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THE INTERACTION OF GRAM NEGATIVE BACTERIA AND *S. MANSONI* IN MICE WITH EXPERIMENTAL SCHISTOSOMIASIS

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Animals (122 mice) were infected each with eighty cercariae of S. mansoni *and subsequently challenged intravenously eight weeks later with the following gram-negative organisms:* S. typhi, E. coli, *Klebsiella-enterobacter species,* Proteus mirabilis *and* Pseudomonas aeruginosa. *Enumeration of bacteria in the liver, spleen and blood and* S. mansoni *from the portal sistem was performed from one to four weeks later in infected animals. A significant difference between infection produced by* S. typhi *and other gram negative organisms was observed:* S. typhi *persisted longer in the spleen and liver and could be recovered from* S. mansoni *worms up to three weeks following bacterial infection. Other gram negative bacteria disappeared from* S. mansoni *worms after two weeks of initial challenge.*

Additional animals (51 mice) infected with S. mansoni *were given* S. typhi, E. coli *or sterile saline. After two weeks, animals were sacrificed and the recovery rate of worms from the portal system, and the mesenteric and hepatic oogram were determined. In animals infected with* E. coli *a significant decrease in the number of worms was observed compared to the saline control group; thirty worms were recovered in the control group compared to two worms in* E. coli *infected animals. In addition, the patterns of oviposition was significantly different in these latter animals suggesting complete inhibition of this process. Following* S. typhi *infection the difference in recovery of worms and pattern of oviposition was minimal. These findings suggest a difference in the interaction of various gram negative bacteria and* S. mansoni *and are consistent with the clinical observation of prolonged salmonella bacteremia in patients with schistosomiasis.*

Several observations demonstrating an altered host-parasite relationship in patients with salmonella infections and underlying schistosomiasis have been reported. Prolonged salmonella bacteremia with distinct clinical and laboratory features has been described in patients with schistosomiasis Neves & Martins (1967), Rocha et al (1971), Tay et al (1958) e Teixeira (1960); a chronic urinary carrier state of enteric bacteria has also been described in patients infected with *S. hoemeatobium* Neva (1949), Hathout (1966).

The basis for this altered relationship has been the subject of some debate. An increased susceptibility of schistosome-infected mice to *S. typhimurium* has been demonstrated Rocha et al (1968). In addition, *S. enteritidis* causes a severe infection with

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a rapid increase in bacteria in organs of mice with mild, chronic *S. mansoni* infection Collins, Boros & Warren (1972). Previous reports have also shown that certain gram negative bacilli, particularly those belonging to the genus Escherichia, Klebsiella, Enterobacter and Serratia, have an antischistosomal effect following intravenous challenge of *S. mansoni* infected animals Ottens & Dickerson (1972). A possible explanation for this phenomenon is that injected bacteria colonizes the gut of schistosomes and massive multiplication causes death of the worm. Subsequently, it has been shown that the injection of *S. typhi* in mice infected with *S. mansoni* results in a self-limited disease, bacteria persisting in tissues of some animals for a period of four weeks. Culturing the pool of *S. mansoni* collected from the portal system of animals demonstrated that worms carry large numbers of bacteria for periods of at least two weeks Young et al (1973). The site where salmonella are carried by the schistosome is unclear. By using immunofluorescence techniques and repeated direct streakings of worms recovered from infected animals and humans, it has been suggested that *S. typhimurium* is attached to the surface of the worm Young et al (1973).

In the present study certain characteristics of gram negative bacterial infections in mice infected with *S. mansoni* were examined especially the comparative antiworm effects of *E. coli* and *S. typhi*. The purpose of this study was to detect differences that could explain the characteristic properties of salmonella in the schistosome infected host.

MATERIAL AND METHODS

Animals – Adults male albino mice were used throughout the study. They were fed commercial pellets and water *ad libitum*. Animals were infected with *S. mansoni* by exposing the subcutaneous tissue to approximately 80 cercariae for a period of 30 minutes. Following eight weeks of the original exposure, mice demonstrating viable eggs of *S. mansoni* in a direct stool examination were used in the study. A total of 122 mice were used in the present study.

