THE EFFECT OF ANTIBACTERIAL DRUGS ON THE WEIGHT OF MICE*

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The oral administration to mice of certain antibacterial drugs brings about profound and lasting changes in their fecal flora; the duration of these changes is conditioned by the composition of the diet fed the animals (1). It will be shown in the present paper that the growth rates of mice and some at least of their nutritional requirements are also affected by the administration of antibacterial drugs.

Materials and Methods

The two colonies of Swiss mice, NCS and Ha/ICR, used in the present study, as well as the experimental diets, were described in preceding papers (1, 2).

All experiments to be reported here were carried out with mice housed individually in stainless steel cages, with wire grids. All NCS mice were of the same age at the beginning of each experiment (30 to 33 days old); within this age group, they were selected for uniformity in weight (within 18 to 22 gm). These requirements for age and initial weight could not be fulfilled for mice of the Ha/ICR colony because their exact date of birth was not known, and also because they were less uniform than NCS mice at the time of receipt from the producer.

All animals were weighed individually between 9 a.m. and 10 a.m. This detail is of importance since most mice progressively lose weight during the daylight hours as a result of the fact that they do not consume any significant amount of food or water between 8 a.m. and 8 p.m. (2). The results presented in the figures correspond to arithmetic averages for 5 to 10 mice.

RESULTS

1. Effect of Antibacterial Drugs on the Weight Gain of NCS Mice Fed a Diet Deficient in Lysine and Threonine.—Purified wheat gluten is deficient in several amino acids, particularly in lysine and threonine; in consequence, diets made from this protein usually do not permit good growth unless adequately supplemented. This is true for ordinary Swiss mice as well as for other types of experimental animals. However, we have consistently observed in many different experiments carried out over the past four years that NCS mice gain weight

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rapidly when fed semisynthetic diets containing 15 per cent wheat gluten as sole source of amino acid (3). As shown in preceding papers, there is reason to believe that this peculiar characteristic of NCS mice is related to the fact that their intestinal flora is profoundly different from that of ordinary mice (3). This explanation is suggested by the fact that, when animals of the NCS colony are placed shortly after birth in contact with ordinary mice and thereby contaminated with the microbial flora of the latter, they lose the ability to grow well on the gluten diet unsupplemented with amino acids (3).

In view of the fact that oral administration of certain antibacterial drugs results in an extensive multiplication of a variety of microbial species in the

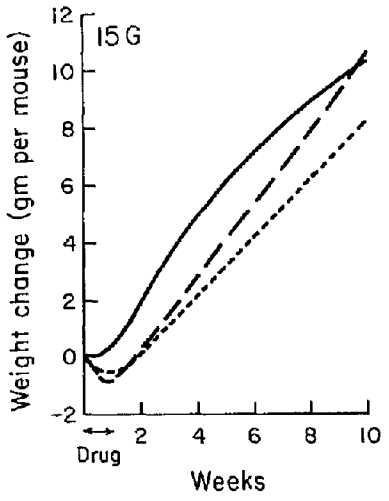

FIG. 1. Effect of Antimicrobial Drugs on Weight Gain of NCS Mice Fed Gluten Diet. Mice fed a synthetic diet containing 15 per cent gluten (15G) as sole source of amino acids (except for supplementation with cystine). The antibacterial drugs were added to the drinking water for one week at the beginning of the experiment, then discontinued. Control, ————; penicillin G, — — —; terramycin, - - - - -.

Results.—Both drugs caused retardation of weight gain in mice fed gluten diets.

intestinal tract of NCS mice (1), it appeared possible that such drug treatments would also change the ability of the latter to grow on the gluten diet. Indeed, as shown in Figs. 1, 2, and 3 NCS mice given penicillin or terramycin in their drinking water were commonly found to lose weight if fed a diet containing 15 per cent gluten supplemented with cystine as sole source of amino acids. Although drug treatment did not always cause actual weight loss, it always retarded weight gain in comparison with that of untreated mice.

