ovum. The 4S band and the bands that migrate just ahead of and behind the 4S RNA marker are slightly less dense in some samples from injected ova than in controls. The difference in radioactivity is not statistically significant but could represent the appearance of competition of the injected 5S gene for some component of the polymerase III transcription machinery that handles both 5S and 4S genes. On the basis of the radioactivity in the 5S band, the specific activity of guanosine, and the amount of DNA injected, it is estimated that at least two 5S RNA molecules are synthesized each hour from each *Xenopus* gene in an injected oocyte. This is similar to the transcription rate for 5S DNA injected into the germinal vesicle of the *Xenopus* oocyte (*5*, *14*).

The significant increase in 5S RNA synthesis by the mouse oocyte after injection of the plasmid containing the *Xenopus 5S* gene and the very precise migration of the radioactive material with the nonradioactive 5S RNA marker provide evidence that the gene has been accurately transcribed by the oocyte nucleus.

Mammalian oocytes and fertilized ova may respond to injected DNA in exactly the same way as *Xenopus* does. However, while mouse and *Xenopus* oocytes have at least a similar capability for response, there may be differences in transcription ability, as has been demonstrated for mRNA translation (*6*). It is possible that our technique can be extended or modified to allow incorporation of the injected gene into the chromosomes of the ovum and thereby into the mouse, an extension that would allow a wide range of studies related to differentiation and carcinogenesis.

Note added in proof: By employing the techniques described here and in collaboration with Dr. Carlo Croce of Wistar Institute, we have obtained incorporation of injected genes into DNA of the mouse.

RALPH L. BRINSTER
HOWARD Y. CHEN
MYRNA E. TRUMBAUER
Laboratory of Reproductive Physiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia 19104

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A Metronidazole Metabolite in Human Urine and Its Risk

Abstract. *Metronidazole is a drug used for the treatment of trichomonal vaginitis, amebiasis, giardiasis, and certain anaerobic bacterial infections in humans. Acetamide and* N-*(2-hydroxyethyl)oxamic acid are metabolites of metronidazole in the rat, and we find small amounts of both metabolites in the urine of human patients taking the drug. Although acetamide is carcinogenic for rats, we do not believe that our finding further defines metronidazole's risk for humans. That risk can only be estimated from surveillance of people previously exposed to the drug.*

Metronidazole is useful in the treatment of trichomonal vaginitis, amebiasis, giardiasis, and certain anaerobic bacterial infections in humans. Either alone or in combination with other antibiotics, metronidazole can decrease the incidence of bacterial infections after intestinal and gynecological surgery (*1*). Acute toxicity is rarely of significance at doses necessary for these indications, but concern has been expressed that metronidazole may pose a risk of human cancer (*2*). Long-term administration of metronidazole at high doses causes tumors in mice and rats (*3*), and the drug and some of its metabolites are mutagenic for the histidine auxotrophs of *Salmonella typhimurium* (*4*). Nevertheless, two recent studies failed to detect any increased incidence of cancer in relatively small groups of patients who had been treated a decade earlier with metronidazole for trichomonal vaginitis (*5*). Thus the benefits of metronidazole are discernible from direct clinical observation, whereas its risks are estimated solely on the basis of laboratory data (*2*).

We found previously that metronidazole is metabolized in the rat to the weak carcinogen acetamide (*6*). In the study described here, we examined the urine of patients taking metronidazole to determine whether or not acetamide is present. In addition to finding small amounts of acetamide we found small amounts of another metabolite, *N*-(2-hydroxyethyl)oxamic acid (HOA) (Fig. 1), which confirms that the imidazole ring of metronidazole is cleaved in the human as it is in the rat (*6*, *7*).

For this study we used metronidazole (melting point, 158° to 160°C) and [1′,2′-$^{14}C_2$]metronidazole (11.7 mCi/mmole) (gifts from G. D. Searle and Co.); [1-^{14}C]acetamide (3.0 mCi/mmole) (California Bionuclear Corporation); [1,2-$^{14}C_2$]ethanolamine hydrochloride (2.44 mCi/mmole) (New England Nuclear); and *N*-[1,2-$^{14}C_2$-(2-hydroxyethyl)]oxamic acid (11.7 mCi/mmole) which was synthesized as described previously (*7*). All other chemicals were purchased from Fisher Scientific Company unless otherwise specified.

