ANTIBACTERIAL MECHANISMS OF THE MOUSE GUT

II: THE ROLE OF EH AND VOLATILE FATTY ACIDS IN THE NORMAL GUT

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WHEN *Salmonella typhi-murium* is administered to normal mice by mouth, the number of viable organisms recovered from the gut steadily falls following their inoculation. In streptomycin-treated mice, on the other hand, the viable count rises to a maximum of *ca.* 10^9 per gut, and the average lethal dose is reduced from $>10^6$ to 1 organism. Streptomycin therefore abolishes a mechanism responsible for elimination of salmonellae from the gut which, the kinetics suggest, is weakly bacteriostatic and weakly bactericidal but is nevertheless present in every mouse to some extent (Meynell and Subbaiah, 1963).

The explanation favoured here is that inoculated salmonellae fail to multiply in the normal mouse gut because of the combined inhibitory effects of a low oxidation-reduction potential (Eh) and short-chain fatty acids produced by the normal gut flora. The predominant organism in the caeca of the mice used in these experiments were fusiforms, known to be strict anaerobes, which produce acetic and butyric acid in concentrations sufficient to inhibit division of inoculated salmonellae and possibly to kill them at a low rate. Most of the inoculated organisms rapidly reach the caecum where they stay for 0–3 hr. and then pass into the colon. There the gut contents are progressively dehydrated so that conditions become steadily less favourable to division. After perhaps 2 hr. in the colon, the organisms are voided. Streptomycin virtually abolishes the caecal flora and although residual fatty acid remains, its antibacterial effect is rendered insignificant by dilution and by a rise in pH. Moreover, the Eh increases by 0·4 V. to +0·2 V. so that conditions in the caecum approximate to those of an aerobic broth culture, and the true division rate increases. Thus, not only do the organisms encounter far lower concentrations of inhibitor but division is encouraged. These changes appear sufficient to account for the profound effect of streptomycin. It is supposed that the outcome of oral challenge is decided in the caecum and that if the organisms do not establish themselves there, they fail to do so in other parts of the gut. The significance of short-chain fatty acids in determining the fate of ingested organisms has been extensively discussed by Bergeim and his colleagues but the changes in Eh have not been previously reported.

MATERIALS AND METHODS

Most have been described in the preceding paper.

Counts.—Viable counts were made by spreading 0·1–0·2 ml. of diluted culture on peptone agar plates as well as by a drop method (Miles and Misra, 1939). Viable enterobacteria were

counted on McConkey agar. Total counts were made in a Helber chamber and turbidities measured nephelometrically (Evans Electroselenium Ltd., Halstead, Essex).

Films.—A loopful of the caecal contents suspended in 10 ml. buffer was spread over an area of *ca.* 1·5 sq. cm. on a slide, dried in air, heat-fixed, cleared with acetic acid and ethanol (Eyre, 1930) and stained by Gram's method.

pH and Eh measurements.—The pH of the caecal contents with *ca.* 1 ml. saline added was measured with a hanging-drop electrode system (Doran Instrument Co. Ltd., Stroud, Glos.). In measuring Eh, the caecum was exposed *post mortem*, clamped off from the colon and ileum, and 0·75 ml. freshly autoclaved distilled water injected into the lumen to suspend the contents which were dispersed by compression of the caecal wall. The contents were then tipped into a plastic cup, capacity 2 ml., transferred to a chamber gassed with nitrogen containing 0·001 per cent oxygen (" oxygen-free nitrogen " : British Oxygen Co.) and brought under a Ag/ $AgCl_2$ reference electrode and a platinum foil electrode (area of 0·5 sq. cm.). Potentials were measured with a null-point potentiometer (Cambridge Instrument Co.) and usually became constant in 3–8 min. and never took longer than 15 min.