Bacteria – A strain of *S. typhi* from a patient with *S. mansoni* infection with prolonged salmonella bacteremia was used. Strains of *E. coli*, *Ps. aeruginosa* and *Proteus mirabilis* had been isolated from patients with chronic urinary tract infections. All bacterial species were maintained on trypticase-soy-agar slants and prior to infection transferred to trypticase soy broth and grown overnight. Before challenge, the culture was diluted in trypticase soy broth, inoculated for four hours at 37°C, and serially diluted in saline (1/10 dilutions) immediately before intravenous injection. All mice were challenged intravenously with approximately 10^6 organisms.

At intervals of one to four weeks, groups of mice which received a bacterial challenge were sacrificed for bacterial enumeration in the blood, spleen, liver and schistosome worms from the portal area.

Enumeration of bacteria in tissues of mice and recovered schistosome worms.

Groups of animals were sacrificed by cervical dislocation and, under sterile conditions, 0.9ml of blood was taken by direct heart puncture. Specimens of liver and spleen were homogeneized in sterile 0.9% saline solution in a Ten Broeck grinder (TRI instruments). Schistosome worms from the venous system and from livers were removed aseptically. At weekly intervals, the schistosome worms collected from a group of mice were pooled and cultured. For culturing blood, 0.1ml of blood was added to melted agar and poured into a Petri dish; 0.1ml was diluted into 0.9ml of distilled water and 1ml was immediately used for pour plates with plain agar. Ground liver and spleen were serially diluted in distilled water and serial agar pour plates were made for appropriate counting. To culture schistosome worms, a series of 5 washings in sterile distilled water were performed. Worms were then placed into grinding tubes in a 10ml volume of distilled water

and after thorough mixing, tubes were centrifuged for 5 minutes at 1,500rpm. A total of 8ml of the supernatant was discarded and substituted by a similar volume of distilled water. After five consecutive washing, the remaining 2ml of liquid were divided into two parts: the supernatant (1ml) and the pellet (1ml) which contained the worms. Following a thorough grinding of worms in a Ten Broeck homogeneizer, both supernatant and pellet fractions were 10 fold serially diluted and Trypticase soy agar pour plates made. All plates were incubated at 37°C for 24 hours, and the number of colonies counted in a Quebec-Spencer colony counter. At least three colonies on each significant plate was appropriately identified by standard bacteriologic procedures.

In a few instances, single schistosome worms recovered from mice were placed in separate grinding tubes and subjected to similar washing and culture procedures.

Enumeration of worms recovered from the portal system of infected mice; egg counts in liver and mesentery.

Eight weeks after infection, worm counts were made by the perfusion method of Duval and De Witt (1967). The enumeration of different types of eggs in liver and mesentery was performed in animals following the perfusion. The oogram was classified as follows Pelegrino & Faria (1965):

N = Normal
\+ = Immature eggs predominate (with a reduction in eggs layed)
++ = Equal numbers of immature (later stages) and mature eggs
+++ = Mature eggs predominate
++++ = Mature and dead eggs only

Comparison of in vivo *and* in vitro *exposure of* S. mansoni *to* S. typhi

S. mansoni worms collected from the portal system of infected mice were exposed *in vitro* to a saline suspension of *S. typhi*, containing 10^5 organisms per ml for a period of 5 minutes. Following five consecutive washings in normal saline (as previously

