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Dynamics of infection of the blood stream and internal organs of white mice with *Salmonella typhi* by intraperitoneal injection

By Ch. B. Gerichter and D. L. Boros
Vaccine and Serum Institute, Government Central Laboratories, Ministry of Health, Jerusalem, Israel.

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INTRODUCTION

The parenteral inoculation of different salmonella strains into white mice, and the subsequent quantitative determination of the fate of the injected micro-organisms, has been the subject of investigations by several research workers.

The main strains employed were: *Salmonella paratyphi A*, *S. typhimurium*, *S. enteritidis* and *S. typhi*. The doses administered differed according to the aims of the experiments: LD 100, LD 50, or sublethal doses were employed.

Pike & Mackenzie (1940) compared a highly virulent strain of *S. typhimurium* with a strain of low virulence by quantitative determinations of the number of micro-organisms in the blood stream, spleen and liver.

Hobson (1957), and Mitsuhashi, Kawakami, Goto, Yoshimura & Hashimoto (1959) examined the presence and duration of micro-organisms in the blood stream and internal organs of mice, following intraperitoneal injection. Hobson worked with *S. typhimurium*, while Mitsuhashi *et al.* employed an attenuated strain of *S. enteritidis*; both investigators based their observations upon the quantitative determination of the total bacterial counts in the mice.

Schewe (1958) investigated the period of survival of mice injected intravenously with *S. typhimurium* together with various antimetabolites such as malonate or fluoroacetate, comparing them with control mice, injected with bacteria in normal saline.

Olitzki, Fleischhacker & Olitzki (1957) contrary to the previous investigators employed sublethal doses of different strains of *S. paratyphi A*, and arrived at certain conclusions, concerning the difference in virulence of the strains by means of viable bacterial count of the blood stream and internal organs. In another experiment, Olitzki & Olitzki (1958) applied the above method to the demonstration of the bactericidal effect of the immune serum upon the organisms present in the blood and internal organs. Olitzki, Sharon & Godinger (1960) extended this method still further when, by administering sublethal doses of *S. typhi*, they attempted to determine the difference between phenol-treated and acetone-treated typhoid vaccines in the active immunization of mice. By comparing the viable counts in the spleen, liver, mesenteric glands and blood of immunized and control animals, after measured challenge doses, the authors tried to reach conclusions concerning the efficiency of the two types of vaccine. Ushiba, Saito, Akiyama, Nakano,

Sugiyama & Shirono (1959) employed basically the same method for comparing the efficiency of live and heated vaccines prepared from *S. enteritidis* but without the use of sublethal dosage.

As more and more investigators became concerned with the estimation of the number of salmonella organisms in the test animals as an experimental tool, we found it of interest to examine more closely the dynamics of the infections of the blood stream and internal organs of the mouse, employing the LD 50 dose of *S. typhi* (strain Ty_2), the latter dosage seemed to us very appropriate for our observation since it enabled us to use the quantitative method for determining the difference in the number of bacilli in the blood stream and internal organs, of very sick and moribund mice on the one hand, and surviving ones on the other.

Following the intraperitoneal injection of LD 50 dose of *S. typhi* (Ty_2) into mice, the animals after the first post-infective hours manifest distinct signs of illness: their fur becomes ruffled, the eyes partly or entirely closed, and unlike the healthy animals they become indifferent to their surroundings and cease to eat. As the illness progresses the animals begin to totter and sway, and finally huddle together in a corner of the cage. After 6–7 hr. had elapsed, we observed a change in the external appearance and behaviour of some of the sick animals: the eyes reopened, the fur became smooth and the animals again began to take an interest in their food, however their activity and vigour still differed from those of the healthy animals.

This rather sudden change in the outward appearance of some of the animals suggesting 'recovery' from the illness, encouraged us to find out whether this difference in the external appearance of the animals could be correlated with a parallel difference in the bacterial count of the blood and internal organs of the mice.

