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Research Article

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REGULATION OF SPLEEN GROWTH AND SEQUESTERING FUNCTION *

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A major function of the reticuloendothelial system is to remove defective cells and other particulate matter from the blood stream. Red cells that have been severely altered, as by complement-fixing or agglutinating antibodies (1, 2), agglutinating metals (3), prolonged incubation (4), or severe chemical injury (5), are sequestered throughout the reticuloendothelial system, much as are foreign particles such as bacteria, carbon, or metallic colloids (6, 7). When this is so, the liver, with its great blood flow, is the major site of sequestration, the spleen simply assisting to the extent of its own blood supply. In other instances, the spleen, despite its modest blood flow, is the dominant sequestering organ. Thus a variety of pathological red cells (8–11) and red cells mildly altered by chemical (3–5, 12), physical (5), or immune (1, 2) agents are susceptible to selective splenic sequestration and destruction. Comparatively little is known of the participation of the lungs and bone marrow in particle clearance. The lung does not sequester experimentally altered red cells (13), unless the cells are very coarsely agglutinated (2). Thus far, the marrow has been observed to play only a small role in the clearance of experimentally altered red cells (13), although it may possibly sequester cells that have been very subtly injured.

The factors that regulate the growth and function of the reticuloendothelial system, as well as the relative activities of such component parts as the spleen, liver, and bone marrow, are obscure. That splenic enlargement occurs in chronic hemolytic anemias suggests that spleen growth may be stimulated by an increase in its "work load." Admittedly, some of this increased splenic bulk may represent trapped red cells and their debris. It is probable, however, that functional splenic tissue actually increases, since in long-standing hemolytic anemias, such as thalassemia, the spleen may progressively increase its capacity to destroy transfused normal red cells (14). Further indirect evidence that the spleens of chronic hemolytic disorders may become hyperfunctional may be inferred from the depression in the levels of blood elements other than red cells as splenic enlargement progresses. This sort of "hypersplenism" has also been observed in patients with infections (15) and in animals injected with various macromolecular or particulate materials (16–21). In these examples, the increased load of particulate or poorly solubilized material to be cleared by reticuloendothelial tissue has stimulated the spleen to overgrow and hyperfunction. Conversely, the finding that splenic atrophy occurs in animals subjected to repeated venesections (22) has led to the suggestion (23) that reducing the load of effete red cells delivered to the spleen may inhibit its growth. To investigate these matters, studies were made in rats of the growth and function of spleens, livers, and spleen autotransplants under a variety of experimental "work loads."

The ability of transplanted spleen tissue to regenerate was reported almost fifty years ago by Manley and Marine (24, 25). That such transplants could in some way function was suspected by Perla and Marmorston-Gottesman (26) from observations that spleen autotransplants protected otherwise splenectomized rats from bartonellosis. Later, Palmer, Kemp, Cartwright, and Wintrobe (27) found that spleen autotransplants in rats suppressed the leukocytosis that normally follows splenectomy. In a report (28) preliminary to this communication, regenerated spleen autotransplants were found capable of sequestering Cr^{51}-labeled red cells that had been coated with an

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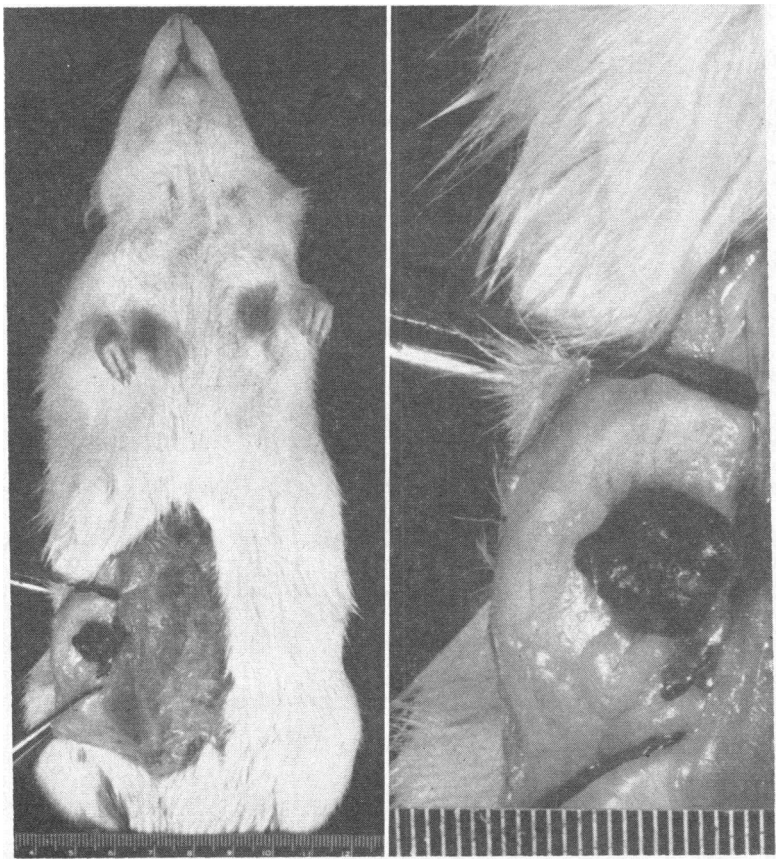

