SOME PHYSIOPATHOLOGICAL PARAMETERS OF NATURAL RESISTANCE TO INFECTION IN MURINE SALMONELLOSIS*

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PLATES 2 TO 7

(Received for publication, January 7, 1959)

Salmonellosis in the mouse is an infectious disease which, both as an unwanted natural event in mouse colonies and as an experimental "model" infection in the laboratory, has received considerable investigative attention.

In spite of this interest much remains unknown. It is not our purpose here to itemize or review the list of unsolved problems in this disease but, from a platform of present information, to focus on the primary phenomenon of survivorship as it is achieved or not achieved in mouse hosts which have had no previous experience with this infectious disease. From this selection it follows that we are concerned with "natural resistance," a phenomenon for which several parameters have been previously identified and others suggested. For example, in mouse salmonellosis, predicted differences in host survivorship can be arranged by either genetic (1, 2) or nutritional (3–5) means. In the present investigation we have elected the genetic arrangement of host differences in survivorship. Now, although the physiological mechanism responsible for such genetically arranged differences is unknown it has been shown that these very survivorship differences are operationally dependent on the genetic structure of the pathogen population, demanding a genetic heterogeneity in terms of virulence (6). It is still moot whether this operational necessity can be generalized to embrace other infectious diseases, but in the present concern with mouse salmonellosis we have felt obliged to retain this prescription and have employed the double strain inoculation infection test (7) as a precise means of providing the appropriate pathogen populational structure.

With survivorship, or lack of it, thus predictively arranged it would seem logical enough next to inquire concerning the physiological basis of the character thus manipulated. However, there is a caveat here which is recommended to us by the work of Webster (1) who showed that genetic resistance to one infectious disease can be accompanied in the identical animal by susceptibility to a second infectious disease. This, it seems to us, raises some difficulties for the concept of a manipulable panresistance as a host attribute and, at the same time, introduces an element of specificity which present notions of "non-specific resistance" seemingly do not include. For mouse salmonellosis, suffice it to say, such specificity as the above consideration would require have not been met by correlation of antibody levels with survivorship

* This investigation was supported in part by a research grant, B-1565, from the National Institute of Neurological Diseases and Blindness, Public Health Service.

(8–10). The possibility remains, however, that in some special, as yet unappreciated way, survivorship in mouse salmonellosis might be dependent on and correlated with the often postulated increased activity of the reticulo-endothelial system either prearranged or as a latent elicitable capacity called forth by the infection itself.

It was the purpose of the present investigation to examine the parametric relations conceivably involved between host genetically determined survivorship and the hypothetical roles of "the activity of the reticulo-endothelial system" including dynamics of change in the peripheral circulating white cells. For improved analysis of the kinetics of salmonellosis, and thus for purposes of interpretation, the progress of the disease was estimated by examination of internal organs both macroscopically and histologically as well as the direct estimation of survival time and survivorship frequencies.

Materials and Methods

Animals.—Two inbred mouse strains were used, salmonellosis susceptible BSVS and resistant BRVR. The origins of these strains have been described elsewhere (1). In the present experiments only 2 month old males were used and were housed in individual cages (3) in a special air-conditioned room at 80°F., 50 per cent relative humidity, and with a 12 hour (fluorescent) light day. The animals had been reared and were continued on a commercial (Dietrich and Gambrill) pasteurized, pathogen-free stock diet and tap water *ad lib.*

Infection.—The experimental animals were infected with *Salmonella typhimurium* by the double strain inoculation procedure (7). Both bacterial strains, RIA-avirulent, and SR-11–virulent have been described in detail elsewhere (11). For the avirulent infection phase, 1000 cells of 16 hour culture of the RIA strain in Penassay broth were suspended in a final volume of 0.25 ml. of normal, sterile saline and injected intraperitoneally. This was followed 2 days later by the virulent infection phase, which consisted of 100,000 cells of a 16 hour culture of the SR-11 strain in Penassay broth, likewise suspended in 0.25 ml. of normal, sterile saline and injected intraperitoneally. The infective doses were verified both turbidimetrically and by plate count on Penassay agar.

RES Activity.—The activity of the reticulo-endothelial system (RES) was tested by a method based on rate of clearance following intravenous injection of India ink (12, 13). Under the conditions of this technique and with the proper amounts of ink, the particles are taken up almost exclusively in the liver and spleen. As the results of the technique are influenced by the size of the particles, it was important to use a stable suspension of homogeneous size (approximately 250 Ångström) (14). The material used in this study was prepared by Dr. Baruj Benacerraf of New York University Medical School from the commerically available suspension C 11 1431a[1] and was generously supplied by him. This standardized preparation was devoid of toxicity.

On the days indicated in the experimental plan below, test mice received by the intravenous route a dose of 16 mg. of carbon per 100 gm. body weight. After 2, 4, 6, 8, 10, and 15 minutes 0.025 ml. of blood was withdrawn from the retroorbital venous plexus in the nasal angle of the eye. The blood samples were immediately hemolyzed in 2 ml. of a 1:1000 solution of sodium carbonate (Na_2CO_3) in distilled water. The amount of carbon present in the sample was then determined electrophotometrically in a Coleman colorimeter set at 675 mμ. From this reading the log of the true concentration of carbon particles was calculated. Once the carbon clearance

[1] Commercially available from Günther Wagner, Hannover, Germany.

test had been completed, the animals were killed with ether and their livers and spleens weighed.

The following values were determined for each animal:

(*a*) Body weight.

(*b*) Combined weights of liver and spleen.

(*c*) The quotient $\frac{\text{Body weight}}{\text{Liver + spleen weight}}$

(*d*) The clearance factor k. This factor was determined by plotting the log of the carbon concentration in each blood sample against the time of bleeding. The points thus obtained give an approximately straight line. The slope of this curve is the value k, expressed by the equation

$$k = \frac{(\log \text{ concentration } 1) - (\log \text{ concentration } 2)}{t_2 - t_1}$$

in which t_1 and t_2 represent the time in minutes when samples 1 and 2 were withdrawn.

(*e*) The corrected phagocytic index alpha, which is the value corrected for the weight of liver and spleen. This factor serves as an index of the activity of the RES in relation to quotient (*c*) and is described by the equation (references 12, 13):

$$\text{alpha} = \sqrt[3]{k} \times \frac{\text{Body weight}}{(\text{Liver + spleen}) \text{ weight}}$$

For each test, five infected mice were compared with an equal number of untreated controls and the arithmetic means and their standard deviations were calculated for factors 2, 3, 4, and 5. Significance of differences between means was evaluated in the usual way (15).

White Cell Counts.—Immediately before the carbon pickup test the animals were weighed and a blood sample was obtained for a white blood count and two smears. For the white count, the sample was diluted with 1 per cent acetic acid and counted by two independent observers in a Hausser hy-lite chamber with improved Neubauer ruling. The smears were stained after Giemsa and likewise examined by two different observers. From the results of the absolute and differential white blood counts the values for each cell type were calculated.

Gross Pathology and Histopathologic Examination.—After the carbon pickup test was completed, liver, spleen, kidneys, heart and lungs, lymph nodes, and intestines were inspected in the gross and then removed and fixed in formalin-alcohol. They were processed in the usual manner and stained with hematoxylin-eosin. When required Gram's method, Giemsa's method, and Weigert's fibrin stain were applied in addition.

EXPERIMENTAL PLAN

In brief, the experimental plan consisted of two parallel and simultaneous sets of test animals and controls. The first set was designed to test the validity of the predicted, genetically arranged survivorship differences. This included 25 BRVR and 25 BSVS male mice 7 weeks of age which were infected and observed for 32 days to estimate final survivorship frequencies. Five uninfected animals of each strain were included as controls.

The second set, infected at the same time as the first and for which the identical *Salmonella* cultures were employed, provided animal samples for a time course study of those other parameters demanding sacrifice of the animals. Specifically, 90 male BSVS mice, 7 weeks of age, were divided into two equal groups of 45 animals each. One group served as controls and

remained untreated, while the second one was infected. One mouse was lost out of the control group during the clearance tests, while twelve animals of the infected group died before they could be tested. On post-infection days 1, 2, 3, 4, 5, 7, 8, and 32, five mice each were taken from the infected and non-infected groups and the RES activity tested as described under Materials and Methods.