With this aim in view, we began, at intervals from the sixth hour and onwards after infection, to separate mice showing signs of recovery from those remaining sick. From these two groups we tried to select only those mice that manifested distinct evidence of either serious illness or recovery; those about which there was some doubt were discarded. At various times during the first 24 hr. (a period during which the vast majority of those mice that eventually would succumb to the infection might be expected to die) selected animals were killed for quantitative determinations of the number of bacteria in a measured volume of blood or a weighed amount of spleen or liver tissue.

Since it was our intention to follow the course of the receding illness, by the viable count of the blood and organs of the surviving animals, we continued to examine the number of micro-organisms up to 34 days after the infection.

The examination of the blood stream began 1 min. after the injection of the infective dose, while that of the spleen and liver was not commenced until 3 hr. after the infection. Altogether thirty-two experiments were carried out, in which the blood and internal organs of more than 1000 mice were examined.

MATERIAL AND METHODS

Mice weighing 18–20 g. of both sexes were used in the experiments; however in the individual experiments only mice of the same sex were used. An LD 50 dose of *S. typhi* (Ty_2) in saline suspension was intraperitoneally injected, the suspension being obtained from an 18 hr. culture of *S. typhi* on trypticase soy agar (Baltimore Biological Laboratory) washed off in saline. The concentration of the organisms, after diluting the original suspension, was adjusted to the desired quantity by means of a Coleman Junior spectrophotometer. The reading was done at 450 mμ. The spectrophotometer reading was at first confirmed by repeated plate counts. The LD 50 dose corresponded to $4{\cdot}0 \times 10^7$ organisms.

The virulence of the strain was maintained by repeated passages through mice.

The animals were anaesthetized with ether, and for the examination of the blood, the chest was aseptically opened, and blood was drawn by cardiac puncture into a tuberculin syringe. Measured quantities of the blood were diluted tenfold in saline. The final dilutions were pipetted on to MacConkey agar plates and spread homogeneously by means of an angled glass rod spreader. The whole spleens were aseptically removed, weighed and ground in a mortar with the addition of saline and afterwards the suspension was serially diluted. The livers were also aseptically removed and homogenized in an M.S.E. Atomix type homogenizer and afterwards were also serially diluted. The procedure for plating the diluted suspension was the same as for the blood count. All the counts were made in duplicate.

RESULTS

Preliminary experiments were carried out with the intention of determining the LD 50 of *S. typhi* (Ty_2) after the intraperitoneal injection. To this end we injected graded doses of $2{\cdot}0 \times 10^7$, $4{\cdot}0 \times 10^7$, $6{\cdot}0 \times 10^7$, $8{\cdot}0 \times 10^7$ and $1{\cdot}0 \times 10^8$ micro-organisms suspended in saline. The mean result of several experiments showed that the LD 50 corresponds to $4{\cdot}0 \times 10^7$ organisms. In the four following experiments, we injected only the LD 50 dose in order to find out the percentage of deviation from the 50 % average. Out of 272 mice injected, 150 died which corresponds to 55 %. (The values ranging from 47–58 %.) This dose of 40×10^6 bacteria was accepted as the LD 50 for mice to be inoculated by the intraperitoneal route with *S. typhi* (Ty_2) and was employed throughout our experiments reported here.

Table 1 presents the number of micro-organisms found in the blood stream from 1 min. after the inoculation of the LD 50 dose up to 24 hr. later. The numbers represent the average of several experiments. As it will be seen, 1–3 min. after the injection, $3{\cdot}2 \times 10^3$ bacilli were found in 1 ml. blood. The number of the bacteria increased steadily, thus after 3–5 min. the count was $8{\cdot}8 \times 10^3$, and after 90 min. $5{\cdot}8 \times 10^4$ in 1 ml. blood. At the end of 3 hr. the count was $2{\cdot}5 \times 10^5$, and this figure remained constant up to 11·5 hr. After 6 hr. it was possible to distinguish between sick and 'recovering' animals by their external appearance. The bacterial count of the two groups also displayed a considerable difference. In the group of the sick animals the mean blood count at 6–11 hr. was $2{\cdot}5 \times 10^5$ bacilli/ml. blood, while the

count in the 'recovering' group at the same period was only about $\frac{1}{8}$th of that of the sick animals: $2{\cdot}9 \times 10^4$ bacilli/ml. blood.