The differences in weight between drug-treated animals and the controls, and especially the duration of the differences, were related to the concentration of the drug administered in the drinking water. For example, Fig. 2 shows the changes induced by various concentrations of penicillin in the weight curves of mice fed the gluten diet. Fig. 4 of the preceding paper shows the changes induced by the same drug concentrations in the fecal flora (lactobacilli, enterococci, Gram-negative bacilli). It will be observed that with regard to both effects, the

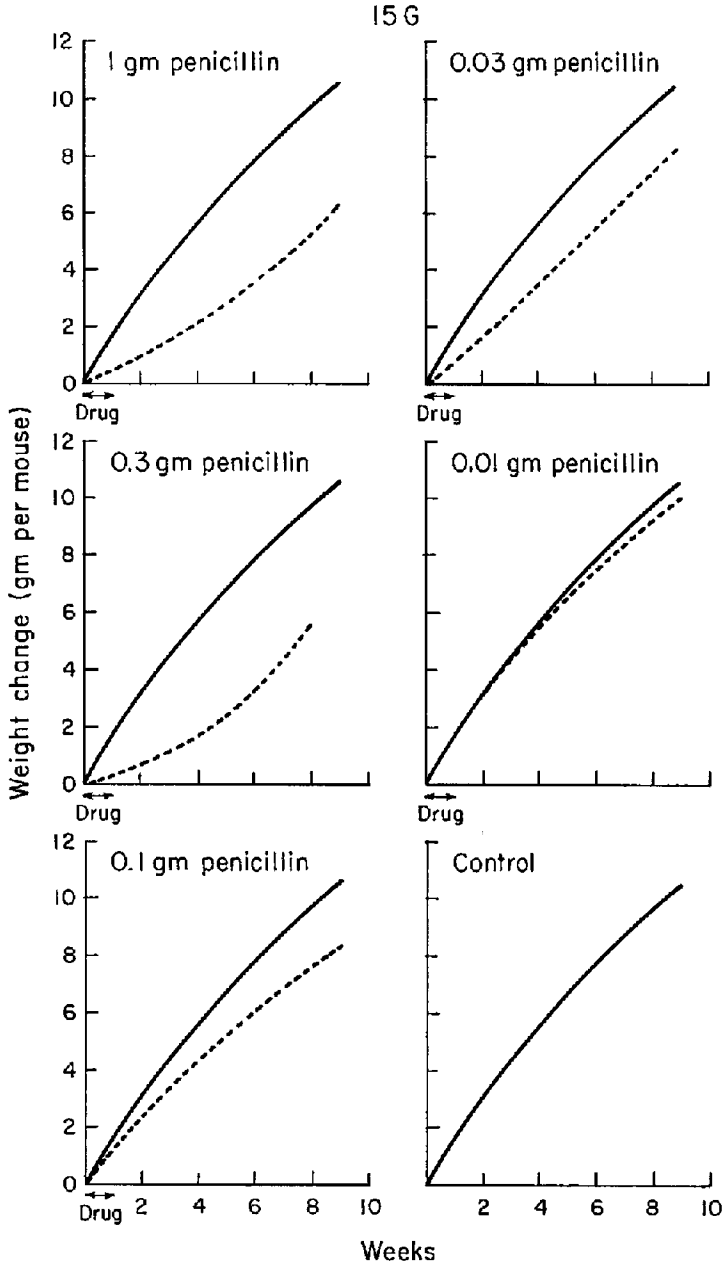

FIG. 2. Effect of Penicillin on the Weight Gain of NCS Mice Fed Gluten Diet. All animals were fed a synthetic diet containing 15 per cent gluten (15G) as sole source of protein. Penicillin G was added to the drinking water in concentrations of 1 gm, 0.3 gm, 0.1 gm, 0.03 gm, or 0.01 gm per liter of water. The drug was administered for 1 week at the beginning of the experiment, then discontinued. The weight gain (in grams per mouse) is compared for each concentration of penicillin (- - - - -) with the weight gain of untreated animals on the gluten diet (————).