Under a protocol approved by the Human Studies Committees at Harvard Medical School, the Peter Bent Brigham Hospital, and the Beth Israel Hospital, urine was collected for a 24-hour period from five hospitalized patients who received metronidazole (Flagyl, Searle) at a dose of 750 mg per 24 hours on their physician's orders, and from six normal subjects (two males, ages 26 and 32 years; four females, ages 20, 28, 30, and 54 years). Urine was kept at 0° to 4°C during collection and was then stored at − 15°C until analyzed. Patient characteristics are listed in Table 1.

The HOA and acetamide in urine were separated by cationic exchange chromatography. The HOA was further purified by anionic exchange chromatography and then hydrolyzed and quantified as the dinitrophenyl derivative of ethanolamine by high-pressure liquid chromatography. Acetamide was quantified by gas-liquid chromatography and its identity confirmed by mass spectroscopy. The analysis was performed on 125 to 150 ml

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Table 1. Study subjects and urinary metabolites found.

Subject			Drugs administered concurrently*	Urinary constituents (milligrams per 24 hours)		
Age (years)	Sex	Admission diagnosis		Acetamide	HOA	Metronidazole
54	F	Chest pain	Furosemide, digoxin, oxazepam, heparin, allopurinol, trimethoprim, sulfamethoxazole, warfarin, chlorpropamide, methyldopa	3.6	10.8	43
20	F	Sarcoidosis	Sulfanilamide, aminoacrine, allantoin (vaginal cream)	4.7	4.5	91
41	M	Lumbar disc herniation	Cefazolin, diazepam, meperidine	3.3	9.5	39
46	F	Lung nodules	Methyldopa, hydrochlorothiazide, theophylline, pentobarbital	4.7	8.2	61
61	F	Fracture	Digoxin, isosorbide dinitrate, dioctyl sodium sulfosuccinate, folate, heparin, hydralazine, spironolactone, bisacodyl, penicillin VK	0.8	1.7	4

*Only organic molecules are listed.

of urine, a volume that represented 5 to 10 percent of the day's collection.

To each sample we added 1.6 mCi (18 ng) of HOA and 6.9 nCi (136 ng) of acetamide as internal standards to monitor the recovery of these metabolites. The urine was reduced in volume to 10 ml by rotary evaporation and added to an AG 50W-X4 column (15 by 2.4 cm, H^+ form, Bio-Rad) that was eluted with 120 ml of water. The eluate between 36 and 66 ml contained HOA as well as other neutral and acidic compounds, and that between 82 and 116 ml contained acetamide as determined by the added radioactive label. The fractions containing HOA were combined, neutralized with NaOH, reduced to a volume of 2 to 3 ml by rotary evaporation and then added to an AG 1-X4 column (10 by 1.0 cm, acetate form, Bio-Rad). The column was eluted with 30 ml of water and then 40 ml of 0.5*N* HCl. The eluate between 38 and 56 ml contained HOA from which ethanolamine was released by hydrolysis in 6*N* HCl for 1 hour at 122°C. After lyophilization, the residue was dissolved in 1 ml of 0.5 $KHCO_3$ and the dinitrophenyl derivative was formed by the addition of 2,4-dinitrofluorobenzene (20 mg in 1.5 ml of acetone) and heating at 50°C for 1 hour. Excess 2,4-dinitrofluorobenzene was then removed by continued heating for 30 minutes in the presence of 1 ml of 5 percent aqueous glycine. The solution was made alkaline with 5 ml of 0.5*M* $KHCO_3$ and the ethanolamine derivative was extracted with diethyl ether. The ether was evaporated under a stream of nitrogen and the remaining yellow residue dissolved in 1 ml of methanol. A 5-μl portion was analyzed with a high-pressure liquid chromatograph (Waters Associates, model ALC/GPC 204) equipped with an ultraviolet absorbance detector (model 440) that was operated at 254 nm. The μBondapak C_{18} column was eluted at a flow of 2 ml/min with 50 or 30 percent methanol, or with 50 percent tetrahydrofuran in 0.005*M* phosphate buffer at *p*H 4.0. The ethanolamine derivative, with retention times, respectively, of 4.1, 13.3, and 2.8 minutes was quantified from its area under the curve in comparison with authentic standards. By this method we could detect as little as 0.1 μg of HOA per milliliter of urine. Between 72 and 95 percent of the added labeled HOA was recovered as ethanolamine by this procedure.