The viable bacterial counts in the blood of sick animals at 12–18 hr. showed a decrease over the figures obtained at 6 hr. This decrease was evident at 9–10 hr. but was more pronounced at 12–18 hr.; at 6 hr. the mean count was $2{\cdot}5 \times 10^5$ and at 12–18 hr. was $3{\cdot}3 \times 10^4$/ml. blood. In the mice that were in the recovering stage the relative decrease was, however, more striking: at 12–18 hr. the mean count

Table 1. *Bacterial count of the blood stream between* 1 *min. and* 24 *hr. after the intraperitoneal injection of* 40 *millions* Salmonella typhi (Ty_2)

Time of examination after infection	Sick or recovering mice	No. of bacteria in 1 ml. blood	Numerical ratio between the count of sick and recovering mice
1–3 min.	Sick	$3{\cdot}2 \times 10^3$	—
3–5 min.	Sick	$8{\cdot}8 \times 10^3$	—
47–82 min.	Sick	$5{\cdot}8 \times 10^4$	—
112–180 min.	Sick	$2{\cdot}5 \times 10^5$	—
3–5·5 hr.	Sick	$2{\cdot}4 \times 10^5$	—
6–11·5 hr.	Sick	$2{\cdot}5 \times 10^5$	—
7–11 hr.	Recovering	$2{\cdot}9 \times 10^4$	1/8
12–18 hr.	Sick	$3{\cdot}3 \times 10^4$	—
12–18 hr.	Recovering	$1{\cdot}5 \times 10^3$	1/22
18–23·5 hr.	Sick	$1{\cdot}9 \times 10^5$	—
18–23·5 hr.	Recovering	$6{\cdot}0 \times 10^2$	1/300
24 hr.	Moribund	$7{\cdot}0 \times 10^5$	—
24 hr.	Recovering	$1{\cdot}7 \times 10^3$	1/400

of these recovering animals was only $1{\cdot}5 \times 10^3$/ml. blood, that is to say, those recovering had a mean bacterial count which was $\frac{1}{22}$nd of the figure for the sick animals in the same period.

The picture changed at 18–23·5 hr. The number of bacteria in the blood of the sick animals increased again and reached almost the level found at 6–12 hr.— approximately $2{\cdot}0 \times 10^5$/ml. blood. In the recovering mice, however, the bacterial count of the blood at the same period, instead of showing an increase further decreased and the mean count was $6{\cdot}0 \times 10^2$/ml. blood, i.e. 300 times less than that of the sick animals.

The increase in the count of the sick animals, which started at 18–23·5 hr., progressed further and after 24 hr. became more obvious, sometimes reaching a peak of several millions, especially in the moribund mice. This final peak was very pronounced and occurred just before death, the mean count at this period being $7{\cdot}0 \times 10^5$/ml. blood. In contrast to this the counts on the blood of recovering animals remained fairly constant at about $1{\cdot}7 \times 10^3$/ml. blood which was approximately that found at 6–12 hr. Compared with the counts of moribund mice there were 400 times fewer bacteria in the blood of recovering mice.

Fig. 1 demonstrates clearly the two peaks in the counts in the blood stream of the sick animals: the first at 8–9 hr. and the second after 24 hr. just before death. After the first peak ($3{\cdot}5 \times 10^5$ organisms/ml. blood) at 8–9 hr. the count fell con-

tinuously up to 18 hr. when the secondary rise commenced and this progressed without interruption until the death of the animal.

Table 2 summarizes the mean bacterial count of the recovering mice between 48 hr. and 34 days. A gradual decrease in the number of micro-organisms is evident; on the twenty-fifth day of the experiment the blood became sterile.

