Antibiotics Alter Methotrexate Metabolism and Excretion

Abstract. *The chemotherapeutic agent methotrexate is metabolized by the intestinal flora of normal mice. This metabolism is reduced either by treatment of mice with the antibiotics neomycin and sulfathiazole, or in germ-free mice. In addition to affecting metabolism, these antibiotics alter physiological distribution of methotrexate so that excretion by the intestinal route is significantly enhanced.*

Methotrexate (MTX) has been studied to a great extent experimentally and used extensively in the treatment of various malignant neoplasms and certain skin disorders in man. However, some questions still exist as to whether the metabolism of this drug plays a significant role in affecting its toxicity. In certain species (rabbit and guinea pig), which are markedly resistant to MTX toxicity, hepatic aldehyde oxidases have been shown to convert significant amounts of MTX to the 7-hydroxy derivative (*1*, *2*). Past studies in other species (rat, dog, mouse, and man) suggest that insufficient metabolism occurs to account for differences in toxicity (*3*).

Although metabolism of chemotherapeutic agents in animals and man has been widely investigated, studies of the metabolism of drugs by the flora of the intestinal tract have been few and far between. A recent review deals with the possible importance of this route of metabolism (*4*). With many chemotherapeutic agents, in which enterohepatic recirculation plays a prominent role in their physiological distribution, toxicity is often first manifested in the intestinal tract. For these drugs, intestinal metabolism may be important.

The administration of neomycin prior to MTX increases the lethality of MTX in normal mice (*5*). Since the intestinal toxicity of this drug is a major contributing factor in causing death in mice, one possible explanation for the increased lethality is a decrease in intestinal flora that normally metabolizes MTX to a nontoxic form. For these reasons we have taken a closer look at the metabolism of MTX in normal CDF_1 male mice and in a similar strain of mice with a germ-free intestinal tract created by treatment with neomycin (4 mg/ml) and sulfathiazole (1 mg/ml) in their drinking water for 5 to 7 days. These mice were then injected intraperitoneally with 3 mg of chromatographically pure 3′,5′-tritiated MTX per kilogram of body weight.

The urine and feces were collected separately for 6 hours and then the animals were killed. The contents of the bladder were added to the collected urine, and the contents of the cecum and rectum were added to the collected feces. The urine was lyophilized immediately, and the dried residue was dissolved in 0.1*M* NH_4HCO_3 buffer, *p*H 8. Approximately 1 mg of unlabeled carrier MTX was added, and the solution was chromatographed on a diethylaminoethyl ion-exchange cellulose column for separation into its various components (*6*). Feces were frozen and thawed to disrupt cells and then boiled for 5 minutes to free any bound MTX. The fecal mixture was centrifuged and the supernatant was treated in the same way as the urine. In control experiments 94 percent of MTX was recovered chromatographically when added to frozen urine or feces and then run through the above treatment procedure.

Table 1 shows that in normal mouse urine only 70 percent of the radioactivity is associated with MTX, while most of the remainder, 27 percent, is associated with substances eluted prior to MTX. In normal feces only 19 percent of the radioactivity is associated with MTX, whereas 72 percent is associated with a fraction eluted before MTX. Figure 1c shows 19 percent (shaded area) of the tritium is in the tail end of the radioactive peak and probably is not MTX, even though it overlaps with the MTX peak. These findings support the hypothesis that MTX is metabolized in the intestinal tract, particularly since past studies (*1*) showed that MTX is not significantly metabolized in the liver and kidney of mice. Other workers (*7*) have shown that in the urine of man a similar rapidly migrating fraction is present following administration of a low dose of tritiated MTX. They attribute this fraction to conversion of tissue-bound MTX to an unbound metabolite that is subsequently excreted in the urine.