FIG. 1. GROSS APPEARANCE OF A SPLEEN AUTOTRANSPLANT. Two views are shown of a fully grown transplant after it had been dissected free of overlying fat and fascia.

incomplete antibody. Similarly, in the present studies, the capability of reticuloendothelial tissue to trap altered red cells has been utilized for quantitating the function of this tissue under various experimental conditions. Preliminary reports of these findings have been published (28, 29).

MATERIALS AND METHODS

Preparation of animals. All studies were made on a *Bartonella bacilliformis*-free, Caesarean-derived strain of Sprague-Dawley rat (Charles River C. D.).[1] Weanling rats, weighing from 50 to 75 g at the time of operation, were used because of the report (25) that transplanted spleen tissue grows best in young animals. Under ether anesthesia and aseptic conditions, a small, weighed, transverse section of spleen was autotransplanted into a subcutaneous pocket fashioned in the anterior abdominal wall. At the time of transplantation, one group of animals underwent total splenectomy, whereas in the second, "hemisplenectomized" group, only half the parent spleen was removed, the other half remaining in its original site. For comparison, a third group of rats was splenectomized without autotransplants, and a fourth group was subjected to a sham operation.

In those experiments involving the use of Millipore filters, a transverse section of spleen was aseptically suspended in sterile Eagle's (30) tissue culture medium[2] within a cylindrical chamber. The cylinder wall consisted of a nylon-reinforced Millipore filter having a pore size of 0.45 μ.[3] After the ends of the cylinder were sealed with plastic plugs, it was placed free in the peritoneal cavity of the original donor rat. At the time of sacrifice, several weeks later, the filter chamber was embraced by omentum. The host animal at this time was of normal appearance and size.

Measurement of the function of reticuloendothelial tissues. At various intervals after transplantation, animals were injected by tail vein with 1 ml of a 50% washed

[1] Obtained from Charles River Laboratories, Boston, Mass.

[2] Generously supplied by Dr. A. Martin Lerner.

[3] Available through the courtesy of Mr. Richard A. Cotton, Millipore Filter Corporation, Bedford, Mass., as 4 cm × 5 mm nylon-reinforced H A filter tubes.

