E. coli LPS administration. In experiment 4, both of lethal rates (4/14, 4/14) 4 and 6 h after LPS administration were significantly larger than that (0/14) of adrenaline control. The time response indicated the gradual appearance of the hyperreaction and the peak of response between 4 and 6 h after LPS administration. In experiments 5 and 6, the evident adrenaline-hyperreaction maintained till 6 h after LPS administration, would gradually decrease and finally disappear 24 h thereafter.

In experiments 1-6, the LPS (W) and LPS (B) preparations of *E. coli* were studied. Furthermore, both preparation types, Westphal (W) and Boivin (B) type, of LPS derived from *Salmonella typhimurium, Salmonella typhosa, Shigella flexneri* and *Serratia marcescens* were studied including Bovin type of *E. coli* LPS. Some data are presented as experiment 7 in the table. All preparations in doses of 10-100 µg per mouse always showed the lethal adrenaline-hyperreacting activity 4 h after LPS administration, regardless of the LPS extraction methods and the origin of bacterial cells. Little or no activity was detected 24 h after LPS administration. These data were consistent with those of experiments 4-6.

We arbitrarily adopted the 4-h interval between the endotoxin administration and the challenge of adrenaline for the analysis of dose-response relationship. Thus, we obtained linear dose-response relationships with varying endotoxin doses and a constant adrenaline dose, as well as with varying adrenaline doses and a constant endotoxin dose[4]. The characteristics of these dose-response lines will be presented in detail elsewhere.

The mechanism of the adrenaline-hyperreaction described above is not understood. Some workers reported the release of adrenaline in vivo by endotoxin administration[7-16]. Such a release of adrenaline may be involved in the lethal adrenaline-hyperreaction in endotoxemia. The exogeneous addition of a sublethal adrenaline dose to the possibly increased adrenaline contents in blood by endotoxin may result in the shock-like death of mice. The hypothesis also suggests that the clinical therapeutical use of adrenaline may bring unexpected prognosis to patients in shock accompanying endotoxemia, who may have high adrenaline content in blood.

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Metabolism of hydrazines and hydrazides by the intestinal microflora

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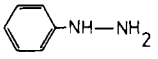
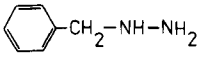
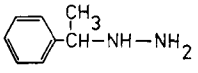
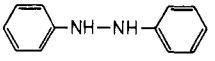
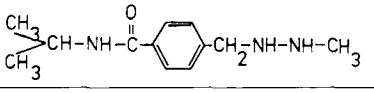
Summary. Intestinal microorganisms are able to effect the metabolic reductive fission of hydrazines but not hydrazides during incubation in vitro.

Since the intestinal microflora is known to be of considerable importance in the metabolism of many azo-compounds, including both food colours[2] and the drugs, neoprontosil[3], salicylazosulphapyridine[4] and phenazopyridine[5] it appeared of interest to establish whether drugs possessing the related hydrazo-linkage were also susceptible to reductive fission by the intestinal microflora. Studies are now reported upon the ability of the microflora of the rat intestine to degrade a number of hydrazo-substituted compounds under conditions of anaerobic incubation in vitro.

The incubations were carried out under anaerobic conditions employing a glucose-peptone-yeast extract medium[6]. Each drug was added to the sterile medium to give a concentration of 0.36 mg/ml; the drug preparations previously being sterilized either by autoclaving under controlled conditions or in the case of labile compounds, by passage through millipore filters. Mixed inoculae of rat intestinal organisms were obtained by sterile section of the rat intestine when a small amount of the withdrawn material was suspended in sterile 0.9% aq. NaCl. Following inoculation the incubation mixtures were held at 37 °C for 7 days under N_2. Appropriate noninoculated controls were incubated under similar conditions. Following incubation the tubes were centrifuged and the supernatants passed through millipore filters and submitted either directly or following a dansylation treatment to TLC. Dansylation was carried out employing a modification of the procedure described by Smith[7]. To 1.0 ml of the supernatant, 0.1 ml of a 1 M solution of sodium bicarbonate was added. A solution of saturated dansyl chloride in acetone was added and the tube sealed and allowed to stand in the dark for 12 h. Acetone (8 ml) was added and the precipitate of protein and inorganic salts removed by centrifugation. The supernatants were then applied to TLC plates for investigation.

