Hepatic Drug Metabolism in Rats: Impairment in a Dirty Environment

Abstract. *Reduction of aniline hydroxylase activity, ethylmorphine N-dementhylase activity, and cytochrome P-450 content occurred in hepatic microsomes of rats kept under dirty conditions, defined as accumulation for 1 week of urine and feces in pans under the wire mesh cages. In comparison with rats that had urine and feces removed twice daily from such pans, rats kept over Kimpak bedding or over Litter Green, changed twice daily, also showed reduced drug-metabolizing activity in hepatic microsomes, but to a lesser degree than the dirty rats. Placement of a filter top on cages for 1 week also decreased drug-metabolizing activity. These experiments suggest that the relative cleanliness of an animal's environment can influence hepatic microsomal drug metabolism.*

Softwood beddings commonly used to house rodents can enhance mixed-function oxidases in hepatic microsomes (*1*), whereas hardwood beddings generally cause no increase. Differences in the type of bedding employed to house rodents constitute one source for large discrepancies among laboratories in the results of investigations on drug metabolism. Another source of such variations is illustrated by the present studies on drug metabolism in clean and dirty rats.

Adult male Sprague-Dawley rats weighing 150 to 170 g were kept for 1 week in our animal facilities before these experiments. They were housed without bedding in wire mesh cages, and the pan under each cage to collect urine and feces was cleaned twice daily. The rats were given free access to water and Purina Chow. Four groups of animals, five rats in each group, were used in each of two experiments. In experiment 1, group 1 rats were kept without bedding; urine and feces were cleaned twice daily from a pan placed 7.2 cm under the wire mesh cage. Group 2 rats had Kimpak (*2*) bedding in the pan under their cages. This bedding was replaced twice daily. Group 3 rats were maintained over Litter Green (*3*) bedding, also replaced twice daily. Group 4 rats were without bedding; their urine and feces accumulated in the pan under the wire mesh cage. However, in order to expose group 4 rats to the same external stimuli as were the other animals, rats in group 4 had their pans moved back and forth twice daily to the same extent as for rats in the other groups.

Table 1. Effect of bedding and change of pan under cage on hepatic drug metabolism. Pans under wire cages were cleaned twice daily for rats in groups 1, 2, and 3; feces and urine accumulated for 7 days in pans beneath cages for rats in group 4. For each protein, groups 2, 3, and 4 were each significantly different from group 1 by Student's *t*-test ($P < .05$). Means ± standard error of the mean are given.

Group	Enzyme activity (nanomoles per milligram of protein per minute)		Cytochrome P-450 (nanomoles per milligram of protein)
	Aniline hydroxylase	Ethylmorphine *N*-demethylase	
1, Control	1.00 ± 0.05	11.2 ± 0.8	1.58 ± 0.09
2, Kimpak	0.83 ± .06	7.5 ± .3	1.17 ± .06
3, Litter Green	.64 ± .03	9.0 ± .6	1.10 ± .04
4, Dirty	.33 ± .04	6.6 ± .3	1.06 ± .04

Table 2. Effect of bedding and change of pan under filter-top cage on hepatic drug metabolism. All animals had filter tops over solid cages; other conditions were as described for Table 1. Groups 2, 3, and 4 differed significantly from group 1 only for aniline hydroxylase ($P < .05$, Student's *t*-test). Group 1 was significantly less than group 1 in Table 1 for each of the three proteins ($P < .05$). Means ± standard error of the mean are given.

Group	Enzyme activity (nanomoles per milligram of protein per minute)		Cytochrome P-450 (nanomoles per milligram of protein)
	Aniline hydroxylase	Ethylmorphine *N*-demethylase	
1, Control	0.77 ± 0.06	8.3 ± 0.5	1.05 ± 0.06
2, Kimpak	.60 ± .03	7.6 ± .8	1.09 ± .06
3, Litter Green	.53 ± .05	7.5 ± .4	1.07 ± .08
4, Dirty	.51 ± .02	8.8 ± .4	1.09 ± .12

In the second experiment, the four groups of rats were placed under the same conditions as in experiment 1, except that solid cages were used instead of wire cages and a filter top was placed over each solid cage. This filter was composed of porous paper that permitted passage of particles 3 μm or less in diameter; such a filter top is occasionally employed to house rodents to help exclude various contaminating materials while permitting entry of oxygen and diffusion of CO_2. Each experiment was repeated twice.

At the end of 7 days, rats in each group were killed. The livers were immediately removed and homogenized at 4°C with 1.15 percent KCl in 0.02*M* tris(hydroxymethyl)aminomethane, *p*H 7.4 (5 ml per gram of tissue), and microsomes were prepared (*4*). Aniline hydroxylase activity, ethylmorphine *N*-demethylase activity, and cytochrome P-450 were measured as described previously (*2*). All the animals in experiments 1 and 2 were killed on the same day, and the assays were also performed on a single day to avoid large day-to-day variations frequently encountered in these determinations.

In experiment 1, rats in dirty cages had only one-third the aniline hydroxylase activity, a little more than half the ethylmorphine *N*-demethylase activity, and two-thirds the cytochrome P-450 activity of rats kept in wire cages over pans that were cleaned twice daily (Table 1). Bedding composed of Kimpak (cellulose air-dried fiber) for group 2 or Litter Green (dehydrated alfalfa) for group 3 produced results intermediate between those of groups 1 and 4 (Table 1).