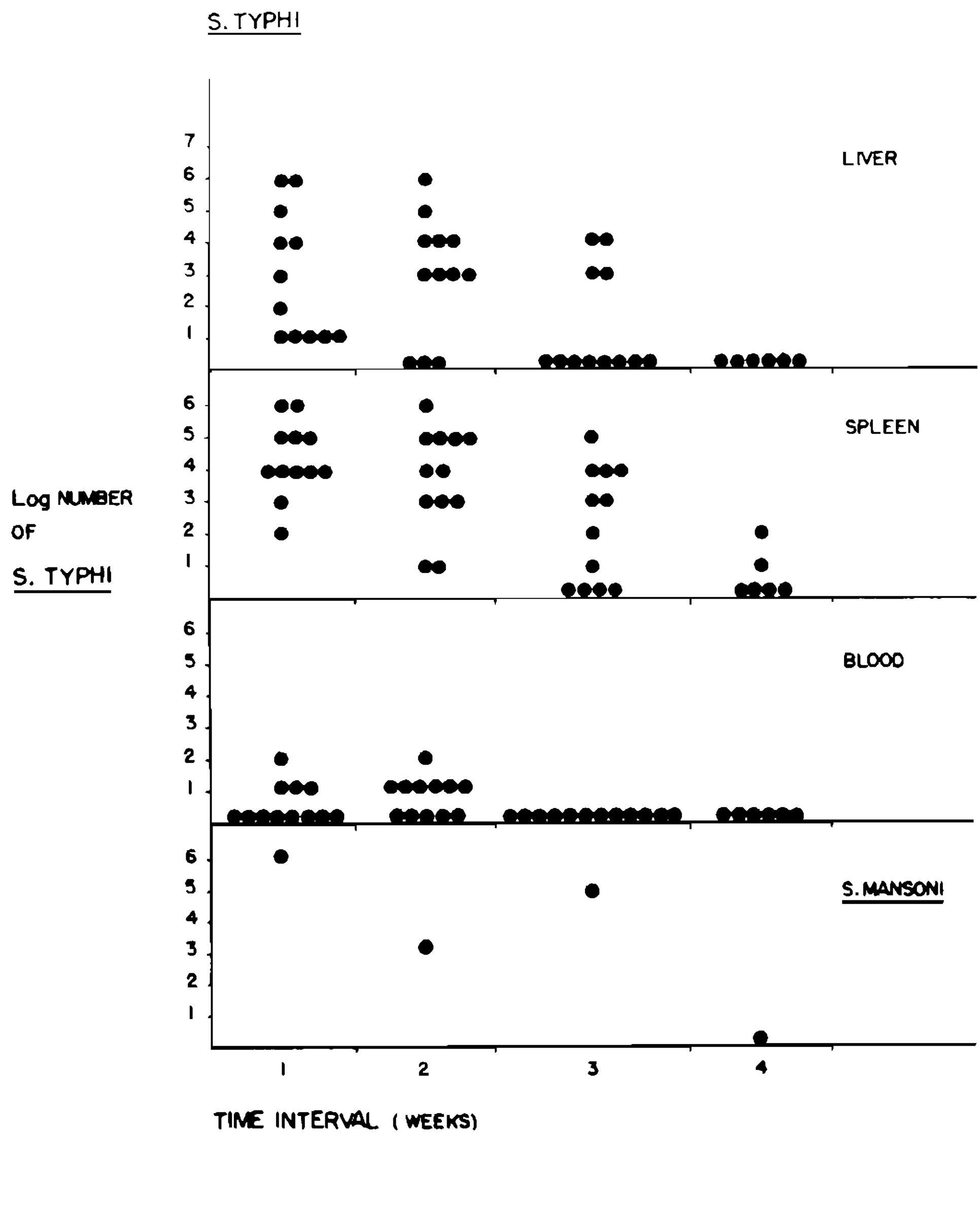

Figure 1. Recovery of *S. typhi* from liver, spleen and *S. mansoni* worms collected from the portal system of infected mice.