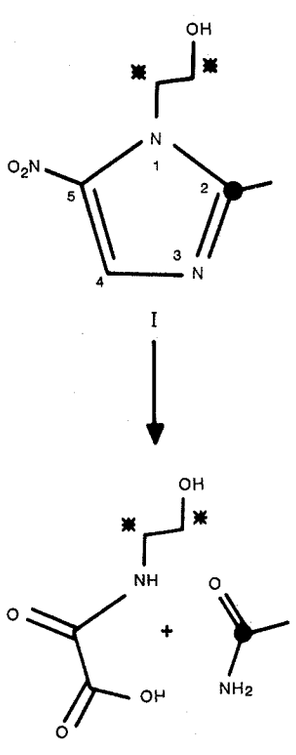

Fig. 1. Metronidazole (I) reacts with the flora to form *N*-(2-hydroxyethyl)oxamic acid (II) and acetamide (III). Solid circles indicate the fate of radioactively labeled carbon originally at the 2-position of the imidazole ring; asterisks show the fate of radioactive carbon in the ethanol side chain of metronidazole.

The acetamide fractions from the cationic exchange chromatography that contained between 93 and 100 percent of the added radioactive acetamide were concentrated to a syrup by rotary evaporation and then lyophilized for 1 hour. Acetamide was then extracted by two 1-ml volumes of chloroform; the extracts were combined and evaporated to dryness under a stream of nitrogen, and the residue was redissolved in 0.2 to 0.5 ml of methanol. Recovery of the added radioactive acetamide was between 24 and 52 percent. A 5-μl portion was removed and analyzed on a Hewlett-Packard gas chromatograph (model 7620A) equipped with a 6-foot (1.8 m), 10 percent SP 1000/1 percent H_3PO_4 (Supelco) analytical column operated isothermally at 180°C with N_2 (55 ml/min) as the carrier gas. Acetamide, with a retention time of 4.8 minutes, was quantified from the area under the curve as determined by triangulation. This method was capable of detecting as little as 0.5 μg of acetamide per milliliter of urine. The identity of acetamide was confirmed by gas-liquid chromatography–mass spectroscopy on a Hewlett-Packard 5992 GC/MS system by selective ion monitoring of mass to charge ratios of 59 and 44, with the same column packing being used but with the N_2 flow at 20 ml/min and the oven temperature at 200°C. Under these conditions, acetamide had a retention time of 5.7 minutes. Complete mass spectra were taken to compare samples with the standards.

To assay the urine for metronidazole content we adjusted a 5-ml sample to *p*H 8.0 and transferred it to a test tube containing 1.8 g of NaCl. The solution was extracted twice with equal volumes of

ethyl acetate, and the extracts were combined and evaporated to dryness under a jet of N_2. The residue was then redissolved in 0.5 ml methanol and analyzed by high-pressure liquid chromatography (*7*).

Both acetamide and HOA were detected in the urine of all five subjects taking metronidazole (Table 1). Neither metabolite was found in the urine of the control subjects. Thus these metabolites of metronidazole occur in the human as they do in the rat. Presumably, as in the rat (*6, 7*), they represent metabolites formed by the intestinal microflora.

Together, HOA and acetamide account for all the carbon and nitrogen atoms of metronidazole except for the nitrogen atoms in the nitro group. Approximately 1 to 2 percent of the daily 750-mg doses of metronidazole was recovered either as HOA or acetamide in patients, a recovery that is similar to that in the rat (*6, 7*). Because the yield of acetamide is approximately tenfold greater than that of HOA when metronidazole is either metabolized by bacteria (*6*) or reacted with xanthine oxidase (*8*), some acetamide must be lost by further mammalian metabolism (*6*), which implies that the patient is probably exposed to approximately tenfold more acetamide than is actually recovered in his or her urine.