The determination of the number of bacteria in the spleen and liver started 3 hr. after the inoculation. We refrained from the immediate or earlier quantitative determination, because we feared contamination of these organs with the peritoneal fluid rich in the injected micro-organisms.

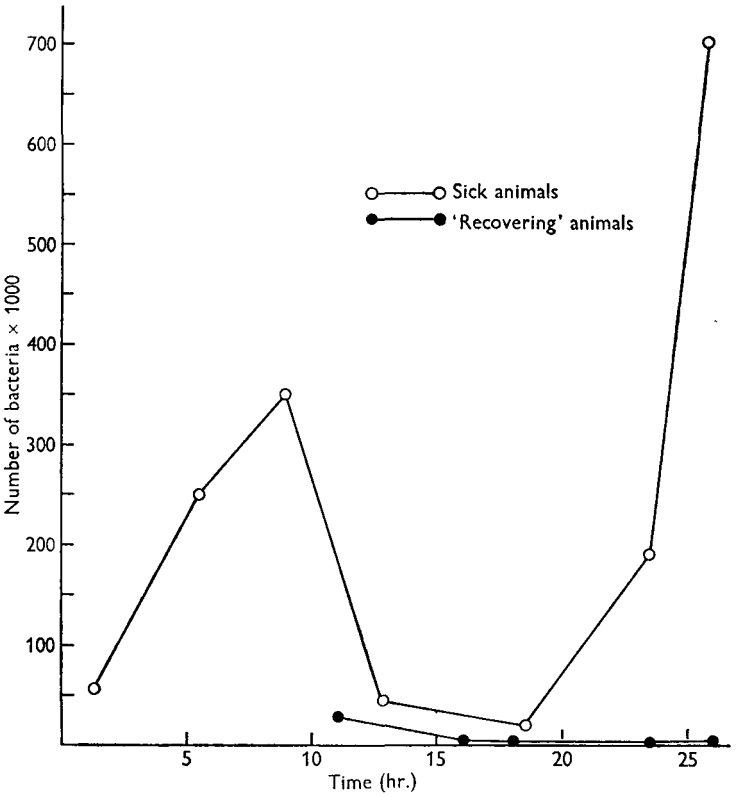

Fig. 1. Bacterial count of the blood stream during the first 26 hr. after the intraperitoneal injection of 40 millions *S. typhi*

Table 3 shows the number of bacteria recovered from the spleen and liver between the period of 3–24 hr. after the inoculation. The numbers in every determination refer to the count per 100 mg. tissue. The mean count of the spleen between 3 and 5·5 hr. (per 100 mg.) was $1{\cdot}7 \times 10^6$ bacteria, and that of the liver $5{\cdot}7 \times 10^5$. Between 6 and 11·5 hr. the trend of the count in the sick and 'recovering' animals differed sharply. In the sick animals a definite increase was evident; about $7{\cdot}0 \times 10^6$ and over $5{\cdot}0 \times 10^6$ organisms per 100 mg. tissue, were found in the spleen and liver respectively. In sharp contrast to this, a decrease in the number of organisms in the organs of the 'recovering' animals during the same period was seen. Here only $1{\cdot}8 \times 10^5$ organisms in the spleen and $2{\cdot}2 \times 10^5$ in the

Table 2. *Bacterial count of the blood stream between* 48 *hr. and* 34 *days after the intraperitoneal injection of* 40 *millions of* Salmonella typhi (Ty_2)

Time of examination after infection	Number of micro-organisms in 1 ml. blood
48 hr.	$1{\cdot}3 \times 10^3$
3 days	$1{\cdot}0 \times 10^3$
4 days	$1{\cdot}5 \times 10^3$
5 days	$1{\cdot}1 \times 10^3$
6 days	$7{\cdot}0 \times 10^2$
7 days	$5{\cdot}2 \times 10^2$
8 days	$3{\cdot}6 \times 10^2$
10 days	$1{\cdot}7 \times 10^2$
11 days	$1{\cdot}3 \times 10^1$
13 days	$8{\cdot}4 \times 10^1$
15 days	$2{\cdot}6 \times 10^1$
18 days	$4{\cdot}1 \times 10^1$
21 days	$1{\cdot}7 \times 10^1$
23 days	5
25 days	0
32 days	0
34 days	0