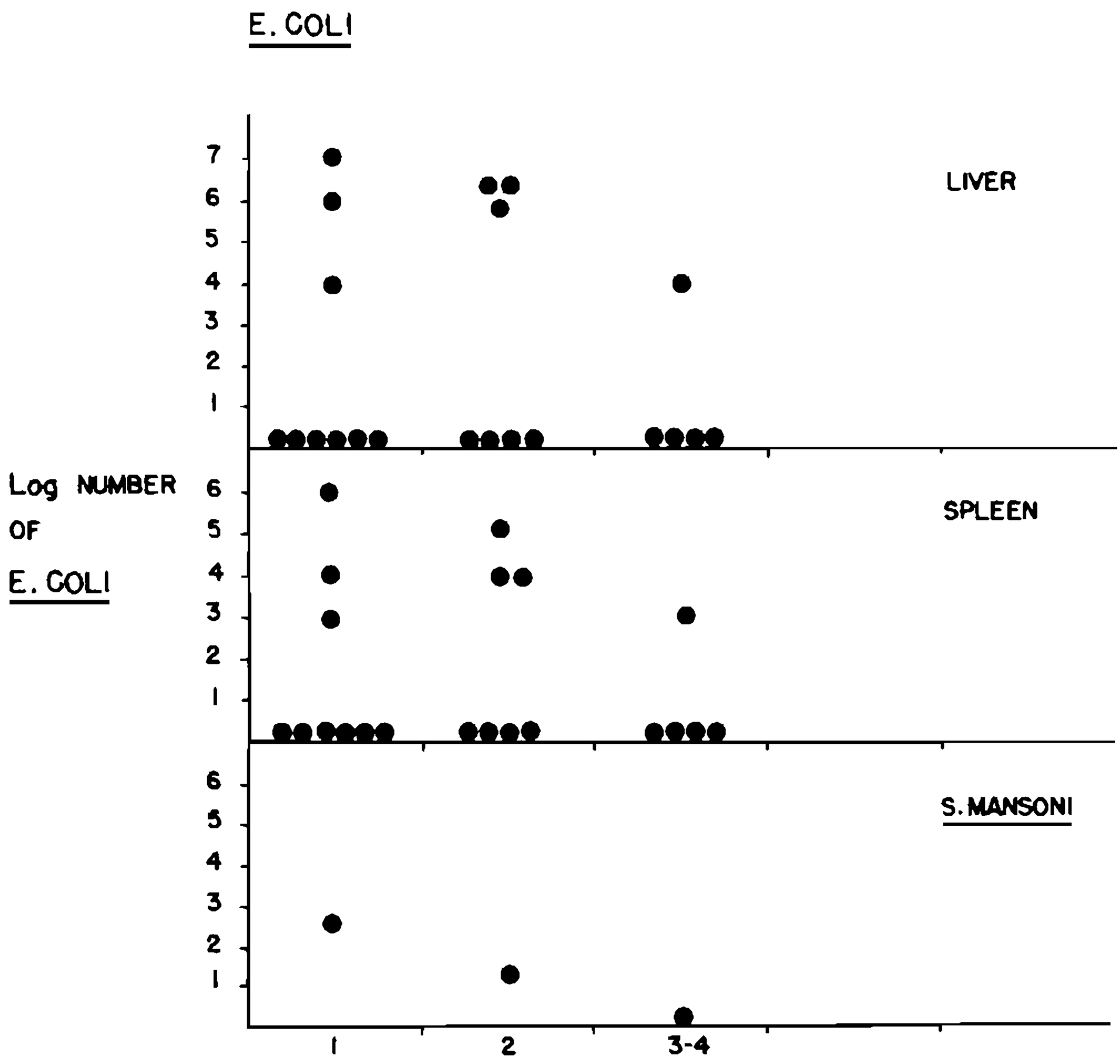

Figure 2. Recovery of *E. coli* from liver, spleen and *S. mansoni* worms collected from the portal system of infected mice.

2. *Enumeration of bacteria recovered from single* S. mansoni *worms following* S. typhi *and* E. coli *challenge.*

A total of 46 *S. mansoni* recovered from mice injected with *S. typhi* and 38 from animals injected with *E. coli* were cultured as single organisms. Worms collected from mice injected with *S. typhi* showed a greater number of bacteria up to three following initial challenge. However *E. coli* was recovered from *S. mansoni* in small numbers for only the first two weeks after challenge (Fig. 3). Not all worms were colonized with bacteria and the number of bacteria decreased in the second and third week. Indeed, following *S. typhi* challenge, 10 of 14 worms were infected in the first week; 3 of 13 in the second, 3 of 9 in the third week and none of 4 in the fourth week. Following *E. coli* challenge, only 4 of 18 worms were colonized in the first week and 2 of 12 in the second week.