Results.—All animals treated with penicillin gained weight more slowly than untreated animals. The differences increased with the concentration of drug. Compare these results with those presented in Fig. 4 of the preceding paper in which the effects of the various drug concentrations on the fecal flora are presented.

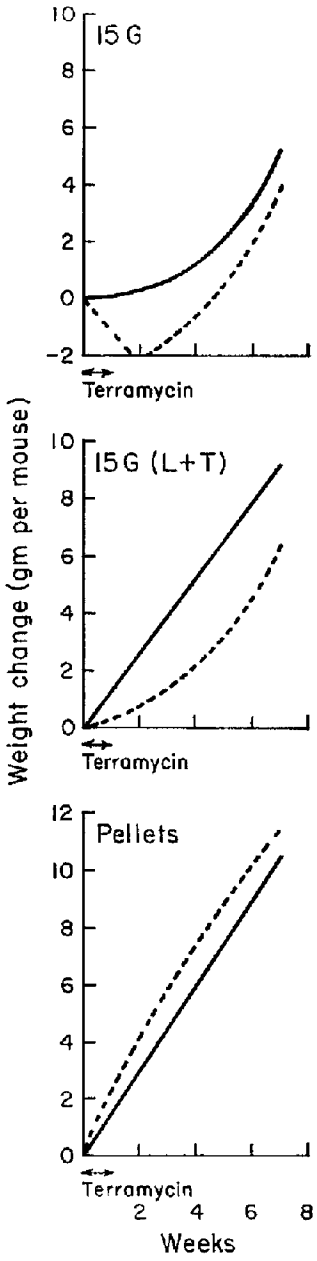

FIG. 3. Effect of Diet on Weight of NCS Mice Treated with Terramycin. Terramycin was added for 1 week to the drinking water in concentration of 0.3 gm per liter, then discontinued. Controls, ————; terramycin, - - - - -. One group of mice was fed a synthetic diet containing 15 per cent gluten (15G); a second group was fed a synthetic diet containing 15 per cent gluten supplemented with lysine and threonine (15G L+T); a third group was fed D&G pellets.

Results.—Terramycin caused an initial weight loss in mice fed unsupplemented gluten diet and a retardation of weight gain in mice fed gluten diet supplemented with lysine and threonine. The drug caused a slight acceleration of weight gain in mice fed D&G pellets.

lowest concentration of penicillin found to be effective was approximately 0.01 gm of drug per liter of drinking water. Even though penicillin was administered for only 6 days, the bacteriological and the weight effects were maintained for prolonged periods of time after discontinuance of treatment. It seems legitimate to conclude therefore, that the intestinal contamination resulting from treatment with penicillin, terramycin, or chloramphenicol renders NCS mice similar to ordinary mice with regard to growth on the deficient gluten diet.

2. Effect of Diet on the Weight Gain of NCS Mice Fed Antibacterial Drugs.—As illustrated in Fig. 3 NCS mice did not actually lose weight following administration of antibacterial drugs when the gluten diet was supplemented with lysine and threonine. However, their rate of growth was consistently smaller than that of mice fed the same diet but without drug treatment. A similar retardation of growth was observed when NCS mice were fed a semisynthetic diet containing 15 per cent casein supplemented with cystine. In contrast, the growth rate of mice fed D&G pellets was much less affected by antibacterial drugs; in general penicillin caused a small depression of growth (Fig. 5) whereas terramycin caused a slight enhancement (Fig. 3).

In experiments not reported here, chloramphenicol (0.3 gm per liter of drinking water) gave results similar to those obtained with terramycin. In contrast, it is of interest to note that isoniazid did not influence the weight gains of the animals irrespective of their diets, just as it did not influence the composition of their fecal flora.

The results presented in Fig. 4 make it apparent that the retardation of weight gain caused by penicillin (either 1 gm or 0.1 gm per liter) was more prolonged in mice fed either the gluten or casein diets than in mice fed D&G pellets. Indeed, as already mentioned, administration of terramycin or chloramphenicol to NCS mice fed the D&G pellets commonly increased the rate of growth. The greater resistance to weight loss exhibited by pellet-fed animals, in comparison with those fed the synthetic diets, is probably related to their greater ability to recover from the change in fecal flora induced by the drugs (1).