Table 3. *Bacterial count per* 100 *mg. spleen and liver between the period of* 3 *and* 24 *hr. after the intraperitoneal injection of* 40 *millions* Salmonella typhi (Ty_2)

Time of examination after infection (hr.)	Sick or recovering mice	No. of bacteria per 100 mg. spleen	No. of bacteria per 100 mg. liver	Numerical ratio between the count of sick and recovering mice	
				Spleen	Liver
3 – 5·5	Sick	$1{\cdot}7 \times 10^6$	$5{\cdot}7 \times 10^5$	—	
6 –11·5	Sick	$6{\cdot}9 \times 10^6$	$5{\cdot}3 \times 10^6$	—	
7·5– 8·5	Recovering	$1{\cdot}8 \times 10^5$	$2{\cdot}2 \times 10^5$	1/40	1/25
12 –18	Sick	$1{\cdot}1 \times 10^7$	$4{\cdot}7 \times 10^6$	—	
14·5–15·5	Recovering	$4{\cdot}8 \times 10^5$	$1{\cdot}9 \times 10^5$	1/20	1/24
18 –19	Very sick	$1{\cdot}2 \times 10^8$	$5{\cdot}6 \times 10^7$	—	
20 –23	Recovering	$2{\cdot}2 \times 10^5$	$3{\cdot}2 \times 10^5$	1/500	1/170
24	Moribund	$1{\cdot}3 \times 10^8$	$9{\cdot}1 \times 10^7$	—	
24	Recovering	$3{\cdot}5 \times 10^5$	$1{\cdot}1 \times 10^6$	1/400	1/80

liver were found, constituting 1/40 and 1/25 part respectively of the count found in the sick animals' organs. It is noteworthy that in the recovering animals the number of bacteria, at this period, was slightly less in the spleen than in the liver (per 100 mg. of tissue). This phenomenon will reappear in a later period. In the viable count of the spleen of sick animals there was a further increase after 12–18 hr. The mean count was $1{\cdot}0 \times 10^7$ bacteria. The liver count showed almost no change: $5{\cdot}0 \times 10^6$ micro-organisms.

In the recovering animals the spleen count also showed an increase: $4{\cdot}8 \times 10^5$ organisms, while the liver demonstrated only a slight change: $1{\cdot}9 \times 10^5$. The ratio between the spleen count of sick and recovering animals was now 1/20 while the liver count ratio remained almost without change: 1/24.

As the illness progressed a very steep rise in the spleen and liver count of the sick animals was observable. In the period of 18–19 hr. after the inoculation almost $1{\cdot}2 \times 10^8$ bacteria in the spleen and half this number $5{\cdot}6 \times 10^7$ in the liver, may be found. It is noteworthy that in contrast to this steep rise in the sick animals, the count in the spleen of the recovering animals between 20 and 23 hr. decreased to $2{\cdot}2 \times 10^5$ and the ratio between the sick and recovering animals' count was now: 1/500. The viable count of the liver in the recovering animals was again higher than in the spleen: $3{\cdot}2 \times 10^5$ organisms. The ratio of the two liver counts was now 1/170.

