THE EFFECT OF ANTIBACTERIAL DRUGS ON THE FECAL FLORA OF MICE*

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As reported in preceding papers, the NCS colony of Swiss mice developed at the Rockefeller Institute is characterized by a fecal flora poor in enterococci and Gram-negative bacilli, but very rich in lactobacilli (1, 2). This fecal flora naturally changes when newborn NCS animals are exposed to ordinary mice. Unexpectedly however, it remains essentially unchanged when adult NCS mice are placed in a room containing contaminated animals, provided the NCS mice are fed an adequate diet and are not subjected to physiological disturbances. In contrast, as will be shown in the present paper, the composition of the flora changes profoundly when the animals are treated with certain antibacterial drugs. Furthermore, the nutritional regimen markedly affects the extent and duration of the changes in the fecal flora induced by these drugs. Similar effects, although quantitatively less striking, have been observed with other Swiss mice possessing an intestinal flora more complex than that of NCS animals.

Materials and Methods

Animals from two different colonies of so called Swiss mice were used in these experiments, namely NCS and Ha/ICR. The NCS animals were produced at The Rockefeller Institute. Their origin, maintenance and characteristics have been reported in several previous papers (1, 2). The Ha/ICR mice, also derived from the original colony of "Swiss" mice, are produced under ordinary conditions; they were obtained from the Millerton Research Farm, Millerton, New York. Only male mice were used in the present study, they were approximately 4 weeks old at the beginning of each experiment.

The diets used are stated for each particular experiment; their composition was described in earlier publications (3, 4). In brief they were of three different types: (*a*) commercially produced pellets (D&G) distributed by Dietrich and Gambrill, Frederick, Maryland; (*b*) commercial white wheat bread, stated by the producer to be supplemented with milk and egg products; (*c*) semisynthetic diets containing either 15 per cent casein or 15 per cent wheat gluten, supplemented with cystine; dextrin was the carbohydrate source, and vitamins and minerals were added in the amounts stated in references 3 and 4; the casein diet will be designated henceforth as 15C and the gluten diet 15G.

The culture media and methods used for the enumeration of stool cultures were the same as described in reference 2.

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The results presented here were obtained with mice housed individually in stainless steel cages, with wire grids and no litter. On many occasions, parallel experiments were carried out with animals kept in groups of 5 or 10 with wood shavings as litter. No significant difference could be recognized between the two types of maintenance, except for the unexpected finding that untreated animals kept in groups on wood shavings usually had fewer Gram-negative bacilli and enterococci in their stools than did those kept on wire grids.

The curves representing the results were drawn from the arithmetic averages of bacterial counts obtained with 5 or 10 mice.

RESULTS

1. The Effect of Antibacterial Drugs on the Fecal Flora of NCS Mice.—The results summarized in Fig. 1 illustrate the changes in the numbers of lactobacilli, enterococci, and Gram-negative bacilli that occurred in the fecal flora of NCS mice following addition for only 1 week to their drinking water of either penicillin, terramycin, or chloramphenicol. Treatment with these antibacterial drugs caused a rapid disappearance of the lactobacilli, followed by an increase in the numbers of enterococci and Gram-negative bacilli.

As was to be expected, the intensity and duration of the effects on the fecal flora were related to the type and amount of drug administered. In this regard, it is worth mentioning that addition of isoniazid to the drinking water did not affect the composition of the fecal flora. This finding is in accord with the fact that while isoniazid is highly effective against tubercle bacilli, it is remarkably inert against other bacterial groups.

With large doses of penicillin (1 gm or 0.3 gm per liter of drinking water) the stool cultures became at first almost negative for the three groups of bacteria; even Gram-negative species were reduced to extremely low levels during the 1 week period of treatment. Then, immediately upon withdrawal of the drug, the enterococci and Gram-negative bacilli multiplied in an explosive manner; the lactobacillus population returned to its initial level at a variable but usually slow rate. Smaller concentrations of penicillin (0.1 gm, 0.03 gm, or 0.01 gm per liter of drinking water) also caused for a time complete disappearance of the lactobacilli from the stool cultures. In these cases, however, the enterococci and Gram-negative bacilli began to increase in numbers even during the course of treatment. The results obtained with the various doses of penicillin are illustrated in several of the figures in the present paper.

While the effects of terramycin and chloramphenicol on the lactobacillus population were similar to those produced by penicillin, the pattern was different with regard to enterococci and Gram-negative bacilli. The increase in the Gram-negative population was smaller following treatment with terramycin or chloramphenicol than it was with penicillin. In contrast, the numbers of enterococci increased even during administration of these two drugs and usually remained elevated for a long time after the end of treatment.

