Regulation of Spleen Growth and Portal Pressure in Hepatic Schistosomiasis

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Growth rate and histology of splenic autotransplants in subcutaneous pockets were compared with those of autotransplants in the extrahepatic portal bed in splenectomized mice infected with cercariae of *Schistosoma mansoni* and in splenectomized uninfected controls. By the fifteenth week after transplantation (and tenth week after injection of cercariae) subcutaneous transplants gained 6.5 times and omental transplants 8.2 times more weight in infected animals than corresponding transplants in uninfected controls. Weight of the intact spleen increased fourfold in nonoperated infected animals. Portal pressures averaged 11 to 13 cm of water in infected animals with transplants and 17 cm in those with intact spleens (compared to that of 6 to 7 cm in controls). Hyperplasia of white pulp with increase in germinal center activity characterized transplants as well as intact spleens of infected animals. The results suggest that a) During the first 10 weeks of experimentally induced infection, portal congestion is not the predominant mechanism regulating increased spleen growth; and b) An intact enlarged spleen appears to contribute to elevated portal pressure. (Am J Pathol 78:211–224, 1975)

IN PATIENTS with hepatic schistosomiasis, extrahepatic portal congestion often accompanies enlargement of the spleen, and for this reason the convenient term "congestive splenomegaly" is usually applied to the disorder. The term implies that portal congestion underlies splenic enlargement, a concept flawed by certain clinical and experimental observations which are usually overlooked. It is curious, for example, that although splenomegaly is a characteristic feature of this disease, elevated portal pressure is actually found in only 60% of such patients.[1] Moreover, successful porta caval shunt fails at times to reduce the size of the spleen even though portal pressure falls.[2] In animals with other forms of experimentally induced hepatic dysfunction, elevated portal pressure is not a requirement for increased growth of spleen[3,4]; nor does sustained splenomegaly follow experimental interruption of splenic venous drainage.[5]

Extrahepatic portal hypertension develops in mice with experimen-

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tally induced hepatic schistosomiasis and, as in the case of man, it is generally assumed that this alteration is the major factor underlying development of splenomegaly in such animals. Thus, Cameron and Bhattacharyya state: "There is no denying the fact that portal hypertension plays a dominant role in initiating the splenic changes, especially in the later phases of infection."[6] An attempt to clarify the significance of this hemodynamic derangement in the pathogenesis of splenomegaly was the basis for this study. As free autotransplants of splenic tissue regenerate and function when placed in the systemic circulation,[7-9] regeneration and growth of such autotransplants were compared with transplants in the portal system in mice chronically infected with *Schistosoma mansoni.*

Materials and Methods

Preparation of Animals

All studies were performed in adult Swiss-Webster albino female mice weighing 18 to 22 g. Under intraperitoneal pentobarbital anesthesia and using aseptic precautions, the spleen was completely removed and a 1- to 2-mm weighed transverse section averaging 40 mg in weight was immediately autotransplanted into either a subcutaneous pocket in the anterior abdominal wall or, in a second group, into the peritoneal cavity within an omental envelope. Five weeks later each animal in these two groups was infected by subcutaneous injection of 20 cercariae of a Puerto Rican strain of *Schistosoma mansoni.*[10] A third and fourth group of mice also underwent splenectomy and spleen autotransplantation in the same two locations but without injection of cercariae; they served as controls. A total of 58 mice, divided into four approximately equal groups, were studied in this manner. A fifth group, consisting of 8 infected mice with intact spleens, and a sixth group of 18 normal mice served as additional controls.

To avoid Gram-negative sepsis and endotoxinemia, a development which could in itself increase growth of spleen, oxytetracycline was administered to all animals in their drinking water.

Measurement of Spleen Growth and Portal Pressure

Sixteen mice were randomly selected for study from the first four groups 10 to 11 weeks after spleen autotransplantation (and in the case of infected mice, 5 weeks after injection of cercariae). All remaining mice in these four groups were studied at 15 to 16 weeks after spleen autotransplantation. Three infected mice with intact spleens were also selected for study and comparison with normal mice 5 weeks after injection of cercariae, and the remainder were examined 5 weeks later (Group 5).

Mice were weighed, anesthetized with intraperitoneal pentobarbital and examined via laparotomy incision. Portal pressure was measured by direct puncture of the portal vein with a number 18 needle connected via a saline-filled plastic cannula to a Statham P23AA transducer; pressures were recorded on a Gould-Brush Model 220 recorder.

Immediately following sacrifice of mice by cervical dislocation, liver and spleen autotransplants and/or intact spleen were removed and weighed. Sections were

then placed in phosphate-buffered formalin at pH 7.25 and prepared for histologic study. Serial sections were stained with hemotoxylin and eosin and by the trichrome technic for determination of connective tissue. All sections were examined without reference to a particular experimental group.

