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Antibiotic Residues and Drug Resistance in Human Intestinal Flora

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The effect of residual levels of ampicillin on the drug resistance of fecal flora was studied in human volunteers given 1.5 mg of ampicillin orally per day for 21 days. This treatment failed to have any significant reproducible effect on the number of resistant *Escherichia coli* in their feces. The effect of continuous administration of small doses of ampicillin, chlortetracycline, or streptomycin in the drinking water was studied in gnotobiotic mice inoculated with a human fecal flora. In this animal model, which is free of many interfering factors, an increase in the fecal concentration of resistant *E. coli* was observed when the mice were given 0.5 μg of ampicillin or chlortetracycline per ml of water. This model is therefore a sensitive system for testing the effect of antimicrobial drugs on the resistance characteristics of the intestinal flora.

Antibiotics are used in food animal production for the treatment and prevention of disease and for growth promotion. This may result in the formation of antibiotic residues in meat or milk, if withholding times are not adhered to. Surveys indicate that these residues are generally found at levels below 1 ppm (micrograms per gram) (8, 22), but even at these low concentrations they may have an effect on the human gut flora. From the standpoint of setting antibiotic residue tolerance levels, it is important to consider the no-effect level of antimicrobial drugs on the drug resistance characteristics of enteric microorganisms (18, 29).

Studies with experimental animals have been carried out in an attempt to examine this problem. The feeding of certain antimicrobial drugs to chickens and pigs results in a population of resistant fecal *Escherichia coli*. It is difficult, however, to obtain a source of these animals with sufficiently low base-line levels of resistant *E. coli* to give meaningful results (21). Rodents are considered unacceptable because of the small number of *E. coli* isolates found in their guts. Dogs were used as an animal model for determining the effect of low levels of oxytetracycline on drug resistance of the lactose-fermenting enteric bacteria of the gut, but no effect was observed at doses below 10 μg/g of diet (27). Nevertheless, this concentration is higher than those ordinarily found as residues in food. When human volunteers were given low levels of oxytetracycline for long periods (11, 17), resistant enterobacteria occurred sporadically in some individuals from the administration of 10 mg of the drug per day, but smaller intakes had no effect. This daily dose, which in practice would correspond to 50 ppm of antibiotic in 200 g of meat, is still higher than the residues usually found in food.

During these in vivo studies, the investigators could not avoid at least one of the following experimental problems: (i) a high base line of resistant bacteria; (ii) differences between the flora of the experimental subjects at the start of the experiment; (iii) bacterial contamination during the trial, especially by resistant enterobacteria arising from the diet or by the staff in charge of the animals, or between the control and experimental antibiotic-supplemented groups; (iv) great variability with time in the percentage of resistant bacteria in the control flora; and (v) a microflora different from human gut flora (except for studies described in references 11 and 17).

Egger and Lebek (7, 18) investigated the minimum antibiotic concentrations that selected an R factor in continuous flow cultures of three clones of *E. coli* K-12 strains, one of which carried an R plasmid. The drugs tested were tetracycline, chloramphenicol, gentamicin, and ampicillin. They found that selection occurred when the medium contained a drug concentration that was one-tenth of the MIC for the susceptible clones used. In vivo, however, the following factors may interfere: (i) the drug may be absorbed or inactivated by chelation or enzymatic degradation; (ii) other ecological factors may govern the *E. coli* interactions, such as adhesion to the gut wall or the concentration of a limiting substrate; and (iii) the antibiotic may modify the dominant intestinal microflora, which is mainly composed of strictly anaerobic bacteria. The resulting effect on populations of *E. coli* constituting less than 1% of the gut bacteria might be different from the effect on pure *E. coli* strains.

In this study an animal model was used to overcome the experimental difficulties that are usually encountered in studies with conventional animals or humans and to investigate the effect of drugs in an ecological situation as close as possible to that of the human gut. As gnotobiotic mice have been used to study the effects of antibacterial drugs on complex microflora (1–3, 13, 25), I decided to examine the effect of low antibiotic concentrations on drug-resistant *E. coli* isolates in the feces of germfree mice dosed with a human fecal flora. Accordingly, ampicillin, chlortetracycline, or streptomycin was given to the mice in drinking water at the residual dose of 0.5 ppm and at the a priori R-factor-selecting dose of 8 ppm. These drugs were chosen because (i) they are widely used in veterinary practice and they have more chance than new expensive molecules to be used without control and hence to be found as food residues and (ii) resistances to these drugs are frequent among fecal bacteria (19, 20). These criteria applied as well to chloramphenicol and sulfonamides, which were not included in this study. The results indicate that antibiotic concentrations of 0.5 ppm may act in vivo at the level of the resistant fecal *E. coli* population.