In the six experiments carried out so far with chloramphenicol (0.3 or 0.1 gm

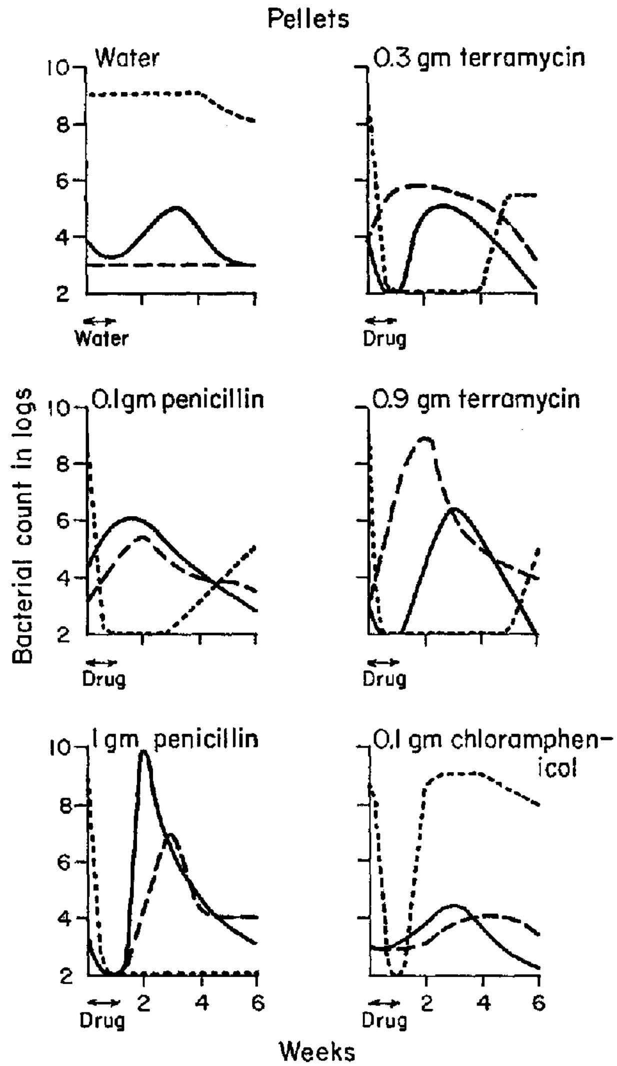

FIG. 1. Effect of Various Antimicrobial Drugs on the Fecal Flora of NCS Mice. All animals were fed D&G pellets throughout the experiment. The drugs were added to the drinking water during the 1st week of the experiment, then discontinued. The following concentrations of drugs were used: penicillin G, 1 or 0.1 gm per liter; terramycin, 0.9 or 0.3 gm per liter; chloramphenicol, 0.1 gm per liter.

Results.—All drugs caused a rapid disappearance of the lactobacilli from the fecal flora, and an increase in enterococci and Gram-negative bacilli. The numbers of lactose-fermenting Gram-negative bacilli were not recorded in this experiment. The fecal flora progressively returned to its initial state after discontinuance of the drugs. The results obtained with mice given isoniazid for 1 week (not shown here) were identical with those obtained with control mice given plain water.

Legend.—The results are given as logarithms of numbers of colonies obtained per gm of stool (collected between 9 a.m. and 12 a.m.). Only figures above 10^2 are shown because the enumeration techniques did not permit detection of smaller numbers.

Lactobacilli, - - - - -

Enterococci, — — —

Gram-negative bacilli, ————; ● indicates the presence of lactose fermenters.