In all the experiments considered so far, the drugs were administered for only 1 week. However, even mice fed D&G pellets eventually grew more slowly than the controls when drug treatment was continued for longer periods of time. Fig. 5 shows that whereas mice treated with penicillin for only 1 week grew as rapidly as the controls, obvious depression of growth was evident in those receiving the drug for 7 weeks. When treatment was discontinued after this period, the weight curve rapidly resumed the slope observed with untreated animals. As shown in the preceding paper, the fecal flora of these animals also began to return to its initial state shortly after discontinuance of the drug. (See Fig. 5 of the preceding paper.)

3. Comparative Effects of Antibacterial Drugs on the Weight Curves of Swiss Mice From Two Different Colonies.—It is well known from experimental studies

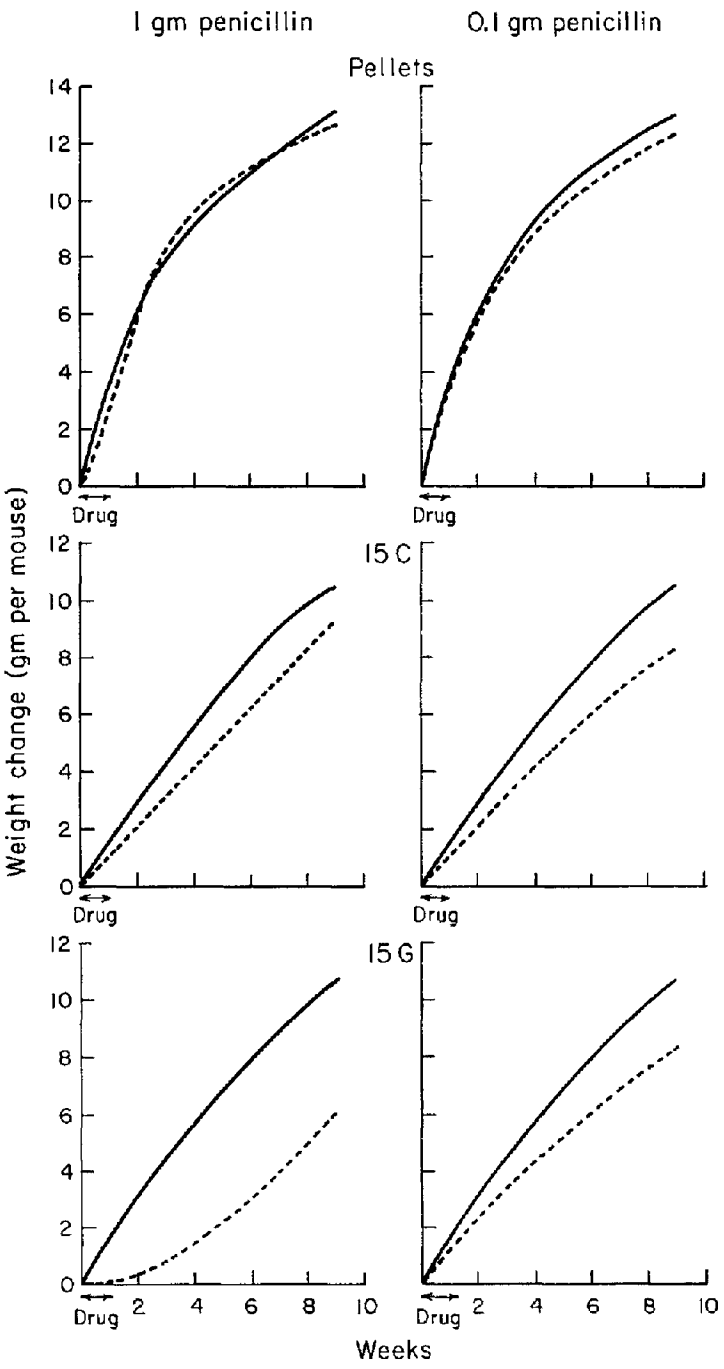

FIG. 4

and practical experience, that addition of certain antibacterial drugs to the diet of farm animals commonly increases their rate of growth, and their efficiency in the utilization of food. This well established fact seems at variance with the results reported above which show that penicillin, terramycin, and chloramphenicol can retard weight gain in NCS mice. Evidence that both statements are true despite their apparent incompatibility is presented in Fig. 6.