Similarly, 80 BRVR males, 7 weeks old, were divided into two groups of 40 animals, one of which served as controls, while the other one was infected. Two animals of the latter group died before they could be tested. Five survivors of the survivorship test were added to this group the 32nd day after infection for sacrifice and test, at which time the experiment was terminated. On post-infection days 1, 2, 3, 4, 7, 8, 15, 22, and 32 five mice each were taken from the infected and non-infected groups and tested as above.

TABLE I

Effect of Intraperitoneal Infection with S. typhimurium upon Survival of Genetically Susceptible (BSVS) and Genetically Resistant (BSVR) Mice*

Mouse strain	No. of mice	Cumulative deaths, on days									
		5	6	7	8	9	12	14	20	22	32 (final)
BSVS (susceptible)	25 infected	2	10	21	24	24	25	25	25	25	25
	5 non-infected	0	0	0	0	0	0	0	0	0	0
BRVR (resistant)	25 infected	0	0	1	2	3	6	10	11	12	12
	5 non-infected	0	0	0	0	0	0	0	0	0	0

* The animals received first 1,000 viable cells of the avirulent RIA followed 2 days later by 100,000 cells of the virulent SR-11. The infecting doses were given in a final volume of 0.25 ml. of normal sterile saline. Both the avirulent and the virulent strain had been grown in Penassay broth for 16 hours before use. The infective dose was determined both by plate count and turbidimetrically.

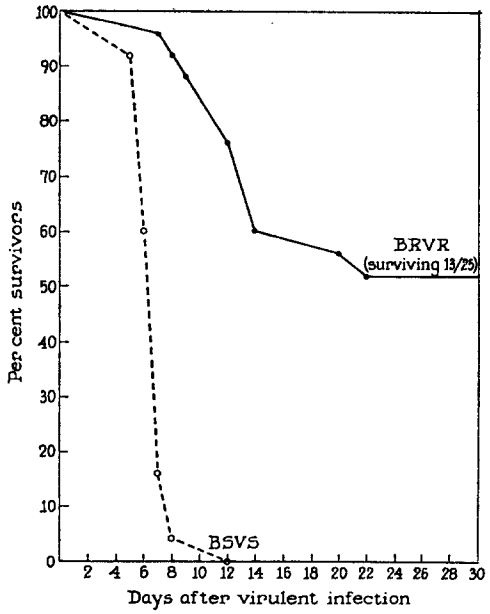
RESULTS

The results of the survivorship test are presented in Table I and Text-fig. 1. A significant difference in survivorship was achieved, for all the BSVS mice succumbed while the BRVR mice showed a survivorship of 52 per cent ($\chi^2 = 14.97$; $P < 0.001$).

Text-fig. 2 presents the results obtained in the concomitant carbon clearance tests. It will be noted, that the first test was run 24 hours after injection of the avirulent RIA strain, while the second one was performed 1 hour after challenge with the virulent SR-11. Each point on the curves corresponds to the arithmetic mean of five test animals; the standard deviation of the controls is indicated by the broken lines.

It will be noted that the rate of carbon clearance, epitomized by the k values, exhibits no significant divergence when BSVS uninfected controls are compared with uninfected BRVR controls. A comparison between the behavior

of the two mouse strains when infected reveals several important differences. In the BSVS group, the rate of carbon clearance as expressed by factor *k*, rose to significantly higher levels 2 days after infection with the avirulent strain RIA (*i.e.*, immediately after the virulent infection). It appears, however, unlikely that increased clearance was caused at this time by the presence of virulent bacteria in the tissues, because only 1 hour had elapsed between injection and test. On the 3rd day, *k* attained its maximum and fell during the next 2 days to control levels. Most animals which were studied on the 5th and 7th

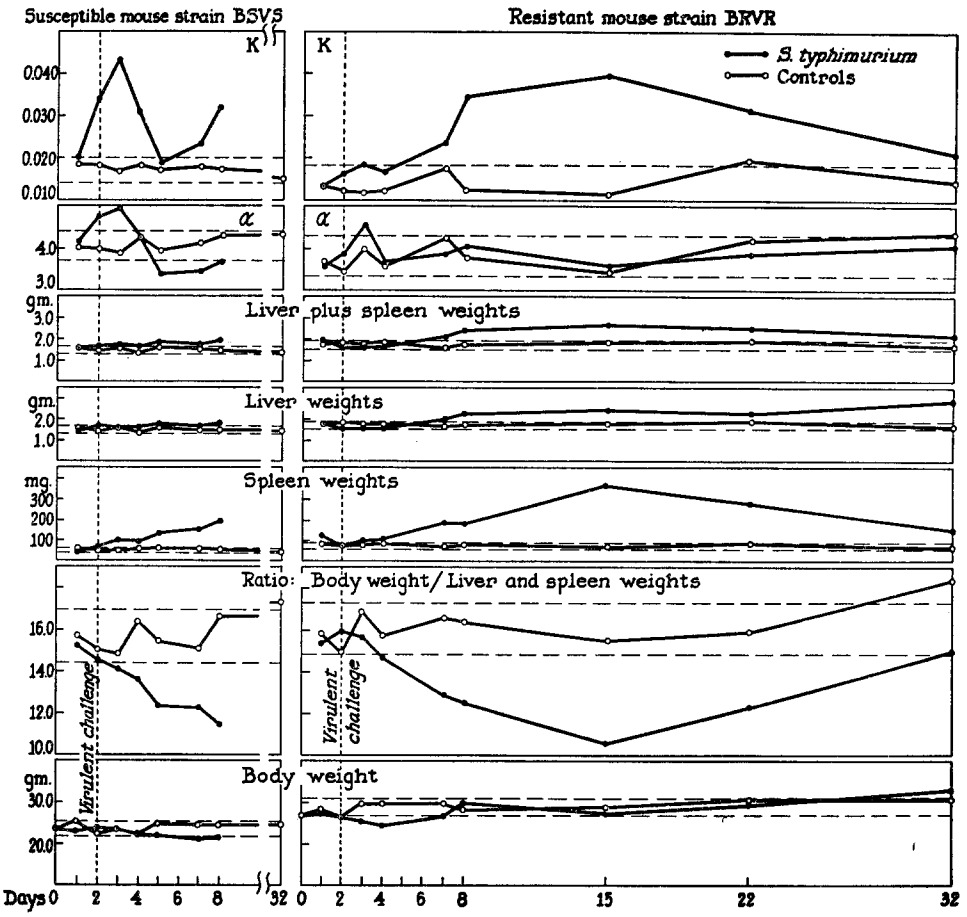


TEXT-FIG. 1. Survivorship after intraperitoneal infection with avirulent, followed by virulent *S. typhimurium*. Infected intraperitoneally with 1,000 viable cells of avirulent *S. typhimurium*, strain RIA, followed 2 days later by 100,000 viable cells of the virulent strain SR-11.

day appeared to be severely ill and it seems therefore surprising that on the 8th day a new rise in clearance rate was detected. No animal survived beyond the 8th day.

The corrected phagocytic index alpha behaved much the same like the global clearance value *k*, reaching a peak on the 3rd day and dropping subsequently to control levels. A significant increase in spleen weight in the presence of a constant body weight prevented it following the upward trend recorded for *k* on the 8th day. Except for the 3rd day, the changes of alpha never reached statistical significance. Spleen weights began to rise after the 5th day, as already stated above, but the liver weights remained practically constant. This

observation can at least in part be interpreted as the result of circulatory changes, as will be discussed later.



TEXT-FIG. 2. Time course studies, in infected susceptible and resistant mice, of carbon clearance rates (k) and associated measurements. Broken lines indicate the standard deviation of the uninfected controls.

The ratio body weight/liver + spleen weight dropped rapidly with the rise in spleen weight, since both the body and liver weights remained essentially unchanged.

In contrast to the susceptible BSVS group, the resistant BRVR mice failed to display any significant rise in their ability to clear intravenously injected particulate matter from the blood stream during the first 7 days after infection. Thereafter, k reached and held a higher level of activity until the 22nd day, but dropped to control levels at the time the experiment was terminated,

namely the 32nd day after infection. The corrected phagocytic index alpha did not show any changes worth mentioning, which must be understood as a consequence of a significant drop in the ratios body weight/liver + spleen weights.

The drop in the ratios can be analyzed without difficulty on examination of the curves for liver, spleen, and body weight. As in the case of the BSVS mice, most changes can be accounted for by a significant, however transient, rise in the spleen weights which reached a maximum on the 15th day. This peak corresponds to the lowest point of the ratio curve. At the time when the experiment was terminated spleen weights returned to control levels and the ratio curve reached accordingly normal values as determined by the standard deviations of the controls. A definite, but not very marked rise in liver weights was in part responsible for the described alterations.