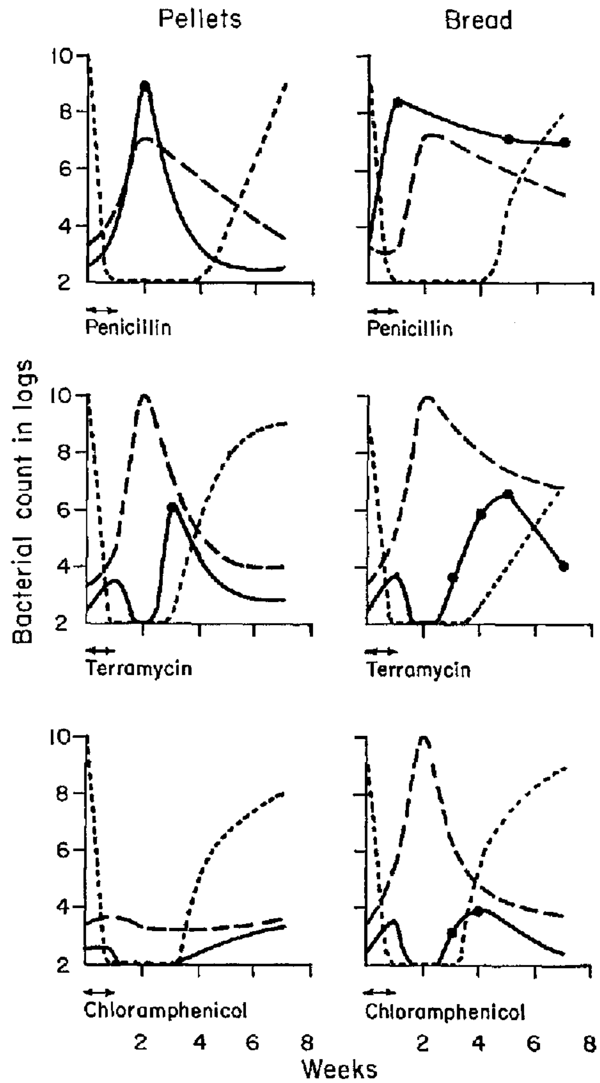

FIG. 2. Effect of Various Antimicrobial Drugs and of Diet on the Fecal Flora of NCS Mice. Mice were fed either D&G pellets or enriched bread throughout the experiment. Penicillin G, terramycin, or chloramphenicol was added in a concentration of 0.3 gm. per liter to the drinking water during the first week, then discontinued.

Results.—Administration of penicillin or terramycin was followed by disappearance of lactobacilli and by a marked increase in the numbers of Gram-negative bacilli and enterococci. Lactose fermenters were abundant in mice fed bread and treated with penicillin. The disturbances in fecal flora were least pronounced with chloramphenicol. In all mice, the fecal flora progressively returned to its initial state following discontinuance of the drugs; this occurred more rapidly in animals fed pellets. For further details see Legend, Fig. 1.

of drug added per liter of drinking water for 1 week) the disturbance of the fecal flora was less pronounced and of shorter duration than with either penicillin or terramycin (see Figs. 1, 2, and 6). A comparison of the effects on the fecal flora exerted by penicillin, terramycin, and chloramphenicol (in concentrations of 0.3 gm per liter of drinking water) is presented in Fig. 2. In this experiment some of the animals were fed D&G pellets whereas the others received a commercial preparation of wheat bread.

Whatever the drug, and irrespective of the concentration of it added to the drinking water, the population of enterococci and Gram-negative bacilli in mice fed D&G pellets began to decrease 2 to 3 weeks after discontinuance of treatment. The lactobacillus population returned to its initial state in 3 to 6 weeks.

2. Effect of Diet on the Changes in Fecal Flora Produced by Antibacterial Drugs.—The results illustrated in Fig. 2 indicate that the disturbances produced by the drugs in the populations of lactobacilli, enterococci, and Gram-negative bacilli were more pronounced and lasted longer in the mice fed bread than in those fed D&G pellets. Even more striking differences were observed with mice fed other diets, either commercially available, or prepared in our laboratory.

Fig. 3 summarizes the results of comparative experiments in which mice fed either D&G pellets or a semisynthetic diet containing 15 per cent purified casein (15C), were treated with penicillin for one week (1 gm or 0.1 gm per liter of drinking water). With both diets, penicillin treatment promptly caused a disappearance of lactobacilli from the stools; this was followed by a pronounced increase in the numbers of enterococci and Gram-negative bacilli. As found in the experiments reported in the preceding section of this paper, the fecal flora of mice treated with penicillin and fed the D&G pellets had returned to its initial state within 6 weeks after discontinuance of the drug. In contrast, the stool cultures of mice treated with penicillin and fed the casein diet were at that time still negative for lactobacilli. Moreover, they yielded many more enterococci and Gram-negative bacilli than did the stools of control mice. Other experiments of similar design have revealed that, following treatment for 1 week with penicillin or terramycin, the composition of the fecal flora of NCS mice fed the casein diet, remains for several months very different from that of untreated mice.