In this experiment, NCS mice were compared with other Swiss mice of approximately the same age, but obtained from a colony of Ha/ICR maintained under ordinary conditions and therefore having the usual complex intestinal flora. It is seen that whereas administration of penicillin retarded the growth of NCS mice, it accelerated the growth of the Ha/ICR animals. The effect was the same whether the animals were fed D&G pellets or the semisynthetic casein diet.

The difference between the two groups of Swiss mice with regard to their response to penicillin can probably be explained by the theory commonly held to account for the growth enhancement of farm animals fed antibacterial drugs (for relevant literature see references 5–8). According to this theory, the enhancing effect on growth is due to the fact that the drugs interfere in some way with the noxious activities of microbial agents which either have toxic effects on the animals, or compete with them for nutritional factors. Compatible with this theory is the finding that in general, enhancement of growth by antibacterial drugs is greater when farm animals are kept under unsanitary conditions, or when their diet is somewhat deficient. In this regard, it is of interest to note that whereas the growth of Ha/ICR mice was enhanced by penicillin when the animals were fed the semisynthetic casein diet, the drug had no detectable effect when the nutritional regimen consisted of D&G pellets which constitute a better nutritional regimen.

It is known that the administration of antibacterial drugs does not alter the rate of growth of germ-free animals (9, 10). In contrast, as we have seen, these drugs interfere with the growth of NCS mice probably by eliminating lactobacilli and permitting the establishment in these animals of a noxious microbial flora (1). As shown in the preceding paper, treatment with penicillin brings about the establishment in the intestine of lactose-fermenting Gram-negative

FIG. 4. Effect of Diet on The Weight Change Induced in NCS Mice by Penicillin. The animals were fed either D&G pellets, or synthetic diets containing either 15 per cent casein (15C) or 15 per cent gluten (15G). Penicillin was added to the drinking water in concentrations of 1 gm or 0.1 gm per liter. It was administered during the 1st week of the experiment, then discontinued. The weight gains for each diet and each concentration of drug are presented in comparison with those of untreated animals on the same diet. Control, ————; drug, - - - - -.

Results.—Irrespective of the concentration of penicillin used, the depressing effect of penicillin on the weight was most pronounced in animals fed the gluten diet, and least pronounced in those fed D&G pellets. Compare these results with those in Fig. 3 of the preceding paper which presents the effect of penicillin on the fecal flora.