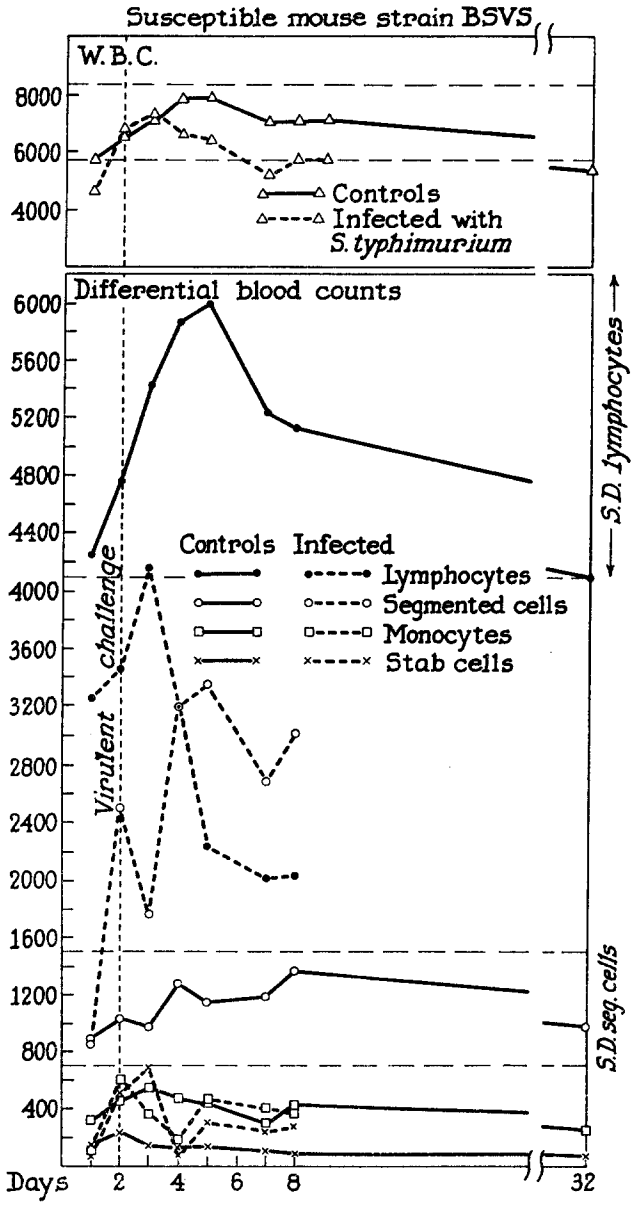
As in the BSVS mice, the body weights of the infected animals failed to show any changes.

Text-figs. 3 and 4 illustrate the behavior of the white blood cells in the peripheral circulation. It is interesting to note that neither in the susceptible nor in the resistant mice did any shift in the white blood count occur which exceeded the limits set by the standard deviation of the controls. The variation encountered in these measurements was considerable and these variations were even greater in the instance of the differential blood picture. Text-fig. 3 shows, for the BSVS mice, that the segmented neutrophils rose at the expense of the lymphocytes although these changes did not attain the level of statistical significance before the 5th day. Neither monocytes nor stab cells exceeded the standard deviations of the control animals.

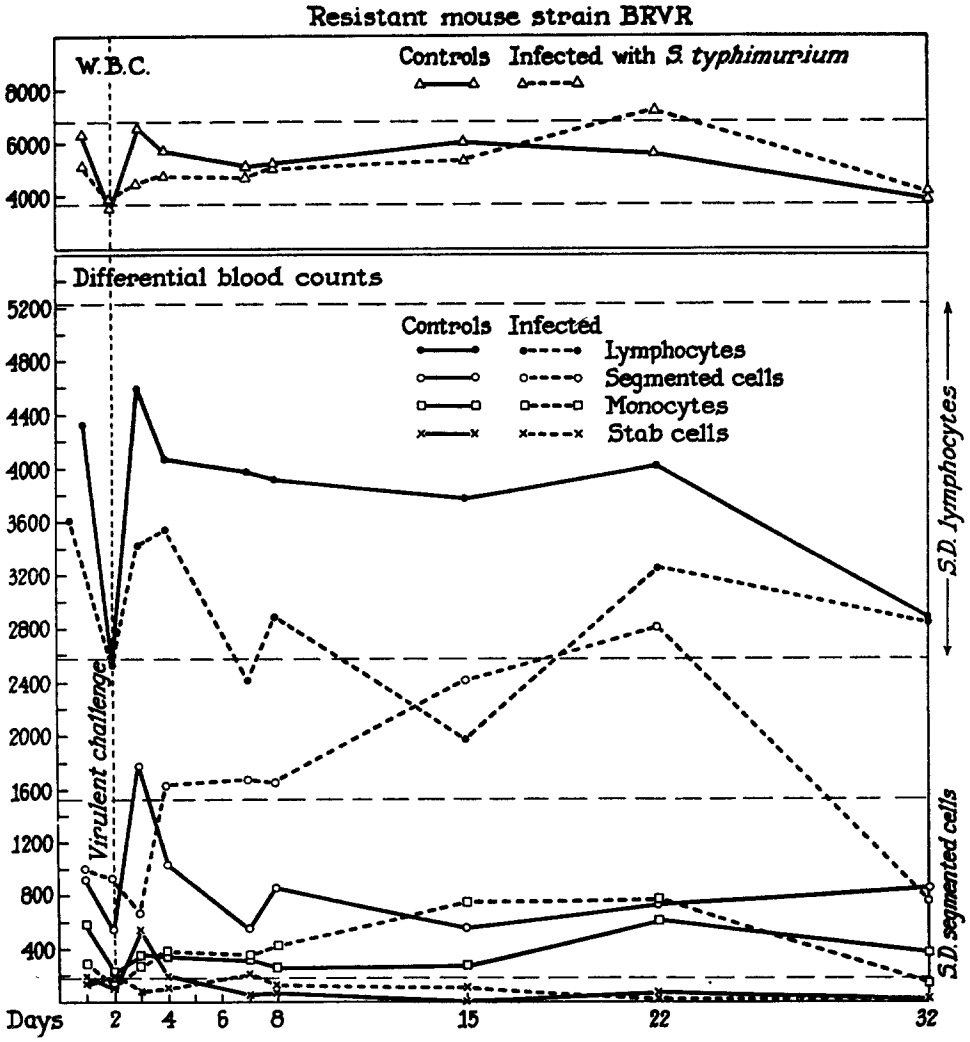
The course of events was similar but more protracted in the resistant BRVR group, as can be seen in Text-fig. 4. The number of segmented cells started to rise after the 8th day, was still found considerably elevated on the 22nd, but had dropped to control values on the 32nd day. There was a small, but not consistent and never statistically significant drop in the number of lymphocytes, whereas neither monocytes nor stab cells displayed any changes.

Macroscopic Findings.—Only a few and unimportant alterations were observed during the first 2 days after infection. Beginning with the 4th day, however, enlargement of the spleen and spotty necroses of the liver were seen in increasing numbers. A clear difference between the BSVS and the BRVR mice existed in so far as pathologic findings were more frequently observed in the former than in the latter group, and which became more and more obvious towards the 8th day when the livers of the susceptible animals were literally covered with miliary abscesses. The predominant lesion in the resistant animal consisted of few relatively large areas of necrosis easily recognizable with the naked eye. In the further course of disease, *i.e.* on the 8th and 15th day, the the BRVR mice also showed predominantly miliary abscesses and the characteristic necroses were seen less frequently.

Histopathological Findings.—Microscopic examination revealed that patho-



TEXT-FIG. 3. Time course studies, in infected and non-infected susceptible mice, of white blood cell concentrations, absolute and differential. Broken lines indicate the standard deviation of the uninfected controls.



TEXT-FIG. 4. Time course studies, in infected and non-infected resistant mice, of white blood cell concentrations, absolute and differential. Broken lines indicate the standard deviation of the uninfected controls.

logical alterations were limited almost exclusively to liver and spleen. Only very rarely lesions in kidney or heart were encountered. Pathologic changes in thymus and lymph nodes were exceptional and doubtful, although the lymphatic ducts of the liver were occasionally seen to contain lympho- and monocytes during the height of the disease.