Antibacterial action of fatty acids.—The fatty acids or their sodium salts were made up in 2 M solutions, adjusted to pH 7·2, and sterilized by autoclaving at 10 lb. per sq. in. for 10 min. For tests, 3 ml. fatty acid solution was mixed with 6 ml. buffer and 3 ml. culture medium (either tryptic broth or 4 × normal strength Oxoid No. 2 Nutrient Broth adjusted to pH 7 when necessary) in a 6 × $\frac{1}{2}$ in. glass tube closed with a metal cap. Tubes for anaerobic cultures also contained either (*a*) 0·01 ml. thioglycollic acid and were heated to 100° for 5 min. and covered with 1·5 ml. liquid paraffin ; after cooling the tubes were incubated in a McIntosh and Fildes jar in oxygen-free hydrogen ; or (*b*) 0·02 ml. thioglycollic acid and were covered with 3 ml. paraffin after heating to 100° for 5 min. : these tubes were incubated in air. Either method produced a medium that completely reduced indigo di-sulphonate ($E_0 = -0{\cdot}125$ V. at pH 7) so that the Eh was $< -0{\cdot}185$ V. initially. The buffer at pH 7 was 0·1 M sodium glycerophosphate, and at pH 7·5, 0·05 M sodium glycerophosphate with 0·05 M tris. Both buffers were adjusted with concentrated HCl and sterilized by autoclaving.

Chemical analyses.—The caecal contents were suspended in distilled water (5 ml./caecum) and the solids removed by 2 centrifugations at mark 5 for 15 min.

Volatile fatty acids were estimated after steam distillation of *ca.* 12·5 ml. aqueous caecal extract added to 3 ml. 30 per cent H_2SO_4 and *ca.* 5 g. Na_2SO_4. Total volatile acids were titrated with 0·1 N NaOH using phenolphthalein. Total fatty acids were also estimated by extracting the acidified aqueous extract 6 times with 10 ml. di-ethyl ether, concentrating the extract by evaporation and titrating with 0·1 N NaOH. This method usually failed owing to the passage of relatively large amounts of H_2SO_4 into the ether. The composition of distillates was determined by chromatography on Whatman No. 1 paper using *iso*-propanol saturated with 2 N NH_4OH as solvent and spraying after 24 hr. with alcoholic bromo-cresol purple with development in ammonia vapour (Reid and Lederer, 1952). Known acids were included in each run.

Ninhydrin-positive material was estimated by heating 3 ml. diluted aqueous caecal extract at 100° for 15 min. with 1 ml. ninhydrin solution (0·4 g. ninhydrin + 0·06 g. hydrintantin in 15 ml. 2-methoxy ethanol mixed with 5 ml. 4 M sodium acetate, pH 5·5: Moore and Stein, 1954).

RESULTS

Enterobacteria in the Normal Mouse Caecum

One of 10 mice examined yielded no viable enterobacteria ($<5 \times 10^2$/caecum) and the other 9 contained between $5{\cdot}5 \times 10^4 - 9 \times 10^7$/caecum. Since the corresponding number of salmonellae was between $1{\cdot}8 \times 10^7$–$1{\cdot}5 \times 10^9$ in 6 streptomycin-treated mice counted 2 days after challenge, the growth of normal enterobacteria was probably partly suppressed. This supposition was confirmed by inoculation of two strains of *Escherichia coli*, made streptomycin-resistant after isolation from 2 of the above normal mice, into 3 mice given streptomycin 24 hr. before. Two days later the viable count of the coliforms in the caecum was $3{\cdot}6 \times 10^8 - 4{\cdot}1 \times 10^9$, values far higher than those in normal mice and similar to

those of salmonellae in streptomycin-treated mice. These results suggest that enterobacteria, like inoculated salmonellae, are inhibited in the normal mouse caecum.