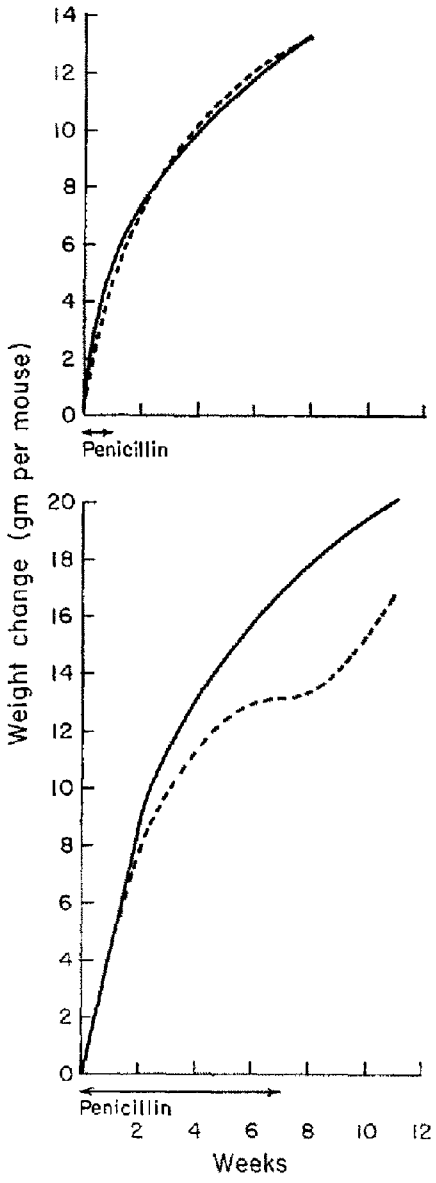

FIG. 5. Effect of Prolonged Penicillin Treatment on Weight of NCS Mice. All animals fed D&G pellets. Penicillin was added to the drinking water in concentration of 0.3 gm per liter; in one group of animals the penicillin was discontinued after 1 week; in the other group it was discontinued after 7 weeks. Control, ————; penicillin, - - - - -.

Results.—The retardation of weight gain by penicillin in NCS mice fed D&G pellets became more pronounced after several weeks' treatment.

bacilli which are not found in untreated NCS mice. This lactose-fermenting flora is particularly abundant in animals fed the semisynthetic casein diet, and through ill understood mechanisms its presence is associated with a marked depression of the weight curve. When, on the other hand, NCS mice fed D&G pellets are given for 1 week a mixture of terramycin and chloramphenicol, the

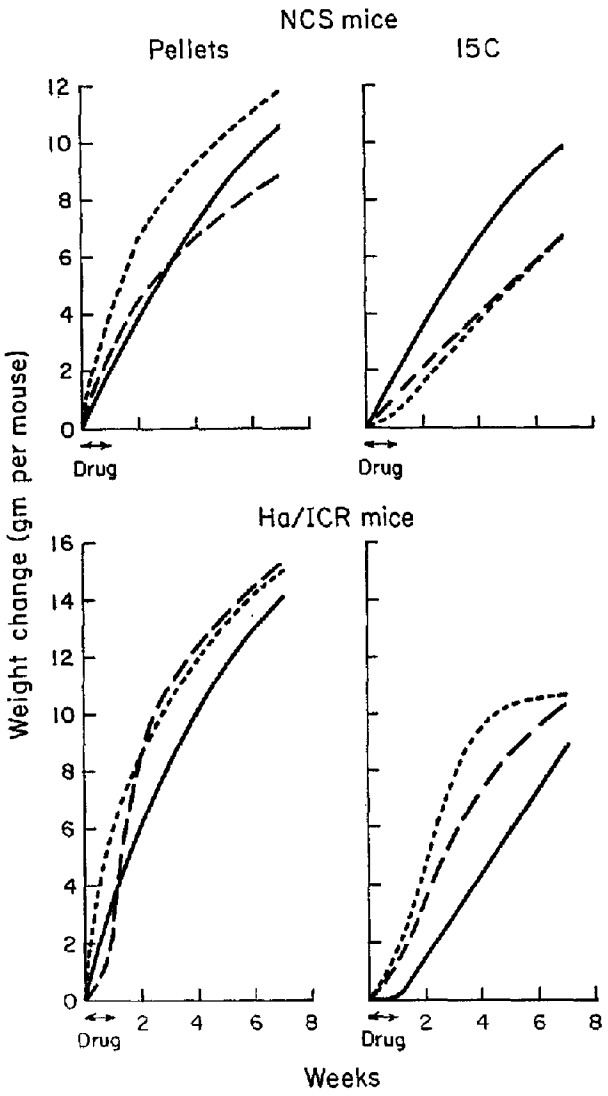

FIG. 6. Comparative Effects of Drugs on Weights of Two Strains of Swiss Mice. Drugs added to drinking water in concentrations of 0.3 gm per liter, during the first week of experiment then discontinued. Control, ————; penicillin, — — —; terramycin, - - - - -. In contrast to NCS mice, the Ha/ICR mice had a complex fecal flora, with large numbers of Gram-negative bacilli and enterococci, at the beginning of the experiment. Half of the mice of each strain were fed D&G pellets; the other half were fed a synthetic diet containing 15 per cent casein (15C).