BSVS. Days 1 and 2.—On day 1, four out of five, and on day 2 all five examined BSVS mice showed localized lesions in their livers in addition to capillary dilatation, stasis, and swelling of the Kupffer cells. Three types of lesions were mainly observed: first, a simple intracapillary conglomerate of lymphocytes, monocytes, and histiocytes as seen in Fig. 1; second, large areas of parenchymal necrosis with apparently intact Kupffer cells and conspicuous absence of an inflammatory reaction as demonstrated in Fig. 2; and third, foci which consisted exclusively of segmented cells (Fig. 3).

The spleens displayed strikingly few changes on the 1st day, but on the 2nd in one mouse a solitary subcapsular focus could be observed, which was predominantly composed of histiocytes (Fig. 4).

Days 3 to 8.—From the 3rd day on until the 8th, when the last BSVS mice were tested, there was no liver in which the changes described above could not be found. Usually all three types could be observed, although from the 3rd to the 5th day the number of segmented cells within the foci steadily increased (Fig. 5).

Fig. 6, taken on the 3rd day, demonstrated phagocytosis by Kupffer cells. Some of them are seen to lie freely within the capillary lumen and to show their typical round form. In one of the five animals examined on the 3rd day, a peritubular accumulation of lymphocytic and monocytic cells could be found in the kidney (Figs. 7, 8) together with a perivascular infiltrate of lympho- and monocytes, but this remained a very rare finding.

Day 4.—On day 4, another type of focus was seen for the first time, which bore a certain resemblance to the typhoid nodules of man (Fig. 9). It consisted mainly of histiocytes and emerged as a predominant lesion on the 7th and 8th day. Besides this, numerous foci contained mixed populations of neutrophils and histiocytes (Fig. 10). None of the spleens examined on the 4th day was devoid of pathological changes. These consisted mainly of considerable stasis and enlargement of the sinuses, whose endothelium had almost completely stopped taking up carbon. In addition, histiocytic or outright purulent foci (Figs. 11, 12) were found in great numbers.

Day 5 to 8.—On the 5th day, three out of five, and on the 7th day, all five inspected animals showed large patches of necrosis in their livers, together with numerous thrombi in branches mostly of the portal, but occasionally also of the hepatic vein. These necrotic patches could not be distinguished from infarctions and had in two instances the typical appearance of a hemorrhagic infarct (Fig. 13). A correlation between the occurrence of necrosis and thrombus formation could not be convincingly established, mainly because most of the thrombi did not obstruct the vascular lumen and were never found inside of arteries. The spleens uniformly displayed findings of the type already described; *i.e.* stasis, gross enlargement of the sinuses which were filled with segmented cells and monocytes, inactivity of the follicles, complete absence of leucopoiesis, and uncountable foci of inflammation. Venous thrombosis was not infrequently encountered. (Figs. 14 to 16.)

One severely ill animal tested on the 7th day died during the test. Microscopic inspection showed the glomerular loops of both kidneys filled with carbon (Fig. 17). Virtually no carbon had been ingested by the Kupffer cells or the endothelial linings of the splenic sinuses (Fig. 18). The number of thromboses was striking (Fig. 19).

On the 8th day after infection when the last surviving animals of the BSVS strain were examined, the predominant hepatic lesion was the histiocytic nodule. Thrombi were encountered again, but only one out of five livers showed obstruction of a main branch of the portal vein. Necroses were rare, and the livers appeared in better condition than those examined on the 4th, 5th, and 7th day.

BRVR.—The character of the anatomical lesions observed in the resistant group was the same as in the BSVS mice, but they differed in time of appearance, number, and extent from those found in the susceptible group.

Days 1 to 3.—On the 1st and 2nd day after infection in only two out of five mice occasional monocytic-histiocytic foci (Fig. 20) were encountered and one animal showed a large subcapsular patch of necrosis. Neither kidney nor spleen appeared to be changed. The findings encountered on the 3rd day were identical with those of the first 2 days.

Days 4 to 6.—On the 4th day rather extensive areas of hepatic necrosis were found in three out of five animals. Many foci—although not all—showed both neutrophils and histiocytes. The liver capillaries were dilated and the Kupffer cells swollen. A great many could be seen lying free inside the capillary lumen after having lost their attachment to the vascular wall. There were surprisingly few changes in the spleens, but, as in the case of the BSVS mice, carbon uptake by the endothelial cells of the splenic sinuses became less and less the further the infective process advanced.

Day 7.—On the 7th day, *i.e.* at a time when carbon clearance started to increase, the most conspicuous alterations in both liver and spleen were numerous, mostly non-obstructing thrombi in the portal and splenic veins and their branches. These were observed in one out of five mice. All livers were literally studded with foci made up of a mixed cell population of neutrophils and histiocytes. It was mainly by the absence of the purely purulent foci that the resistant animals differed from the susceptible ones. In one of the kidneys a peritubular infiltrate of lymphocytes and monocytes was found, but all the others were free of anatomical changes. The majority of the spleens were enlarged and heavier than the control organs. This observation can be explained by the uniformly dilated splenic sinuses, which were stuffed with segmented and monocytic cells and had increased to approximately three times their normal size. Only in two out of five animals focal infiltrates of neutrophils or typhoid nodules were found.

Days 8 to 15.—Between the 8th and 15th day after infection, the disease process apparently reached its peak. All animals—with one exception—had livers which offered a mottled appearance, and there was practically no hepatic unit free of foci, either histiocytic or mixed histiocytes, and segmented cells. On the 15th day, however, the histiocytes began to prevail, particularly in the spleens, in which neutrophils had completely disappeared. As on the 7th day, numerous thrombi were encountered, some of them already in the process of organization (Fig. 21). Only one kidney displayed signs of peritubular nephritis (Fig. 23).

Day 22.—By the 22nd day, when the next group was examined, the resistant animals had apparently overcome the disease. The number of foci had considerably decreased, and those which were found consisted exclusively of monocytes and histiocytes (Fig. 22). There was no scar formation in spite of the extensive necroses seen on previous days, and the only signs that these mice must have undergone some severe disease were numerous thrombi (in one out of five spleens and in four out of five livers), most of them organized and covered with endothelium. Only one spleen exhibited a histiocytic focus and dilatation of the sinuses. Both the numbers of plasma and reticular cells were impressively greater than those found in normal animals. This fact was apparently mainly responsible for the increase in organ weight. Myelopoietic activity, which had practically ceased at the height of the disease, was found again. The splenic follicles, which were entirely inactive on previous days, had regained a certain activity as demonstrated by the presence of mitotic figures and tingible bodies.

Day 32.—The last group was examined on the 32nd day. In the livers, histiocytic foci had become less frequent than on the 22nd day, and bore a close resemblance to those seen on the 2nd day of the experiment. Two of them showed large abscesses walled off by fibrocytes and containing a marginal layer of segmented cells. It was only in these two animals that thrombi were still encountered. Four spleens were found somewhat enlarged. They had, as was to be expected, stored less carbon than the controls, but one spleen, which weighed only 78 mg., exhibited the same degree of carbon storage as the controls. The kidneys were free of anatomical changes.

It must be emphasized that the necrotic foci were never found to contain stainable Gram-negative bacteria, a fact which held true for both BSVS and BRVR mice.

DISCUSSION

The experiments reported above seem to us to indicate that no simple or facile relationship springs readily into view in the general question of the possible association of natural resistance to infection and activity of the reticulo-endothelial system. In order to extract what meaning we can it may be well if we examine the operational bases on which these experiments rest and then take up certain specific questions, inquiring when we can discern unambiguous answers and when we can glean only clues.

In the present communication a predicted difference in survivorship in mouse salmonellosis was arranged, and confirmed within the experiment, by genetic means. This offered the obvious advantage of providing additional and identical samples of the disparate host populations which could be tested, infected, and non-infected, during the time course of the disease, in terms of carbon clearance from the circulation. All this is straighforward. What is perhaps not so obvious, and needs some comment, is the use of the double strain inoculation method in producing the test infection. This use was dictated by the finding (7) that for these identical mouse stocks and for the very cultures of *S. typhimurium* used here, survivorship differences are achieved only when both avirulent and virulent *S. typhimurium* are admitted into the host. Infection with only a clone of virulent *S. typhimurium* obliterates the survivorship difference, all mice succumbing, "resistant" and "susceptible" alike. The achievement of survivorship is thus operationally dependent on the double strain phenomenon and without pursuing its meaning further we can note that its use resulted here in the desired end.