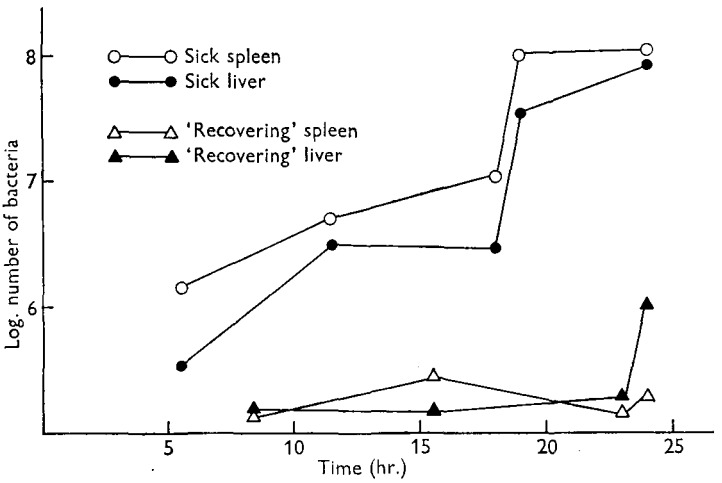

Fig. 2. Bacterial count per 100 mg. liver and spleen during the first 24 hr. after the intraperitoneal injection of 40 millions *S. typhi*.

The climax of bacterial multiplication in the sick animals' organs was reached at the twenty-fourth hour or a little later. In the moribund animals the mean spleen count and the mean liver count were $1{\cdot}3 \times 10^8$ and $9{\cdot}1 \times 10^7$ respectively.

At the same period an increase was evident in both organs of the recovering animals. The spleen count was $3{\cdot}5 \times 10^5$ organisms and the liver count now reached $1{\cdot}1 \times 10^6$ bacteria, which was almost three times more than in the spleen. The numerical ratios compared with the moribund animals were: for the spleen, 1/400; and for the liver, 1/80.

The numerical ratio of the blood count in the moribund as compared to the recovering animals, after 24 hr. was the same as for the spleens: 1/400. After 24 hr. the preponderance of bacteria in the liver count of the recovering animals in comparison to the spleen, became very distinct.

Fig. 2 is a graphical presentation of the number of micro-organisms found in the spleen and liver of the two groups of animals during the first 24 hr. The difference in the curves of the sick and recovering animals is worthy of note.

Table 4 shows the number of bacteria in the spleen and liver of the animals from 48 hr. after the infection until 34 days. The data representing the weight of the spleens are the averages of the spleens weighed. The increase in their weight is

Table 4. *Bacterial count per* 100 *mg. spleen and liver between* 48 *hr. and* 34 *days after the intraperitoneal injection of* 40 *millions* Salmonella typhi (Ty_2)

Time of examination after infection (days)	Mean weight of spleen (mg.)	Relative increase in the spleen weight (%)	No. of bacteria per 100 mg. spleen	No. of bacteria per 100 mg. liver
2	170	—	$1{\cdot}6 \times 10^6$	$1{\cdot}4 \times 10^6$
3	203	35	$3{\cdot}6 \times 10^6$	$2{\cdot}8 \times 10^6$
4	286	90	$5{\cdot}9 \times 10^6$	$2{\cdot}2 \times 10^6$
5	357	137	$4{\cdot}6 \times 10^6$	$1{\cdot}1 \times 10^6$
6	428	185	$3{\cdot}0 \times 10^6$	$1{\cdot}7 \times 10^6$
7	450	200	$2{\cdot}1 \times 10^6$	$1{\cdot}1 \times 10^6$
8	493	228	$7{\cdot}6 \times 10^5$	$9{\cdot}1 \times 10^5$
10	359	140	$1{\cdot}3 \times 10^5$	$1{\cdot}5 \times 10^5$
11	268	85	$4{\cdot}5 \times 10^4$	$1{\cdot}0 \times 10^3$
13	282	89	$2{\cdot}9 \times 10^4$	$1{\cdot}5 \times 10^4$
15	275	83	$3{\cdot}1 \times 10^4$	$2{\cdot}0 \times 10^3$
17	224	49	$4{\cdot}0 \times 10^3$	$2{\cdot}0 \times 10^3$
21	—	—	$5{\cdot}0 \times 10^3$	$2{\cdot}5 \times 10^2$
23	250	60	$6{\cdot}5 \times 10^3$	$3{\cdot}0 \times 10^2$
25	280	80	$3{\cdot}0 \times 10^3$	$1{\cdot}4 \times 10^3$
32	250	60	$1{\cdot}5 \times 10^2$	0
34	184	22	$4{\cdot}0 \times 10^2$	$2{\cdot}0 \times 10^2$