MATERIALS AND METHODS

Human subjects. Six healthy adult volunteers who had not taken any antimicrobial drug for at least 3 months were used. Volunteers gave informed consent to participate in the trial.

TABLE 1. Examples of *E. coli* biotypes, based on acid production from six carbohydrates and resistance to three antibiotics[a]

Acid production from:			Octal code (first digit)	Acid production from:			Octal code (second digit)	Resistance to:			Octal code (third digit)	Resulting biotype code
Rhamnose	Sucrose	Sorbose		Raffinose	Dulcitol	Sorbitol		Ampicillin	Chlortetracycline	Streptomycin		
+	−	−	1	−	+	−	2	−	−	+	4	124
+	+	−	3	+	−	+	5	−	+	+	6	356
+	+	+	7	+	+	+	7	−	−	−	0	770

[a] A numerical value was attributed to each test: 1 for the first positive test in each group of three tests, 2 for the second positive test, and 4 for the third. Negative tests were scored as zero. The three values in each group of three tests were added, yielding one digit (between 0 and 7) for the biotype code.

After a control period of 15 to 35 days, one of them (volunteer A) was given 15 mg of ampicillin and 15 mg of streptomycin daily for 17 days, and the other five (volunteers B to F) were given 1.5 mg of ampicillin per day for 21 days. The antibiotics were taken orally in freshly prepared solutions every day at noon. Because the bacteria in human feces reflect the flora of the large colon (24), fecal samples were collected 3 to 5 times a week; a mean number of 18 samples per volunteer was examined before drug treatment, and 16 samples were examined during treatment.

Gnotobiotic mice. Ninety-five adult OF1 germfree mice (IFFA Credo, Lyon, France) were divided into four groups, each of which was maintained in a plastic isolator. They were fed ad libitum a commercial drug-free diet sterilized by irradiation (4 Mrad) and supplied with autoclaved drinking water (pH 2.5). Water intake was recorded daily for each group.

The fecal flora of four untreated human donors (volunteers A and B and subjects G and H) were transferred to the mice in the four groups. Each mouse was inoculated intragastrically with a dilution of the original flora, as described previously (3). All the mice in each group were given the same fecal suspension within 60 min of collection.

Experimental design. After 4 days without any treatment, each group of mice was divided into control and experimental subgroups of five mice, and each subgroup was kept in one cage in a separate isolator. The drinking water in the isolators was drug free for the control groups and supplemented with either 0.5 or 8 μg of ampicillin, streptomycin, or chlortetracycline per ml for the experimental groups. Chlortetracycline and streptomycin were given twice a week, and ampicillin was given daily always after sterilization by filtration on a filter (pore size, 0.45 μm). These supplemented drinking water samples were assayed by the disk plate method with *Bacillus subtilis* for streptomycin and ampicillin and with *Bacillus cereus* for chlortetracycline.

Because the number of *E. coli* isolates in mouse feces reflects the number in the cecum (9), fecal samples were examined for resistant *E. coli* isolates by comparative plate counting. For this purpose, freshly voided feces were collected directly at the anus of the mice, pooled for the five mice in each subgroup, and processed within 30 min of collection. Fecal samples were collected 3 to 5 times a week during the 5 weeks of continuous drug treatment.

Microbiological methods. A comparative counting procedure was used to determine the incidence of resistance to ampicillin, chlortetracycline, and streptomycin in *E. coli* populations. For this purpose, fresh feces were diluted 10-fold in sterile saline. Duplicate 0.2-ml fractions were plated onto deoxycholate agar (DCA; Difco Laboratories, Detroit, Mich.) in petri dishes containing either no antibiotic or 20 μg of ampicillin, streptomycin, or chlortetracycline per ml of agar. These breakpoints were chosen in accordance with usually accepted standards of resistance. Dilutions of feces from two subjects (D and F) were also plated on media containing 5 μg of the drugs per ml, and a very high correlation was observed between the number of bacteria resistant to 5 and 20 μg/ml. The total viable number of *E. coli* isolates per gram of fresh feces was determined from drug-free DCA plates, and the number of resistant *E. coli* isolates from the media containing antibiotics was determined.

