Effect of Malonate on Bacteremia Developed in Mice Artificially Infected with *Salmonella typhimurium*.*† (20832)

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Survival time of mice artificially infected by intraperitoneal injection of a standardized suspension of *Salmonella typhimurium* is reduced from an average of more than 72 hours to less than 24 hours by injections of sublethal quantities of certain inhibitors and intermediates of the tricarboxylic acid cycle (1-3). Malonate is the most effective compound (10 have been tested but results with only 5 have been reported) in reducing survival time in infections not only with this particular organism but also with *Proteus morganii, Micrococcus pyogenes var. aureus,* and *Streptococcus pyogenes*(4). Implied in these observations is a degree of specificity between the particular type of alteration in the tricarboxylic acid cycle of the host and the ability of the bacterial parasite to "exploit" it.

Some years ago, Webster(5) cultured at intervals the blood of mice infected intraperitoneally with *S. enteritidis*. Positive cultures were obtained after 2 to 4 hours but they became negative at about 12 hours. They remained negative until the onset of the terminal stages of the disease. Since Webster's procedure was not designed to yield quantitative data, a modified dilution count procedure was devised which permits repeated bacterial counts on the blood of individual mice.

Methods. Ten mice were infected intraperitoneally with 0.5 ml of a saline suspension containing about 250,000 cells of *S. typhimurium*. Starting immediately thereafter and continuing at hourly intervals for a total of 8 injections, half the mice were injected with 20 mg sodium malonate (Eastman) contained in 0.5 ml of sterile saline and the remaining half, to serve as controls, were injected concomitantly with the same volume of sterile saline. Each mouse was marked by color code in such a way that it could be readily identified. As soon as the second injections of malonate or saline had been completed, a blood sample was taken, as described below, from each animal in turn and when all 10 mice had been bled, they were sampled again in the same order, over and over for a period of about 12 hours. Each time an animal was bled, the end of the tail was amputated and blood was drawn to the "1.0" mark in a small red blood cell diluting pipette. The absolute volume of this portion of the pipette was determined in advance by filling it to the mark with mercury and then weighing this amount of mercury on an analytical balance. Only pipettes with a volume of 1.0 cu mm ± 5% were used. The blood was diluted to the "101" mark of the pipette with sterile heparinized saline. For each dilution a different tube of saline was employed. The pipette was thoroughly shaken and its contents were then blown onto the surface of SS agar (Difco) contained in a Petri dish. The diluted blood

TABLE I. Number of Mice Dying from Intraperitoneal Infection with *S. typhimurium* followed Immediately by Hourly Injections of Either 20 mg Sodium Malonate Contained in 0.5 ml of Sterile Saline for a Total of 8 Injections or a Comparable Number of Injections of 0.5 ml of Saline. (15 mice per group.)

Time post-infection (hr)	Death of mice injected with Malonate + Saline	
7	0	0
7½	1	0
8	4	0
9	5	0
10	6	0
13	10	0
24	15	0
72	15	2
96	15	4
120	15	13
144	15	15

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was spread over the agar with a wire loop, the plate was incubated over night at 37°C and the number of colonies times 1,000 (since 0.001 ml of blood was used) was taken as the number of bacteria per ml of blood. When preliminary counts indicated that the number of bacteria was likely to approximate 200,000 or more per ml of blood, a second dilution was necessary. This was accomplished by discharging the contents of the red cell pipette onto a clean glass slide and then drawing it up into a white blood cell diluting pipette (similarly calibrated) to the "1.0" mark. The white cell pipette was then filled to the "11" mark with saline, the contents were shaken and discharged onto SS agar. The white cell pipettes were selected with capillary volumes of 9.0 $mm^3 \pm 5\%$. Therefore, the number of colonies developing on the SS agar times 11,000 gave the estimated number of bacteria per ml of blood. The factor of 11,000 is obtained since 9/101 mm^3, or about 1/11.000. ml of blood was employed.

Results. As a check on the method, quadruplicate counts were made by both the single and the double dilution technics on appropriate dilutions of a broth culture of *S. typhimurium*. The culture used for the latter count was diluted 1:10 for the former. The average count obtained by the single dilution was 26,300 with a range of 21,000 to 29,000. The average count for the double dilution was 255,000 with a range of 231,000 to 297,000. These results are adequate for the purposes of the present experiments.

