brief exposure to sub-inhibitory concentrations and the progressive acquisition of the maximum resistance in an environment where the concentration of streptomycin dwindles from a sub-inhibitory level to still lower values are not as readily explained by genetic selection.

No concise range for the management of the constant-treatment technic systems can be given. However, in a given series, involving the same organism and the same antibiotic, the maximum fold change will be virtually equal, although reached, perhaps, by one system in a shorter period of time and/or with exposure to smaller concentrations of antibiotic.

Summary. 1. A new method is described for the production of resistant bacteria by continuous exposure to an antibiotic in a liquid environment. 2. The constant-treatment technic rapidly produces streptomycin-, aureomycin-, or bacitracin-resistant variants of an isolated streptococcus, but fails to produce any significant decrease in streptococcal sensitivity to penicillin. 3. The responses of streptococci to varying degrees of exposure to streptomycin are discussed.

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Effects of Antibiotics in Mice on Weight and Thorium Dioxide Uptake of Spleen and Liver.* (19845)

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It has been demonstrated in a number of studies(1-3) that the antibacterial host defense mechanisms play a supporting role in the antibiotic treatment of bacterial infections. In addition to independent participation of the host defense mechanisms, some studies suggest(4,5) that certain antibiotics may act directly upon the cellular defenses of the body to increase their effectiveness. It is also possible that antibiotics may, through direct action decrease the effectiveness of certain host defense mechanisms. In this regard Munoz and Geister(6) have shown that aureomycin inhibits the phagocytic activity of leukocytes of human blood. Lepin, Barski and Maurin(7) have observed that both aureomycin and chloramphenicol, in concentrations attainable in the body, retard the rate of growth of fibroblasts.

In the present investigation several antibiotics were tested for their effect, whether of enhancement or inhibition, on the phagocytic activity of the reticulo-endothelial cells of the liver and spleen of the mouse. The ability of these cells to take up ThO_2 from the circulating blood was used as an indication of their phagocytic ability.

Materials and methods. Laboratory albino mice of mixed sexes were used in all experiments. Prior to each experiment the mice,

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TABLE I. Effect of Antibiotics on Weight and ThO_2 Uptake of Mouse Spleens.

Treatment and dose per 12 hr	Avg spleen wet wt, mg	P for wt diff.	mg ThO_2/g dry wt	P for uptake diff.	mg ThO_2/ spleen
P,* 10000 units	133	<.7	120	<.3	4.10
S	127	—	124	—	3.86
C, 2 mg	143	<.2	113	<.01	3.83
St, 2 mg	148	<.3	114	<.8	4.10
S	159	—	116	—	4.32
A, 2 mg	126	<.05	131	<.05	4.15

* P = penicillin; S = saline; C = chloramphenicol; St = streptomycin; A = aureomycin.

weighing 24 ± 4 g, were pooled and placed into groups at random. Each group contained from 15 to 20 mice; an equal number of animals was included in each experiment for control. Antibiotic treatment with arbitrarily chosen high concentrations was begun 4 hours before the intravenous injection of 0.1 ml of Thorotrast.‡ Subsequent injections were given every 12 hours for a total of 7 antibiotic injections. All antibiotics were dissolved or suspended in saline. A small amount of Tween 80 was used to help suspend the insoluble antibiotics and the same amount was added to all other solutions. The antibiotic injections were given subcutaneously in a volume of 0.25 ml. Mice in the control groups received the same volume of saline plus Tween 80 according to the above schedule. Seventy-two hours after the injection of Thorotrast the mice were sacrificed by ether anesthesia, their livers and spleens removed and the wet weights of these organs determined by weighing 3 or 4 organs in tared beakers. After weighing, the organs from a group were pooled, minced with scissors and thoroughly mixed. Aliquots not exceeding 400 mg dry weight from this pool were added to tared beakers, dried, weighed and the ThO_2 content determined according to the technic described by Gordon and Katsh(8). The accuracy of the analytical procedure was checked by running analyses on known amounts of ThO_2. One ml of Thorotrast diluted 1:10 was added to each of a series of beakers containing a small amount of normal rabbit liver. The series of 6 values obtained varied between 22.0 mg and 23.3 mg with a mean of 22.7 ± .19 mg of ThO_2. According to the manufacturers, 0.1 ml of Thorotrast contains 24-26 mg of ThO_2. Errors resulting from variation among lots of Thorotrast were minimized by using one sample throughout a given experiment. Results of ThO_2 analyses were expressed as mg ThO_2 per gram dry tissue. Five independent analyses were made on each tissue pool. The significance of differences in ThO_2 uptake between treated and control groups was determined by the "t" test as described by Fisher(9). The significance of changes in weight resulting from treatment was estimated in the same manner. The antibiotics used in this investigation were: sodium penicillin G; dihydrostreptomycin sulfate; aureomycin hydrochloride§ and pure chloramphenicol.‖