Results

Appearance of Spleen Autotransplants

Spleen autotransplants survived in every mouse, with two exceptions. One of the mice injected with cercariae failed to develop schistosomiasis. A well-formed capsule facilitated dissection from surrounding tissue, and by the tenth week after transplantation, vascular channels to and from each transplant were visible grossly. In the case of omental transplants, venous connections to nearby omental veins were regularly identified and were particularly prominent in mice with portal hypertension. In uninfected as well as infected animals, the gross appearance of transplants in the systemic circulation did not differ significantly from that of transplants in the portal circulation (Figure 1).

Growth of Spleen and Changes in Portal Pressures

At 10 weeks after spleen autotransplantation the weight of transplants had decreased in 7 of 8 control animals. Contrasting findings were noted in mice infected with cercariae. Three subcutaneous transplants in these animals had gained an average of 17 mg, while five in the omentum averaged a gain of 26 mg. Weight of the intact spleen in 3 other infected mice averaged 237 mg, compared to that of 158 mg in controls. Portal pressures at this time were approximately normal (6 to 7 cm of water) in all mice, infected as well as controls.

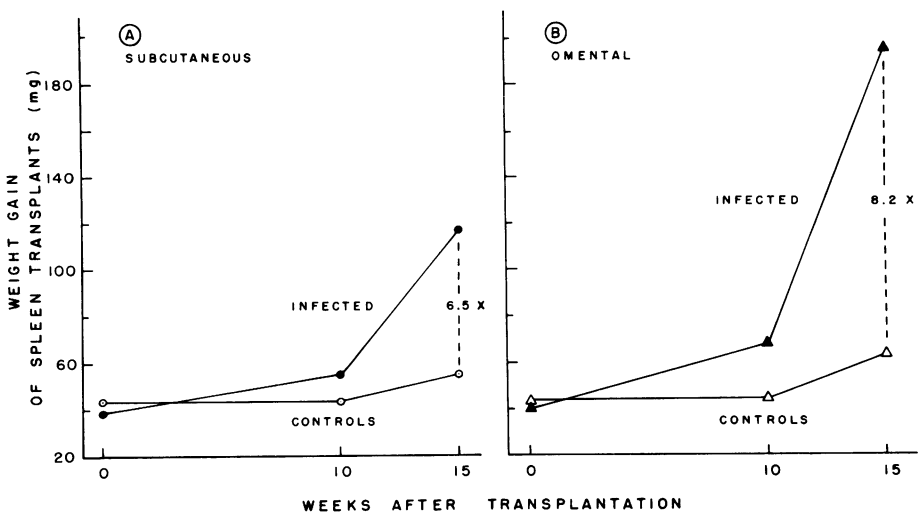
By the fifteenth week of autotransplantation, transplants in subcutaneous pockets of control animals had gained an average of 12.2 mg and those in the omentum 18.9 mg. In mice injected with cercariae, growth of transplants in the systemic as well as in the portal circulation was again strikingly greater than in corresponding controls. Subcutaneous transplants gained an average of 79.4 mg; those in the omentum, 155.5 mg. Thus, in infected animals the growth rate of subcutaneous autotransplants was 6.5 times and that of omental transplants was 8.2 times that of corresponding transplants in uninfected controls (Text-figure 1). Weight of the regenerated spleen in 4 of the injected animals exceeded 158 mg, the average weight of a normal intact spleen in animals similar in weight and age. As shown in Text-figure 2, the intact spleens of 5 infected mice weighed an average of 590 mg by the tenth week of infection, or about four times the average weight of an intact

spleen in controls. At this time portal pressures were highest and averaged 17 cm of water in these infected mice with an intact spleen (Text-figure 3). Pressures averaged 11 to 13 cm of water in infected animals with spleen autotransplants regardless of whether the transplant was in the portal or systemic circulation. In uninfected animals with transplants, pressures averaged a normal level of 6 to 7 cm of water.

