position of the imidazole ring. The correlation of one electron reduction potential and biological properties observed among the nitroimidazoles also includes the 5-nitrofurans, drugs which are even more potent for certain applications than any of the nitroimidazoles.

Obviously, however, the potency of a nitroheterocyclic compound depends on the biological system as well as on its properties that determine its one electron reduction potential. Nitroreductase activity is one factor in determining bacterial sensitivity to a nitroheterocyclic drug. Thus, susceptibility to a nitrofuran antibiotic, for example, is diminished in an *E. coli* mutant that has lost one of its nitroreductase enzymes [6]. Furthermore, a strain of *B. fragilis* found to be relatively insensitive to metronidazole [7] has nitroreductase activity that is diminished in comparison with a sensitive strain [8, 9]. Clearly, then, the bacteria's capacity to carry out nitro group reduction, as well as the ease with which the drug is reduced, contributes to the determination of a drug's bactericidal potency.

The same factors seem also to determine the potency of the nitroheterocyclic compounds as bacterial mutagens. Thus, the mutagenicity of a series of nitroheterocyclic compounds for the Ames histidine auxotrophs of *Salmonella typhimurium* varies as their one electron reduction potentials [2] and the susceptibility of such strains is diminished when they lose a nitroreductase enzyme [10]. Such studies affirm that metronidazole is activated by an organism's nitroreductase and in addition imply that DNA is a target for the activated molecule. That DNA is a target for metronidazole and other nitroimidazoles is in accord with chemical studies [11, 12] as well as studies suggesting that nitroheterocyclic compounds are more potent for *E. coli* mutants that are deficient in DNA repair enzymes [13–15]. It seems likely, therefore, that the biological properties of a nitroheterocyclic compound depend first on activation by nitro group reduction and then on the susceptibility of the cell's DNA to this activated form.

It is generally assumed that nitroheterocyclic compounds such as metronidazole and misonidazole (a 2-nitroimidazole used experimentally as a radiation sensitizer [16]) are reduced through a series of additions of either one or two electrons to yield the intermediates shown in Fig. 1. In the case of metronidazole, only the amino derivative (**V**) has been synthesized [17] and is therefore the only proposed intermediate that is available for chemical and biological evaluation. The existence of other proposed intermediates must be based on indirect evidence, particularly as conditions suitable for monitoring biological activity are usually inappropriate for monitoring unstable intermediates. Thus the formation of the radical anion (**II**), is consistent with electron spin resonance data, while kinetic data suggest that this intermediate dismutates to the nitroso derivative (**III**) [18, 19]. That an uptake of 4 electrons is observed when metronidazole is reduced [11, 20] suggests the formation of an intermediate with the oxidation state of the hydroxylamino derivative (**IV**). Such stoichiometry is also consistent with the formation of a nitrene derivative, an intermediate that has been suggested to occur in the reduction of the 2-nitroimidazoles [21].

What is the reactive species responsible for the biological effects of the nitroheterocyclic compounds? Do such compounds form in mammalian