The compounds selected for investigation were of the hydrazine (table 1) or hydrazide type (table 2). Following incubation, all of the hydrazine compounds were found to have given rise by reductive fission to primary amines detected on TLC plates as their dansyl derivatives (table 3). Since the intensity of fluorescence and size of each of the spots were similar to those given by the dansyl derivatives of the corresponding primary amines at the same molar levels as the hydrazine substrates initially present it was

Table 1. Hydrazines and their corresponding metabolites formed by the intestinal microflora in vitro

Compound	Structure	Metabolite detected
Methylhydrazine	$CH_3-NH-NH_2$	Methylamine
Phenylhydrazine		Aniline
Benzylhydrazine		Benzylamine
1-Phethylhydrazine (mebanazine)		1-Phenethylamine
1,2-Diphenylhydrazine		Aniline
Procarbazine		Methylamine

concluded that extensive degradation of the hydrazines had occurred. Trace amounts only of primary amines were found in the uninoculated control tubes probably due to the presence of naturally occurring reducing agents in the culture medium.

It will be noted (table 1) that both mono-substituted and disubstituted hydrazines are metabolized whilst the metabolism of methylhydrazine indicates that the presence of an aromatic substituent is not essential for hydrazo-fission to take place.

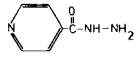
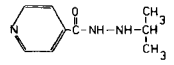
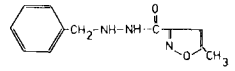
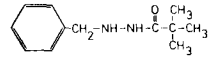
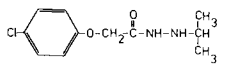
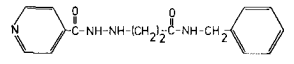
Although the disubstituted hydrazine, procarbazine gave rise to large amounts of methylamine the primary amine corresponding to the other fragment of the molecule was not detected by the dansylation procedure which suggests that it may have been further metabolized. The possibility of release of the isopropylamine moiety by hydrolysis of the amide linkage was explored but isopropylamine was not detected.

In contrast to the hydrazines studied, the hydrazides (acylhydrazines) investigated (table 2) did not apparently undergo reductive fission in the presence of the intestinal microflora as none of the expected fission products (table 2) were detected in the inoculated media under the conditions of the in vitro experiments, although added standards were satisfactorily separated and located.

The possibility that the $-CO-NH-$ linkage of the hydrazides might be susceptible to metabolic hydrolysis by intestinal bacteria was also considered, since evidence for the microfloral N-deacylation of a number of acetanilides[8] and of certain acetylsulphonamides[9,10], has been reported. No evidence for the formation of hydrolysis products of the hydrazides under investigation was however obtained. The possibility that the hydrazide, iproniazid might undergo bacterial N-dealkylation was also considered, as evidence has been presented of the microfloral N-dealkylation of imipramine[11] and of methamphetamine[12], but isoniazid, the N-dealkylation product of iproniazid, was not detected after incubation. Visual inspection of the chromatograms moreover revealed no apparent decrease in the amounts of the hydrazides remaining in the supernatants from inoculated tubes compared with those from uninoculated tubes.

A possible explanation for the observed difference in susceptibility of hydrazines and hydrazides to microfloral reduction is that in a hydrazide bond, resonance stabiliza-

Table 2. Hydrazides and their theoretical fission products found to be absent following microfloral incubation

Hydrazides	Theoretical fission products
 Isoniazid	Isonicotinamide, isonicotinic acid
 Iproniazid	Isopropylamine, isonicotinamide, isoniazid, isonicotinic acid
 Isocarboxazid	Benzylamine, benzylhydrazine
 Tersavid	Benzylamine, benzylhydrazine
 Iproclozide	Isopropylamine
 Nialamide	Isonicotinamide, isoniazid, isonicotinic acid, benzylamine

tion would be expected to take place by interaction of the lone electron pair on the nitrogen atom and the π electrons of the carbonyl group. This would impart a partial positive charge on the nitrogen atom which would in turn decrease the nucleophilic character of both nitrogen atoms and thus stabilize the molecule. Alternatively the resistance to reduction could be due to a steric effect of the carbonyl group but this seems less likely as the hydrazide grouping is known to be usually planar along all 4 atoms.