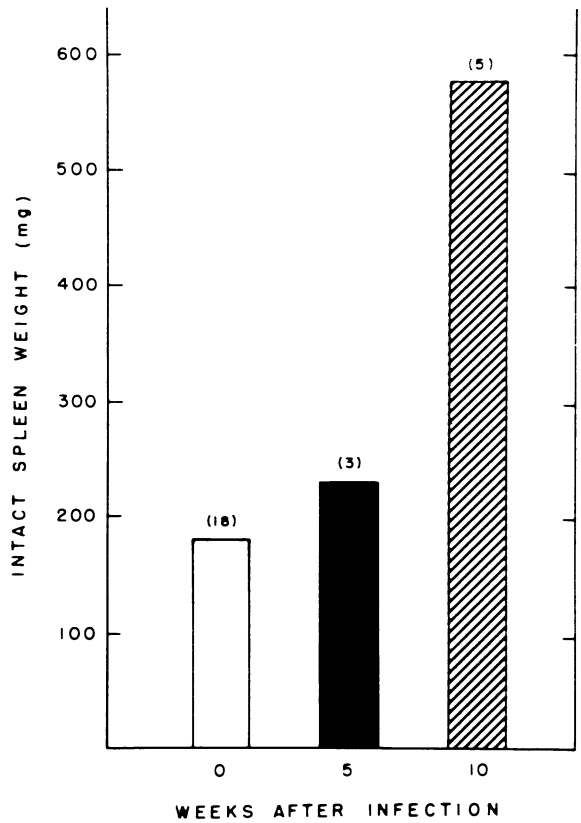
Liver weights increased by an average of 1.2 g in all infected animals, regardless of location of the transplant or presence of an intact spleen.

Histologic Findings

Livers and intact spleens of control noninfected mice were normal (Figure 2). Regenerating spleen transplants in infected as well as control animals were characterized by a thickened capsule, vascular adhesions, numerous megakaryocytes and some hemosiderin-like pigment. In noninfected control mice transplants contained red pulp which was clearly distinct from the white; follicles scattered throughout the latter tended to be irregular in outline and usually lacked germinal centers as well as nuclear activity (Figures 2 and 3). In such animals, subcutaneous transplants were indistinguishable from those in the omentum



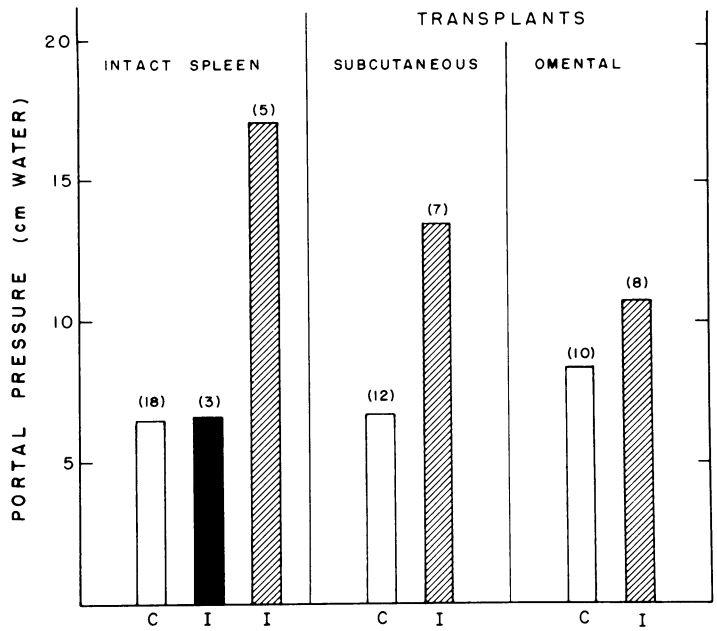
TEXT-FIG 1—Effect of infection with *Schistosoma mansoni* on growth of spleen autotransplants. By the fifteenth week of transplantation, subcutaneous transplants (*A*) in infected animals had gained 6.5 times more weight than did corresponding controls. Omental transplants (*B*) had gained eight times more weight than did corresponding controls. The small number of animals studied at 10 weeks after transplantation precludes meaningful statistical comparison. Each point at 15 weeks represents the mean values from 8 to 12 animals; $P < 0.005$.



TEXT-FIG 2—Comparison of weights of intact spleens in mice after 5 and 10 weeks of infection with the weight of spleens in control animals. $P < 0.005$ applies to spleen weights after 10 weeks of infection.

and, by the tenth week, as noted by others, closely resembled normal spleen (Figures 2 and 4).[8]

Livers of infected animals displayed numerous perivascular accumulations of ova and inflammatory granulomata by the fifth week of infection, and by 10 weeks many of these areas were fibrotic (Figure 5). Adult worms were found in the portal tracts of 2 animals. Spleen transplants in infected mice were strikingly different from those in control animals. White pulp was markedly increased, and red pulp proportionately diminished and indistinct. Accumulations of lymphocytes and reticular cells were usually so dense in these transplants that it was difficult to determine whether a significant portion of the reticular cells were located in cords and sinuses, a finding noted previously by others in the intact spleens of infected animals.[11] In many sections, aggregations of lymphocytes were noted extending through the capsule. Germinal centers were massive and numerous and nuclear activity widespread (Figures 2 and 6). These characteristics were particularly