With the two infected mouse populations thus moving to divergent outcomes we may first inquire whether this difference was anticipated by a pre-existing difference in status of their respective RES systems. The answer to this query is clearly in the negative for the ability to clear injected carbon from the circulation was statistically indistinguishable in uninfected BRVR and BSVS mice at the outset and subsequently during the course of the experiment. This finding is similar to that of Berry (16) who found BRVR and BSVS mice identical in their ability to clear intravenously injected thorotrast. He concluded that the cellular defense systems of these mouse stocks were of equal potential, but failed to examine infected animals in this respect. In a similar vein, in the instance of circulatory clearance of bacteria, Rogers (17) was persuaded that differences in host susceptibility were not explicable by differences in the initial clearance mechanisms.

If BRVR and BSVS mice thus entered our experiment with their respective RE systems in equal status, we are led next to inquire whether under the im-

pact of the infection the two stocks showed any changes in their ability to clear circulating carbon and if changes occurred, whether they exhibited any differences in extent or in onset.

The results show that changes did occur, that they were of approximately equal magnitude in terms of increased clearance, but were different in time of onset. Thus, BSVS mice showed a steady rise in k in the first days of infection, responding immediately on injection of the avirulent *S. typhimurium* (RIA). In contrast, BRVR mice showed no such rise in carbon clearance at this early time, but did show a later rise at a time when all of the BSVS mice had since succumbed. At this later time the BRVR mice showed extensive liver damage on histological examination.

While it is thus true that BRVR and BSVS mice *are* different in the kinetics of their response to infection in terms of carbon clearance, there remains considerable doubt as to the meaning of this difference. Classically (18), an increased RES activity is associated with an enhanced capacity to sequester bacteria, the first step toward an increased resistance. But paradoxically, in the present instance the indubitably more *susceptible* BSVS mice showed the earlier response, and of a magnitude similar to that which the BRVR mice eventually exhibited. Indeed, confined to this single model, the suggestion might be made that a rise in clearance has the implication of a poor prognosis, and since *some* BRVR mice eventually died, the rise of k at the later date was an indication of impending death for these unfortunates. Further experiments with much larger numbers of animals would be needed, however, to examine this hypothesis.

That a rise in k can follow injection of small amounts (*ca.* 20 micrograms) of bacterial endotoxin has been shown for endotoxins from a variety of organisms, including those from *Salmonella typhi* (19), *Salmonella abortus-equi*, and for pertussis vaccine and a variety of extracts obtained from *Mycobacterium tuberculosis* (20). Whether the rise in k observed in the present experiment is attributable to the release of endotoxin from disintegrating bacteria remains conjectural.

The susceptible BSVS mice differed from the resistant BRVR mice in a second respect, namely with regard to changes in the white blood count. The BSVS mice showed a quick drop of their neutrophils at the expense of their lymphocytes, a change which occurred in the BRVR animals only much later and never reached any significant degree. These findings stand in contrast with those obtained with more or less purified endotoxins derived from different *Salmonella* strains which produce a distinct leucocytosis, usually followed by leucopenia (21, 22).

The histological observations conformed generally to the reports of earlier investigators (23–30). Oakberg (23), who worked with resistant and susceptible mouse strains, postulated the constitution of liver and spleen as the basis for

genetic resistance. He was able to show that resistant mice are more likely to develop liver necrosis than the susceptible ones, and that this relationship is just the reverse with regard to the spleen. The present results support this view up to a certain point. It seems, however, that following the 8th day the predominant lesion of the liver was the localized, necrotic focus rather than an extensive necrosis. As to the spleens, the difference between the two mouse strains seemed to lie not so much in the extent of damage as in the time of its appearance. Carbon uptake was uniformly very poor, which stands in contrast to the experiences of the above mentioned author (23).

The widespread occurrence of thrombi in liver and spleen has not been emphasized in other reports, but emerged as an important feature in the present study. It appears that the weight gain recorded for infected organs was not only caused by an actual increase in the number of parenchymal and other cells, but also by the trapping of a considerable blood volume.

The rare occurrence of renal lesions has been commented upon before and is surprising in view of the fact that *S. typhimurium* has been cultivated from the kidneys as early as the 4th day after infection (24).

Bacteria have practically never been visualized inside of hepatic lesions with the help of histological staining methods (23, 24) which corresponds to the conditions encountered in man (26, 27). It was therefore assumed that the necrotic foci are produced by endotoxins rather than by the intact bacillus (26, 27), and it has been shown and repeatedly confirmed that liver necrosis can be brought about by the injection of *Salmonella* endotoxin (28–31). It must, however, be borne in mind that cultures taken from the liver after the 4th day were found uniformly positive (30), and that therefore, the presence of bacteria cannot be excluded.

In view of the extensive liver damage observed around the 15th day, it seems astonishing that the liver parenchyma of the resistant animals was found, 1 week later, to be largely free from pathological alterations. The resistant animal was apparently capable of overcoming its disease, at least in the anatomical sense, because the necrotic foci disappeared without leaving any trace. There is, however, impressive evidence that the animal fails to be cured bacteriologically, and remains a carrier for the rest of his life (10).

SUMMARY

Susceptible (BSVS) and resistant (BRVR) mice were experimentally infected with *Salmonella typhimurium*. The double strain inoculation technique was used in which both avirulent and virulent representatives of *S. typhimurium* were admitted into the hosts. The BSVS mice succumbed without exception while the BRVR mice survived to 52 per cent. No deaths occurred during the experimental period in non-infected control mice.

Parallel to the survivorship test, and concurrent with it, the activity of the

reticulo-endothelial system (RES) was measured at frequent intervals in identically infected BSVS and BRVR mice and in their non-infected controls. For this measurement the carbon clearance method was used. No pre-infection difference of activity of the RES could be discerned in susceptible BSVS mice *vs.* resistant BRVR. Following infection, increases in the RES activity were detected in about the same magnitude in both mouse stocks. However, a difference was found in the time of onset. The susceptible animals showed an early and short increase in the activity of the RES, followed by a drop to control levels at the time of death. The resistant group exhibited a considerably delayed, but significant increase in RES activity, which returned to control levels approximately 4 weeks after infection.

The absolute white blood count did not undergo significant change in either of the two infected groups, but the susceptible animals showed a relative increase of their neutrophils at the expense of their lymphocytes.

Extensive anatomical changes were observed in both mouse strains, mainly confined to liver and spleen. These consisted of stasis, swelling of Kupffer cells, necrotic foci, histiocytic-monocytic nodules, widespread thrombosis of branches of the portal and splenic veins, and extensive areas of necrosis. These changes appeared earlier in susceptible than in resistant animals.

The implications of these findings are discussed.