Well-isolated lactose-fermenting colonies were selected from drug-free and drug-supplemented media. The biochemical types of 1,838 clones were determined by replica plating on nutrient agar containing 66 mg of bromothymol blue per liter and 10 g of one of the following sugars per liter: dulcitol, raffinose, rhamnose, sorbitol, sorbose, or sucrose (Sigma Chemical Co., St. Louis, Mo.). Duplicates were also plated on DCA plates containing 20 μg of ampicillin, chlortetracycline, or streptomycin per ml. A quasioctal code of three digits was used to identify the different biotypes (Table 1). Similar fermentation patterns were previously used in ecological studies of *E. coli* strains and found to be as discriminant as the serotyping of the strains (4, 15). These particular sugars were chosen after preliminary experiments because of their ability to discriminate between the strains from human volunteers. At least one representative clone of each biotype was tested for its ability to produce indole, ferment lactose and glycerol, produce gas from glucose, and degrade urea. All strains tested gave reactions typical of *E. coli*.

Liquid subcultures of 30 different resistant strains were counted on Mueller-Hinton agar (Difco), DCA, and MacConkey (Difco) and Tergitol 7 (Merck & Co., Inc., Rahway, N.J.) agars containing antimicrobial drugs. As no significant difference was found between the number of colonies on the different media, it was considered that these media and the antibiotics tested against *E. coli* strains had no synergistic inhibitory effects. DCA therefore was considered suitable for these experiments.

Statistical analysis. Owing to the wide range of the percentages of resistant *E. coli* in the feces (from 0.00006 to 100%), results were expressed as the decimal logarithms of the number of total *E. coli* isolates per gram minus the log number of resistant *E. coli* isolates per gram. This log difference, which is the log of the proportion that is resistant, without the minus sign, eliminated the effect of the low but significant correlation between the respective log numbers of total and resistant *E. coli* isolates. When the skewness and kurtosis (28) of these data were calculated, their distribution was found to be far from normal ($P < 0.01$). Hence, nonparametric tests were used. Nevertheless, the standard deviations (SD) of the data were calculated and shown to give a rough indication of the dispersion of the data. In experiments with human volunteers, the Mann-Whitney rank test (28) was used to compare, in the same individual, the occurrence of resistant *E. coli* isolates before and during

TABLE 2. Effect of ampicillin on the drug-resistant *E. coli* isolates in human fecal flora

Human volunteer	Drug and dose (mg/day)[a]	Duration of test (days)	No. of daily counts	Total no. of *E. coli* isolates/g of feces (log ± SD)[b,c]	Log ± SD difference between total *E. coli* isolates and *E. coli* isolates resistant to[c]:		
					Ampicillin	Chlortetracycline	Streptomycin
A	Control	28	22	7.96 ± 0.76	3.07 ± 1.55	4.24 ± 1.36	3.80 ± 1.79
	15 mg of AMP–15 mg of STR	18	14	8.16 ± 0.58	0.41 ± 0.44***	0.79 ± 0.54***	0.44 ± 0.44***
B	Control	35	24	7.26 ± 0.79	1.27 ± 0.87	2.12 ± 1.06	1.11 ± 0.58
	1.5 mg of AMP	25	19	7.43 ± 1.01	0.98 ± 0.91	2.14 ± 0.99	1.04 ± 1.01
C	Control	28	19	7.23 ± 0.85	3.40 ± 1.42	2.54 ± 1.32	2.21 ± 1.26
	1.5 mg of AMP	21	16	6.33 ± 1.10*	2.35 ± 1.60	2.47 ± 1.19	2.14 ± 1.27
D	Control	21	16	8.48 ± 0.72	2.75 ± 1.33	3.60 ± 1.74	2.90 ± 1.76
	1.5 mg of AMP	22	16	8.98 ± 0.58*	1.44 ± 0.53**	2.46 ± 0.78*	1.59 ± 0.68*
E	Control	15	11	7.57 ± 0.79	2.26 ± 1.14	3.18 ± 1.21	2.40 ± 1.43
	1.5 mg of AMP	18	14	8.03 ± 1.12	2.54 ± 1.61	1.71 ± 1.15**	1.32 ± 1.18
F	Control	28	21	7.46 ± 0.61	3.36 ± 1.78	3.35 ± 1.10	2.18 ± 0.72
	1.5 mg of AMP	21	16	7.16 ± 0.78	2.53 ± 1.45	2.53 ± 1.16	1.90 ± 1.09
Total (except volunteer A)	Control	127	91	7.60 ± 0.51	2.61 ± 0.88	2.96 ± 0.61	2.16 ± 0.65
	1.5 mg of AMP	107	81	7.59 ± 0.99	1.97 ± 0.71	2.26 ± 0.34	1.60 ± 0.44