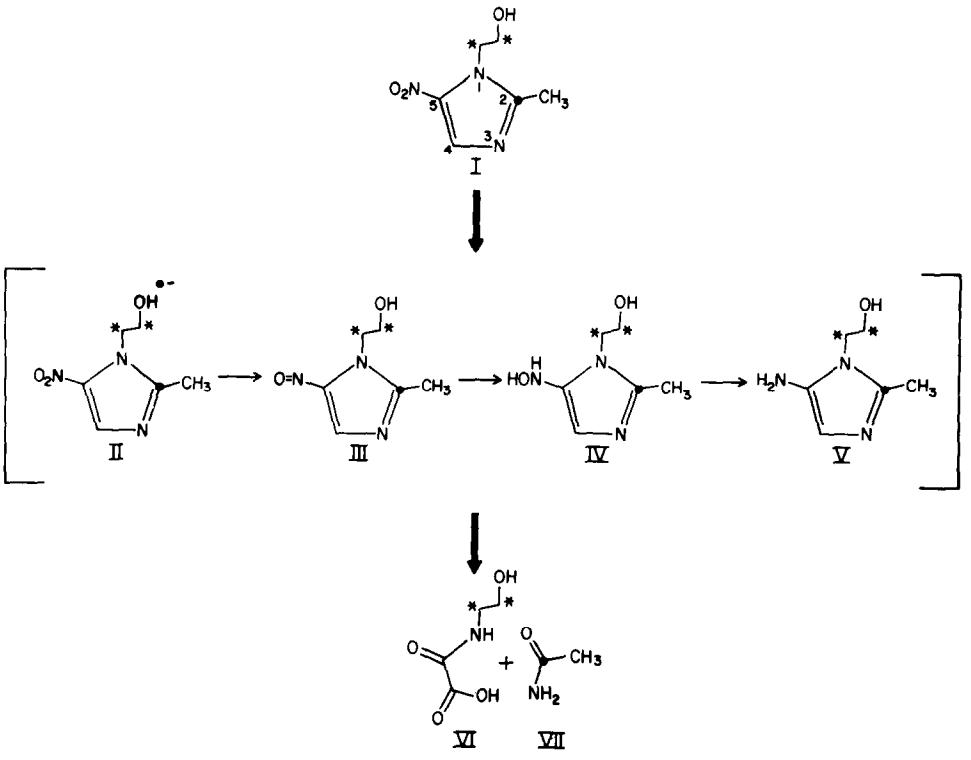

Fig. 1. Reduction of metronidazole (**I**) to form *N*-(2-hydroxyethyl)oxamic acid (**VI**) and acetamide (**VII**). Possible intermediates include the radical anion (**II**), nitroso (**III**), hydroxylamine (**IV**), and amino (**V**) derivatives of metronidazole. Solid circles indicate the fate of the radiolabel in [2-^{14}C]metronidazole, and asterisks the fate of radiolabel in [1′,2′-$^{14}C_2$]metronidazole, the two forms of radiolabeled metronidazole used in the experiments described in this paper. Reproduced with permission of the Cold Spring Harbor Laboratory.

tissues as well as in bacteria? Such were the questions we set out to answer several years ago. Although we were to use several nitroheterocyclic compounds in these studies, initial emphasis was placed on the widely used drug, metronidazole.

BACTERIAL REDUCTION OF THE NITROIMIDAZOLES

When we started our studies it was known that metronidazole disappeared as the result of metabolism by the anaerobic flora. Yet its fate had not been determined [22]. The 2-nitroimidazole, misonidazole, on the other hand was found to be reduced metabolically to the amine [1-(2-aminoimidazole-1-yl)-3-methoxypropan-2-ol] in both man and laboratory animals [23].

In our initial studies we found that incubation of metronidazole with the anaerobic flora yielded both *N*-(2-hydroxyethyl)oxamic acid [24] and acetamide [25], two metabolites that are complementary in the sense that together they contain all the carbon and nitrogen of metronidazole except that in the nitro group. These two metabolites appear to arise from a cleavage of the imidazole ring between nitrogen atom 1 and carbon atom 2 as well as between nitrogen atom 3 and carbon atom 4 (Fig. 1). It seemed likely, therefore, that fragmentation of the imidazole ring proceeded by means of a nucleophilic attack by water on an intermediate derived from the reduction of metronidazole. If so, the intermediate which reacted with water might also be the electrophile whose existence was implied by the weak carcinogenicity of metronidazole.

The conversion of metronidazole to these complementary metabolites, however, was not stoichiometric. When reduced by the anaerobic flora only approximately 20% of the metronidazole that disappeared was recovered as acetamide and less than 2% as its "complementary" metabolite, *N*-(2-hydroxyethyl)oxamic acid (Table 1). We sought to characterize the remaining products by carrying out the reduction of metronidazole with xanthine oxidase [26]. This purified enzyme system, which eliminated side reactions that might be present in the anaerobic flora, enabled us to characterize a number of additional metabolites as well as to affirm the incomplete yield of acetamide and *N*-(2-hydroxyethyl)oxamic acid. The products isolated and their yields are listed in Table 2.

Of the metabolites listed in Table 2, several others might also be considered "complementary" pairs (Fig. 2). One is *N*-acetylethanolamine and glycine, which can be considered to arise by cleavage of the imidazole ring between carbon 2 and nitrogen 3 and between carbon 5 and nitrogen 1. Other complementary pairs may be more difficult to discern. For example, the presence of acetate indicates that carbon 2 was separated from both nitrogen 1 and

Table 1. Stoichiometry of the formation of acetamide and *N*-(2-hydroxyethyl)oxamic acid*

Incubation	Metronidazole consumed (%)	Metabolites formed (%)	
		Acetamide	*N*-(2-Hydroxyethyl) oxamic acid
C. perfringens	90	8.5	1.3
Cecal contents	100	15.5	1.7