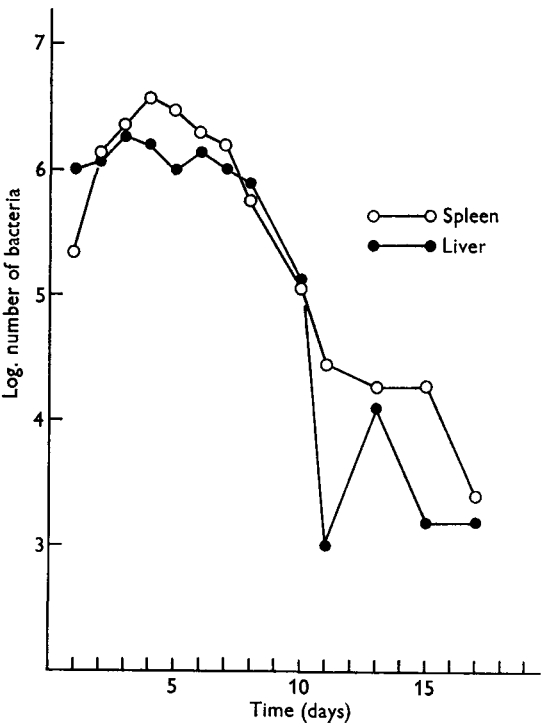

Fig. 3. Bacterial count per 100 mg. liver and spleen between 1 and 17 days after the intraperitoneal injection of 40 millions *S. typhi*.

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computed in relation to the normal weight of this organ. The mean weight of 243 spleens during the first 24 hr. of the experiment was 150 mg. Between 24 and 48 hr. almost no increase in the spleen weight was observed.

In both organs of the recovering animals, a steady increase in the number of bacteria was evident after 48 hr. In the spleen this increase continued during the following days, and reached its peak on the fourth day of the experiment. The mean count of the spleen was $6{\cdot}0 \times 10^6$ bacteria. In this period a considerable hyperplasia was observable which was expressed in the mean weight of the spleens. The same trend of increase in bacterial count was also seen in the liver, although the peak was reached one day earlier, and the count only amounts to half of that of the spleen: about $3{\cdot}0 \times 10^6$ organisms. Having reached the peak in the bacterial multiplication a gradual decline was observed in both organs, during the rest of the period. The hyperplasia of the spleen continued until the eighth day of the infection, when it reached its maximum weight and henceforth decreased gradually. The trend of the decrease in the viable count was steadier in the spleen than in the liver, in which the decrease was interspersed with occasional increases. Even after 34 days a small number of bacteria still persist in the organs. Fig. 3 demonstrates the viable count of both organs during the first 17 days of the experiment.

DISCUSSION

About 6–7 hr. after the intraperitoneal injection of an LD 50 dose of *S. typhi* (Ty_2) into mice we were able to divide the animals into two groups by relying upon the change in the external appearance, one group consisting of sick animals and the other consisting of the recovering ones. The result of the quantitative determinations of the bacteria in the blood stream, spleen and liver confirmed this difference, showing that a correlation existed between the outward appearance and behaviour of the animals and the changes in the viable count of the infecting organisms. In order to find out how far these external signs were reliable, we infected 172 mice with 40 millions of *S. typhi* in three separate experiments, separating the animals 6–9 hr. after inoculation into two groups, again on the basis of their outward appearance. It was found that 71 % of the mice judged sick died within 72 hr., while 84 % of those appearing to be recovering survived. Presumably, in the actual experiment in which the animals had to be sacrificed to obtain the viable count of the organs, the proportion of errors would have been still lower because we picked out only those animals manifesting definite signs of illness or recovery, discarding the doubtful ones.