Like mice fed the casein diet, mice fed a semisynthetic diet containing 15 per cent wheat gluten (15G) as source of protein proved inefficient in overcoming the disturbing effects of antibacterial drugs on the fecal flora (Figs. 3 and 4). Supplementation of this diet with lysine and threonine naturally increased somewhat the growth rate of normal control animals, but did not significantly change the rate at which the fecal flora returned to its initial state. Despite many attempts, it has not yet proved possible to identify the factor(s) in the commercial D&G pellets which endow mice with the ability to reverse rapidly the disturbances produced by the administration of antibacterial drugs.

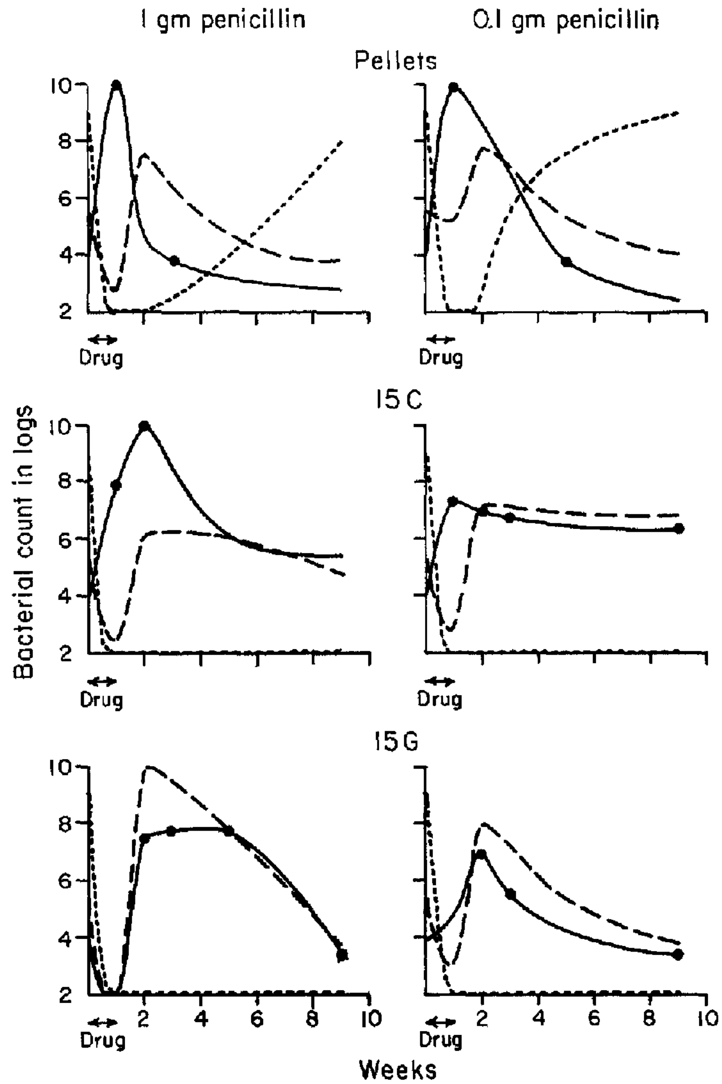

FIG. 3. Effect of Diet on the Fecal Flora of NCS Mice Treated With Penicillin. Penicillin was added to the drinking water in concentrations of either 1 gm or 0.1 gm. per liter. The drug was administered during the 1st week of the experiment, then discontinued. For each concentration of drug, one group of animals was fed D&G pellets; a second group was fed a synthetic diet with 15 per cent casein (15C), and a third group was fed a synthetic diet with 15 per cent gluten (15G).

Results.—The disturbances in fecal flora caused by penicillin, irrespective of the concentration used, were more rapidly corrected after discontinuance of the drug in mice fed pellets than in those fed the synthetic diets (15C and 15G). For further details see Legend, Fig. 1.

