Summary. X-irradiation was followed by evidence of marked damage to mast cells in mesentery, skin and cheek pouch of rat and hamster. Daily intraperitoneal injection of pyrilamine maleate largely prevented damage to mast cells in the mesentery but not to those in skin and cheek pouch of the irradiated hamster. Pyrilamine maleate offered no protection against damage to mast cells in tissues of the irradiated rat. Intraperitoneal injections of promethazine hydrochloride in low dosage failed to protect mast cells against radiation damage in any tissue of the hamster. In high dosages this compound destroyed mast cells of mesentery of the nonirradiated hamster, but did not affect those of skin and cheek pouch.

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Effect of Tetracyclines on Formation of Amines by Bacteria.* (23906)

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Many intestinal bacteria are known to produce amines from amino acids(1). Melnykowicz and Johansson(2) found that rats fed small amounts of tetracyclines contained in their gastrointestinal tract less amines than control animals. They found that tetracyclines interfered with formation of some amines by the intestinal flora.

The effect of tetracyclines on formation of amines by single strains of bacteria isolated from human feces was therefore studied. Formation of isoamyl amine and isobutyl amine by *Proteus vulgaris* and of tyramine and phenyethyl amine by *Streptococcus fecalis* is the subject of the present communication.

Methods. Test organism and antibacterial assay. Str. fecalis and *Proteus vulgaris*, isolated from feces of patients suffering from gastro-enteritis were used. Sensitivity towards antibiotics was determined by 2-fold dilutions in nutrient broth (Difco). *Str. fecalis* was inhibited by 2 μg/ml oxytetracycline hydrochloride, tetracycline hydrochloride or chlortetracycline hydrochloride after 24 hours of incubation. *Proteus vulgaris* was inhibited by 50 μg/ml tetracycline hydrochloride or by 20 μg/ml chlortetracycline hydrochloride after 24 hours of incubation. *Culture media.* For amine production a casein hydrolysate medium of the following composition was used:

Casein hydrolysate vit. free (NBC) 10%	3	ml
Yeast extract (Difco)	.5	g
Glucose	.1	g
Sodium chloride	.5	g
Tryptophan	.002	g
Salt solution	1	ml
Distilled water to	100	ml

The salt solution was composed of: $MgSO_4 \cdot 7H_2O$ – 22.5 g, $CuSO_4 \cdot 5H_2O$ – 0.5 g, $ZnSO_4 \cdot 7H_2O$ – 0.4 g, $MnCl_2 \cdot 4H_2O$ – 0.15 g in distilled water to 500 ml pH adjusted to 5.5 and the medium autoclaved 20 minutes. For manometric experiments the strains were grown on brain heart infusion medium (Difco) with 1.5% agar. After incubation at 37°C for 14-18 hours the cells were harvested, washed 3 times with 0.85% sodium chloride solution and kept at –5°C until used. *Analytical methods. Determination of volatile amines by DNFB method.* Volatile amines were quantitatively determined by colorimetric method based on dinitro fluorobenzene (DNFB) reaction(3): 1 ml of a sample was alkanized by addition of 2 ml 40% potassium hydroxide solution and steam distilled

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TABLE I. Effect of Tetracyclines on Growth of *Proteus vulgaris*.

	Time (hr)				
	0	48	72	96	144
Control (no antibiotics)	5×10^{4}*	7×10^{7}	4×10^{7}	1.5×10^{7}	7×10^{6}
Chlortetracycline, 30 μg/ml	"	6 "	4 "	1 "	4 "
Tetracycline, 100 μg/ml	"	8 "	9 "	5 "	5×10^{7}

* No. of viable cells/ml of casein hydrolysate medium.

in a Markham still. The distillate was collected in a tube containing 0.5 ml of saturated sodium tetraborate solution. This was then treated with 0.25 ml of DNFB solution (0.65 ml of DNFB in 50 ml acetone), heated 10 minutes at 60°C and 0.5 ml of 5 N HCl added (4). After cooling to room temperature the yellow color was extracted with ethyl acetate and estimated in a Klett-Summerson photoelectric colorimeter at 420 mμ. *Paper chromatography.* Measured quantities of solution to be tested were applied to Whatman No. 1 filter paper. Butanol-acetic acid-water (2:1:1) was the solvent and the paper was sprayed with 0.2% ninhydrin in butanol. Quantitative determinations of the material on each spot were made(5). *Determination of amines by ion exchange method.* Amines were separated from amino acids by Amberlite IRC 50(6). The amines were eluted from resin by addition of 1 N H_2SO_4 and the eluate tested by paper chromatography or the DNFB method. *Manometric experiments.* The conventional Warburg method(7) was used for manometric determination of CO_2. Warburg flasks contained 50 mg freeze dried cells or cell-free preparation, 1 ml phosphate buffer pH 5.8 and 0.5 ml substrate. Total volume in each flask was 3.2 ml and the atmosphere was hydrogen to obtain anaerobic conditions. Freeze dried cells were prepared by drying over phosphorus pentoxide. Cell free preparations were obtained by shaking suspensions in a Mickle tissue disintegrator for 1 hour, and centrifuging at 11,000 rpm for 10 minutes.