Results.—Whereas penicillin and terramycin accelerated the weight gain of Ha/ICR mice fed the semi-synthetic casein diet, the drugs retarded the weight gain of NCS mice fed the same regimen. The differences were less striking in mice fed D&G pellets.

numbers of fecal Gram-negative bacilli increase only slightly, no lactose-fermenting coliforms appear in the stool cultures, and the lactobacilli are eliminated for only a short period of time; under these conditions the drugs cause an acceleration of weight gain.

TABLE I

Effect of Contamination on Weight and Fecal Flora of NCS Mice

Days after Birth	Controls*			Contaminated‡		
	Weight	Fecal flora		Weight	Fecal flora	
		SLF§	*E. coli*‖		SLF§	*E. coli*‖
	gm			*gm*		
	First generation					
22	10.5¶			8.9**		
25		10^8	0		10^8	10^7
32		10^8	0		10^8	10^8
36	21.4¶			20.3**		
38		10^8	0		10^8	10^6
40	22.8¶			22.0**		
46		10^2	0		10^2	0
	Second generation					
10	5.8‡‡			4.7§§		
21	11.5‡‡			9.4§§		

* These animals were obtained from the NCS colony described in reference 3.

‡ On day 9, the NCS mothers of these animals were sprayed with a suspension of homogenate of intestine of CFW mice; the animals of the first generation received no further treatment. The contaminated animals of the second generation were obtained by cross mating contaminated animals of the first generation, (without further treatment). The control animals of the second generation were derived from controls of the first generation. 30 per cent of the contaminated animals died (cause unknown) in the course of this experiment.

§ SLF = Gram-negative bacilli (slow lactose fermenters or non-fermenters).

‖ *E. coli* = rapid lactose-fermenting Gram-negative bacilli. The figures indicate the numbers of colonies recovered per gram of stool.

¶ Arithmetic average for 80 animals.

** " " " 60 " .

‡‡ " " " 45 " .

§§ " " " 39 " .

There is some evidence that the intestinal flora can depress growth by interfering with absorption of fats and carbohydrates, and perhaps also of vitamins and amino acids (8, 11). The remarkable ability of NCS mice to grow well on gluten diets deficient in lysine and threonine, and even on poorer diets, and the fact that they lose this ability when contaminated after birth, or following treatment with penicillin, may be explained in part by the differences in absorption brought about by changes in the intestinal flora. Coliform organisms may play an important role in this respect since normal fecal strains as well as

enteropathogenic strains tend to colonize both the upper intestinal tract and the lower region, even when their presence remains asymptomatic (12).

Whatever the mechanisms involved, it is apparent that the effect of antibacterial drugs on the weight gain of NCS mice fed a given diet can be manipulated at will, and rendered positive or negative by operations which modify the composition of the fecal flora. Consistent with this finding is the fact that the rate at which NCS mice gain weight can also be depressed by contaminating them early in life. As shown in Table I NCS mice contaminated 9 days after birth with the intestinal contents of ordinary mice acquired the fecal flora of the latter and gained weight more slowly than uncontaminated animals. Furthermore, the second generation of the contaminated animals also grew more slowly than uncontaminated NCS mice. The differences in weight reported in Table I acquire greater significance from the fact that 30 per cent of the contaminated animals died of unknown causes in the course of this experiment.

SUMMARY

NCS mice gained weight rapidly when fed a gluten diet deficient in several amino acids, but their weight gain on the same regimen was very much retarded if they were given antibacterial drugs, even for a short period of time. This retardation of growth could not be entirely corrected by supplementing the gluten diet with lysine and threonine.

The decrease in growth rate brought about by antibacterial drugs could probably be traced to the alteration in intestinal flora resulting from drug treatment. The intensity and duration of both types of changes were related to the dose of drug administered, and to the length of the treatment period.