Results. The effects of singly administered antibiotics on the spleen weight and the ThO_2 uptake of splenic tissue are given in Table I. It is seen from Table I that chloramphenicol caused a significant reduction in the uptake of ThO_2 per unit weight of tissue; however the average weight of spleens from chloramphenicol treated mice was greater than that of control mice and this weight change may account for the apparent decrease in ThO_2 uptake. Similarly, the significant reduction in spleen weight produced by aureomycin may account for the increase in ThO_2 uptake observed with this antibiotic. In the spleen analyses all of the material in each organ pool was analysed and hence the amount of ThO_2 taken up by each spleen can be calculated. These values, shown in the right-hand column of Table I do not suggest that either chloramphenicol or aureomycin had any marked effect on the uptake of ThO_2 per spleen.

The effects of antibiotics singly and in

‡ Thorotrast is a stabilized colloidal solution of ThO_2 (24-26% by volume). This chemical was generously supplied by Heyden Chemical Corp., New York.

§ Generously supplied by the Lederle Laboratories.

‖ Generously supplied by Parke Davis and Co.

TABLE II. Effect of Antibiotics on Weight and ThO_2 Uptake of Livers.

Treatment and dose per 12 hr	Avg liver wet wt, g	P for wt diff.	mg ThO_2/g dry wt	P for uptake diff.
St,* 2 mg	2.13	<.4	29.4	<.3
S	2.30	—	28.2	—
A, 2 mg	1.59	<.01	31.7	<.7
S	1.63	—	30.9	—
P, 10000 units	1.67	<.2	34.7	<.01
S	1.79	—	30.3	—
C, 2 mg	1.77	<.8	33.6	<.05
A, 2 mg + C, 2 mg	1.81	<.01	30.9	<.02
+ St, 2 mg	1.74	<.01	35.4	<.001
+ P, 2 mg	1.71	<.01	32.4	<.01
S	2.07	—	24.2	—
C, 2 mg + St, 2 mg	1.95	<.2	25.9	<.5
+ P, 10000 u	1.92	<.2	23.9	<.9
St, 2 mg + P, 10000 u	1.76	<.01	28.1	<.05

* St = streptomycin; S = saline; A = aureomycin; P = penicillin; C = chloramphenicol.

combinations of two upon the weight and ThO_2 uptake of livers are given in Table II. It will be noted that aureomycin alone and all combinations which included aureomycin caused a significant reduction in the wet weight of livers. The combination of streptomycin + penicillin also significantly reduced liver weight although this effect was not noted with either antibiotic alone. In all instances in which antibiotics produced significant changes in the ThO_2 uptake per unit weight of tissue the weight changes produced were in the proper direction to account for such a change in uptake.

Summary. The antibiotics penicillin, streptomycin, aureomycin and chloramphenicol were studied for their effects upon weight and ThO_2 uptake by spleens and livers of mice. These antibiotics were studied singly in the case of spleens and singly and in combinations of two in the case of livers. Aureomycin singly and in combination with other antibiotics caused a significant reduction in the weight of spleens and livers. The combination of streptomycin + penicillin caused a reduction in liver weight. The uptake of ThO_2 was expressed as weight of ThO_2 per unit weight of tissue. Apart from the organ weight changes produced there is no evidence that any of the antibiotics in the concentrations used, had a significant effect upon the ability of either spleens or livers to take up ThO_2.

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