Comparison of normal and streptomycin-treated mice

At *post mortem*, the caecum was obviously unusual in appearance if streptomycin had been given 24 hr. earlier. Normally, it is relatively small, tan-coloured, and filled with a brown paste. After streptomycin, however, the caecum was nearly always enlarged and filled with gas and greenish-yellow liquid. The difference

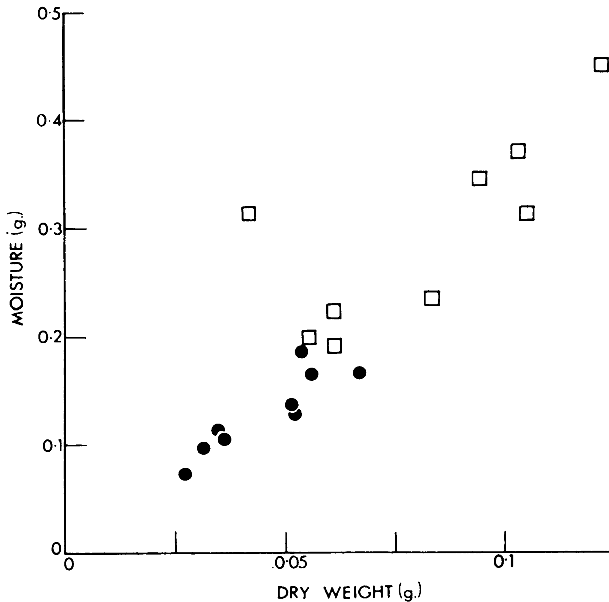

Fig. 1.—Showing the increase in moisture in the caeca of streptomycin-treated (□) as compared with normal mice (●); the mean volume of liquid being estimated as 0·325 ml. and 0·125 ml. respectively.

can be measured by determining the content of solids and moisture by weighing (Fig. 1). The mean volume of liquid per caecum 24 hr. after streptomycin was *ca.* 0·325 ml., nearly 3 times the normal of *ca.* 0·125 ml., and the ratio of liquid/solid was slightly increased.

Striking differences were also obvious when the caecal contents were examined in Gram films. The normal caecum contains very large numbers of fusiforms of varying sizes; relatively few lactobacilli, coliform-like organisms, Gram-positive cocci and bacilli (including spore-formers); and scanty yeasts. The bacterial concentration is so high that a density satisfactory for films is only obtained by suspending the caecal contents in 10 ml. diluent. After streptomycin, all these organisms have disappeared save extremely scanty Gram-positive bacilli, presumably arising from spores, and occasional yeasts.

The change in flora markedly affects the pH and Eh of the caecal contents (Fig. 2). The mean pH rises after streptomycin from *ca.* 7·0 to *ca.* 7·5 while the mean Eh increases from *ca.* —0·2 V. to *ca.* +0·2 V. The change in Eh is easily

detected if the caecum is clamped off from the colon and ileum *post mortem* and injected with 0·2 ml. of 0·02 per cent solution of methylene blue. The dye remains blue in treated mice but is decolourized in 1–2 min. in a normal caecum, and, when the caecal contents are tipped into a watch-glass, the surface exposed to the air rapidly turns blue while the layer next the glass remains brown.

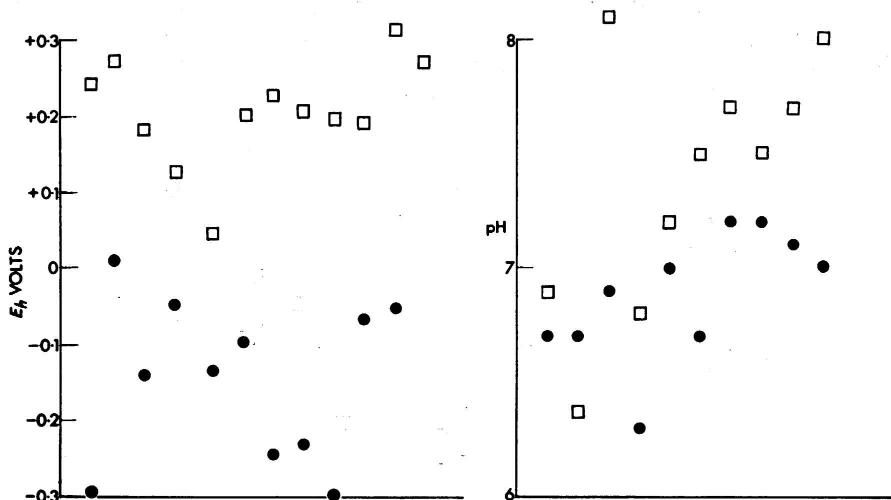

FIG. 2.—The oxidation-reduction potential (Eh) and pH of the caecal contents of normal (●) and streptomycin-treated (□) mice. Each point is the value for one animal.