TEXT-FIG 3—Effects of infection with *Schistosoma mansoni* and splenectomy on portal pressure. Pressures in control (*open columns*) and animals infected for 5 (*solid column*) and 10 weeks (*hatched columns*) with intact spleens are compared with those in control and infected animals with splenic autotransplants. Pressures at 10 weeks of infection represent the mean values from 6 to 10 animals. For intact spleen, $P < 0.005$; for subcutaneous transplants, $P < 0.005$; and for Omental transplants, $P < 0.03$. (Number of animals in each group in parentheses.)

prominent after 10 weeks of infection but were also evident in sections examined 5 weeks after injection of cercariae. Follicles in omental transplants tended to occupy a peripheral position rather than a central one, and this was the only feature which tended to distinguish such transplants from subcutaneous ones. The number of megakaryocytes and the amounts of hemosiderin-like pigment were similar to controls. Ova were found in only one regenerating spleen, an omental transplant examined after 10 weeks of infection.

The intact spleens of infected mice closely resembled spleen autotransplants in infected animals and showed increased white pulp, numerous and massive germinal centers which were central in location and considerable nuclear activity. Specimens from 2 animals in this group also contained granulomata.

Discussion

Authoritative statements concerning the pathophysiology of hepatic schistosomiasis in man and in experimental animals usually identify

portal stasis as the predominant mechanism underlying splenic enlargement.[12] Application of a convenient catch phrase "congestive splenomegaly" to the splenic disorder reflect general agreement with this view. Specific evidence for the relationship implied by the term has been lacking, however; an association between portal hypertension and splenomegaly has provided the only support for the presumed role of venous stasis.

In the experiments with infected mice here reported, omental transplants, subject to venous pressures which averaged almost twice normal levels, grew significantly larger than subcutaneous transplants. On the other hand, the latter gained 6.5 times more weight than corresponding transplants in uninfected animals. Thus, during the first 10 weeks of hepatic schistosomiasis in mice, portal congestion is not a requirement for increased spleen growth but may contribute to the process or enhance it. Support for this view also derives from data included in a recent report by Bloch, Wahab and Warren on growth of intact spleen in experimentally infected animals. They noted a fivefold increase in spleen weight by the tenth week and an additional increase of only one-half of this amount during the next 10 weeks.[10] Moreover, in their study, as well as in the experiments described here, omental autotransplants and/or intact spleens of infected animals demonstrated increased growth as early as 5 weeks after injection when portal pressures were still at normal levels.

Hyperplasia of white pulp with an increase in number and size of germinal centers together with a corresponding diminution in red pulp distinguished autotransplants as well as intact spleens of infected animals. Previous descriptions of experimentally induced hepatic schistosomiasis in mice stressed development of a prominent red pulp together with fibrosis and atrophy of lymphoid follicles in enlarged intact spleens of animals examined later than the tenth week of infection, an interval not included in the observations reported here.[11] Prominent red pulp is usually considered a manifestation of venous congestion, and it is possible, therefore, that the first 10 weeks of infection might be too early for the detection of an additional contribution to spleen growth arising from congestion alone. On the other hand, as the rate of splenic enlargement is known to decrease after the tenth week of infection such a contribution would not appear to be of overriding importance.

Hyperplasia of lymphocytes as well as reticuloendothelial (RE) cells in hepatic schistosomiasis is also an early feature of rapidly growing intact and subcutaneously transplanted spleens in animals with other

forms of hepatic dysfunction.[4] Multiplication of these cells probably reflects the combined effects of RE plus immunologic stimulation.[13] In this regard, it may be important to recall a suggestion by Cameron and Bhattacharyya [6] and by Andrade[11] that enlargement of the spleen in hepatic schistosomiasis might be regulated in part by increase in RE and immunologic activity secondary to release of antigenic material from ova and/or worms. An additional finding in this study which will be described in detail in a separate report appears to bear on such a possibility. Lymph nodes in two separate regions, the upper anterior mediastinum (retrosternal nodes) and the root of the small bowel mesentery (mesenteric nodes) were strikingly larger in animals infected with *Schistosoma mansoni* than in corresponding uninfected controls. Histologic examination of these enlarged nodes disclosed that the normal architecture was obscured by a remarkable increase in the number of lymphocytes throughout the gland. Considered together with the predominant histologic finding of hyperplasia of splenic white pulp, the immunologically competent component of spleen, this observation supports the view that sustained antigenic stimulation is an underlying factor in regulating increased spleen growth in this disorder.