* Incubation mixtures contained 5.8 μmoles of metronidazole together with 10.1 μCi[2-^{14}C]metronidazole (to measure acetamide formation) and 10.1 μCi [1′, 2′-$^{14}C_2$]metronidazole (to measure *N*-(2-hydroxyethyl)oxamic acid) with the bacterial preparation indicated in a total volume of 10 ml.

nitrogen 3, a cleavage from which such a complementary fragment as glycylethanolamine might be expected. Although we did not find this fragment, we were able to identify its deaminated product, glycoylethanolamine (Table 2). Similarly, the finding of ethanolamine suggests that the imidazole ring also cleaves so as to separate nitrogen 1 from both carbon 2 and carbon 5. Although *N*-acetylglycine, a likely complement for ethanolamine, was not identified, we did find its components, acetate and glycine. It is possible that the imidazole ring may fragment into three as well as two metabolites.

ASSOCIATING BACTERIAL REDUCTION OF METRONIDAZOLE WITH BACTERICIDAL ACTIVITY

Reduction of the nitro group therefore seems to result in fragmentations of the imidazole ring that might be attributed to a nucleophilic attack at various positions by water. The intermediates that react with water, however, might also be the activated form of the nitroimidazole that is postulated to react with DNA. As a means of trying to link the reductive metabolism of metronidazole to its biological activity we postulated that the proposed partially reduced intermediate might be considered to have one of two possible fates. On the one hand, it could react with a macromolecule such as DNA to initiate biological activity. On the other hand, it could interact with water to yield a stable metabolite. The partial reduction of a drug like metronidazole to form a reactive intermediate and the subsequent fate of this intermediate is shown schematically in Fig. 3. Although particular attention in this representation is accorded to acetamide, the argument that follows would be equally valid for any of the metabolites mentioned in Table 2.

Figure 3 provides a basis for predicting a relationship between the biological effect of metronidazole and the accumulation of acetamide (or any other stable metabolite). Consider a biological effect such as the failure of a bacterium to propagate. Such an effect should depend on the total exposure of the culture to the reactive form of the drug. Although exposure of the culture to the reactive form of metronidazole cannot be measured directly, the proposed model provides a basis for measuring it indirectly. In terms of the model, the culture's exposure to the reactive form is measured indirectly by the accumulation of acetamide or another such metabolite that reflects the accumulated results of the reaction between the reactive intermediate and water. Since the concentration of water remains constant, the accumulation of acetamide reflects the total concentration of reactive intermediate to which the culture is exposed. If we further assume that the

Table 2. Metabolites derived from the reduction of metronidazole mediated by xanthine oxidase*

Metabolite	Yield in terms of metronidazole metabolized (%)
N-(2-Hydroxyethyl)oxamic acid†	2.2
N-Glycoylethanolamine†	2.7
N-Acetylethanolamine†	2.4
Ethanolamine†	13.0
Acetate‡	4.8
Acetamide‡	14.1
Glycine	8.0
Glycine found after acid hydrolysis	39

* The sterile incubation mixture contained 5.85 mM metronidazole (labeled with ^{14}C at either the 1′, and 2′ or the 2 position), 9.85 mM hypoxanthine, 0.032 M KPO_4 buffer at pH 7.4 and 3.5 units of xanthine oxidase in a volume of 3.15 ml. Incubation was for 24 hr at 37°. Further experimental details can be found in ref. 26.

† Based on 1′,2′-$^{14}C_2$ radiolabel.

‡ Based on 2-^{14}C radiolabel.