[a] Abbreviations: AMP, ampicillin; STR, streptomycin.
[b] Mean ± standard deviation (SD) of log counts.
[c] Significance of the difference between control and experimental periods in the same individual: ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$ (Mann-Whitney test).

drug treatment. In experiments with mice, the Wilcoxon test (28) was used to compare the paired groups of treated and control mice.

RESULTS

Effects of antibiotics on resistant *E. coli* isolates in human feces. Except for volunteer D, total log counts of *E. coli* were not different from one individual volunteer to another and were affected little or not at all by antibiotic treatment (Table 2). In contrast, the mean proportions of resistant *E. coli* isolates differed among individuals. Daily administration of 15 mg of ampicillin and 15 mg of streptomycin significantly raised the concentration of *E. coli* resistant to the three drugs tested (e.g., the percentage of chlortetracycline-resistant *E. coli* in the feces of volunteer A increased from 0.0057 to 16%; the percentages were calculated from the means of 22 and 14 daily counts, respectively). The dose of 1.5 mg of ampicillin per day gave much less significant results. Thus, the population of resistant *E. coli* increased a little in two individuals (D and E) but did not change significantly either in the three other volunteers or in the pooled results for the five individuals who took 1.5 mg of ampicillin per day (Table 2).

Variations with time in the concentration and biotypes of *E. coli* in human feces. An example of typical kinetics of total and resistant *E. coli* in human feces (i.e., total and ampicillin-resistant *E. coli* in volunteer F; Fig. 1) indicates that the total *E. coli* concentration remained stable, whereas the resistant *E. coli* concentration varied with time. During certain periods there were no detectable resistant *E. coli* isolates, but peaks of resistance occurred from time to time. Similar kinetics were observed for all the volunteers. The mean number of days between two peaks of resistance was 9.3 ± 3.5 (a total of 31 periods were studied). The biotypes of the dominant resistant *E. coli* strains were determined from time to time in each individual, at least at each peak of resistance. For example, in Fig. 1 the biotype codes (Table 1) of the ampicillin-resistant *E. coli* isolates found in fecal samples from volunteer F are shown. At peak resistance, most of the 20 colonies that were randomly selected from the drug-supplemented plates belonged to a single biotype, which was mostly the same for the three plates containing ampicillin, chlortetracycline, or streptomycin. In this connection, there was a significant coefficient of correlation between the concentrations of *E. coli* isolates that were resistant to the three drugs per individual, because for the six volunteers, the median of 18 coefficients of correlation between the numbers of *E. coli* isolates that were resistant to the three drugs taken 2 by 2 was 0.87 ($P < 0.01$, in the r test of the Fisher test). This reflects the fact that most of the dominant resistant strains were triply resistant (Fig. 1).