It is tempting to speculate that the enlarged splenic pool of immunologically active cells might have contrasting effects on hepatic granuloma formation, depending on the stage of the disease. As sensitization to ova can be transferred with either lymph node or spleen cells,[17] and as a cell-mediated immune reaction appears to initiate granuloma formation,[18,19] the latter could be augmented early in the disease by proliferation of immunologically active cells in an enlarging spleen. In contrast, at a later stage, an enlarged pool of antibody-forming cells in the spleen might help to suppress granuloma formation by blocking cell-mediated immunity.[20]

Studies reported by Jacob, McDonald and Jandl indicate that increased splenic RE activity is a most important influence regulating spleen growth.[8] Further, decreased blood flow through hepatic sinusoids, the hemodynamic alteration associated with many forms of liver injury, is accompanied by reduction in Kupffer cell activity and corresponding increase in splenic RE function.[4] Development of splenomegaly in animals following injection of silica particles into the portal vein, an experimental maneuver used by Rousselot and Thompson [14] to simulate the hemodynamic effects of schistosomiasis, might thus be explained by compensatory increase in splenic RE activity secondary to reduction in transsinusoidal blood flow rather than by increased portal pressure, which develops concomitantly. To consider that stimulation of splenic

RE activity secondary to decreased hepatic sinusoidal blood flow underlies enlargement of the spleen in hepatic schistosomiasis is, however, to ignore data which indicate that transsinusoidal blood flow is relatively undisturbed in this disease even though portal flow becomes impaired. Thus, in mice with portal hypertension, varices and splenomegaly secondary to experimentally induced hepatic schistosomiasis, Cheever and Warren demonstrated that hepatic uptake of isotope-labeled colloid was only slightly decreased.[15] Subsequent studies disclosed that sinusoidal perfusion was maintained by increased diversion of blood from the hepatic artery.[10]

Portal hypertension is often considered the direct result of impairment to transhepatic portal blood flow. It seems surprising, therefore, that portal pressures in infected mice with omental transplants, while being almost twice normal levels, were at the same time lower than values found in infected mice with enlarged intact spleens. This finding may bear directly on recent clinical observations which indicate that in some patients with splenomegaly abnormally increased arterial inflow via an enlarged splenic artery contributes to elevated levels of portal pressure.[16] Ligation of an enlarged splenic artery and/or splenectomy in such patients stops variceal hemorrhage and lowers portal pressure. Abnormal enlargement of the splenic artery may also develop in patients or experimental animals with hepatic schistosomias, and the intact enlarged spleen may then contribute much more than the normal 30 to 40% to portal flow. Based on these considerations, it is likely that in infected animals the levels of portal pressure were lower in mice with autotransplants than in animals with intact spleens because arterial inflow to the former is limited by the small size of newly established vessels and much less than that possible through intact splenic arteries.

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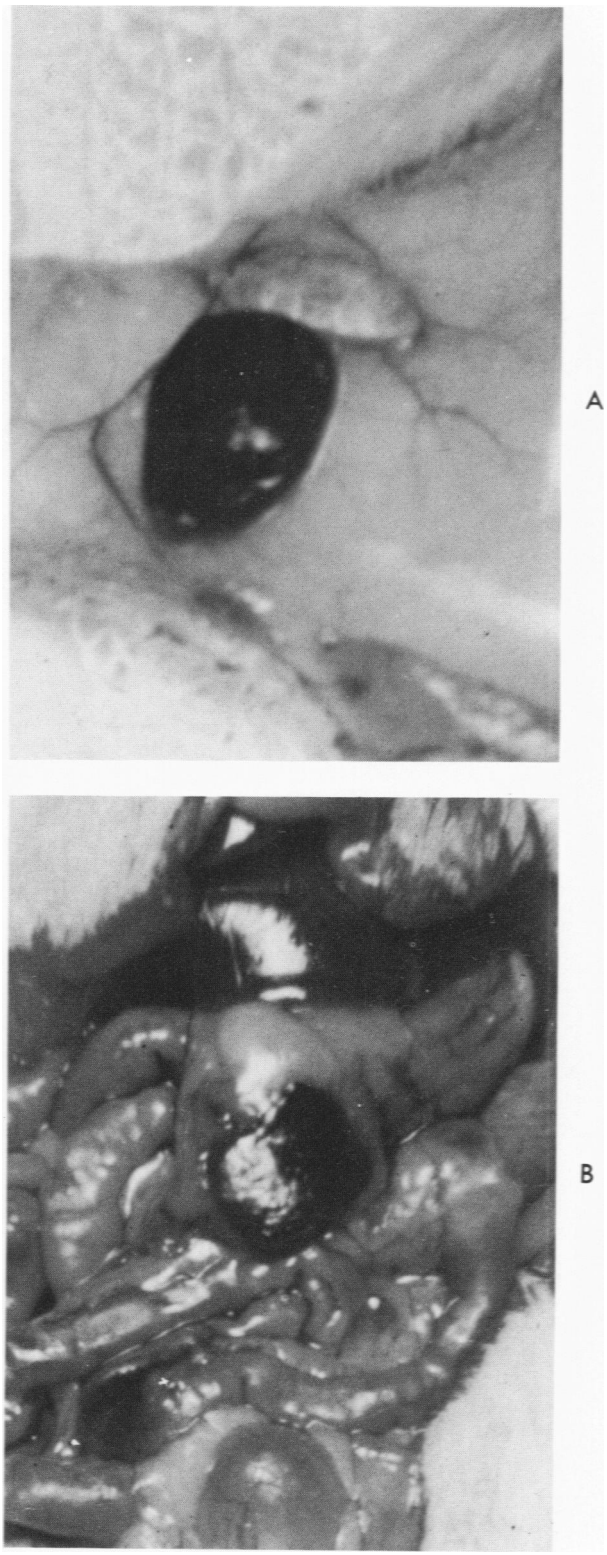