The change in flora does not seem to affect the amount of nutrient in the caecum, judging from the amount of ninhydrin-positive material present in aqueous extracts. The concentration in the caeca of treated and normal mice was almost equal and was equivalent to a 2·5–3·5 per cent solution of Evans peptone as calculated from a visual comparison of the intensities of the ninhydrin reactions of the diluted extracts with those of various dilutions of peptone water, and using the mean volumes for caecal liquid obtained from Fig. 1. Chromatograms showed traces of glucose and possibly fructose and galactose, the spots being markedly fainter in the samples from normal mice.

Fatty acids

The concentration of volatile acids in the normal caecum was *ca.* 0·2 N (Table I). These are minimum estimates since the determination of free liquid in the caecum by weighing probably gave an unduly high figure for the volume occupied by fatty acids since it includes liquid within food particles and bacteria. Volatile fatty acids probably comprised almost the entire fatty acid present in the acidified aqueous caecal extracts for, although controls showed most of the estimates of total acid to be grossly misleading as noted already, titrations of the distilled and the ether-extracted preparations of 3 samples were essentially equal. The normality of volatile acids fell about 10-fold after giving streptomycin.

Chromatography of distillates showed spots corresponding to the volatile acids, acetic, butyric and propionic (in order of decreasing intensity in each sample), the intensity being greater for samples from normal than from streptomycin-

TABLE I.—*Estimated Concentration of Volatile Acids in Individual Caeca*

Normal Mice

	Experiment 1	2	3	4	Mean
Volatile acids*	0·36	0·30	0·26	0·18	0·28
Estimated normality in caecum	0·29	0·24	0·17	0·14	0·21

Mice given 50 mg. Streptomycin 24 hr. previously

	Experiment 1	2	3	4	Mean
Volatile acids*	0·12	0·054	0·044	0·037	0·064
Estimated normality in caecum	0·036	0·017	0·013	0·011	0·019

* As ml. 0·1 N NaOH. Mean of a pool of 6 caeca. Mean volume of water in caeca of normal and streptomycin-treated mice taken as 0·125 ml. and 0·325 ml. respectively (Fig. 1). As the volatile fatty acids are mono-carboxylic, the normality and molarity of their solutions are equal.

treated mice. This result is to be expected since the human gut with a similar flora also contains considerable quantities of acetic and butyric acids (Olmsted, Duden, Whitaker and Parker, 1930 ; Bergeim, 1940).

The antibacterial effects of the short-chain fatty acids have been discussed by many authors and the consensus of opinion is that their action is due to the undissociated molecule, not the free acid (Winslow and Lochridge, 1906 ; Reid, 1932 ; Hoffman, Schweitzer and Dalby, 1939 ; Levine and Fellers, 1940 ; Albert, 1951). If this be so, their activity will be profoundly affected by pH since this determines the degree of dissociation of the acid. Thus, in a solution of acetic acid (pK = 4·73), the percentage of the total acid dissociated at pH 6·5, 7·0 and 7·5 is 1·56, 0·5 and 0·13 per cent respectively. A rise in pH from 7·0 to 7·5 is therefore equivalent to a 4-fold reduction in the concentration of undissociated acid. The corresponding figures for butyric acid (pK = 4·83) are 1·96, 0·6 and 0·2 per cent. The rise in pH occurring after administration of streptomycin and the greatly reduced normality of acid (Table I) therefore produce about a 40-fold reduction in the concentration of undissociated acid in the caecum.