If the flora of the intestinal tract is involved in this metabolism in mice, and there is evidence that bacteria can metabolize MTX (*8*), then some alteration in the urinary and fecal excretion of MTX after creation of a germ-free gastrointestinal tract by antibiotic treatment might be anticipated. The data in Table 1 indicate that in urine little change is noted in the MTX fraction—77 percent of the radioactivity in "antibiotic urine" is associated with MTX compared to 70 percent in normal urine. However, in feces the major part of the radioactivity, 86 percent, is associated with MTX as compared to only 19 percent in normal mice (see Fig. 1d and compare to Fig. 1c). This

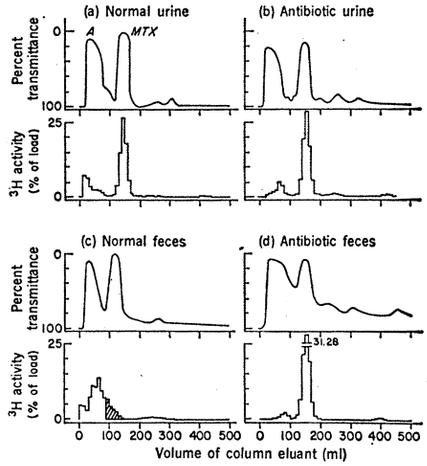

Fig. 1. Column chromatography of urine and fecal material on diethylaminoethyl cellulose. Linear elution with NH_4HCO_3 buffer, *p*H 8.3, gradient 0.1 to 0.4 molar; *A* indicates elution peak of pteridines, pteroic acid, uric acid, bilirubin, and others; *MTX*, elution peak of methotrexate.

Table 1. Summary of the relative excretion and chromatographic fractionation of radioactivity in urine and feces. The average of three mice is presented with the range in parentheses.

Sample	Percentage of dose	Fraction	
		MTX	Pre-MTX
Normal urine	42 (38–45)	0.70 (0.61–0.75)	0.27 (0.21–0.36)
Antibiotic urine	19 (9–25)	.77 (.70– .81)	.18 (.15– .24)
Normal feces	39 (31–43)	.19 (.10– .30)	.72 (.60– .79)
Antibiotic feces	54 (49–60)	.86 (.83– .91)	.08 (.03– .12)

finding indicates that bacteria of the intestinal tract probably play a prominent role in degradation of MTX and may be responsible for the changed MTX toxicity observed during antibiotic treatment in mice.

Another interesting finding is the change in the relative excretion of radioactivity in urine as compared to feces. In Table 1, the percentage of the dose that appears in the urine decreased approximately 50 percent, from 42 percent in normal mice to 19 percent in animals treated with antibiotics. This change in the urine was paralleled by an inverse change in feces, in which there was an increase from 39 percent in normal animals to 54 percent in those treated with antibiotics. A possible explanation for this observation is that neomycin has been shown to alter the intestinal absorption of several substances (*9*) and may have a similar effect on MTX. In order to test whether this inhibition of MTX absorption was due to neomycin, several mice (male DBA/2) that had been raised in a germ-free environment were tested in a similar manner. After an intraperitoneal injection of tritiated MTX, 80 to 99 percent of the radioactive dose appeared in the feces. This abnormal fecal excretion was attributed to the inevitable injection directly into the characteristic, enormously enlarged cecum of these animals (*10*). Similar experiments with a subcutaneous injection into germ-free and normal animals indicate that the distribution of radioactivity between urine and feces of germ-free animals was similar to that of normal mice—52 to 61 percent in the urine and 20 to 34 percent in the feces of germ-free mice. These findings indicate that neomycin probably inhibits the intestinal absorption of MTX. The fraction of radioactivity representing MTX in the urine of the germ-free mice rose to 86 to 94 percent, which suggests that little metabolism of MTX was taking place. Unfortunately, feces of germ-free mice were not amenable to separation into identifiable radioactive components by various organic solvent extraction and chromatographic procedures.

Our findings support the hypothesis that an antibiotic regimen given along with MTX affects absorption from the intestinal tract and the metabolism of MTX by intestinal flora. This kind of drug-host-drug interaction may have far-reaching significance in chemotherapy during which antibiotics are used extensively along with anticancer drugs and perhaps other chemotherapeutic agents. The choice of the proper antibiotic may be crucial for successful chemotherapeutic treatment.