Fig 1—Gross appearance of subcutaneous (**A**) and omental splenic (**B**) autotransplants 15 weeks after transplantation.

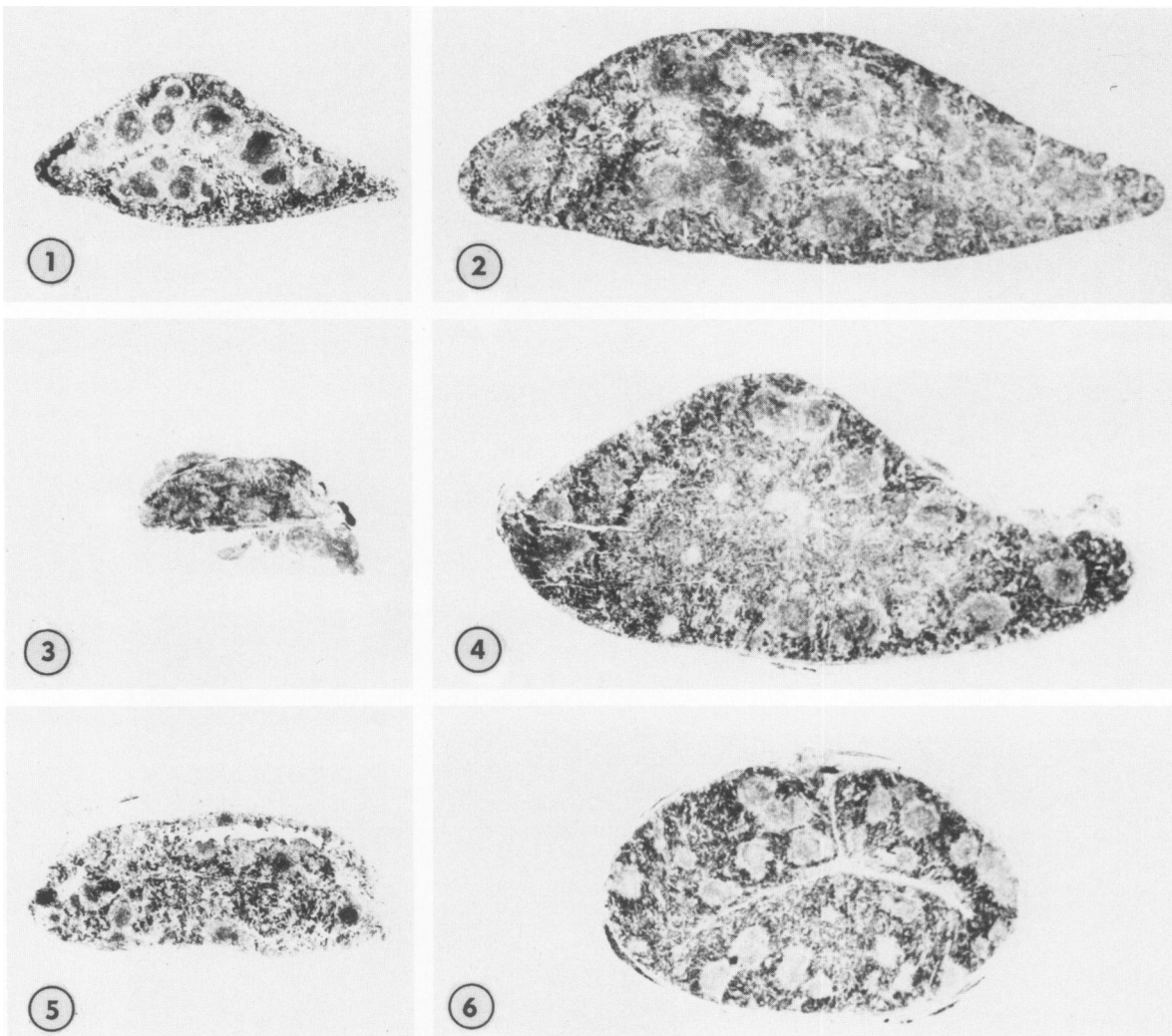
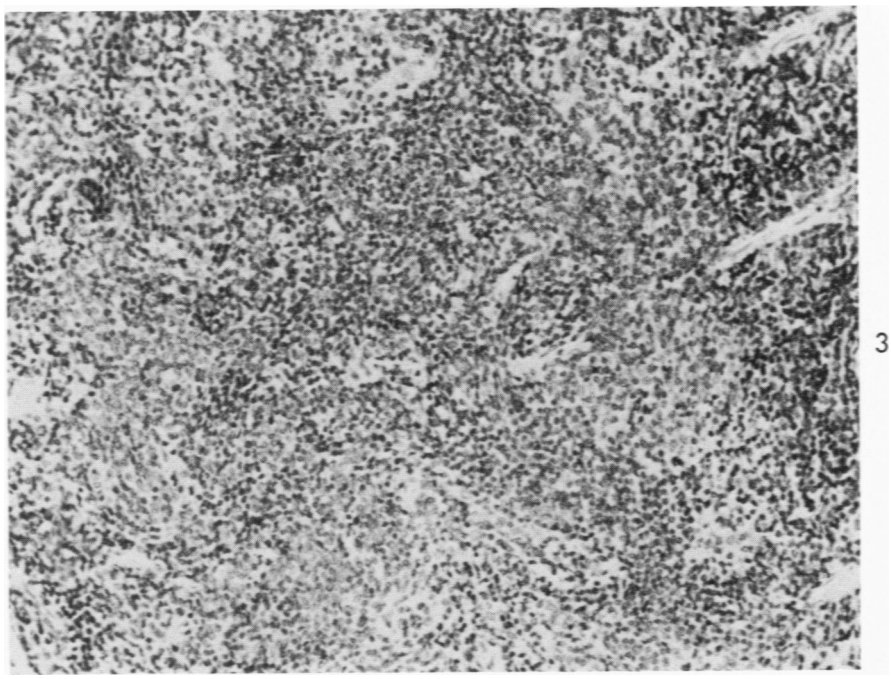