The procedure, outlined above, was repeated three times or until a total of 15 mice in each group was studied. A summary of the results is shown in Fig. 1. The logarithm of the average number of bacteria found in the blood of mice injected with malonate and of mice injected with saline is each plotted against time post-infection. The curve designated as "malonate-treated" rises steadily to a peak value in excess of 3 million bacteria per ml of blood at 9 hours and this high count is maintained for the next 3 hours. The mortality data for the same mice are given in Table I, column 2. Ten of the 15 mice died between 7½ hours and 13 hours post-infection while the remaining 5 were dead at 24 hours,

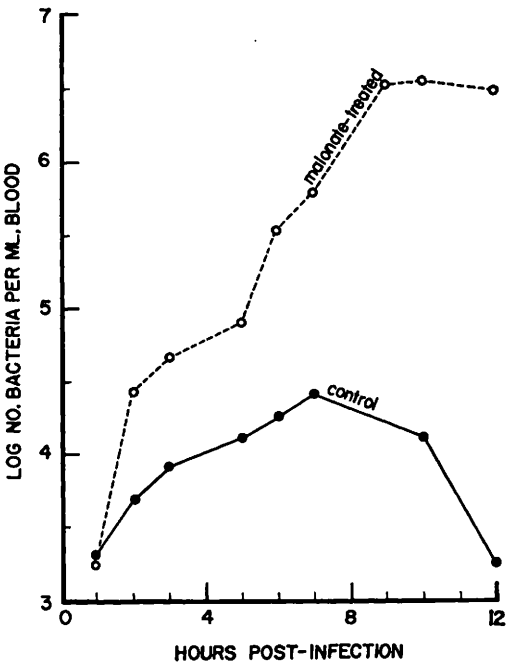

FIG. 1. Logarithm of mean number of bacteria/ml of blood plotted against hours post-infection for 15 mice given hourly injections of 20 mg sodium malonate contained in 0.5 ml of saline for a total of 8 injections (malonate-treated) and for 15 mice given the same number of injections of 0.5 ml saline (controls). All mice were infected intraperitoneally with about 250000 cells of *S. typhimurium* contained in 0.5 ml of a saline suspension.

the exact time of death not having been determined. The constancy of the bacteremia in these mice between 9 and 12 hours post-infection must be considered characteristic of the surviving animals and not necessarily evidence for a static bacterial population. Control mice show a peak bacterial count at 7 hours. By the twelfth hour post-infection the bacteria have practically disappeared from the blood stream (it was zero for 7 of 10 mice on which counts were made). This agrees, in general, with Webster's findings(5). As Table I, column 3 shows, the control mice died between 72 and 144 hours. The average bacterial count for 5 control mice at 72 hours was 406,000. Thus the bacteremia becomes more severe as the infection progresses.

Conclusions. These experiments show that malonate injections in mice artificially infected with *S. typhimurium* results in the development of a bacteremia more than 100 times as

severe as that found within the same time period in control mice injected with equal volumes of saline. Malonate injections in the same mice also reduce survival time, apparently because of the fulminating infection which results. The increased severity of the bacteremia accompanying the malonate injections could be due to a) an impairment of the mouse's defense against bacterial infection, b) the establishment of an internal environment within the mouse which favors bacterial reproduction along the lines postulated by Lewis(6), or c) a combination of (a) and (b). The need for additional experiments thus becomes evident.

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Effect of Glycol Mesylates on Mouse Sarcoma 180.* (20833)

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Haddow and Timmis(1) recently reported on the inhibition of growth of Walker carcinoma 256 in rats by a series of α,ω-dimethanesulfonyloxyalkanes, $CH_3SO_2O(CH_2)_nOSO_2CH_3$. Growth inhibiting activity was found to be greatest when $n = 4$ or 5. Galton (2) tested Myleran, the compound corresponding to $n = 4$, and found it to be of value in the treatment of chronic myeloid leukemia. We are reporting on the ability of a series of methanesulfonyl ("mesyl") esters of primary, secondary and tertiary aliphatic diols and triols, and the mesylates of polyglycols, to inhibit the growth of Crocker sarcoma 180 in white mice.

Materials and methods. Both Carworth Farms and Taconic white mice were employed. Drugs were injected intraperitoneally in normal saline or peanut oil both in the determination of single-dose LD_{50} values and in the evaluation of tumor inhibitory activity. A 7- to 10-day-old tumor was dissected in penicillin-Ringer-Locke solution, and approximately 20 mg portions transplanted subcutaneously into the axillary region of anesthetized mice, groups of 5 experimental and 5 control animals being used with each drug at each dose level. Treatment was begun 24 hours after transplantation, and was continued daily or on alternate days dependent on the reaction of the mice to the drug. Initially the compounds were administered at a level $\frac{1}{3}$ to $\frac{1}{2}$ that of the single-dose LD_{50} value, the level subsequently being reduced if toxic effects became marked. The experiments usually were terminated eight days after transplantation, and the mean weights of the control and treated tumors determined. The compounds were compared on the basis of the mean per cent change in body weight in the experimental and control animals, and the mean per cent inhibition of tumor weight. As a control, mice were treated with 2:4:6-tris-ethyleneimino-1:3:5 triazine (TEM), a known inhibitor of sarcoma 180. In the case of several of the more active compounds, hematoxylin and eosin stained slides were prepared from tumors recovered from treated animals, and compared with tumors from control animals.

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