The dominant biotype at one resistance peak was never the same as the biotype of the preceding or subsequent peak. By contrast, the dominant biotype of the resistant *E. coli* isolates remained the same from one day to the next during the period of decline following each peak. I never observed the same biotype simultaneously in different volunteers, although they worked together and lunched at the same cafeteria. The daily intake of 15 mg of ampicillin plus 15 mg

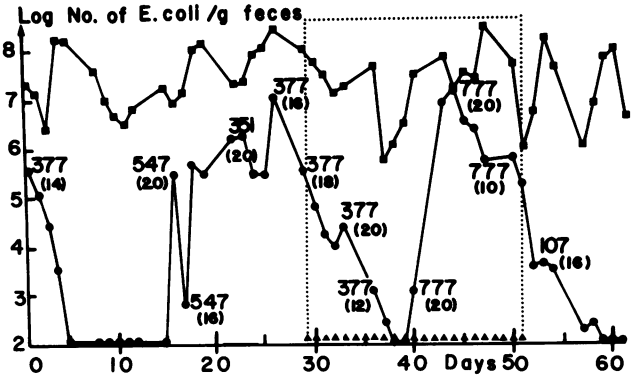

FIG. 1. Fecal *E. coli* isolates in a human volunteer (F) given 1.5 mg of ampicillin orally per day. Symbols: ■, total *E. coli*; ●, ampicillin-resistant *E. coli*; ▲, ampicillin administration. Three-digit figures indicate the biotype code of resistant strains (Table 1), and values in parentheses indicate the number of 20 randomly selected colonies that contained isolates of this biotype.

TABLE 3. Effect of ampicillin on the drug-resistant *E. coli* isolates in germfree mice associated with a human fecal flora

Human volunteer[a]	Ampicillin concn (μg/ml) in water	Total no. of *E. coli* isolates/g of feces (log ± SD)[b]	Log ± SD difference between total *E. coli* isolates and *E. coli* isolates resistant to[b]:		
			Ampicillin	Chlortetracycline	Streptomycin
A	0	7.19 ± 0.42	5.09 ± 0.86	4.64 ± 1.22	4.75 ± 0.75
	0	7.17 ± 0.43	5.04 ± 1.19	4.96 ± 0.89	5.08 ± 0.60
	0.5	7.09 ± 0.47	1.05 ± 0.66***	2.37 ± 0.94***	1.15 ± 0.68***
	8	7.33 ± 0.38	0.80 ± 0.52***	2.25 ± 0.60***	0.71 ± 0.53***
G	0	7.98 ± 0.39	2.83 ± 0.95	3.11 ± 1.32	Not done
	0.5	7.79 ± 0.35	3.68 ± 2.44	2.76 ± 0.60	Not done
	8	8.49 ± 0.50**	3.31 ± 1.80	1.49 ± 0.38**	Not done

[a] A total of 14 counts were made over a period of 35 days from the mice inoculated with the flora of volunteer A. A total of 16 counts were made over a period of 46 days with the flora of volunteer G.
[b] Different from control mice in the Wilcoxon test: ***$P < 0.001$; **$P < 0.01$.

of streptomycin induced the selection of a single resistant biotype in the feces of volunteer A (code 147), but the lower intake of 1.5 mg of ampicillin induced no such clear selection in any subject.

Drug intake in mice. Initial mean water intake was 4.15 ± 0.57 ml per mouse per day and did not change after antibiotics were added to the acidified water. Microbiological assays of the drinking water samples supplemented with 8 or 0.5 μg of ampicillin, chlortetracycline, or streptomycin per ml showed that the following amounts of drug were present (means expressed in micrograms per milliliter of water for more than 15 assays of each concentration): ampicillin, 7.4 ± 3.6 and 0.40 ± 0.17, respectively; chlortetracycline, 6.7 ± 0.6 and 0.43 ± 0.05, respectively; and streptomycin, 9.7 ± 1.7 (the method was not sensitive enough to test 0.5 μg of streptomycin per ml). The food intake of the mice was 4.5 g per day. In humans and mice, drug intake was comparable whether on the basis of the concentrations in the total diet (in parts per million) or of daily intake (in micrograms per kilogram of metabolic weight). In mice, drug intake ranged from 0.35 to 8.6 ppm (or 24 to 595 μg/kg of metabolic weight), and the volunteers were given 1 ppm of ampicillin (or 71 μg/kg of metabolic weight).