Fig 2—Composite photograph of representative whole sections of intact spleens and 15-week-old splenic autotransplants from each of the 6 groups described in this study. Spleens 1, 3 and 5 are from uninfected mice and represent, respectively, an intact spleen, a subcutaneous transplant and an omental transplant. Spleens 2, 4 and 6 are from mice infected for 10 weeks and represent, respectively, an intact spleen, a subcutaneous transplant (H&E, × 3).



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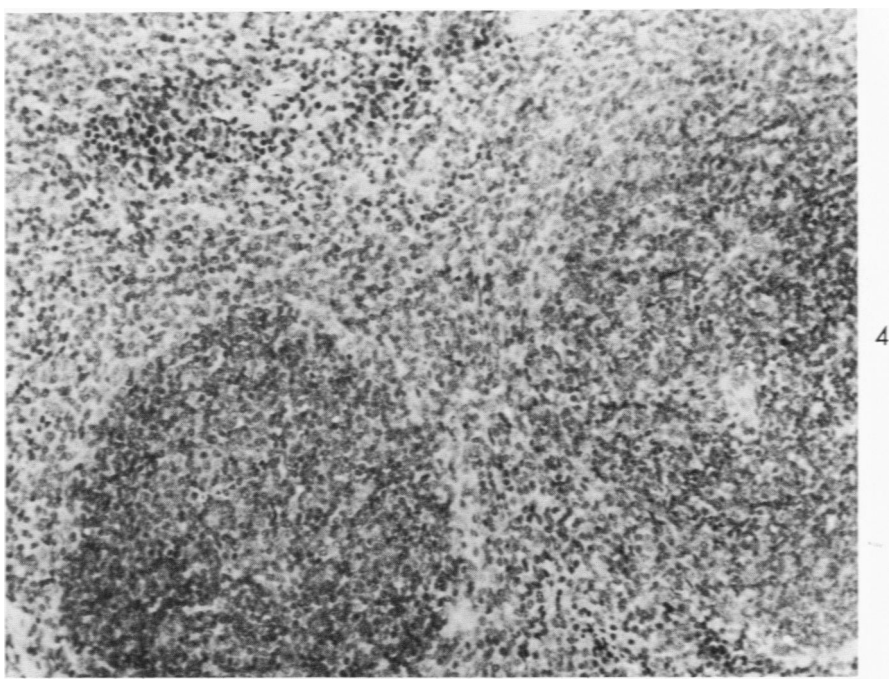

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Fig 3—Section through a 15-week-old subcutaneously transplanted spleen of an uninfected mouse. White pulp is evident but is rather diffuse. Germinal activity is lacking, and red pulp appears normal (H&E, × 150). **Fig 4**—Section through an intact spleen of a normal mouse. There are well-formed follicles with minimal germinal activity. The red pulp is abundant (H&E, × 150).