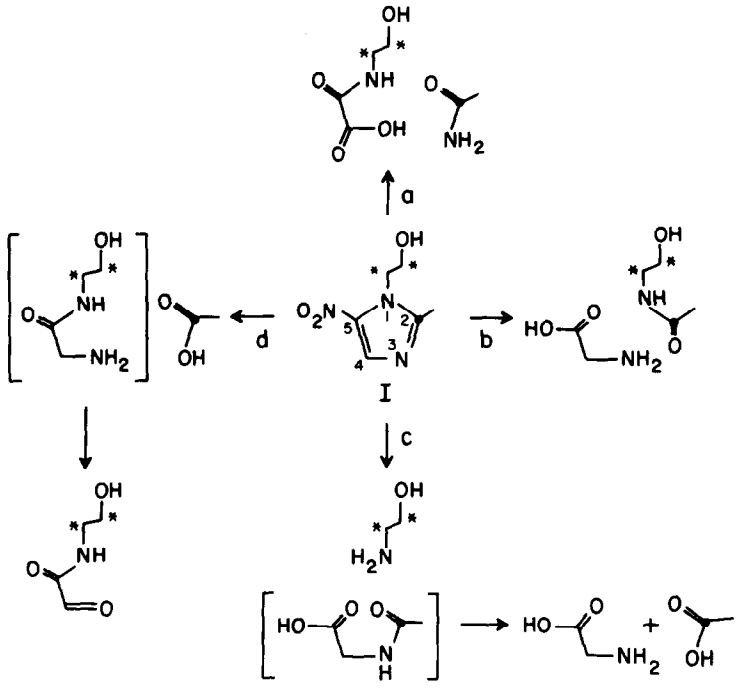

Fig. 2. Fragmentation patterns of metronidazole. The pairs of complementary fragments formed in the cleavage schemes are: cleavage a, *N*-(2-hydroxyethyl)oxamic acid and acetamide; cleavage b, *N*-acetylethanolamine and glycine; cleavage c, ethanolamine, acetate and glycine; and cleavage d, glycoylethanolamine and acetic acid. Compounds in brackets are possible intermediates, which were not actually isolated from the reaction mixture containing metronidazole, hypoxanthine and xanthine oxidase [26].

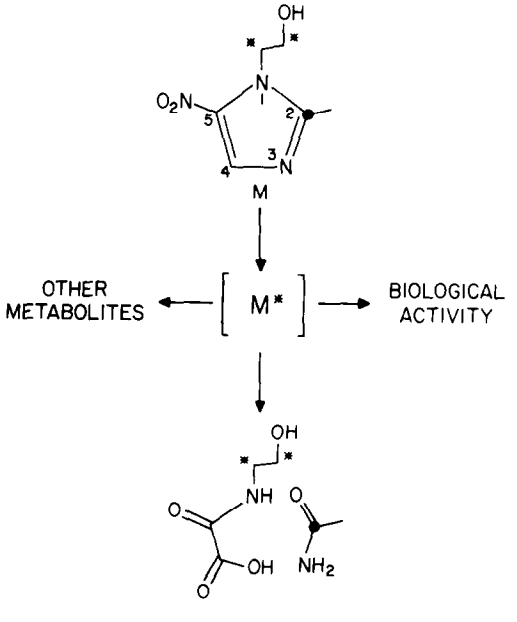

Fig. 3. Model relating metronidazole's metabolism and its lethal effect on bacteria. Metronidazole (M) is metabolized to a labile intermediate (M*) which may cause some biological effect such as bactericidal activity. Alternatively M* may react with water to form acetamide or other metabolites.

drug's reactive form is lethal to bacteria in proportion to the concentration of bacteria in the medium, bacterial survival should be logarithmic, the slope being proportional to the concentration of the reactive intermediate, or, in other words, to the accumulation of acetamide. And, indeed, for several bacteria, a proportionality was found between the accumulation of acetamide and the log of bacterial survival (Fig. 4) [27].

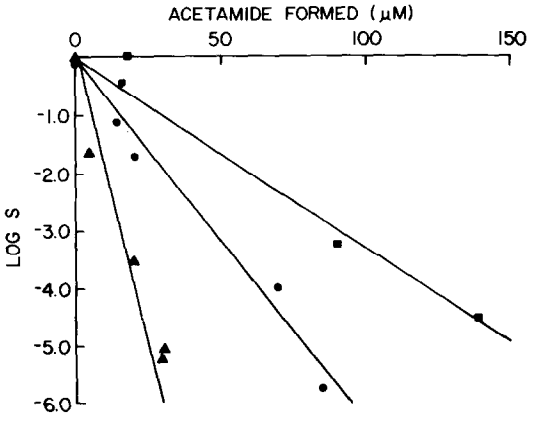

Fig. 4. Relationship between bacterial survival (LOG S) and acetamide formation. Symbols: ▲, *B. fragilis*; ●, *E. coli*; ■, *C. perfringens*. Reproduced with permission of the American Society for Microbiology.

If the model shown schematically in Fig. 3 and affirmed by the experimental results of Fig. 4 is a general one, it should hold for all bacterial strains, and under all circumstances. We therefore examined a strain of *B. fragilis* which had attracted particular attention because it was the first clinical isolate found to be somewhat resistant to metronidazole [7]. As a first step, we confirmed previous findings [8] that nitroreductase activity in this strain was somewhat less than that of the usual more sensitive strains of *B. fragilis*. Our model, would then predict that the reactive derivative of metronidazole would be generated more slowly in the relatively resistant strains than in other strains. If, however, the cellular architecture of the relatively resistant strain is like that of a more sensitive strain, the reactive form of the drug should have the same fate in both strains and react in the same proportion with either water or the biological target. In other words, if their target sensitivity is the same, both strains should obey the same relationship between lethal effect and acetamide formation. This prediction is fulfilled by the finding (Fig. 5) that the relationship between bacterial survival and the accumulation of acetamide is indistinguishable in the resistant and a sensitive strain of *B. fragilis* [28].