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References and Notes

1. D. G. Johns, A. T. Iannotti, A. C. Sartorelli, J. R. Bertino, *Biochem. Pharmacol.* **15**, 555 (1966).
2. H. M. Redetzki, J. E. Redetzki, A. L. Elias, *ibid.*, p. 425.
3. E. S. Henderson, R. H. Adamson, C. Denham, V. T. Oliverio, *Cancer Res.* **25**, 1008 (1965).
4. R. R. Scheline, *J. Pharm. Sci.* **57**, 2021 (1968).
5. H. Bruckner, *Fed. Proc.* **28**, 263 (1969).
6. V. T. Oliverio, *Anal. Chem.* **33**, 263 (1961).
7. D. G. Johns, J. W. Hollingsworth, A. R. Cashmore, I. H. Plenderleith, J. R. Bertino, *J. Clin. Invest.* **43**, 621 (1964).
8. P. Goldman and C. C. Levy, *Biochem. Pharmacol.* **17**, 2265 (1968).
9. E. D. Jacobsen, R. B. Chodos, W. W. Faloon, *Amer. J. Med.* **28**, 524 (1960).
10. M. E. Coates, H. A. Gordon, B. S. Wastmann, Eds., *The Germ-Free Animal in Research* (Academic Press, New York, 1968), pp. 145–151.
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Lissamphibian Origins: Possible Protolissamphibian from the Lower Permian of Oklahoma

Abstract. *A new genus and family of rhachitomous labyrinthodont amphibian, related to the Upper Paleozoic Dissorophidae and Trematopsidae, may be ancestral to some or all of the modern Amphibia.* Doleserpeton *occurs in Lower Permian fissure fill deposits in southwestern Oklahoma. It is unique among nonlissamphibian tetrapods in that it possesses pedicellate, bicuspid teeth together with nearly monospondylous vertebrae in which the main central element is a pleurocentrum.* Doleserpeton *may have been utilizing the food resources of the upland, terrestrial environment in a novel fashion for rhachitomes.*

The phylogeny of most higher taxa of living tetrapods is now known at least in broad outline. The three orders of modern Amphibia (hereafter to be referred to as Lissamphibia for convenience—no phyletic judgment is intended) constitute one of the few exceptions. Various investigators have suggested a taxonomically and structurally diverse series of groups as ancestors of part or all of the Lissamphibia, but no consensus on their origins presently exists. The major reason for this is the lack of recognizable transitional forms in the fossil record. A variety of fragmentary specimens have been hopefully assigned to an ancestral position, but all are open to other interpretations. This paper presents the first report of a species which is represented by abundant and well-preserved material and possesses characters otherwise confined to the Lissamphibia among tetrapods.

All specimens come from Lower Permian fissure fillings in Ordovician limestones from the Wichita Mountains in southwestern Oklahoma. Almost all are from the Fort Sill locality of Gregory *et al.* (*1*), which is the Richards Spur site of Olson (*2*). A few fragmentary specimens came from the South Carnegie locality of Olson (*2*). Both Fort Sill and Carnegie are limestone quarries; the latter is not being worked at present. Preservation at Fort Sill is exceptional; both surface markings and histological details, such as calcified cartilage in the ends of long bones, are easily observed. My material came from fissure fill which had been discarded in a corner of the quarry; no accessible fossil-bearing fissures are presently exposed in the quarry walls. Most specimens came from a single clay mound of the many in the spoil heap. This clay contains remains of many thousands of individuals but virtually no genera other than *Doleserpeton*. I will hereafter refer to it as the "D-concentrate."

Class Amphibia

Subclass Labyrinthodontia

Order Temnospondyli

Suborder Rhachitomi

Superfamily Dissorophoidea, n. superf.

Family Doleserpetontidae, n. fam.

Diagnosis: The same as for the genus; presently a monogeneric family.

Doleserpeton, n. gen.

Diagnosis: Small rhachitomous amphibian. The marginal teeth are nonlabyrinthine, bicuspid with labial and lingual cuspules, and pedicellate in the sense of Parsons and Williams (*3*). Postatlantal vertebral centra consist of two ossifications: (i) a cylindrical pleurocentrum, which may or may not be open dorsally but is always complete ventrally in adults; and (ii) a small, crescentic, ventral intercentrum. The generic name refers to Dolese Brothers Company, the operator of the Fort Sill quarry.