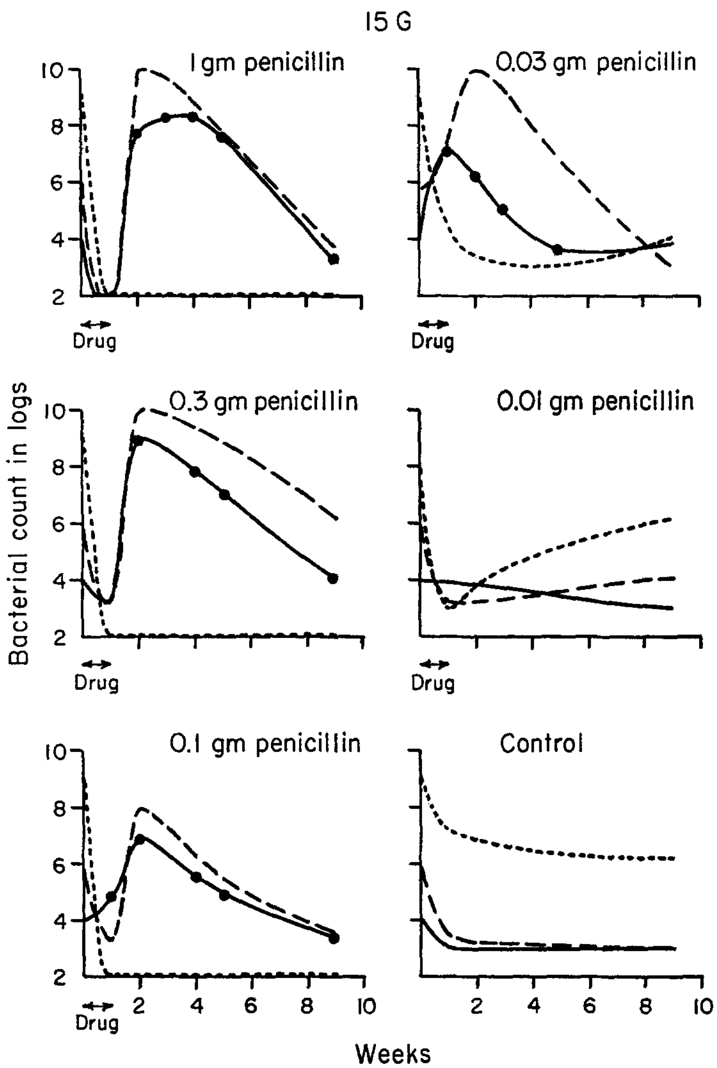

FIG. 4. Effect of Penicillin on the Fecal Flora of NCS Mice Fed Gluten Diet. All mice were fed a synthetic diet containing 15 per cent gluten (15G). Penicillin was added to the drinking water in concentrations of 1, 0.3, 0.1, 0.03, or 0.01 gm per liter. The drug was administered for 1 week at the beginning of the experiment, then discontinued.

Results.—The extent and duration of the effects of penicillin on the fecal flora were related to the concentration of drug administered. Note that with concentrations of penicillin larger than 0.03 gm per liter, the fecal flora had not yet returned to its original state 6 weeks after discontinuance of the drug; indeed, no lactobacilli could be detected at that time. Compare these results with those in Fig. 2 of the following paper, in which the effects of various concentrations of penicillin on the weight gain of the animals are presented. For further details see Legend, Fig. 1.

In the tests recorded above, the drugs were administered at the beginning of the experiment for only 5 to 7 days. The effects on the fecal flora, and the influence of diet on the response, became even more striking when treatment was continued for a longer period of time, namely 7 weeks. Figs. 5 and 6 present