***E. coli* in control mice.** The concentrations of total and resistant *E. coli* isolates in the feces of germfree mice, which were inoculated with the dilution of human fecal flora and given drug-free drinking water, were not significantly different from those measured in the fecal samples from human donors ($P > 0.5$, in the Wilcoxon test) (Tables 3 to 5). The mean log of the total number of *E. coli* isolates was 7.69 ± 0.32 in human donors versus 7.52 ± 0.42 in recipient untreated mice. The difference between the logs of total and resistant *E. coli* (pooled results for the three antibiotics) was 2.65 ± 1.00 in humans versus 3.02 ± 1.56 in control mice.

On two occasions, 10 control, germfree mice dosed with human fecal flora from the same donors (A and B) were divided into two groups and kept without drug treatment in two separate isolators. No differences were found between the two control groups for any of the parameters measured (Tables 3 and 5).

Effects of antibiotics on mouse fecal *E. coli*. The total number of fecal *E. coli* isolates was not affected by the presence in the drinking water of 0.5 μg of any of the three drugs tested per ml. When the drug concentration was raised to 8 μg/ml, this number did not change either, except for a slight increase in two of six experiments (volunteer G, Table 3; volunteer H, Table 4). It was verified that these dominant *E. coli* isolates were fully susceptible to the antibiotics (data not shown).

Treatment with 0.5 μg of antibiotic per ml of water significantly raised the proportion of resistant *E. coli* isolates in 9 of the 17 situations tested. More precisely, at this concentration ampicillin increased triple resistance in the flora of volunteer A but not in that of volunteer G (Table 3), and chlortetracycline increased chlortetracycline resistance in the three flora tested with no effect on ampicillin resistance and a minimal effect on streptomycin resistance in volunteer H (Table 4). The pooled results of experiments with 0.5 μg of ampicillin or chlortetracycline per ml showed increased *E. coli* resistance levels, and the log difference

TABLE 4. Effect of chlortetracycline on the drug-resistant *E. coli* isolates in germfree mice associated with a human fecal flora

Human volunteer[a]	Chlortetracycline concn (μg/ml) in water	Total no. of *E. coli* isolates/g of feces (log ± SD)[b]	Log ± SD difference between total *E. coli* isolates and *E. coli* isolates resistant to[b]:		
			Ampicillin	Chlortetracycline	Streptomycin
G	0	7.89 ± 0.39	2.83 ± 0.95	3.11 ± 1.32	Not done
	0.5	8.24 ± 0.32	1.51 ± 0.28	1.43 ± 0.97***	Not done
	8	8.07 ± 0.42	3.51 ± 1.39	1.05 ± 0.47***	Not done
H	0	7.69 ± 0.77	Not done	3.44 ± 1.68	3.17 ± 1.55
	0.5	8.28 ± 0.48*	Not done	2.03 ± 0.68**	1.94 ± 0.77*
	8	8.81 ± 0.49***	Not done	2.22 ± 1.14***	2.31 ± 1.20*
A	0	7.17 ± 0.43	5.04 ± 1.19	4.96 ± 0.89	5.08 ± 0.60
	0.5	7.19 ± 0.35	5.15 ± 1.07	3.93 ± 1.44**	3.21 ± 1.22***

[a] A total of 16 counts were made over a period of 46 days from the mice inoculated with the flora of volunteer G. A total of 15 counts were made over a period of 35 days with the flora of volunteer H, and 14 counts were made over 35 days with the flora of volunteer A.
[b] Different from control mice in the Wilcoxon test: ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$.

TABLE 5. Effect of streptomycin on the drug-resistant *E. coli* isolates in germfree mice associated with a human fecal flora

Human volunteer[a]	Streptomycin concn (μg/ml) in water	Total no. of *E. coli* isolates/g of feces (log ± SD)	Log ± SD difference between total *E. coli* isolates and *E. coli* isolates resistant to:		
			Ampicillin	Chlortetracycline	Streptomycin
H	0	7.69 ± 0.77	Not done	3.44 ± 1.68	3.17 ± 1.55
	0.5	7.57 ± 0.81	Not done	4.55 ± 1.59	4.13 ± 1.46
	8	8.22 ± 0.62	Not done	0.75 ± 0.39[b]	0.60 ± 0.38[b]
B	0	7.17 ± 0.64	0.74 ± 0.55	1.97 ± 0.51	0.72 ± 0.60
	0	6.97 ± 0.70	0.94 ± 0.58	1.51 ± 0.58	1.00 ± 0.57
	0.5	7.43 ± 0.82	1.93 ± 0.92	2.49 ± 0.88	1.91 ± 0.77
	8	6.67 ± 1.04	0.27 ± 0.41[b]	1.53 ± 0.46	0.34 ± 0.35[b]