Whatever the nutritional regimen, treatment with penicillin caused a retardation of weight gain in NCS mice. The retardation was more pronounced, and longer lasting, when the animals were fed semisynthetic regimens (containing casein or gluten) than when they were fed crude diets (pellets) containing natural materials of ill defined composition. These differences probably had their origin in the fact that the changes in fecal flora induced by the drugs were profoundly influenced by the composition of the diet.

Antibacterial drugs which retarded weight gain of Swiss NCS mice, in contrast increased weight gain in ordinary Swiss mice raised under usual conditions. It is probable that this difference in response to the antibacterial drugs resulted from the fact that ordinary Swiss mice have a much more complex intestinal flora than NCS animals.

Addendum.—As mentioned in this and earlier publications, stool cultures have been consistently carried out every week in our laboratory within 3 days after receipt of NCS mice from the breeding room. The animals are approximately 4 weeks old at the time of receipt. Examples of the results obtained since May 1958 have been presented in reference 4. Unexpectedly the mice received on June 26, 1962, yielded fecal cultures which were very different in composition from those of the preceding years, suggesting

that the breeding colony had become contaminated. The numbers of enterococci and Gram-negative bacilli obtained from these animals ranged from 10^5 to 10^7 per gm of stools, and more importantly lactose-fermenting organisms (chiefly *E. coli*) were obtained from a large percentage of them. After investigation, it was established that this sudden and profound change in fecal flora occurred 3 weeks after an accident had happened in the breeding room. Two of the air conditioners had broken down in the course of a period of very warm and humid weather during the last week of May 1962, and in consequence it had been found necessary to open the windows. Several of the animals died during that period.

Throughout July, August, and early September 1962, all NCS mice raised in the breeding room at the Rockefeller Institute yielded stool cultures with numbers of

TABLE II

Sex	Age	Weight*	Lactobacilli	Enterococci	SLF‡	Lactose fermenters, *Pseudom.* and *Proteus*
	wks.	*gm*				
M	4	18.0				0
"	5	24.6	10^9§	10^8§	10^8§	0
"	6	26.2	10^9§	10^4	10^8§	0
"	7	27.4	10^9§	10^8	10^8§	0
F	4	16.7				0
"	5	20.7	10^9§	10^4	10^8§	0
"	6	21.8	10^9§	10^8	10^8§	0
"	7	23.3	10^9§	10^8	10^8§	0

* Arithmetic averages for 12 mice

‡ SLF = slow or non-lactose-fermenting Gram-negative bacilli

§ The figures correspond to the numbers of colonies obtained per gram of stools; arithmetic averages for 12 mice.

enterococci and Gram-negative bacilli much larger than those recorded in 4 preceding years. Although the number of these organisms were still smaller than those found in ordinary mouse colonies, the change in flora was made more striking by the fact that the fecal cultures of NCS mice now included many lactose-fermenting organisms. Other evidence of change was that (*a*) the average weight of the animals at weaning time was smaller than in preceding years; (*b*) individual variations were much larger and (*c*) the growth response to various experimental diets was different from what had been observed when the colony had a simpler intestinal flora. It is not likely that these changes could be traced to seasonal variations since NCS mice had retained their characteristics during 3 previous summers.

Efforts made since July, 1962, to restore the colony to its original state seem at this stage to have been successful, in part at least. Table II summarizes the results obtained with 12 male and 12 female NCS mice, born on August 17, 1962, and received in our laboratory on September 18, 1962. It will be noticed that these animals yielded fecal cultures, and exhibited weight gains, similar to those found in earlier

studies of this colony. Still more recent tests on large numbers of NCS animals indicate that 2 to 3 per cent of them still yield lactose fermenters when brought from the breeding room after weaning.

Experiments similar to those reported in the present and preceding paper have been carried out with mice born during late August, 1962. The findings have again confirmed that uncontaminated and untreated NCS mice gain weight rapidly when fed a gluten diet, but that their growth is retarded when penicillin is added for 1 week or longer to their drinking water; furthermore, lactose-fermenting Gram-negative bacilli appear in very large numbers in their stools shortly after the beginning of this treatment.

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