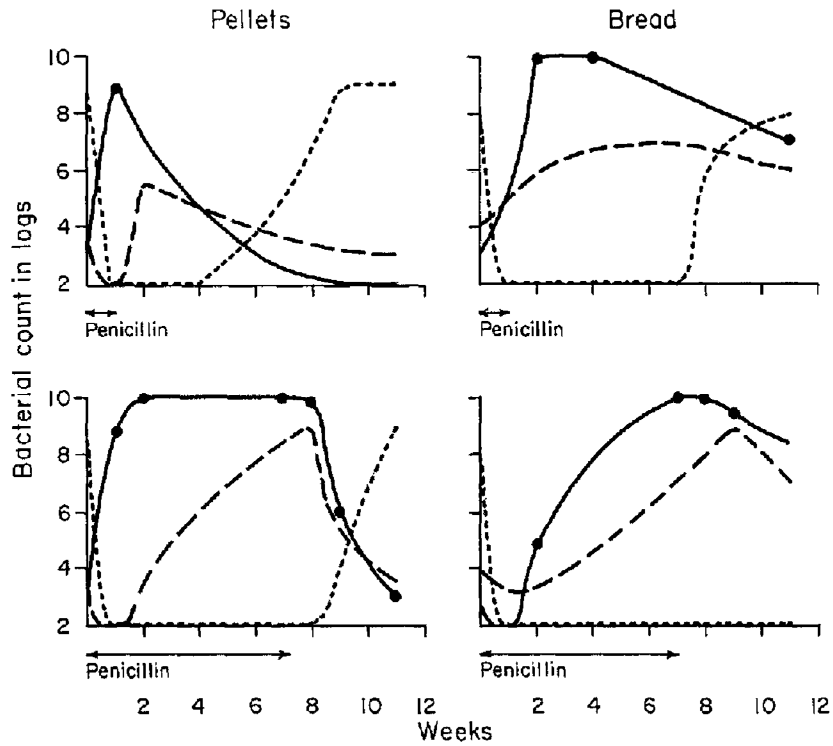

FIG. 5. Effect of Diet on the Fecal Flora of NCS Mice Following Short Term and Long Term Treatment with Penicillin. Penicillin, in concentration of 0.3 gm per liter of drinking water, was administered for either 1 week or 7 weeks. The diet was either D&G pellets or enriched bread.

Results.—The disturbances in fecal flora caused by penicillin were more rapidly reversed upon discontinuance of the drug in animals fed D&G pellets than in those fed bread; the differences between the two diets were more pronounced in animals which had received the drugs for 7 weeks than in those which had received it for only 1 week. For further details see Legend, Fig. 1.

the comparative effects of short (1 week) *versus* prolonged (7 to 11 weeks) drug treatment in mice fed either D&G pellets or bread. The results show that with both diets lactobacilli disappeared from the fecal flora throughout the period of treatment; there was no evidence that these organisms acquired resistance to either penicillin, terramycin, or chloramphenicol *in vivo* under the conditions of these experiments.

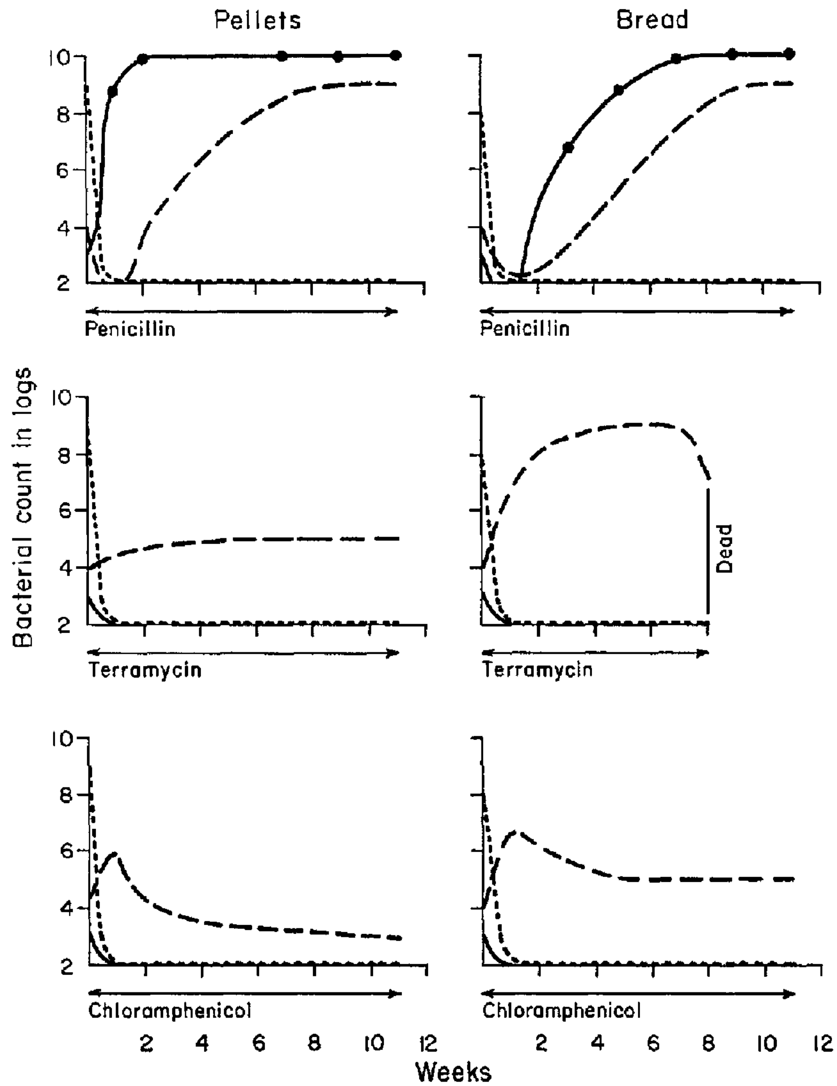

FIG. 6. Effect of Prolonged Treatment with Antimicrobial Drugs on Fecal Flora of NCS Mice. Mice fed either D&G pellets or enriched bread. Penicillin, terramycin or chloramphenicol was added to the drinking water in concentration of 0.3 gm per liter and administered throughout the duration of the experiment.