The viable count of the bacteria in the blood stream during the first 24 hr., in which the majority of deaths occurred, shows that the difference between sick and recovering animals became more and more pronounced as time passed. The numerical ratio between the count of the sick and recovering animals increased rapidly; it was 1/8 between 6 and 11 hr., 1/22 between 12 and 18 hr., climbing to 1/300 within 18–23 hr., and reached the climax: 1/400, 24 hr. after the infection, usually just before the exitus. The viable count of the organs also displayed the sharp difference between the two groups. The number of the micro-organisms in

the organs of the recovering animals was far less than in the sick ones. Our observations indicate that in the sick group of animals the bacteria circulating in the blood are subject to a different fate to those found in the internal organs of the sick animals. In the first post-infective hours a parallel rise was evident both in the blood and the organs of the sick animals. After 9–10 hr. a change commenced—a decrease began in the blood stream, continuing till the eighteenth hour, while at the same time a slow and steady increase was seen in the internal organs. From 18 hr. and onwards, a sharp increase began both in the blood and the internal organs, prognosticating the approaching exitus of the animals. This parallel rise reached its climax during the subsequent 6–8 hr. when the mice were already in the *ante finem* state.

The above-mentioned decrease in the blood stream, 9–10 hr. after the infection, is explained by the 'clearing mechanism' of the blood, which is probably not active in the internal organs. The secondary rise in the bacterial count of the blood after 18 hr. is probably connected with a quick multiplication of the bacteria in the internal organs. We may suppose that the surplus bacteria 'overflow' from the heavily invaded reticulo-endothelial system, and enter the blood stream. This picture does not differ in principle from the secondary bacteraemia which can be seen in the process of natural infection with *S. typhi* in human beings, or with *S. typhimurium* in mice.

From the above observations it may be concluded that the outcome of the type of infection described is probably already decided within 6–7 hr. after the injection of the LD 50 *S. typhi* (Ty_2), that is to say, whether the animals will die or recover. The fact that the bacterial counts of the mice which will die are so remarkably different from those of mice which will recover is of very great interest and is in keeping with the clinical observations on the infected mice in a significant proportion of the cases.

It is still not possible to give any reason for the different behaviour of the mice towards exactly the same dose of the same organism administered in the same way under the same conditions. The LD 50 of *S. typhi* (Ty_2) injected intraperitoneally into mice of the same strain, sex, age and weight is remarkably constant; approximately 50 % will die and 50 % will recover. Research work which aims at providing a satisfactory explanation for this phenomenon is in progress in our laboratory.

SUMMARY

An LD 50 dose *S. typhi* (strain Ty_2) was injected intraperitoneally into white mice, and the number of micro-organisms was determined after 1 min. and subsequently, in the blood, and after 3 hr. and subsequently, in the spleen and liver. The course of the infection was observed for 34 days.

The number of bacteria gradually increased in the blood stream during the first post-infective hours. It was possible to differentiate by external signs from the sixth post-infective hour and onwards between sick animals and recovering ones. This difference corresponded to the bacterial concentration in the blood stream and internal organs.

In the sick animals the increase in the bacterial count of blood stream continued till 9–10 hr. after the inoculation, when it reached its first peak. During the following 8 hr. a clear decrease in the blood count was evident which was attributed to the 'clearing mechanism' of the blood. The onset of a new bacterial rise in the blood stream appeared 18 hr. after the inoculation and reached a second peak during the further 6–8 hr.

In contrast to the observed decrease in the bacterial blood count, a steady and gradual increase in the count of the internal organs was seen; it was steadily progressive till the exitus.

In the recovering animals a steady decrease in the bacterial blood count was recorded. Between 24 and 26 hr. the number of micro-organisms in the blood and spleen was found to be 400 times less in the recovering animals than in the moribund ones. From 48 hr. and onwards a slow and gradual decrease in the bacterial count of the surviving animals was observed. The blood became sterile after 25 days. In the spleen and liver of the surviving animals after 48 hr. a new increase in the bacterial count is evident, accompanied by hyperplasia of the spleen. The number of bacteria reached a new peak in the liver on the third day and in the spleen on the fourth day after the infection.

Subsequently a slow and gradual decrease in the bacterial count was observed in both organs, but even after 34 days a small number of persisting bacteria in the spleen and liver tissue may be found.

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