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EXPLANATION OF PLATES

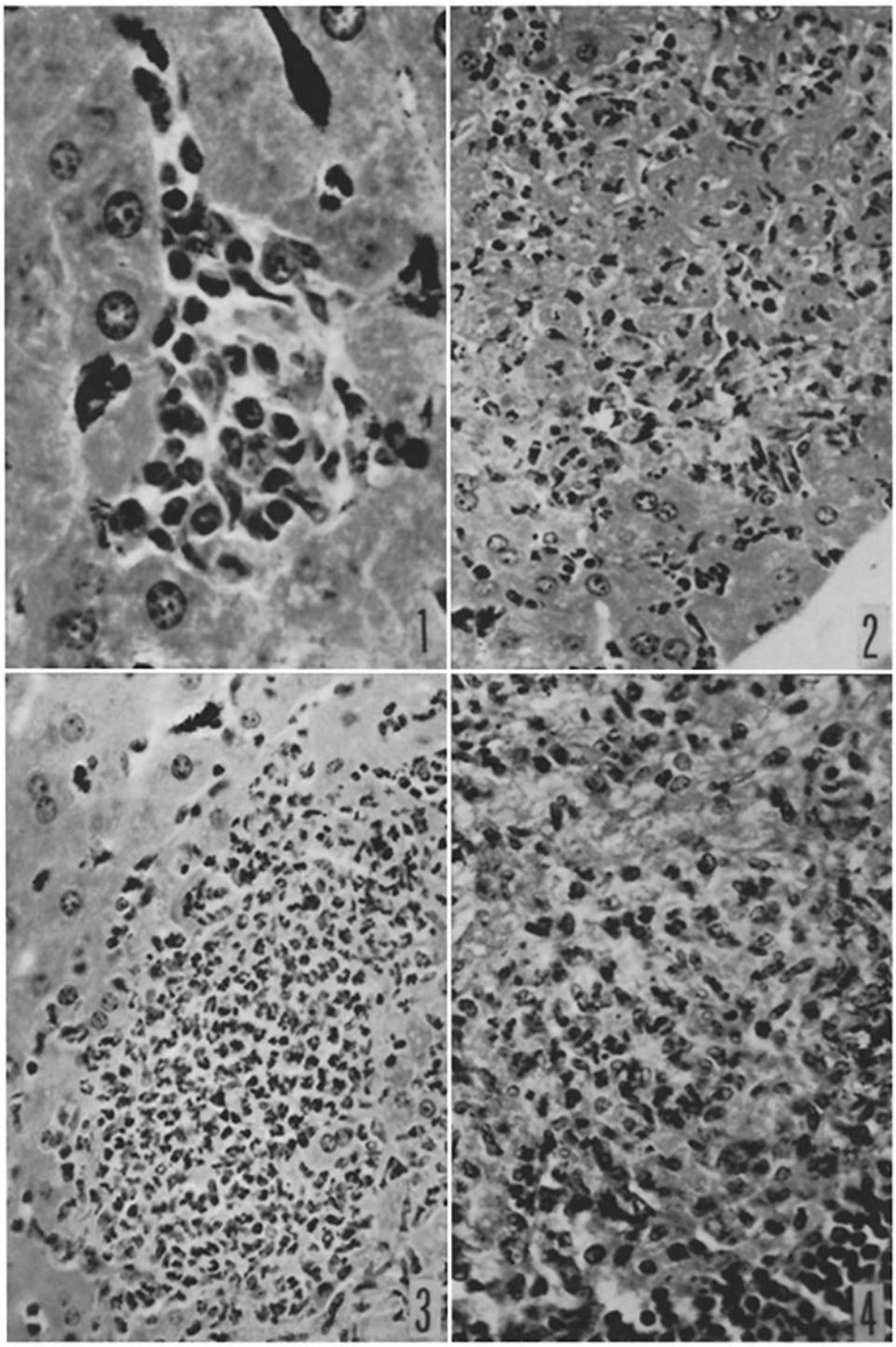
PLATE 2

FIG. 1. BSVS mouse, 2nd day after infection. Monocytic-histiocytic focus in the liver. Hematoxylin and eosin. × 856.

FIG. 2. BSVS mouse, 2nd day after infection. Patchy necrosis of the liver. Note almost complete absence of inflammatory reaction. Hemotoxylin and eosin. × 431.

FIG. 3. BSVS mouse, 2nd day after infection. Focus of inflammation in the liver with predominance of neutrophilic cells. Hematoxylin and eosin. × 419.

FIG. 4. BSVS mouse, 2nd day after infection. Histiocytic nodule in the spleen at the border of white and red pulp. Hematoxylin and eosin. × 526.



(Böhme *et al.*: Natural resistance to infection in salmonellosis)

PLATE 3

FIG. 5. BSVS mouse, 3rd day after infection. Focus of hepatic necrosis with predominantly neutrophilic cells. Hematoxylin and eosin. × 760.

FIG. 6. BSVS mouse, 2nd day after infection. Active phagocytosis by Kupffer cells. The cells are generally swollen and are partly found free in the capillary lumen. Hematoxylin and eosin. × 664.

FIG. 7. BSVS mouse, 3rd day after infection. Accumulation of monocytes and histiocytes in the renal interarteriovenous space. Periarterial edema and phagocytosis of carbon. Hematoxylin and eosin. × 395.

FIG. 8. BSVS mouse, 3rd day after infection. Accumulation of monocytic cells in the peritubular vessels with active phagocytosis of carbon by the endothelium. Hematoxylin and eosin. × 529.