Results.—All animals fed bread and treated with terramycin died between the 8th and 9th week of treatment. During the 11 weeks of treatment with penicillin or terramycin, lactobacilli could not be cultivated from the stools, whereas the numbers of enterococci remained elevated. The stool cultures of mice treated with either terramycin or chloramphenicol did not yield any Gram-negative bacilli. In contrast, these were extremely numerous, and included lactose-fermenting forms in animals treated with penicillin. There was no evidence of development of drug resistance in the cultures recovered from the stools.

For other details see Legend, Fig. 1.

As mentioned earlier, the fecal cultures yielded only very small numbers of Gram-negative bacilli during the early phase of treatment with large doses of penicillin. As the treatment was continued, however, these organisms became very numerous, and most of them were lactose fermenters. In contrast, the Gram-negative flora remained at a low level in mice that received either terramycin or chloramphenicol; with these drugs the Gram-negative flora began to increase only after discontinuance of treatment. In this case again therefore, there was no evidence of development of drug resistance *in vivo*.

The dietary effect manifested itself as in previous experiments, and indeed it was even more pronounced than during treatments of shorter duration. Within 2 weeks after discontinuance of the drugs, lactobacilli were once more abundant in the fecal flora of mice fed D&G pellets, but not in mice fed the bread diet. (Figs. 5 and 6) Furthermore, the numbers of enterococci and Gram-negative bacilli fell much more rapidly in the animals fed pellets than in the others. All mice fed the bread diet and receiving terramycin died after 8 weeks of treatment. The cause of death was not determined except for the fact that the animals receiving this drug progressively lost weight after the first 4 weeks on the bread diet. In contrast, all animals fed D&G pellets survived throughout the duration of the experiment. No deaths occurred with either penicillin or chloramphenicol.

It has been emphasized in a preceding paper that cultures of the fecal flora of NCS mice do not yield lactose-fermenting coliform bacilli (2). However, large numbers of these organisms (*Escherichia coli* and *aerogenes*-like organisms) have been consistently found in most mice within 1 week after treatment with penicillin especially in animals fed bread or the casein diet. In all cases, the lactose-fermenting organisms persisted for many weeks after the drug had been discontinued in animals fed bread or the semisynthetic casein or gluten diets, but they disappeared rapidly from animals fed the D&G pellets.

Three possible explanations can be considered to account for the appearance, multiplication, and persistence of lactose-fermenting Gram-negative bacilli in the feces of mice fed the bread or casein diet and treated with antibacterial drugs: (*a*) These organisms being ubiquitous in the environment, may have invaded the intestinal tract of NCS mice from the outside as soon as the drug had disturbed the normal intestinal flora; (*b*) it is possible that lactose-fermenting Gram-negative bacilli are always present in NCS mice, but in a latent form; according to this hypothesis, they would be unable to multiply and to reach detectable numbers unless other bacteria have been eliminated by the drug; (*c*) they may arise by mutation from the non-lactose fermenters of the intestinal flora, the mutant forms being favored by penicillin treatment. Experiments designed to decide between these three hypotheses are presently under way.

3. Effect of Antibacterial Drugs and of Diet on the Fecal Flora of Ordinary Swiss Mice.—As shown in a preceding paper, the fecal flora of Swiss mice raised under usual conditions differs from that of NCS mice in many respects; of special interest here is the fact that it contains much larger numbers of enterococci and Gram-negative bacilli of various types (2).