TABLE I

S. Mansoni Recovered from Liver and Mesentery of Mice Following I. V. Injection of 10^5 *S. Typhi* of *E. Coli*

Weeks After Bacterial Infection	*Mice Receiving* S. Typhi		*Mice Receiving* E. Coli		*Control Mice*	
	Nº of Mice	*Nº of Worms Recovered (Average)*	*Nº of Mice*	*Nº of Worms Recovered (Average)*	*Nº of Mice*	*Nº of Worms Recovered (Average)*
2	8	13	5	2	6	30
3	8	11	4	1	6	22
4	8	9			6	16

TABLE II

S. Mansoni oogram* in the Liver and Mesentery of Mice After 10^5 I.V. Injection of *S. Typhi* or *C. Coli*

Weeks After Bacterial Injection	*Types of* S. Mansoni *Eggs*								
	Mice Receiving S. Typhi			*Mice Receiving* E. Coli			*Control*** *Mice*		
	Nº of Mice	*Liver*	*Mesentery*	*Nº of Mice*	*Liver*	*Mesentery*	*Nº of Mice*	*Liver*	*Mesentery*
2	8	++	+	5	++++	+++	6	+	+
3	8	+++	+	4	++++	++++	6	+	+
4	8	+++	++				6	+	+

*Oogram expressed as:

\+ Immature eggs predominate
++ Equal numbers of immature and mature eggs
+++ Mature eggs predominate
++++ Mature and dead eggs only

**Mice infected with *S. mansoni* only

4. *Infection produced by* Klebsiella-enterobacter sp, Ps. aeruginosa *and* Proteus mirabilis

A total of 18 mice were injected with *Klebsiella-enterobacter sp,* 12 with *Proteus mirabilis* and 20 with *Ps. aeruginosa.* Distribution of bacteria in the liver, spleen and in *S. mansoni* (pool of worms) recovered from those animals was determined at one and three weeks following bacterial injection. As shown in Figure 4, Klebsiella-enterobacter organisms produced an infection very similar to *E. coli.* However, except in four animals, *Ps. aeruginosa* and *P. mirabilis* were completely cleared within one week after infection. No colonization of *S. mansoni* was detected by these two bacteria.

5. *Enumeration of* S. typhi *in* S. mansoni *following* in vitro *and* in vivo *exposure.*

While the exposure of worms to *S. typhi* in vitro resulted in cultures revealing less than 10^1 organisms, the *S. mansoni* recovered from the portal system of infected mice, at a time of a low grade bacteremia, revealed 10^3 to 10^6 *S. typhi.*