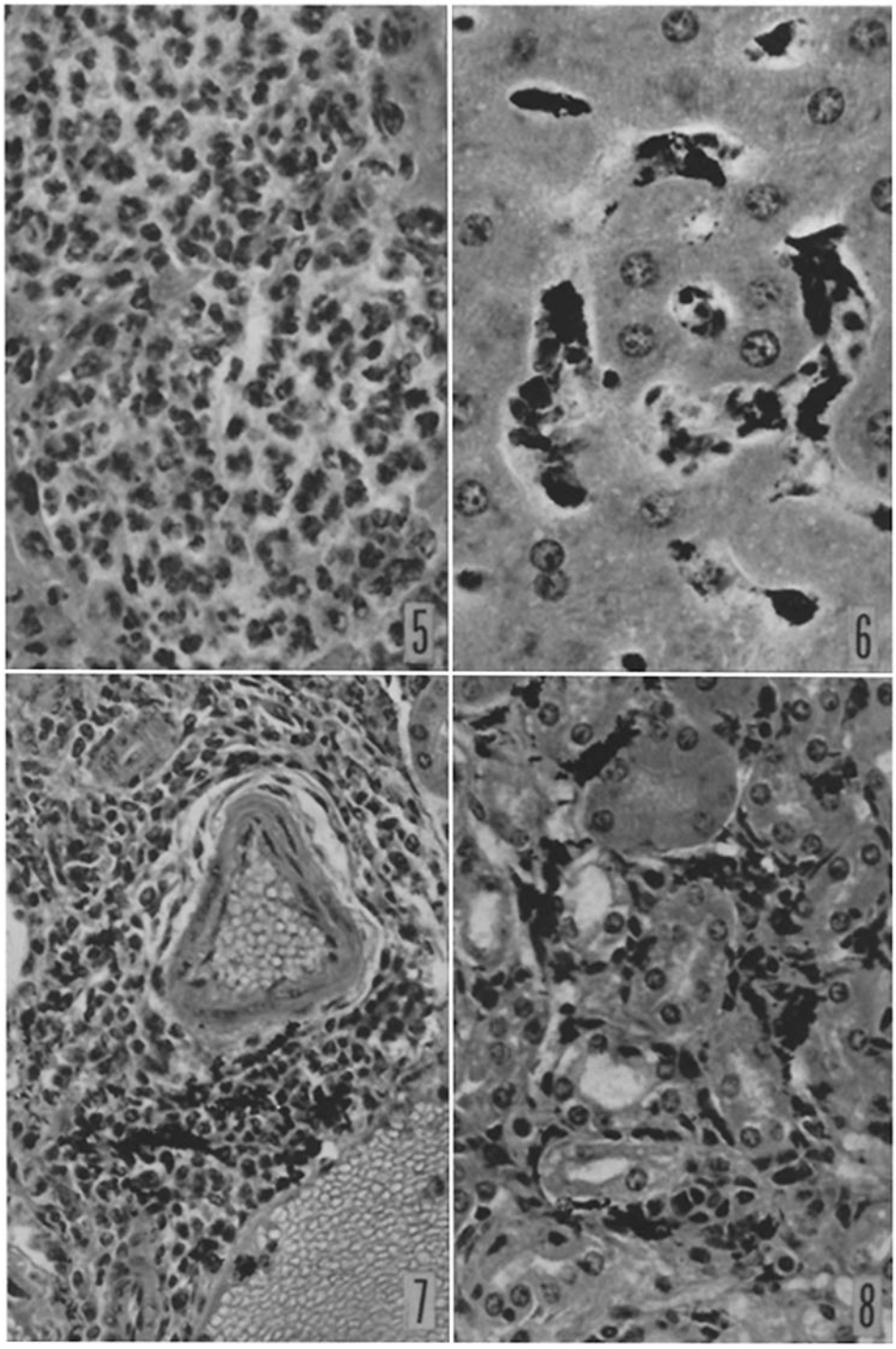


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PLATE 4

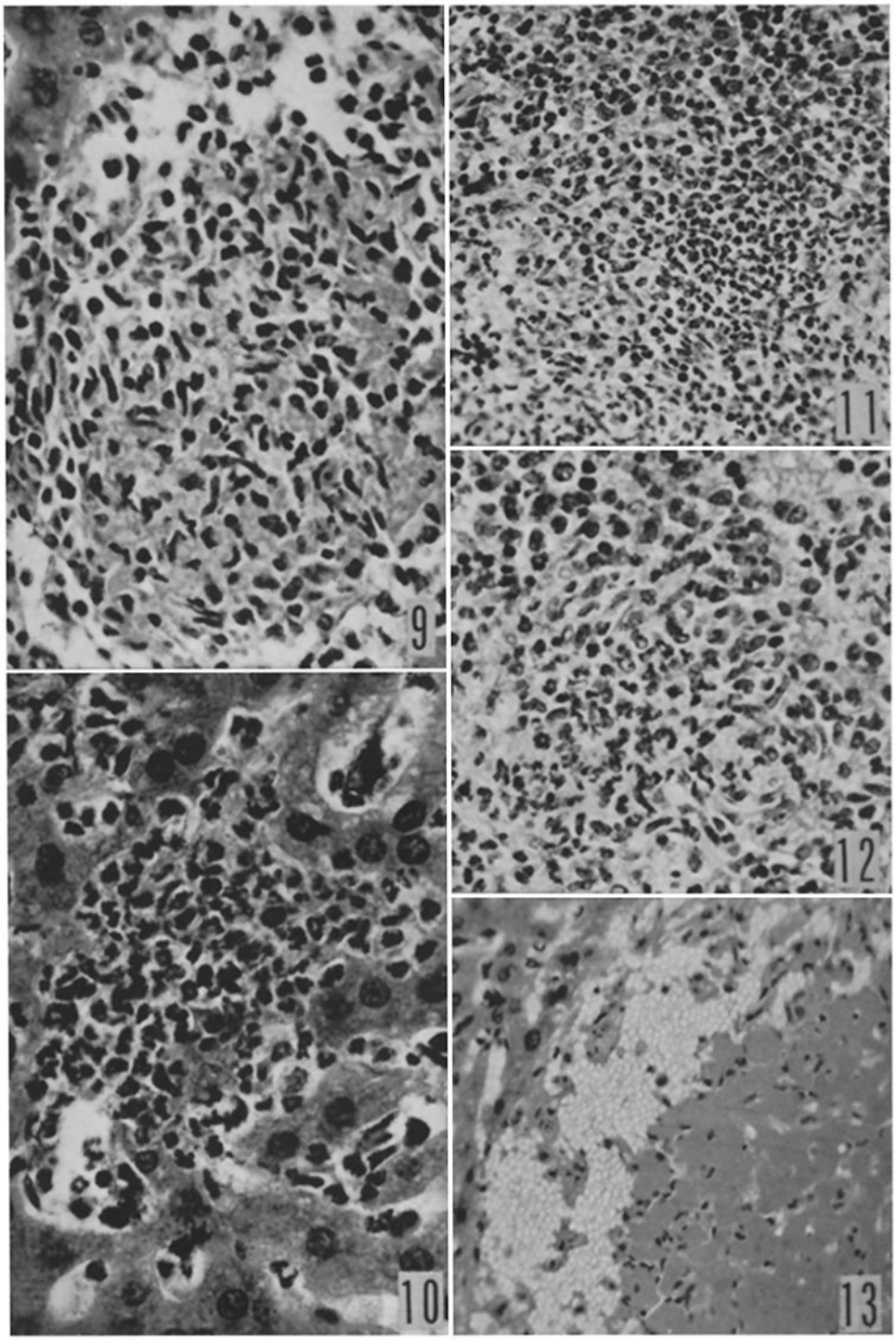
FIG. 9. BSVS mouse, 4 days after infection. "Typhoid nodule" of the liver, surrounded by edema. Hematoxylin and eosin. × 510.

FIG. 10. BSVS mouse, 4 days after infection. Dilatation of capillaries, swelling and detachment of Kupffer cells, necrotic focus consisting mainly of histiocytes. Hematoxylin and eosin. × 587.

FIG. 11. BSVS mouse, 4 days after infection. Stasis in the splenic sinuses. Necrotic focus in center. Note the almost complete absence of carbon in the endothelial linings. Hematoxylin and eosin. × 312.

FIG. 12. The same area as in Fig. 11 under higher magnification. Both a histiocytic (center) and a mixed focus are visible (left margin). Hematoxylin and eosin. × 491.

FIG. 13. BSVS mouse, 5 days after infection. Extensive wedge of hepatic necrosis with hemorrhagic margin. Hematoxylin and eosin. × 255.



(Böhme *et al.*: Natural resistance to infection in salmonellosis)

PLATE 5

FIG. 14. BSVS mouse, 7 days after infection. The splenic follicles are almost lost in an area of generalized stasis, and there are a great many purulent foci visible in the white pulp. Hematoxylin and eosin. × 30.

FIG. 15. BSVS control mouse on the 7th day. Note the carbon particles deposited around the splenic follicles. Hematoxylin and eosin. × 15.

FIG. 16. BSVS mouse on the 7th day after infection. Spleen grossly enlarged, numerous foci of inflammation, stasis, very little carbon deposited in the splenic follicles. Hematoxylin and eosin. × 15.

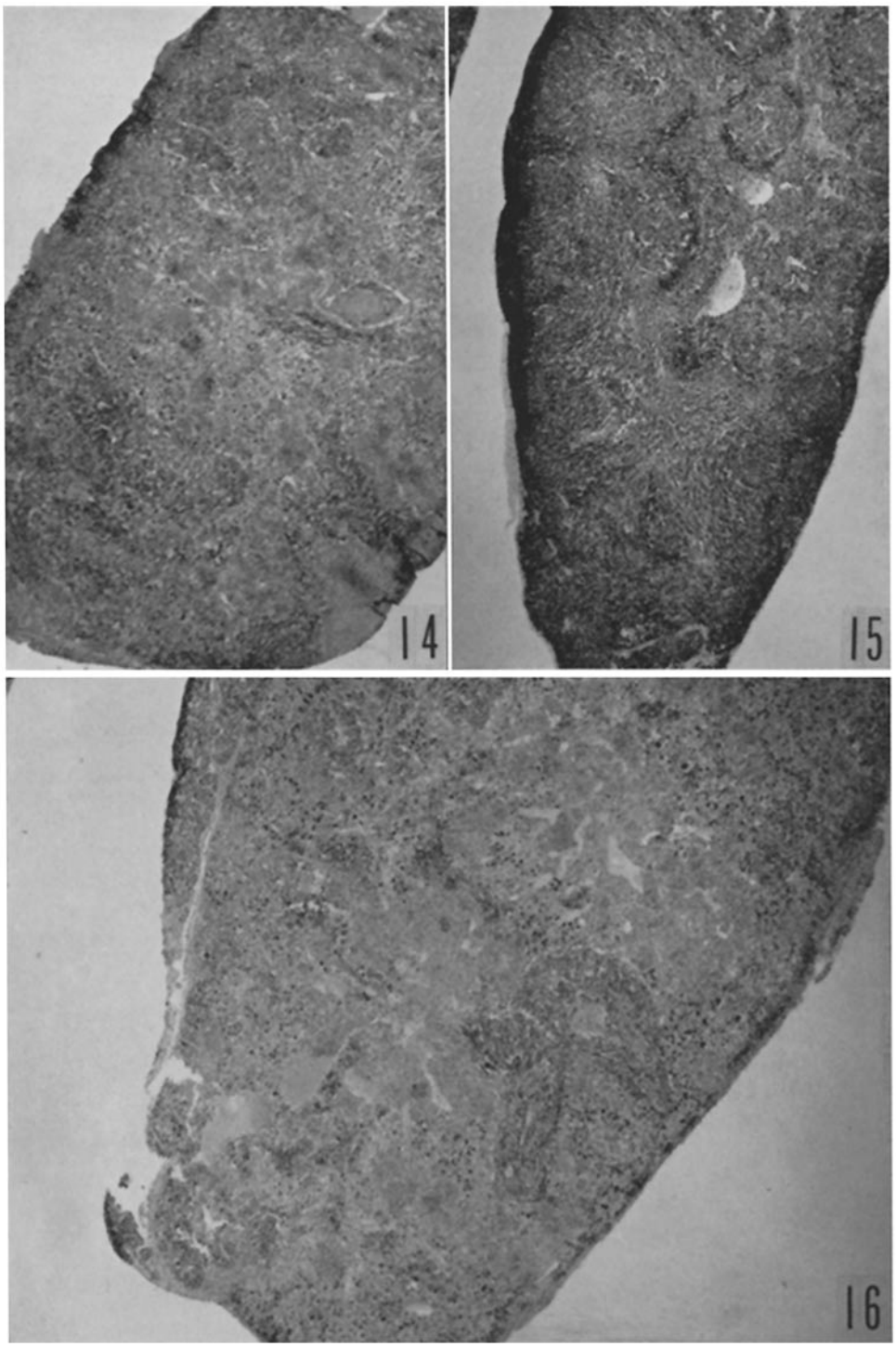


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PLATE 6

FIG. 17. BSVS mouse, 7 days after infection. Animal died during clearance test. Note the injected ink in the glomerular capillaries. Hematoxylin and eosin. × 44.

FIG. 18. Same animal as in Fig. 15. Extensive stasis in the splenic sinuses, no carbon storage. Hematoxylin and eosin. × 255.