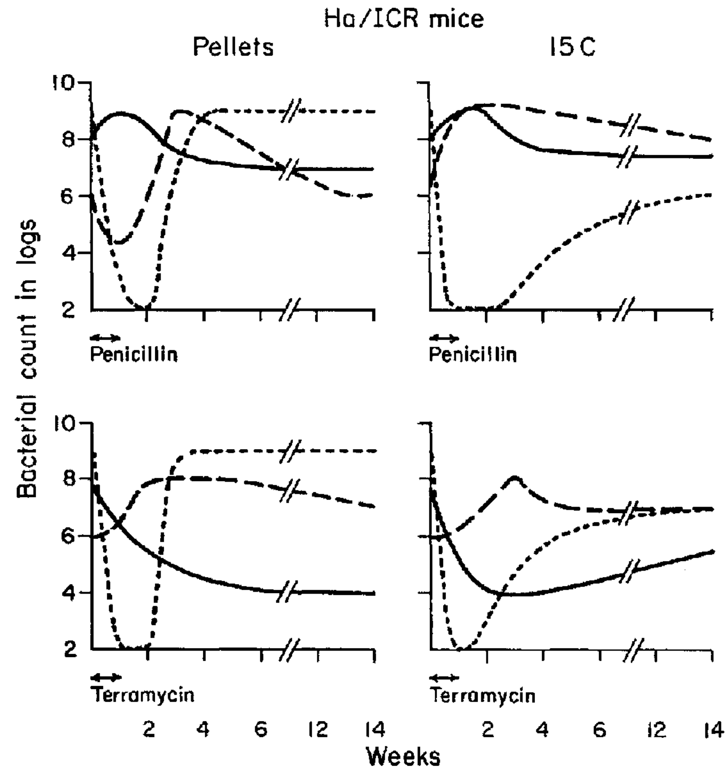

FIG. 7. Effect of Diet and Antibacterial Drugs on The Fecal Flora of Ha/ICR Mice. The animals were approximately 5 weeks old at the beginning of the experiment; they were fed throughout the experiment either D&G pellets, or a synthetic diet containing 15 per cent casein (15C). Penicillin, or terramycin was added to the drinking water in a concentration of 0.3 gm per liter; the drugs were administered during the 1st week then discontinued.

Results.—At the beginning of the experiment, the fecal flora of these mice contained many more enterococci and Gram-negative bacilli than found in NCS mice. The effect of penicillin and terramycin on the flora was less pronounced and less lasting in Ha/ICR mice than in NCS mice. Following discontinuance of the drug the fecal flora returned to its initial state more rapidly in animals fed D&G pellets than in those fed the synthetic diet. For further details see Legend, Figure 1.

The fecal flora of the Ha/ICR Swiss mice, used in comparison with the NCS mice in the present study, contained approximately 10^9 lactobacilli, 10^6 enterococci, and 10^8 Gram-negative bacilli per gram of stool; a large percentage of the Gram-negative bacilli were lactose fermenters. When penicillin and terramycin were added for 1 week to the drinking water of these animals, the general trend of changes in their fecal flora was similar to that observed in NCS mice as described in the preceding sections of this report, but the effects were less pronounced. For example, the lactobacilli were not as completely eliminated by drug treatment, and they returned to their initial level more rapidly after discontinuance of treatment. Likewise, the changes in other components of the fecal flora were more transient (see Fig. 7). In contrast with what has been consistently found with NCS mice, little difference could be detected at first between the animals fed D&G pellets and those fed other diets; however, the difference became apparent in the later phase of the experiment.

Comparison of the results obtained with NCS and Ha/ICR mice strongly suggests that the composition of the fecal flora during the early weeks of life markedly influences the response of the animals to drug treatment. As can be seen in Fig. 6 of the following paper, this difference in response manifested itself also in the weight curves of the animals.

SUMMARY

Oral administration of penicillin, terramycin, or chloramphenicol to NCS mice rapidly brought about profound changes in their fecal flora. The lactobacilli disappeared completely, whereas the numbers of enterococci and Gram-negative bacilli reached very high levels. In contrast, no effect on the fecal flora could be detected following administration of isoniazid in any amount.

The intensity and duration of the effects on the fecal flora were related to the type of drug and to the amount of it administered. Chloramphenicol produced disturbances which were less profound and of shorter duration than those produced by penicillin or terramycin.

The duration of the disturbances in the fecal flora produced by antibacterial drugs was markedly conditioned by the nutritional regimen. The fecal flora returned to its pretreatment state (large numbers of lactobacilli, few enterococci, and few Gram-negative bacilli) within less than 4 weeks after discontinuing the drug when the mice were fed a complex diet of ill defined composition (commercial pellets). Contrariwise, the fecal flora remained markedly different from that of control mice when the animals were fed semisynthetic diets containing as source of protein either 15 per cent casein or 15 per cent wheat gluten (both supplemented with cystine); or 15 per cent wheat gluten supplemented with lysine, threonine, and cystine.

The fecal flora of mice treated with penicillin contained large numbers of lactose-fermenting Gram-negative bacilli, not found in the untreated animals. These lactose fermenters persisted for several months after discontinuance of the drug in mice fed either the casein or gluten diets, but they disappeared rapidly from mice fed pellets.

Similar results, although less striking, were obtained with Swiss mice from

colonies maintained under usual conditions, and therefore having a fecal flora more complex than that of NCS mice.

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