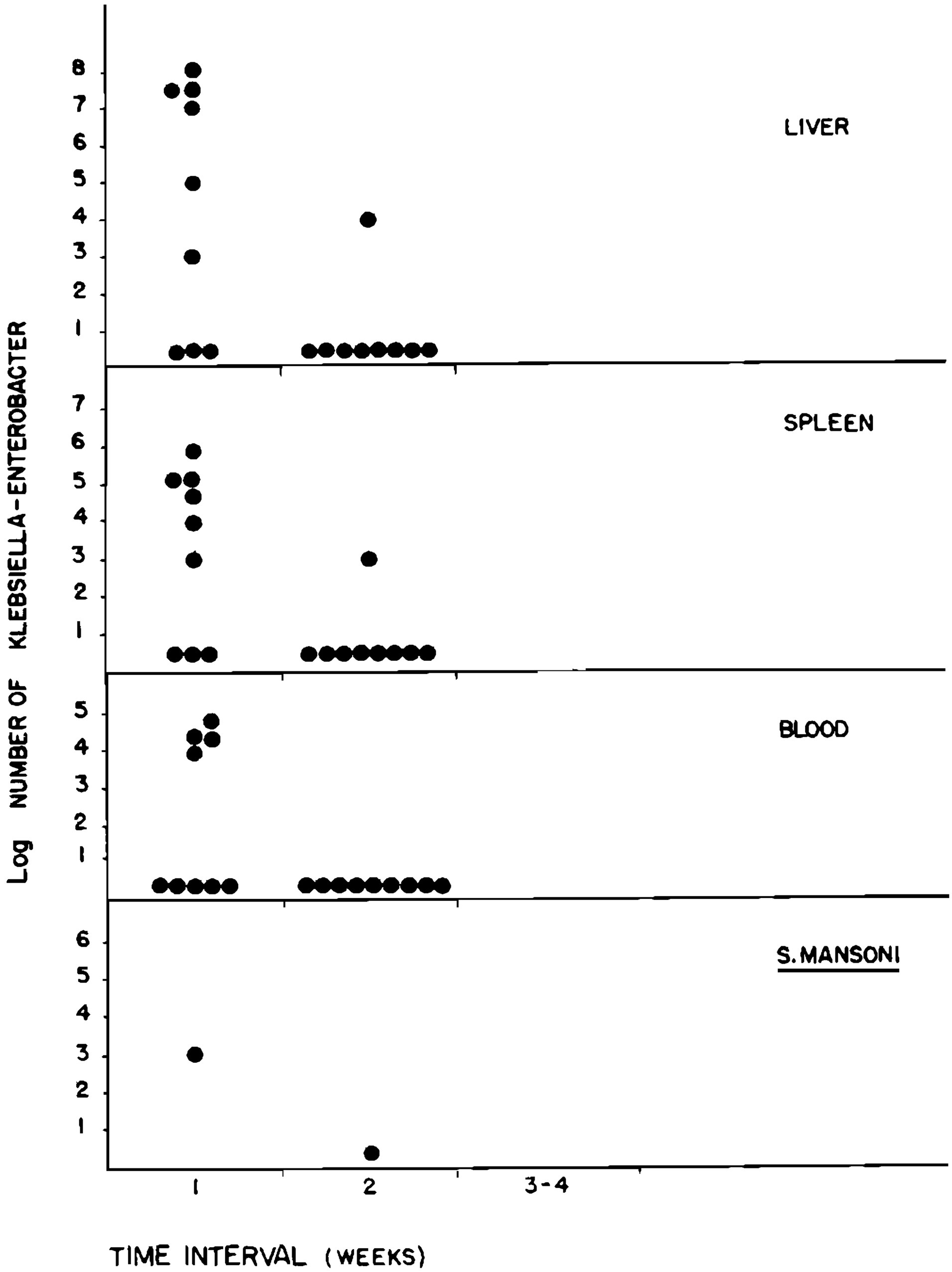

Figure 4. Recovery of Klebsiella-Enterobacter from liver, spleen, blood and pooled *S. mansoni* collected from the portal system of infected mice.

DISCUSSION

The mechanism responsible for chronic salmonella bacteremia in patients with hepatosplenic schistosomiasis has not been well established. It has been shown that a multitude of gram negative bacteria are able to colonize the gut of the worm, some of them being able to kill it as a result of a multiplication and "infection" of the parasite Ottens & Dickerson (1972). It has also been shown that mice infected with *S. mansoni* are more susceptible to *S. typhimurium* Rocha et al (1968), *S. enteritidis* Collins, Boros & Warren (1972) and *E. coli* infection Rocha, Motta & Rebouças (1968). Moreover, it has been demonstrated that *S. mansoni* infected mice when injected with *S. typhi* intravenously not only show a greater number of microorganisms in several organs, but also that *S. mansoni* recovered from the mesenteric system carry *S. typhi* for up to two weeks following the initial injection of this bacterium Rocha et al (1971).

A difference between the pattern of *E. coli* and *S. typhi* infection in mice infected with *S. mansoni* has been clearly shown in the present study. Mice cleared both bacteria from the bloodstrem within two weeks. However, while *S. mansoni* recovered from animals which received *E. coli* showed a small number of bacteria at two weeks, *S. mansoni* from mice injected with *S. typhi* showed a large number of microorganisms for three weeks. In addition, following *E. coli* infection, the recovery of *S. mansoni* from the mesenteric system of mice was greatly reduced, and a change in the pattern of oviposition at the mesentery and liver was observed indicating an interruption in oviposition. In contrast, reduction in the number of worms recovered and an intermediate change in oogram as compared to the control group was seen when *S. typhi* was the infecting bacterium. These data suggest that *E. coli* was probably lethal for the worms, whereas *S. typhi* was much better tolerated. Worms "infected" with *E. coli* as well as Klebsiella-enterobacter organisms most likely died whereas *S. typhi* persisted in viable worms for up to 3 weeks.