[a] A total of 15 counts were made over a period of 35 days from the mice inoculated with the flora of volunteer H. A total of 20 counts were made over a period of 32 days with the flora of volunteer B.
[b] Different from control mice in the Wilcoxon test ($P < 0.001$).

shifted from 4.05 ± 1.03 to 2.52 ± 1.27 ($P < 0.005$, in the Wilcoxon test), i.e., more than a 30-fold increase in resistance (calculation: 4.05 − 2.52 = log[33.9]). No enhancement of resistance was observed, however, with 0.5 μg of streptomycin per ml (Table 5).

The ingestion of 8 μg of any of the three drugs per ml augmented the proportion of resistant *E. coli* isolates in all flora tested (Tables 3 to 5). The exceptions were that none of the drugs affected *E. coli* resistance to ampicillin in the flora of volunteer G and that streptomycin had no effect on this resistance to chlortetracycline in the flora of volunteer B. Pooled results from Tables 3 to 5 show the significant effect of the drugs at 8 μg/ml, which shifted the log difference from 3.12 ± 1.36 to 1.51 ± 1.06 ($P < 0.005$, in the Wilcoxon test). This corresponds to a 40-fold increase in the percentage of resistant *E. coli*.

Variation with time in the number and biotypes of *E. coli* in mouse feces. In a typical illustration (Fig. 2) of the kinetics of total and resistant *E. coli* isolates in fecal samples from mice inoculated with human flora (i.e., *E. coli* isolates resistant to ampicillin in flora from volunteer A), the concentration of total *E. coli* remained stable (between log 7 and 8). The dominant biotypes (140 and 560) did not change during the experimental period and were the same in the different groups of mice inoculated with the flora of volunteer A. One of these biotypes (140) was dominant (in 7 of 10 colonies) in the fecal samples of the human donor which were transferred to the mice. The other biotype (560) was detected only 15 days later in the feces of volunteer A. Similar observations were made from other experimental curves (data not shown).

In the control mice represented in Fig. 2, the population of resistant *E. coli* decreased during the first week of drug-free water ingestion and then stabilized at the detection limit used in this study (between log 2 and 3), whereas in the experimental mice, which ingested water containing antibiotics, this population remained at a high level, although it never predominated over the susceptible population of *E. coli*. In these treated mice, one biotype (577), which was the dominant resistant strain in the feces of donor A at the start of the experiment, remained dominant throughout the experimental period. In all the experiments associating human flora with mice, similar decreases with time were observed in the number of resistant *E. coli* isolates in the feces of control mice, whereas the feces of treated mice always exhibited a large population of resistant *E. coli* isolates that was dominated by a single biotype and that persisted throughout the experimental period. This biotype was always the dominant resistant biotype in the human fecal samples used to inoculate the mice. The peaks of resistance and changes in the dominant biotype observed in human feces were not found in the isolated mice after the first week of stabilization of the resistant *E. coli* population.

DISCUSSION

Some of the main findings in this study resulted from daily monitoring of resistant *E. coli* isolates in human subjects. The data thus obtained show that (i) in the same individual the concentration of resistant *E. coli* isolates in feces varied between 10^8/g and an undetectable level below 10^2/g, and (ii) these drastic variations in the number of resistant *E. coli* isolates were due to changes in the resistant strain, as demonstrated by the changes in biotype (Fig. 1). The same changes have also been observed by other investigators (5, 12), but the mean duration of one strain in the gut was a little longer in this study than in those reported previously (9 versus 6.6 days). The frequent changes in the dominant