The effect of these acids on the viable counts of cultures in buffered broth (*i.e.* on the net rate of change : Meynell and Subbaiah, 1963) is evident from Table II where the entries are the ratio of the counts at the times stated/initial count. In this and the succeeding experiments, the inoculum came from an unaerated overnight broth culture. Two sets of conditions were used : sealed cultures containing thioglycollate at Eh $< -0{\cdot}185$ V. and pH 7·0 ; and open cultures at Eh $> +0{\cdot}15$ V. and pH 7·5 to simulate the conditions in the caeca of normal and treated mice respectively. The counts show, almost invariably, that the higher the concentration of fatty acid, the smaller the increase in viable count, which may indeed be less than the initial figure, and that the increase in viable count is less at low than at high Eh. Butyric acid is more inhibitory than acetic acid. A similar experiment was done with lactic acid (pK = 3·86), a non-volatile fatty acid likely to be present, but even 0·5 M only slightly delayed the increase in turbidity of the culture. Killing is far more rapid at lower pH which are not lethal in themselves (Meynell, 1955). The viable count fell to 10 per cent or less of the initial value in the mixture of acetic, butyric and propionic acids (total M = 0·5) after 2 hr. at pH 4·5 although it increased 1·4 fold in 4 hr. at pH 7·5.

TABLE II.—*Effect of Short Chain Fatty Acids on the Increase in Viable Count in Aerobic and Anaerobic Cultures*

Time (hr.)	Initial Eh(V.)	Moles of acid 0·5	0·25	0·125	0·06	Nil
		Acetic acid				
4	>+0·15*	2·02‡	4·6	9·8	14·5	17·1
4·5	<−0·17†	0·87	3·2	7·7	9·2	10·4
25	>+0·15	12·8	18·9	59·2	60·6	46·7
25·5	<−0·17	0·47	37·6	37·5	36·3	41
		Butyric acid				
4	>+0·15	0·49	2·63	8·8	12·0	18·3
4·5	<−0·17	0·89	0·78	3·18	7·15	13·3
25	>+0·15	0·35	20	49	83·6	75·0
25·5	<−0·17	0·38	2·56	24·7	32·2	37·3
24	>+0·15	0·21	26	—	—	30
24	<−0·17	0·33	0·49	41	—	62·8
		Propionic acid				
4	>+0·15	0·93	3·1	9·5	14·4	14·4
4·5	<−0·17	0·8	0·82	0·92	1·2	4·8
24	>+0·15	1·1	12·0	40·7	42·9	26·4
24·5	<−0·17	0·25	0·68	2·7	6·0	10·7
		Mixture of acids (Molar ratios of acetic : butyric : propionic = 7 : 2 : 1)				
3·75	>+0·15	2·5	—	16·4	17·7	26·7
3·75	−0·03	0·5	1·2	1·6	3·3	4·1
24	>+0·15	15·5	—	—	—	88
24	−0·03	3·8	32·4	—	—	50
24	>+0·15	29	85	—	—	50
24	<−0·17	0·57	19·5	—	—	26
		Lactic acid				
24	>+0·15	46·3	—	—	—	30
24	<−0·17	28·2	—	—	—	62·8

* Toluylene blue not reduced (E_0' at 30°, pH 7·5, = +0·1 V.).
† Indigo di-sulphonate reduced (E_0 at 30°, pH 7·0, = −0·125 V.).
‡ Each entry is the viable count at the stated time/initial viable count, usually *ca.* 5 × 10^6/ml.