The exact location of salmonella organisms recovered from *S. mansoni* worms is unclear. The following observations in this study suggest that microorganisms such as *E. coli* and Salmonella "infect" *S. mansoni* and are not just attached to its surface: 1. Not all worms recovered from mice injected with *S. typhi* were culture positive. It would be logical to find bacteria in the majority of *S. mansoni* if organisms were attached to their surface; 2. The change in oviposition following *S. typhi* suggests that worms were damaged from bacterial challenge. This is consistent with infection of worms and not solely surface colonization; 3. The *in vitro* exposure of worms to large numbers of bacteria did not result in recovery of large numbers of *S. typhi* as compared to the culture of worms recovered from infected mice.

Clinically, there is no question that the schistosome is directly related to the particular syndrome of prolonged salmonella bacteremia observed in endemic areas of this parasitic infection. Salmonella bacteremia in these patients may be cured by effective treatment of schistosomiasis alone Neves et al (1969), probably by the elimination of foci of persisting organisms. In addition, a high relapse rate of salmonellosis occurs in patients with schistosomiasis Hathout et al (1967). These clinical observations suggest that salmonella surviving within the worm may be not as available to host defense mechanisms and to the action of antibacterial agents. This fact emphasizes the need for specific therapy for schistosomiasis in the setting of salmonella bacteremia particularly in the hepatosplenic form of this parasitic infection.

RESUMO

Interação de Bactérias Gram Negativas e Esquistossomose Mansônica em Camundongos com Esquistossomose Experimental

Camundongos (122 animais) foram infectados com 80 (oitenta) cercarias de *S. mansoni* cada, e subseqüentemente receberam, oito semanas depois, injeção intraveno-

sa das seguintes bactérias gram negativas: *S. typhi, E. coli*, uma raça de Klebsiella enterobacter, *P. mirabilis* e *Ps. aeruginosa*. Os animais foram sacrificados a intervalos de uma a quatro semanas depois, sendo feita a contagem de bactérias no fígado, baço, sangue e de *S. mansoni* recolhidas por perfusão do sistema portal destes animais. Foi observada uma nítida diferença entre a infecção produzida por *S. typhi* e pelas outras bactérias gram negativas: *S. thypi* persistiu por mais tempo no fígado e baço e pôde ser recuperada dos *S. mansoni* até três semanas após a injeção bacteriana.

Os outros gram negativos desapareceram dos *S. mansoni* dentro de duas semanas da inoculação inicial.

Um grupo adicional de camundongos (51 animais) infectados de modo similar com *S. typhi, E. coli* foi sacrificado duas semanas depois da infecção venosa, à semelhança de um grupo controle que recebeu solução salina fisiológica – foi feito estudo do número de vermes recolhidos do sistema portal nos três grupos, assim como o oograma do fígado e do mesentério. Nos animais inoculados com *E. coli* houve decréscimo significante no número de vermes, quando comparado ao grupo que recebeu salina (controle): 30 vermes foram recolhidos do grupo controle, em comparação a apenas dois nos animais que receberam *E. coli*. Além disso, o padrão de oviposição foi muito diferente entre estes dois grupos – após a injeção de *S. typhi*, a diferença na recuperação de vermes e no oograma foi relativamente pequena, quando comparado ao grupo controle. Estes dados sugerem uma diferença na interação de várias bactérias gram negativas com o *S. mansoni*, algumas matando os vermes na sua maioria *(E. coli)* outras *(S. typhi)* permitindo a sobrevivência de muitos dos vermes infectantes. De um modo geral estas observações corroboram para o entendimento do quadro clínico de salmonelose sistêmica prolongada em portadores de esquistossomose mansônica.

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