These experiments indicate that under in vitro conditions the intestinal microflora is able to effect extensive reductive fission of all the hydrazines examined, but the extent to which the intestinal microflora participates in vivo can in fact only be assessed by investigations in intact animals, as the rate of uptake of the administered compound from the gut, the period of contact between the compound and the microflora and the physical and chemical conditions obtaining in the lumen of the intestine are clearly important factors.

Table 3. Chromatographic characteristics of dansyl derivatives of the amine metabolites and other compounds

Dansyl derivative	R_f-value in solvent system A	B	C	D
Methylamine	0.17	0.14	0.26	-
Aniline	0.28	0.20	0.38	0.44
Benzylamine	0.25	0.27	0.80	-
1-Phenethylamine	0.27	0.31	-	0.77
Isopropylamine	0.40	0.22	0.60	-

Dansyl derivatives were detected on TLC plates by their fluorescence under $UV_{365\,nm}$ irradiation. All separations were carried out on silica gel G layers employing the solvents indicated: A, benzene-acetone (98:2 by vol.); B, chloroform-benzene (1:1 by vol.); C, chloroform; D, benzene-triethylamine (4:1 by vol.).

Table 4. Chromatographic characteristics of substituted hydrazides and related compounds

Compound	R_f in solvent system E	F	G	H	I	Detection
Iproniazid	0.75	0.95	-	-	-	1
Isoniazid	0.55	0.62	-	-	-	1
Nialamide	0.15	0.95	0.0	0.0	-	1
Isocarboxazid	-	-	0.80	0.77	0.70	2
Iproclozide	0.95	-	0.60	0.65	-	2
Tersavid	0.65	-	0.65	0.85	0.65	2
Isonicotinamide	0.40	0.80	-	-	-	1
Isonicotinic acid	0.05	0.55	-	-	-	1
Benzylhydrazine	-	-	0.40	-	0.15	2

All separations except that employing solvent F were carried out on silica gel G layers. Separations employing solvent F were carried out on cellulose powder layers.
E, chloroform-acetone-diethylamine (5:4:1 by vol.); F, propan-2-ol-water (85:15 by vol.); G, 1,2-dichloro-ethane-ethylacetate-formic acid (3:1:1 by vol.); H, benzene-1,4-dioxane-acetic acid (90:25:4 by vol.); I, chloroform-ethylacetate-formic acid (5:4:1 by vol.). 1, nitroprusside reagent[15]; 2,4-dimethylamino-cinnaldehyde reagent[16].

Although it has been reported by Schwarz[13] that small amounts of methylamine are detectable in the urine of rats dosed with procarbazine, free phenylethylamine and benzylamine were not detected among the urinary metabolites of [^{14}C] mebanazine and [^{14}C] benzylhydrazine respectively in the intact rat[14] although the possible formation of benzylamine as an intermediate in the formation of either benzoyl glucuronide or of benzoylglycine, the major urinary metabolite of benzylhydrazine, cannot be ruled out.

The demonstration that intestinal microorganisms under favourable conditions are capable of degrading hydrazines to their corresponding primary amines may be of particular significance in relation to intestinal diverticulosis in man since this condition is known to result in areas of intestinal stasis which are characterized by considerable proliferation of the bacterial population.

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Separation and quantitative determination of imipramine and desipramine from rat biological samples by high pressure liquid chromatography

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Summary. Imipramine and its metabolite, desipramine, have been determined in several rat biological samples by high pressure liquid chromatography. The method allows one to detect 15 ng in column of both drugs with a lower sensitivity limit of 20 $\mu g\,l^{-1}$.

In recent years, different analytical techniques have been developed for the qualitative and quantitative determination of imipramine and desipramine in biological samples. The techniques so far used can be distinguished in spectrophotometric[1], spectrophotofluorimetric[2], autoradiographic[3], isotopic[4], TLC[5–7] and gas chromatographic[8,9]. In this work we have employed high pressure liquid chromatography (HPLC), in order to separate and detect imipramine and its metabolite desipramine in biological samples; this technique presents, in addition to the advantages of a high specificity and sensitivity (in the range of ng), a considerable simplicity and rapidity of execution.

Materials and methods. Male pellet-fed Wistar rats weighing 190–210 g, were injected i.p. with a standard dose of