Table II shows that fatty acids are bactericidal but it was also necessary to demonstrate bacteriostasis if these acids were to be held responsible for the changes observed *in vivo*. Bacteriostasis is not evident from Table II since a halving of the viable count in 4 hr. (net rate of change of −3) could result, to take the extreme cases, either from division at the maximum true rate observed *in vitro* of +24 with a killing rate of −27 ; or from a true division rate of 0 (*i.e.* complete bacteriostasis) with a killing rate of −3. The second possibility corresponds more closely to conditions in the normal gut since the estimated value for the true division rate was 0 to +2 and for the killing rate, −4·9 (Meynell and Subbaiah, 1963). However, bacteriostasis by fatty acids was demonstrable when cultures similar to those shown in Table II were examined in more detail.

An overnight culture was diluted 1 in 13 in broth at high or low Eh, and at the corresponding pH, containing either 0·5 M acetic acid, 0·5 M butyric acid, 0·25 M

of each, or a mixture of these and propionic acid (0·7, 0·2 and 0·1 M respectively). Colony counts were made initially and after 4 hr. incubation, and the turbidity recorded from 0 to 4 hr. as a measure of cell growth (Fig. 3). The turbidity increased in 3 of the 4 aerobic cultures containing fatty acid, the exception being that containing 0·5 M butyric acid, but not in any of those incubated anaerobically.

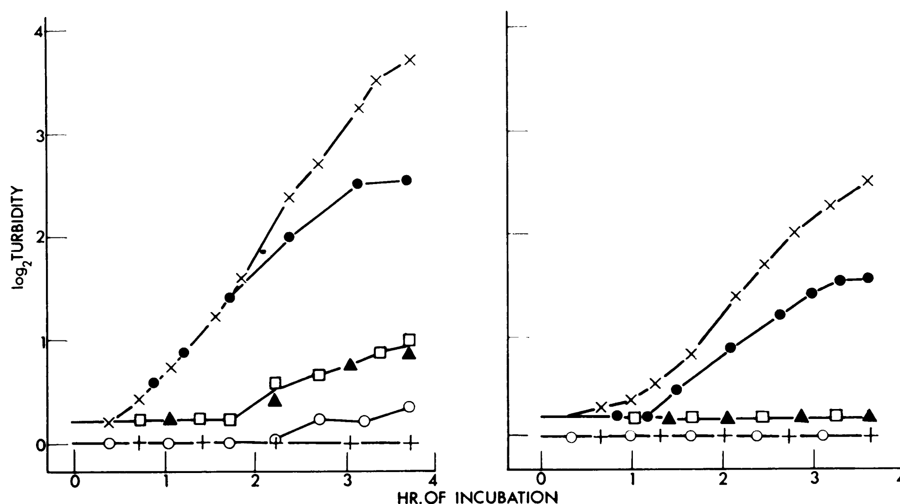

FIG. 3.—Changes in turbidity of aerobic cultures at pH 7·5 (Fig. 3*a*) and anaerobic cultures at pH 7 (Fig. 3*b*). Viable counts were done initially and after 4 hr. incubation with the following results :

Medium	Count at 4 hr./Initial count	
Oxoid No. 2 Broth *plus*	Aerobic	Anaerobic
× 0·4 per cent glucose	11·1	9·0 †
● No additions	5·0	3·8 †
□ Acetic, butyric and propionic acids*	1·43	0·81
▲ 0·5 M acetic acid	1·23	0·66
○ 0·25 M acetic + 0·25 M butyric acids	1·02	0·74
+ 0·5 M butyric acid	0·86	0·67

* The mixture contained 0·35, 0·1 and 0·05 M respectively of each acid.
† From the increase in turbidity.

The viable count of the anaerobic cultures fell to *ca.* 70 per cent of the initial value so that there had been complete bacteriostasis with slight killing (see legend to Fig. 3). The same is true of cells incubated aerobically in 0·5 M butyric acid. In other experiments, cultures in medium containing 0·25 M acetic acid + 0·25 M butyric acid incubated either aerobically or anaerobically did not change in viable or total count during 4 hr., showing that complete bacteriostasis could also occur without killing. Thus, the short-chain volatile fatty acids produced stasis and cell death and therefore behaved like the natural antibacterial mechanism of the gut whose properties were measured by Meynell and Subbaiah (1963).