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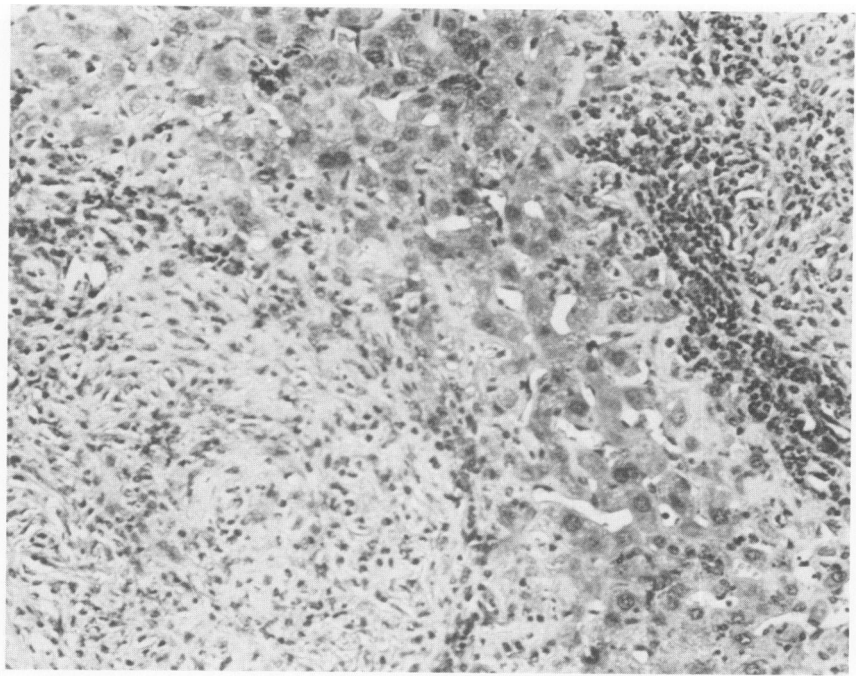

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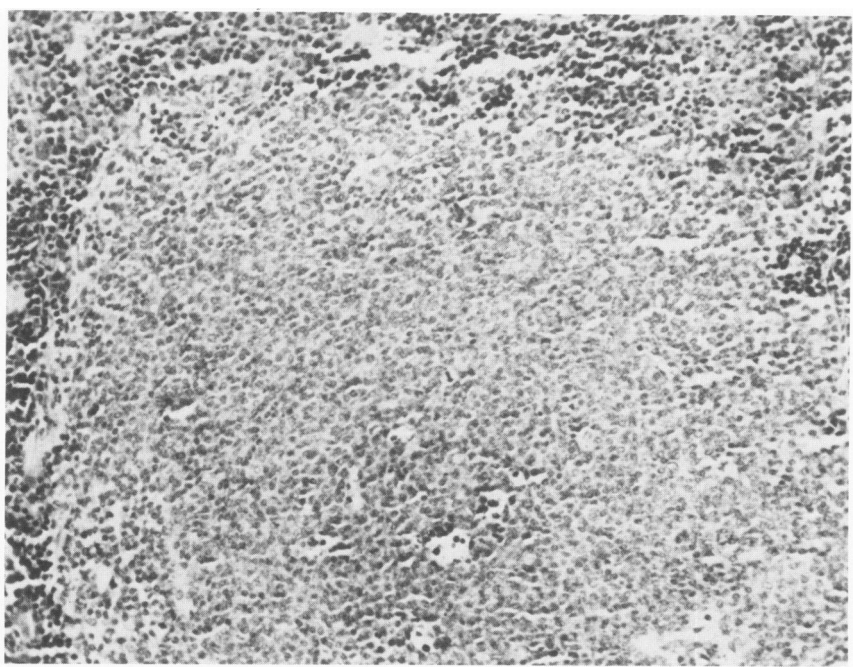

Fig 5—Section through liver after 10 weeks of infection; an acute granulomatous reaction with beginning fibrosis is evident. In most animals more than one-half of liver tissue showed this abnormality (H&E, × 150). **Fig 6**—Section through a 15-week-old subcutaneous autotransplant in an infected animal. An enormous germinal center fills the entire field. Nuclear activity is abundant. Dense aggregates of normal-appearing lymphocytes surround the germinal follicle (H&E, × 150).