Another test of the model can be made in bacteria with defective DNA repair systems. If DNA is damaged by metronidazole and repaired by one of these systems, bacteria with defective DNA repair systems should be more susceptible to metronidazole than those with intact DNA repair systems. The lower mean inhibitory concentration of metronidazole found for point mutants of *E. coli* with defective UvrB or RecA genes (Table 3) seems to verify this prediction. On the other hand, Table 3 also indicates that mutants that have lost these genes by DNA deletions do not manifest a similar susceptibility to metronidazole [29].

This inconsistency in the behavior of these deletion mutants was resolved by considering some of their other properties. When the deletion mutants were given to us, it was indicated that they showed a decreased ability to reduce chlorate and nitrate. When the apparent inconsistency in their behavior was observed, it suggested that these deletion mutants might also have a decreased ability to reduce metronidazole. This proved to be the case; the deletion mutants reduced metronidazole at a rate which was approximately half that of the wild type from which they were derived [29]. Thus, although the targets of the deletion mutants were more sensitive to metronidazole, their lack of nitroreductase predicted that these mutants would also have a diminished ability to generate the reactive form of metronidazole. Therefore the expected increase in sen-

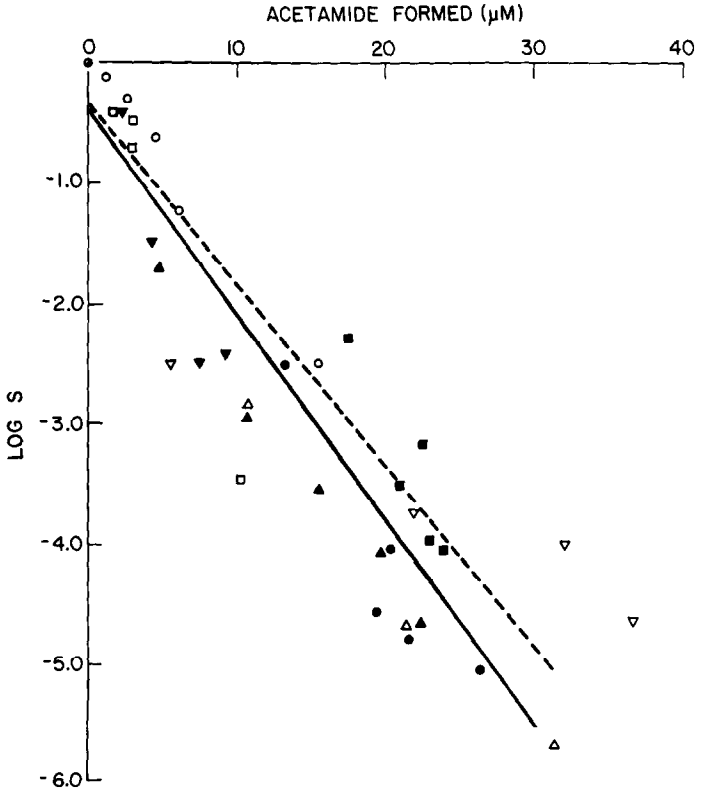

Fig. 5. Relationship between bacterial survival (LOG S) and acetamide formation. The results of a single experiment are shown by the same symbol. Open symbols (and the dotted line) refer to the resistant strain while closed symbols (and the solid line) refer to the susceptible strain. The lines are the least square fit of their respective data points. Reproduced with permission of the American Society for Microbiology.

Table 3. Metronidazole MIC of the *E. coli* strain*

E. coli strain (phenotype)	Metronidazole MIC (μg/ml)
AB1157 (Uvr$^+$ Rec$^+$)	>200
P90C (Uvr$^+$ Rec$^+$)	> 200
AB1885 (UvrB$^-$ Rec$^+$)	100
Sr58 (RecA$^-$ UvrB$^-$)	13
EE128 (Uvr$^+$ RecA$^+$)	25
EE348 (UvrB$^-$Rec$^+$)†	>200
EE349 (UvrB$^-$ Rec$^+$)†	> 200
SM172 (UvrB$^-$Rec$^-$)†	> 200

* MIC (minimal inhibitory concentration) was determined under anaerobic conditions as described in ref. 29.