Results. Effect of tetracyclines on formation of volatile amines by *Proteus vulgaris. Proteus vulgaris* was grown in casein hydrolysate medium in the presence of 30 μg/ml of chlortetracycline or 100 μg/ml of tetracycline. Growth appeared after 30 hours of incubation only. Samples were taken for the count of viable cells and for determination of volatile amines at different time intervals. After 48 and 72 hours incubation there was no significant difference in number of viable cells in the medium with or without chlortetracycline (Table I). Moreover, in the presence of tetracycline, the number of surviving bacteria after 72 and 144 hours was higher than in the control.

In the presence of antibiotics, formation of volatile amines was markedly depressed (Fig. 1). After 6 days, about a quarter of the control amount of volatile amines was formed in the presence of 30 μg/ml chlortetracycline and about a half in the presence of 100 μg/ml tetracycline. Quantitative paper chromatography examination showed that both volatile amines produced, isoamyl amine and isobutyl amine, were affected in the same degree.

Effect of tetracyclines on formation of aro-

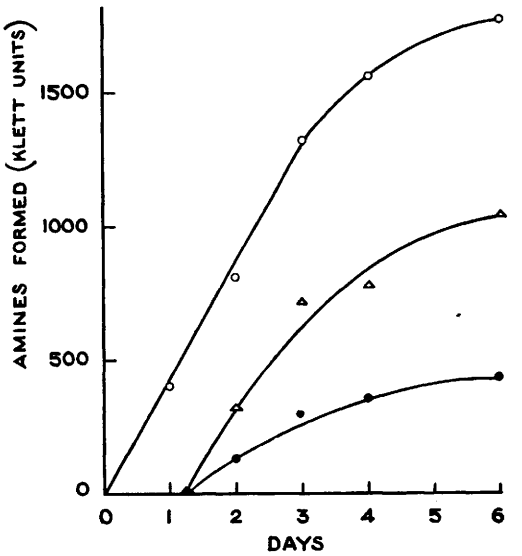

FIG. 1. Effect of tetracyclines on formation of volatile amines by *Proteus vulgaris*. Bacteria grown in casein hydrolysate medium —○—; casein hydrolysate with 30 μg/ml chlortetracycline —●—; or casein hydrolysate with 100 μg/ml tetracycline —△—. Concentrations of amines expressed in Klett units.

TABLE II. Effect of Tetracycline on Decarboxylation of Phenylalanine and Tyrosine by Freeze Dried Cells of *Streptococcus fecalis*.

	Tetracycline, μg/ml				
	1700	170	17	1.7	0
Phenylalanine	0*	17	60	70	77
Tyrosine	39	196	221	215	224

* μl CO_2 evolved/hr.

matic amines by *Streptococcus fecalis*. Tetracyclines in subinhibitory concentrations did not affect formation of aromatic amines by *Str. fecalis* grown on casein hydrolysate medium. High concentrations of tetracyclines, however, inhibited formation of these amines by dried cells or cell free preparations. Manometric determinations showed that 1700 μg/ml tetracycline depressed decarboxylation of tyrosine and 170 μg/ml inhibited decarboxylation of phenylalanine (Table II). Chlortetracycline and oxytetracycline in these concentrations exhibited a similar effect. These findings were confirmed chromatographically.

Discussion. Metchnikoff(8) assumed that bacterial action on proteins in the gut produced harmful amines "ptomaines" which led to enterotoxemia. Evidence is now growing in favor of this view(9). Growth promoting effect of subinhibitory amounts of antibiotics given to young animals might be due to depression of bacterial amine formation(10). The effect of subinhibitory concentrations of tetracyclines on formation of volatile amines by *Proteus vulgaris* might be of clinical interest, even though the effect of isoamyl amine and isobutyl amine on the host has not yet been elucidated. The enzymes responsible for formation of isoamyl amine and isobutyl amine by *Proteus vulgaris* are adaptive(11) and interference of subinhibitory amounts of tetracyclines with formation of some adaptive enzymes has been described(12).

Inhibition of amines produced by *Str. fecalis* has to be looked at from a different point of view. Concentrations of amines about 500 times greater than that necessary for growth inhibition were required to depress formation of phenylethyl amine and tyramine. These high concentrations of antibiotics interfered not only with decarboxylation of tyrosine but also with its deamination(13). Phear and Ruebner(14) found that subinhibitory amounts of tetracyclines had no effect on formation of amines by *Proteus mirabilis*, and had little effect on their formation by *Str. fecalis*. These authors employed a different technic than that used by us.

Our findings that subinhibitory concentrations of tetracyclines slowed down rate of death of cells of *Proteus vulgaris* are in agreement with similar observations reported for chloromycetin(15).

Summary. Tetracyclines in subinhibitory concentrations depressed formation of isoamyl amine and isobutyl amine by *Proteus vulgaris*. High concentrations of tetracyclines inhibited formation of tyramine and phenylethyl amine by *Str. fecalis*.

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