Various concentrations of the mixture of the three acids were examined for their effect on the rate of increase in turbidity (Fig. 4). The curves do not necessarily correspond to the course of the viable counts as killing and division may occur together but it is clear that growth was delayed by 0·25 M fatty acid in both anaerobic and aerobic cultures. Since the excretion rate of the gut contents is —7·5 in normal mice (Meynell and Subbaiah, 1963), the viable count

of inoculated organisms can only increase if their net rate of change is more that +7·5. As the true division rate in anaerobic broth cultures without fatty acid may be only +8 (= a doubling time of 90 min. : Fig. 3), even slight inhibition by fatty acid may determine whether or not inoculated organisms increase in the gut.

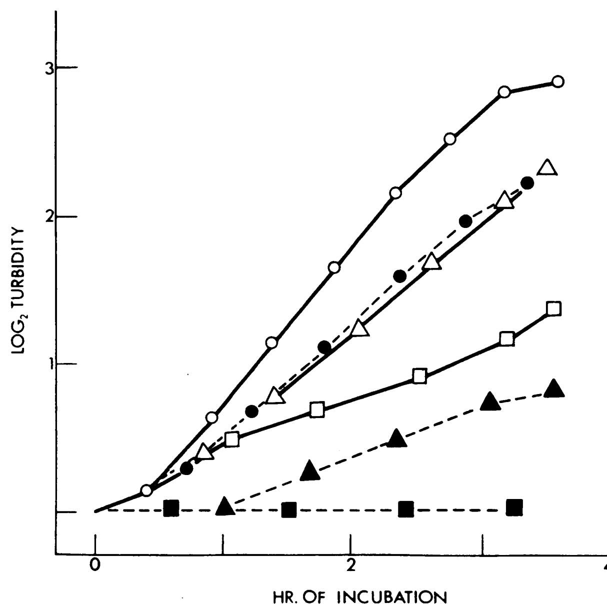

FIG. 4.—Changes in turbidity of aerobic cultures at pH 7·5 (open symbols) and anaerobic cultures at pH 7 (solid symbols) in Oxoid No. 2 Broth (○, ●) containing 0·25 M (△, ▲) and 0·5 M (□, ■) of a mixture of acetic, butyric and propionic acids (molar ratios = 7 : 2 : 1). The curves for 0·125 M or less were the same as those of the controls.

All these experiments show the significance of Eh and fatty acid in determining the outcome of challenge. The controls also show how the true division rate may be increased by glucose. It follows that, without far more precise knowledge of the composition of the gut contents, it is pointless to press reconstruction experiments too far.

DISCUSSION

The joint effects of low Eh and short-chain volatile fatty acid appear to account satisfactorily for the failure of inoculated salmonellae to increase in the normal mouse gut and also for their proliferation after streptomycin has been given. This conclusion rests not only on the demonstration that these acids have an antibacterial action in anaerobic cultures but that it is of the order of magnitude suggested by the kinetics of infection in normal mice. Bacteriostasis is evident from Figs. 3 and 4 which also show moderate killing. The observed survival of *ca.* 70 per cent after 4 hr. exposure to fatty acid *in vitro* corresponds to a killing rate of −1·48, assuming an exponential process, which agrees reasonably well with the rate of −4·8 *in vivo* considering the nature of the experiment (Meynell and

Subbaiah, 1963). It should be recalled that it suffices for the mouse if bacterial division is prevented for only a matter of hours, since the gut contents are voided fairly rapidly.

The role of fatty acids and H_2S in immunity to oral challenge was pointed out by Bergeim who showed that bakers yeast fed to either man or dogs was killed principally in the large intestine since a large proportion of the dose was often recovered from the caecum. Growth of the yeast was inhibited by faeces (Montgomery, Boor, Arnold and Bergeim, 1931) due to its content of hydrogen sulphide, yeasts being relatively resistant to fatty acid (Bergeim, Hanszen, Pincussen and Weiss, 1941). The presence of considerable amounts of these acids was noted, however, and were shown to kill and inhibit many bacterial genera, including salmonellae (Bergeim, 1940). At that time, it was, of course, impossible to examine their importance by removing the normal gut flora, an experiment first recorded by Bohnhoff, Drake and Miller (1954).