† Deletion mutants that also had decreased nitrate and chlorate reducing activities. Other mutants were point mutants.

sitivity of these mutants was apparently masked by their inability to produce metronidazole's lethal form.

When metronidazole's lethal effect is measured in terms of exposure to the activated form of metronidazole, that is by monitoring bacterial survival in terms of the production of acetamide, no distinction was observed between the two types of DNA repair mutants. The behaviour of both types of mutants becomes strictly concordant with their identical genetic defects in DNA repair (Fig. 6). Thus, by eliminating the apparent distinction between point and deletion mutations in the UvrB and RecA genes, the proposed model emphasizes that sensitivity to metronidazole must be monitored in terms of exposure to the drug's activated form rather than simply in terms of the inhibitory concentration of the drug itself. The studies with *E. coli* mutants, therefore, offer further confirmation of the hypothesis that metronidazole's action is mediated by a partially reduced intermediate which either interacts with bacterial DNA to cause a lethal event or with water to form a stable metabolite such as acetamide.

IS THE BACTERIAL MODEL APPLICABLE TO MAMMALIAN TISSUES?

Because such drugs as metronidazole are tumorigens in animals and interact with bacterial DNA, the possibility that they may pose a risk for human patients has received considerable attention. Although there is no direct evidence to indicate a correlation between prior exposure to metronidazole and the subsequent incidence of human metagenicity, carcinogenicity or birth defects [1], the intense interest in such questions provides an incentive for trying to interpret the significance of indirect evidence. Thus, it might be asked whether the model advanced for metronidazole's action in bacteria is applicable to its action in mammalian tissues.

In making such an extrapolation one must ask first whether nitro group reduction occurs in mammalian tissues; the answer to this question is not easy to obtain experimentally. Because such drugs as metronidazole [24, 25] and misonidazole [30. 31] are reduced *in vitro* by preparations of an animal's flora,

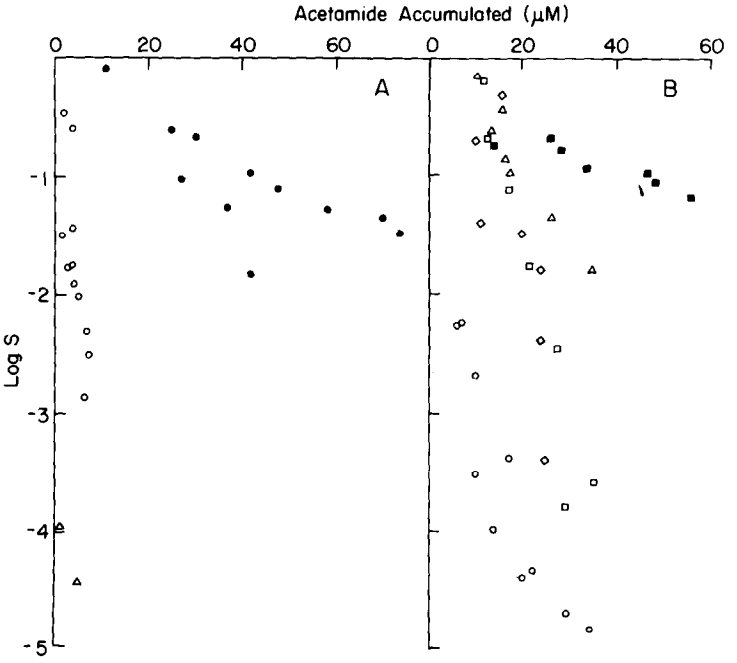

Fig. 6. Relation between acetamide formation and log of the fraction of surviving bacteria for wild types (closed symbols) and DNA repair-deficient mutants (open symbols) of *E. coli*. The concentration of metronidazole was 100 μg/ml. Symbols: (Panel A) ●, AB1157 (Uvr$^+$Rec$^+$); ○, AB1885 (UvrB$^-$Rec$^+$); △, SR58 (UvrB$^-$RecA$^-$); (Panel B) ■, P90C (UvrB$^+$Rec$^+$); ○, EE128 (Uvr$^+$RecA$^-$); △, EE348 (UvrB$^-$Rec$^+$); □, EE349 (UvrB$^-$Rec$^+$); ◇, SM172 (UvrB$^-$Rec$^+$). Strains EE348, EE349 and SM172 were deletion mutations. Reproduced with permission of the American Society for Microbiology.

the metabolites appearing in the excreta that indicate reduction of these drugs can be attributed to the activity of the flora; it is, therefore, impossible to determine whether or not the reactions that occur in bacteria also take place in mammaliam tissues. Of course the effect of the flora may be eliminated by studying mammalian metabolism in tissue cells or perfused isolated organs. The metabolism of nitroheterocyclic compounds in such systems, however, depends on the experimentally imposed oxygen tension [31–33] and therefore may not reflect the reactions that take place under physiological conditions.