Prolonged feeding of high doses of acetamide causes hepatocarcinoma in rats (*9*), and it is of interest that this is similar to one of the tumors reported in the rat after long-term feeding of metronidazole (*3*). Thus the finding of acetamide in the rat may provide insight into the mechanisms of metronidazole's carcinogenicity in the rodent. However, our finding of acetamide in the urine of human patients should not be interpreted as evidence that metronidazole is carcinogenic for humans. It has been known for nearly a decade that long-term feeding of maximally tolerated doses of metronidazole causes tumors in rats and mice (*3*), but two surveys of a limited number of patients treated with metronidazole suggest that the risk of this drug being a potent carcinogen in humans is probably small or negligible (*5*). Further studies of patients previously exposed to metronidazole may provide more accurate estimates of this risk.

RONALD L. KOCH
BERNARD B. BEAULIEU, JR.
EWAN J. T. CHRYSTAL
PETER GOLDMAN
Division of Clinical Pharmacology, Department of Pharmacology, Harvard Medical School, Beth Israel Hospital, Boston, Massachusetts 02215

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Two Novel Classes of Small Ribonucleoproteins Detected by Antibodies Associated with Lupus Erythematosus

Abstract. *The RNP and Sm antigens recognized by lupus erythematosus antibodies are located on discrete particles containing single small nuclear RNA's complexed with proteins. The antigens Ro and La are also on ribonucleoproteins. The small RNA's in ribonucleoproteins with Ro are discrete, like those associated with RNP and Sm; in contrast, ribonucleoproteins with La contain a striking highly banded spectrum of small RNA's from uninfected cells as well as virus-associated RNA from adenovirus-infected cells.*

Antibodies directed against a variety of nuclear and cytoplasmic constituents are characteristic of lupus erythematosus. The molecular identities of some of these antigens are known (for example, DNA), but four have simply been designated Sm, RNP, Ro (also called SS-A), and La (also called SS-B and Ha) (*1–6*). Defining the molecular nature of these antigens is one approach to the difficult problem of understanding why they so often become targets for immune responses in lupus erythematosus.

By examining immune precipitates of nuclear extracts prepared from ^{32}P-labeled mouse Ehrlich ascites cells, we established the molecular identity of RNP and Sm as small nuclear ribonucleoprotein particles, which we call snRNP's (*7*). Antigenically conserved for over 500 million years, snRNP's may play a role in messenger RNA biogenesis (*8*). Antibody to RNP precipitates the most abundant snRNP's, those containing the small nuclear RNA's (snRNA's) U1a (*9*) and U1b (*7*). Antibody to Sm precipitates these snRNP's and four others containing the snRNA's U2 (*10*), U4, U5, and U6. Both antibodies precipitate the same seven polypeptides with molecular weights of 12,000 to 32,000. At least some of these proteins are required for antigenicity, since purified U1a, U1b, U2, U4, U5, and U6 RNA's do not react with either antibody (*7*).

In the present study, we examined 29 serums (25 from patients with lupus erythematosus and four reference serums containing antibody to Sm, RNP, Ro, and La, respectively) for the ability to precipitate small RNA-protein complexes from total cell extracts of ^{32}P-labeled Ehrlich ascites cells (Fig. 1). Seven of the serums gave a pattern of small RNA's consistent with the presence of antibody to Sm (lane 1), and two with the presence of antibody to RNP (lane 2) (*7*). In contrast to these previously defined patterns, three serums precipitated the small RNA's Y1 and Y2 (lane 4), and a fourth serum precipitated Y1, Y2, and Y3 (lane 3). A single serum precipitated a highly banded spectrum of small RNA's (lane 5). Standard serums containing predominantly antibodies to Sm, RNP, Ro, or La yielded the same patterns as those seen in lanes 1, 2, 4, and 5, respectively. Because lane 3 is identical to lane 4, with the addition of Y3 in lane 3, we call this pattern Ro^b and that in lane 4 Ro^a; much darker exposures of lane 4 do not show Y3. Serums from three patients apparently contained combinations of antibodies; two of these precipitated a constellation of small RNA's that suggest the simultaneous presence of antibodies to Sm and Ro^a (lane 7), and the third contained antibodies to Sm, RNP, Ro^a, and La (lane 8). [Although the small RNA's precipitated by antibody to RNP are a subset of those precipitated by antibody to Sm, the intensity of U1a and U1b relative to U2 in lane 8 (compare to lane 1) indicates that antibodies to both RNP and Sm are present in this serum.] Finally, serums from

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