These findings very probably apply also to man on a free diet whose faecal flora resembles that of mice in consisting largely of fusiforms (see Wilson and Miles, 1955 ; Zubrzycki and Spaulding, 1962).

A factor not previously noted is the effect of streptomycin on the Eh of the gut contents. This is normally *ca.* —0·2 V., as expected from the abundance of anaerobes, but rises to *ca.* +0·2 V. in treated mice, presumably because the sterilized contents come to equilibrium with the tissues in which the Eh has a positive value such as +0·246 to +0·126 V. (venous blood ; Hanke and Tuta, 1928), +0·12 V. (subcutaneous tissue ; Fildes, 1929) and +0·119 V. (bone marrow ; Soru and Brauner, 1931). An analogous observation is that the mean Eh in the caecum of germ-free guinea-pigs is —0·09 V. compared with —0·367 V. for normal animals (Phillips, Wolfe and Bartgis, 1958). The caecum is therefore not intrinsically anaerobic but becomes so owing to the reducing activity of its flora, the predominance of anaerobes presumably being due to selection.

The mechanism of natural immunity to oral challenge described here differs completely from that suggested by Freter in his studies of antibiotic-treated animals intentionally repopulated by species such as *Proteus vulgaris* or *Aerobacter aerogenes* and subsequently challenged by *Shigella flexneri* (Freter, 1956*a*, *b*, 1962 ; Hentges and Freter, 1962). Freter concludes from these experiments and from others conducted *in vitro* that " *in vivo* antagonism between the micro-organisms tested was based on competition for carbon sources utilizable in a highly reduced medium " (Freter, 1962). Thus, while inoculated pathogens are here thought to be inhibited in the normal gut, Freter suggests that, in the highly artificial conditions of his experiments, they are in effect starved of nutrient, fail to divide, and are therefore eliminated in the faeces. There are several arguments against Freter's hypothesis being applicable to normal animals : an appreciable proportion of mice and guinea-pigs do not contain enterobacteria, the only organisms tested by Freter (Formal *et al.*, 1961) ; when enterobacteria are present, their growth is suppressed ; the kinetics suggest the presence of a bactericidal mechanism ; the normal caecum contains considerable amounts of fatty acid which has been shown to be bactericidal and bacteriostatic, and also considerable nutrient since the liquid is equivalent to a 2–3 per cent solution of peptone and traces of glucose and other sugars are present. The last observation suggests that the growth of the normal flora is controlled, not by lack of nutrient, but by their metabolic products, including fatty acids.

It does not follow, of course, that fatty acid and anaerobiosis are the only factors that may inhibit ingested organisms : some colicinogenic strains of *Escherichia coli* can kill salmonellae (Fredericq and Levine, 1947) ; some organisms are susceptible to hydrogen sulphide produced in the normal gut (Bergeim *et al.*, 1941) ; and acrylic acid present in algae ingested by arctic animals determines the composition of their intestinal flora (Sieburth, 1961).

SUMMARY

The caecal contents of normal mice contain considerable quantities of volatile fatty acid at an Eh of *ca.* −0·2 V. A similar environment *in vitro* inhibits the growth of *Salmonella typhi-murium* and is weakly bactericidal. When the normal bacterial flora, consisting largely of obligate anaerobes, is eliminated by giving streptomycin, the Eh rises to *ca.* +0·2 V. and the concentration of volatile fatty acid falls, so producing conditions favourable to the growth of salmonellae. The joint action of Eh and these acids resembles that of the antibacterial mechanism previously identified in normal mice and is sufficient to account for their ability to eliminate salmonellae given by mouth and for their greatly increased susceptibility to infection after administration of streptomycin.

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