An experimental system for determining whether a reaction may take place in mammalian tissues under physiological conditions is provided by the germfree rat [34]. We therefore administered nitroheterocyclic compounds to these animals and monitored their excreta for the presence of metabolites that indicate the reduction of these compounds [35]. Our earlier studies in conventional rats indicated that metronidazole is converted to both acetamide and *N*-(2-hydroxyethyl)oxamic acid and that both of these metabolites appear in the excreta [24, 25]. As these metabolites were not found in parallel experiments conducted with germfree rats, it was concluded that the observed reduction of metronidazole could be attributed to the activity of the intestinal bacteria. In other words, metronidazole reduction did not appear to take place in mammalian tissues. Our initial experiments in germfree rats, however, were done with relatively large doses of metronidazole (200 mg/kg), which was of rather low specific activity. Under such circumstances, therefore, reduction might take place but the resulting formation of such metabolites as acetamide and *N*-(2-hydroxyethyl)oxamic acid be too small to detect. To increase the sensitivity of detecting the possible presence of reduced metabolites, smaller doses of metronidazole (0.021 and 10.0 mg/kg), of much higher specific activity, were administered to the germfree rats. As the data of Table 4 indicate, even under these circumstances the metabolites indicating reduction of metronidazole in the tissue could not be detected.

Since acetamide and *N*-(2-hydroxyethyl)oxamic acid were not detectable in mammalian tissues under physiological conditions, there was no evidence for the formation of their presumed precursor, a reactive intermediate derived from the reduction of metronidazole. Of course, this did not necessarily mean that the reactive intermediate response for metronidazole's interaction with bacterial DNA does not occur in mammalian tissues. The intermediate may have formed and had some other fate. For example, oxygen, which is obviously present in mammalian tissues, could react with the radical anion derived from such compounds as metronidazole to form superoxide as well as to restore the parent compound [36]. Restoration of the parent compound by reaction of the radical anion of a nitroheterocyclic compound with oxygen has been termed "futile" [18, 19], as a means of emphasizing how the presence of oxygen blocks the further reduction of nitroheterocyclic compounds. Therefore, oxygen, which is present in mammalian tissues, but not in the bacterial systems mentioned above, may interact with one of the reactive intermediates in metronidazole reduction to prevent the formation of detectable amounts of such metabolites as acetamide. Our model, which was developed and tested in terms of a bactericidal effect under anaerobic conditions, may therefore not be an appropriate one for describing

Table 4. Metabolites recovered in the urines of germfree rats that indicate reduction of nitroheterocyclic compounds

Compound administered*	Dose (mg/kg)	Metabolite†	Radiolabel recovered (%)
Metronidazole	0.021	Acetamide	‡
		HOE	‡
		Ethanolamine	‡
	10.0	Acetamide	‡
		HOE	‡
		Ethanolamine	‡
Misonidazole	0.015	AIM	0.5, 0.5§
		Urea	2.8, 3.1§
		G	0.8, 0.4§
Nitrofurazone	0.13	C	0.8 (0.6, 0.9)‖

* The compound indicated was administered to germfree rats by gavage and the urines collected for 3 days. Experiments with metronidazole used 3 rats, those with misonidazole 2 rats, and those with nitrofurazone 4 rats. Experimental details are explained more fully in ref. 35.

† Abbreviations are as follows: HOE, *N*-(2-hydroxyethyl)oxamic acid, AIM, 1-(2-aminoimidazol-1-yl)-3-methoxypropan-2-ol; G, 2-hydroxy-3-methoxypropyl)guanidine; C, 4-cyano-2-oxobutyraldehyde semicarbazone.

‡ Values indistinguishable from those obtained by adding metronidazole to germfree rat urine.

§ Values for each of two rats.

‖ Mean with range in parenthesis.

the reactions pertinent to an assessment of mammalian risk.