FIG. 19. Same animal as in Figs. 15 and 18. Thrombus in a branch of the hepatic vein in the process of organization. Absence of carbon storage in the dilated liver capillaries. Hematoxylin and eosin. × 287.

FIG. 20. BRVR mouse, 2 days after infection. Prenecrotic stage: aggregation of monocytes in liver capillary. Hematoxylin and eosin. × 924.

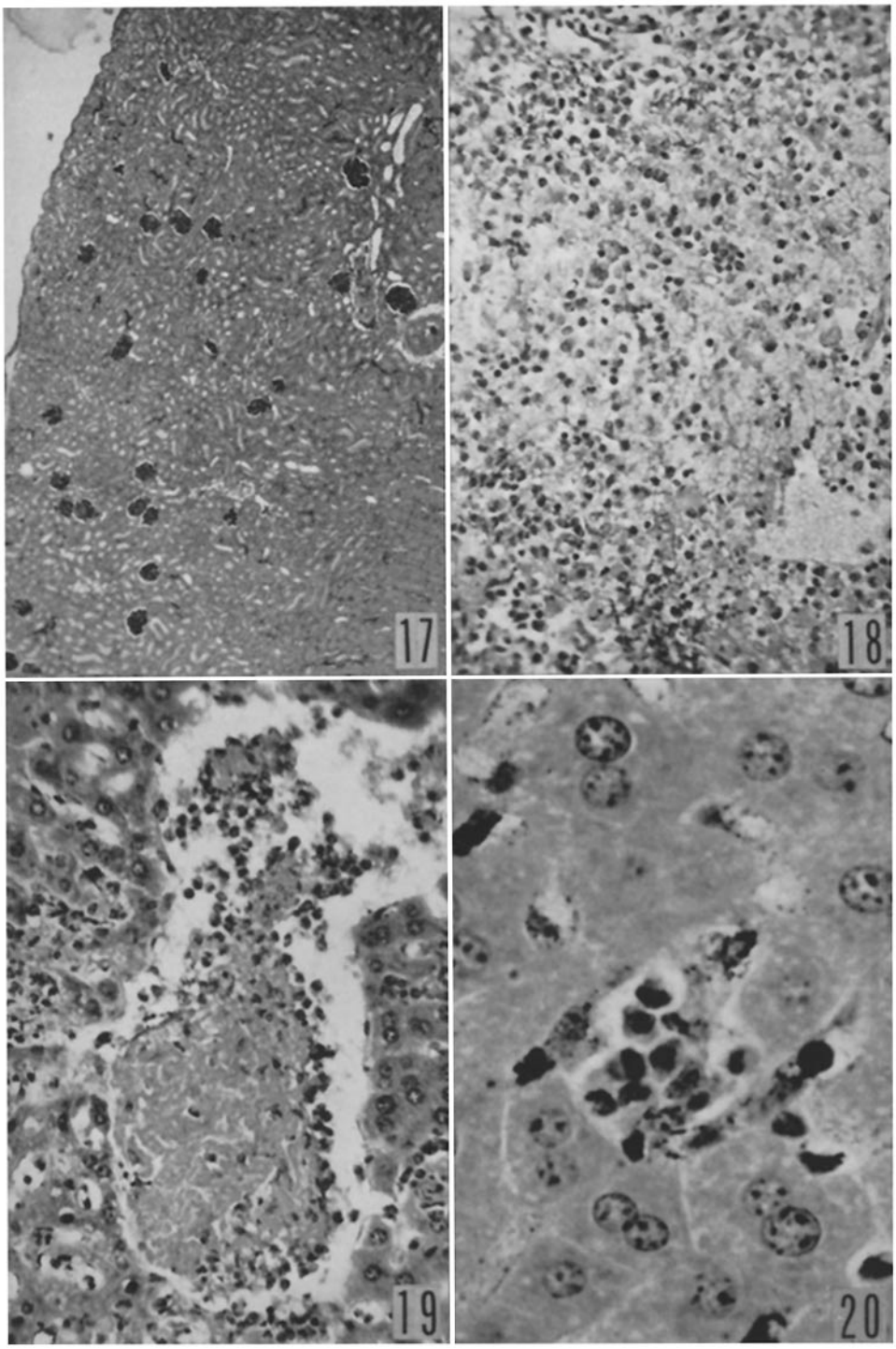

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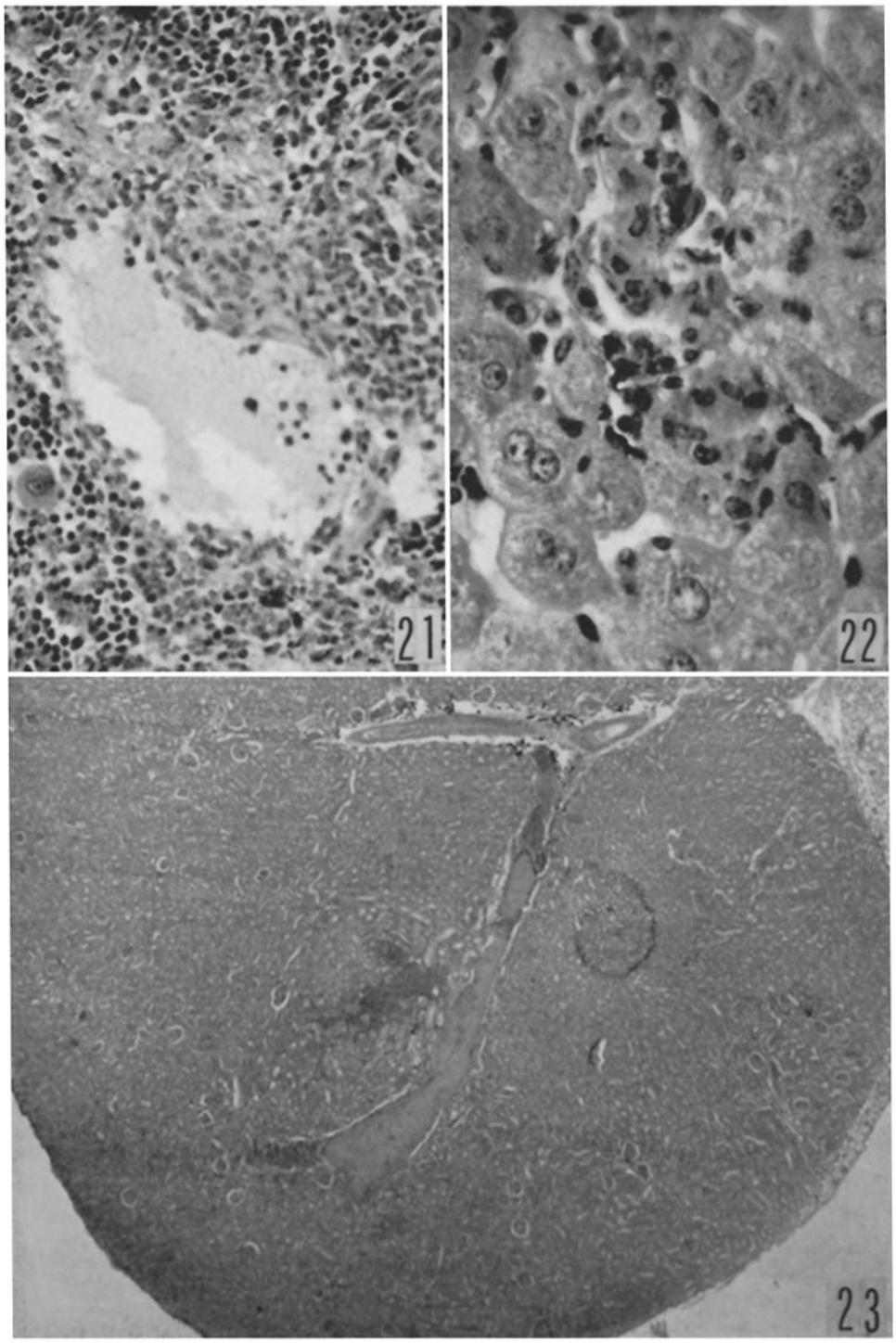
PLATE 7

FIG. 21. BRVR mouse, 15 days after infection. Organized thrombus in splenic vein. Hematoxylin and eosin. × 305.

FIG. 22. BRVR mouse, 22 days after infection. Small histiocytic focus in the liver. Hematoxylin and eosin. × 660.

FIG. 23. BRVR mouse, 15 days after infection. Two foci of peritubular nephritis of the kidney. Hematoxylin and eosin. × 30.



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