Rats maintained under conditions identical to those described above except for the use of solid, filter-top cages instead of wire mesh cages showed no significant differences in ethylmorphine *N*-demethylase activity or cytochrome P-450 content among the four groups (Table 2). However, aniline hydroxylase activity was significantly reduced in dirty rats compared to clean rats (group 1); rats on Kimpak and Litter Green also exhibited reduced activity of this enzyme compared to the rats without bedding (Table 2). Placement of a filter top did decrease microsomal drug metabolism, as indicated by the significant reductions in the three proteins in group 1 animals in cages without filter tops (Table 1) compared to group 1 animals in filter-top cages (Table 2).

The present experiments reveal that

drug metabolism in hepatic microsomes was inhibited when urine and feces of rodents were not removed twice daily but permitted to accumulate for 1 week. Inhibition of drug metabolism in rats kept under these conditions may arise from hepatic toxicity due to increased concentrations of ammonia (*5*) in such environments. Ammonia concentrations in animal rooms depend on several factors, including the presence of urease-positive bacteria (*6*), whose population would be related to accumulation of feces. Although filter tops are designed to permit passage of such gases as CO_2 and ammonia out of the cages, filter tops may not perform this task with complete efficiency. Therefore, inhibition of drug metabolism in group 1 of experiment 2 (Table 2) might also arise from hepatic toxicity secondary to ammonia accumulation under filter tops, even with twice-daily changes of the pans under the cages. Even without filter tops, animal rooms would require adequate ventilation to remove ammonia and prevent some of the effects observed when filter tops were used.

Whatever the explanation of our results, they serve to alert investigators to the potential inhibiting effects of dirty environments on hepatic drug metabolism. Clean and dirty conditions had comparatively narrow definitions in these experiments, since animal rooms in different laboratories vary over a wider range of conditions than those employed in our studies; one measure of this extensive range is the large variation in number of times per week or month that cages are cleaned in different laboratories. Dirty environments should now be added to the growing list of factors that affect the extremely sensitive hepatic microsomal system for metabolizing drugs. Among others, these factors include age; sex; strain; litter of origin; painful stimuli; ambient temperature; degree of crowding; time of day or season of drug administration; hormonal, nutritional, and physiological status; and type of bedding (*7*).

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Amine Metabolites in Human Cerebrospinal Fluid: Effects of Cord Transection and Spinal Fluid Block

Abstract. *Patients with spinal cord transection had normal concentrations of 5-hydroxyindoleacetic acid and low concentrations of 3-methoxy-4-hydroxyphenyl glycol in lumbar cerebrospinal fluid. The presence or absence of spinal fluid block in these patients did not affect concentrations of either amine metabolite. However, the concentration of homovanillic acid was lower in patients with spinal fluid block than in those without block. The results suggest that the spinal cord contributes to concentrations of 3-methoxy-4-hydroxyphenyl glycol and possibly 5-hydroxyindoleacetic acid, but contributes little to that of homovanillic acid in the lumbar spinal fluid of man.*

In an attempt to evaluate brain amine function in man, the concentrations of amine metabolites in the cerebrospinal fluid (CSF) have been studied in a variety of illnesses such as depression, mania, schizophrenia, Parkinson's disease, and dementia. 5-Hydroxyindoleacetic acid (5HIAA), homovanillic acid (HVA), and 3-methoxy-4-hydroxyphenyl glycol (MHPG), the respective metabolites of serotonin, dopamine, and norepinephrine, have received the most attention. Considerable evidence supports the conclusion that brain amine changes are reflected in the concentrations of amine metabolites in ventricular or cisternal CSF (*1, 2*). However, the relation between changes in amine concentrations in the brain and metabolite concentration in the lumbar CSF remains uncertain.

It is likely that HVA in lumbar CSF is derived from sources in the brain because, with the exception of one study (*3*), there is agreement that the spinal cord does not contain appreciable amounts of dopamine (*4–6*). Additional evidence in man comes from Pletscher *et al.* (*7*), who injected [^{14}C]dopamine intravenously and found that peak amounts of labeled HVA in cisternal CSF appeared 2 to 4 hours after infusion, whereas those in lumbar CSF appeared 6 to 8 hours after infusion. Furthermore, Curzon *et al.* (*8*) reported that obstruction to CSF flow in the cervical area was associated with low concentrations of HVA in lumbar CSF.

Little is known about the site of origin of MHPG in the lumbar CSF, and controversy has developed as to whether 5HIAA in the lumbar CSF reflects metabolism in spinal cord rather than in brain. A single injection of 5HIAA into the cistern of cats did not increase 5HIAA concentrations in the lumbar CSF, and values for 5HIAA in the spinal cord after reserpine or serotonin administration closely paralleled those in the CSF (*9*). It was concluded that 5HIAA concentrations in lumbar CSF do not reflect serotonin metabolism in brain. However, several lines of indirect evidence, including accumulation of amine metabolites after probenecid administration and clinicopathologic studies in man (*2*), suggest that some brain contribution to the pool of 5HIAA in lumbar CSF is likely.

In the present study, we investigated the contribution of the spinal cord to the levels of amine metabolites in the CSF of humans. All patients studied had spinal cord transections, and some also had evidence of a block in flow of CSF. Studies of spinal cord transection in rats (*5, 10*), cats (*6, 11*), and rabbits (*12, 13*) suggest that cord serotonin and norepinephrine are localized to nerve endings in descending tracts and that amounts of both amines are dramatically reduced in the cord below the transection. Below the lesion, no serotonin or norepinephrine is recognizable by histofluorescence techniques, and concentrations of amines and their metabolites are reduced to 75 to 86 percent of control values by 1 to 5 weeks after transection.

If similar reductions in spinal cord amines occur after cord transection in man, then it would be expected that metabolites of these amines in CSF would be low in patients with transected spinal cords if the cord normally makes a significant contribution to the levels of amine metabolites in CSF. More-