It was therefore of interest to determine whether other nitroheterocyclic compounds were reduced in mammalian tissues under physiological conditions. Of course all nitroheterocyclic compounds would be expected to be reduced in tissues at rates determined by their one electron reduction potentials, the rates being slower for compounds whose one electron reduction potentials were more negative. Furthermore, the radical anion formed from such compounds in this first step of nitro group reduction reacts with oxygen to restore the parent compound. The rate of this reaction also depends on the one electron reduction potential, being faster for radical anions that form from compounds with more negative one electron reduction potentials [36]. Therefore, both on the basis of their forming the radical anion and on the basis of the reaction of the radical anion with the oxygen, one would expect nitroheterocyclic compounds with more negative one electron reduction potentials to be less reduced in mammalian tissues.

ONE ELECTRON REDUCTION POTENTIAL OF NITROHETEROCYCLIC COMPOUNDS AND THEIR REDUCTION BY MAMMALIAN TISSUES

As the one electron reduction potential of misonidazole ($E_7^1 = -389$ mV) is less negative than that of metronidazole ($E_7^1 = -486$ mV), the observation that misonidazole in contrast to metronidazole is reduced in tissue cultures of anoxic anaerobic Chinese hamster lung fibroblasts [31] is not surprising. The reduction of misonidazole results in the formation of [1-(2-aminoimidazole-1-yl)-3-methoxypropan-2-ol] which in turn is converted to (2-hydroxy-3-methoxypropyl)guanidine [31]. Indeed the products of misonidazole reduction in anaerobic tissue culture cells resemble those that form in the anaerobic flora.

It was therefore of interest to determine whether misonidazole was also reduced in the germfree rat. When misonidazole was administered in relatively high doses (200 mg/kg) [30] to the germfree rat no products of reduction were detected in the excreta. As mentioned previously, however, metabolites indicative of the reduction of a nitroheterocyclic compound may escape detection unless compounds of high specific activity are used. It was therefore not surprising to find that the urine contained a small but definite quantity of the reduced metabolites of misonidazole when misonidazole of high specific activity was administered to the germfree rat in doses as low as 0.015 mg/kg (Table 4) [35].

These results had interesting implications as they indicated that both the germfree rat and anoxic tissue culture cells were capable of reducing misonidazole but apparently incapable of reducing metronidazole. Such results are in accord with what would be expected from the relative one electron reduction potentials of the two compounds. On this basis misonidazole would be predicted to undergo the first step of reduction more readily than metronidazole and, similarly, its radical anion would participate less readily in a "futile" cycle as the result of an interaction with tissue oxygen. It seems unlikely that pharmacokinetic differences might explain the difference in reduction of the two compounds as both metronidazole and misonidazole are distributed fairly uniformly in body fluids [1, 23, 37, 38] and therefore, with similar half lives, would have similar access to any tissues where reduction might occur.

In order to test further the importance of the one electron reduction potential in determining whether a nitroheterocyclic compound is reduced in the germfree rat, studies were conducted with nitrofurazone. Because of its less negative one electron reduction potential ($E_7^1 = -257$ mV), nitrofurazone should be reduced more readily than misonidazole when administered to the germfree rat; as indicated in Table 4, small amounts of 4-cyano-2-oxobutyraldehyde semicarbazone, a product of nitrofurazone reduction, were found in the urine of the germfree rat dosed with nitrofurazone [35].

That the fraction of administered compound recovered in the form of reduced metabolites does not relate more quantitatively to the compound's one electron reduction potential may be explained by the difference between *in vivo* and *in vitro* systems. Thus under anaerobic conditions in such diverse systems as L-929 tissue culture cells, *E. coli* and liver microsomes misonidazole is reduced an order of magnitude faster than metronidazole and an order of magnitude slower than nitrofurazone [39, 40]. A precise quantitative analogy to these experiments would only be expected in the germfree rat if each of the compounds had exactly the same pharmacokinetics and therefore maintained equivalent concentration vs time relationships at the site in the animal where reduction occurs. It seems reasonable, therefore, to attribute the comparative reduction of metronidazole, misonidazole and nitrofurazone in the germfree rat to the relative rate at which their respective radical anions are formed or to the relative rates at which these anions interact with oxygen in the "futile" cycle [18, 19] that restores the parent compound.

The comparative reductive metabolism of metronidazole, misonidazole and nitrofurazone in germfree rats is consistent with other studies on the reduction of these or similar compounds that occurs in the presence of oxygen. Thus the radical anion of nitrofurazone is readily detected in the presence of oxygen [18] and leads to the consumption of oxygen under conditions where it is not observed with the radical anion of metronidazole [41]. Therefore, the presence of oxygen in mammalian tissues makes a clear difference in the ease with which metronidazole is reduced and as a result makes the model proposed for metronidazole's action in bacteria of uncertain value for understanding its action in mammalian tissues.

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