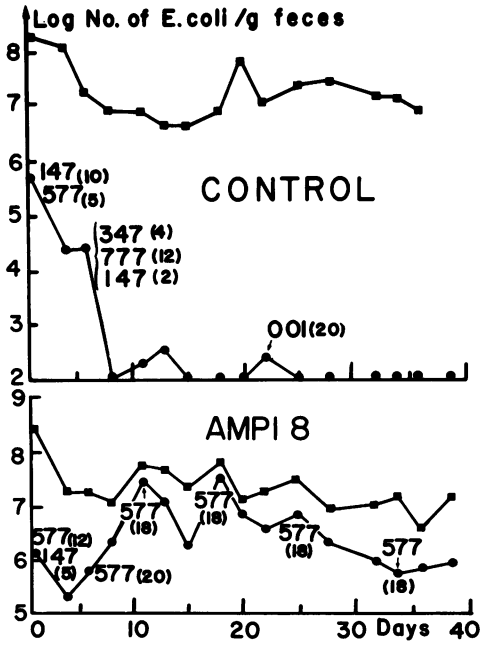

FIG. 2. Fecal *E. coli* isolates in groups of germfree mice associated with a human fecal flora (volunteer A) and continuously given 8 μg of ampicillin per ml of drinking water (AMPI 8) or no drug (CONTROL). For symbols, see the legend to Fig. 1.

resistant strain are probably due to bacterial contamination from food (19, 20) or from the environment, because they were not observed in isolated mice (Fig. 2). These factors may explain the reason that when a large number of people are checked once for the bacterial resistance levels in feces, the data obtained display a wide scatter (16, 19). The result is that in humans it is almost impossible to detect any minor change in the level of resistance due to the presence of antimicrobial residues. A new methodology is needed in this area.

Another purpose of this study was to demonstrate the effect of low levels of antibiotics in humans. The level of ampicillin tested (1.5 mg/day, which could correspond to the amount ingested in either 150 g of meat containing 10 ppm of residue or in 1.5 kg of total food containing 1 ppm of residue) did not have significant reproducible effects on the resistance of *E. coli* fecal flora (Table 2). Assays of ampicillin in the gut might have provided an explanation for the observed difference among the volunteers (Table 2), but this was out of the scope of this study. Because this antibiotic level is higher than that of the residues actually found in meat, the residues left by drugs used in veterinary practice or animal food supplements cannot be a major cause of the development of bacterial resistance in the human gut.

In previous studies attempts were made to transfer the microflora of human subjects to germfree mice and to verify the similarity between the floras of the donor and the recipient. Microscopic examination of these floras (14) and the study of microbial cultures of dominant anaerobes (1, 6, 25, 26) led to the general conclusion that the gross composition of the recipient mouse microflora resembles that of the fecal flora of the donor and remains the same for a few weeks after the transfer of the flora, provided that the mice are isolated from a microbial environment. This somewhat surprising similarity was also observed between the ecology of the gut and that of anaerobic continuous flow cultures of mixed populations of bacteria from mice (10) and of complex fecal flora from humans (23). The data obtained here in germfree mice associated with human gut flora confirm that the complex flora are able to reduce *E. coli* populations to similar levels in the gut of mice and in human donors (1, 14). Thus, when the same flora were given to separate groups of untreated mice, no difference in the resistant *E. coli* in feces could be found between the groups (volunteer A, Table 3; volunteer B, Table 5). This observation confirms what I found in previous work, namely, that there is no difference between the number of total and chlortetracycline-resistant *E. coli* isolates in five groups of mice dosed with the same complex flora and kept in separate isolators (3).

The gnotobiotic mouse is a suitable animal model for studying the effects of low levels of antibiotics, because it meets the following five prerequisites: (i) a low base line of resistant bacteria (Fig. 2); (ii) no difference between the flora of the different groups of animals at the start of the experiments; (iii) no contamination with resistant bacteria during the trial; (iv) a variation with time in the percentage of resistant *E. coli* that is lower than the variation in humans; and (v) a microflora that, according to previously published data (1, 6, 14, 25, 26), resembles human gut flora. In this study a significant increase in the fecal concentration of resistant *E. coli* isolates was observed in all the groups of mice given water containing 8 μg of antibiotic per ml and in more than half the groups given water containing 0.5 μg of antibiotic per ml. This animal model is therefore more sensitive to the effect of low levels of antibiotics than is its human counterpart, probably because it fulfills the five conditions cited above. Due to species differences that do not concern gut flora but that do concern drug metabolism, direct extrapolation to humans should not be considered as such. This animal model could be used for preliminary tests of new compounds, however, especially when their administration to humans is not permitted.

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