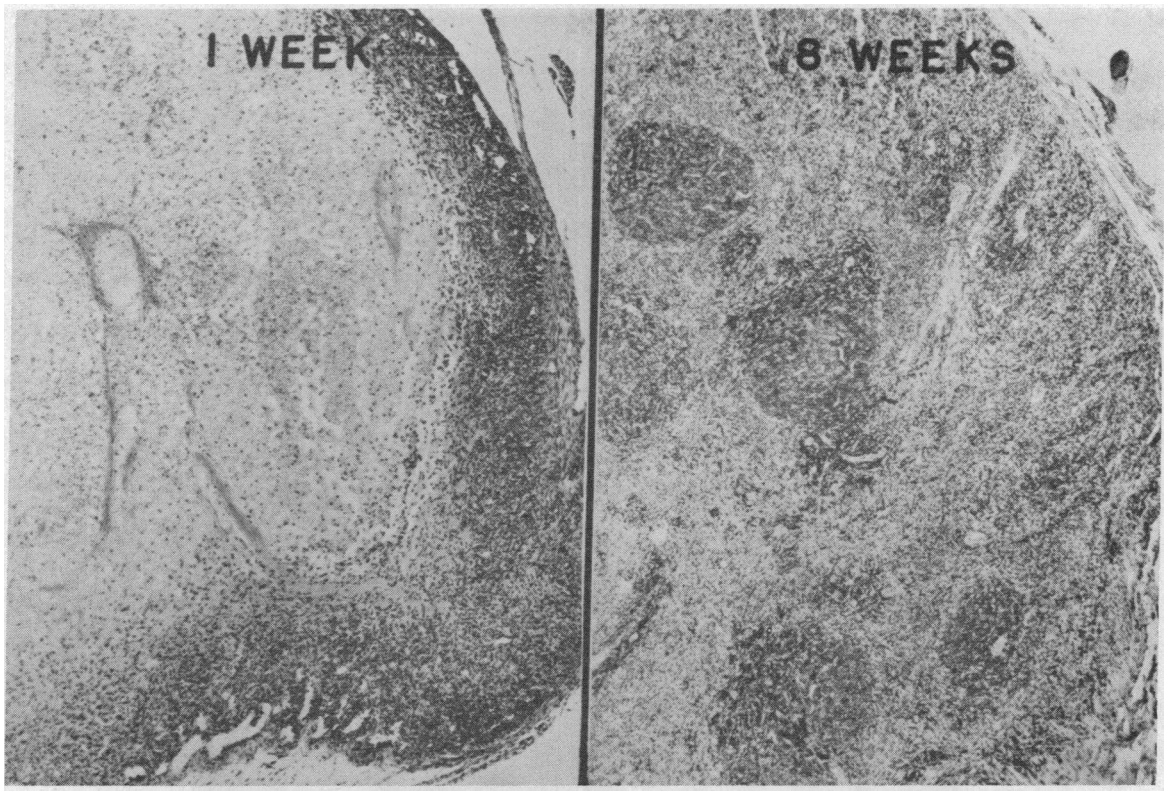

FIG. 2. SEQUENCE OF HISTOLOGIC CHANGE AFTER SPLEEN TRANSPLANTATION. One week after transplantation, as shown on the left, the bulk of the tissue is necrotic; only a thin rim of mononuclear cells containing some disorganized vascular spaces give evidence of viability. After gradual replacement of the necrotic tissue, normal splenic histology is evident in the full-grown 8-week-old transplant seen on the right.

suspension of isologous red cells that had been labeled with 5 μc of $Na_2Cr^{51}O_4$.[4] The red cells were altered either by incubation with an "incomplete" antibody to rat red cells prepared in rabbits (13), or by exposure of a 50% cell suspension to an equal volume of 12 mM *N*-ethylmaleimide,[5] a sulfhydryl inhibitor (31). In both cases, concentrations of antibody or inhibitor were chosen that gave pronounced splenic but little hepatic uptake of the altered cells (12, 13). Three hours after injection, at a time when approximately 50% of the labeled cells had been sequestered, the animals were sacrificed. Cardiac blood, spleens, livers, kidneys, lungs, femurs, and spleen transplants were removed, weighed, and assayed for radioactivity in a well-type scintillation counter as described previously (32). Finally, histologic sections were made from formalin-fixed specimens of spleens, livers, and spleen transplants.

The levels of blood cells were measured by standard techniques (33). Platelets were counted by the method of Pohle (34); approximately 1,000 platelets in two chambers were counted for each determination.

In certain animals, a chronic hemolytic anemia was produced by the subcutaneous injection 3 times a week of 5 mg of β-acetylphenylhydrazine[6] per 100 g body weight. In such rats, hemoglobin levels were about 50% of normal during drug administration, and reticulocyte concentrations averaged about 40%.

RESULTS

Appearance of spleen autotransplants. Spleen autotransplants survived in over 95% of 150 animals operated on. The gross appearance of a fully grown transplant *in situ* is shown in Figure 1. A well-formed capsule allowed for its easy dissection from the surrounding subcutaneous fat. Numerous small blood vessels supplied the tissue, which thereby lacked a hilum and assumed the shape of a seminodular, oblate spheroid of deep purple color. The sequence of histologic changes occurring after spleen transplantation has been reported previously by others (25, 35–38).

[4] Obtained from Abbott Laboratories, North Chicago, Ill.

[5] Obtained from Schwarz BioResearch, Inc., Mount Vernon, N. Y.

[6] Obtained from the Matheson Company, East Rutherford, N. J.

Briefly, in the first week following transplantation, the tissue underwent almost complete necrosis. At the end of this period, as seen on the left side of Figure 2, a large central necrotic area was surrounded by a thin rim of viable, newly vascularized tissue made up of reticulum and lymphoid cells. Thereafter, the necrotic area was gradually replaced by viable tissue with lymphocytes accumulating around arterioles ultimately to form typical Malpighian corpuscles. Characteristic spleen architecture was evident within 3 weeks. Between 6 and 8 weeks, the transplants reached their full growth and histologically resembled normal spleens (right side of Figure 2).

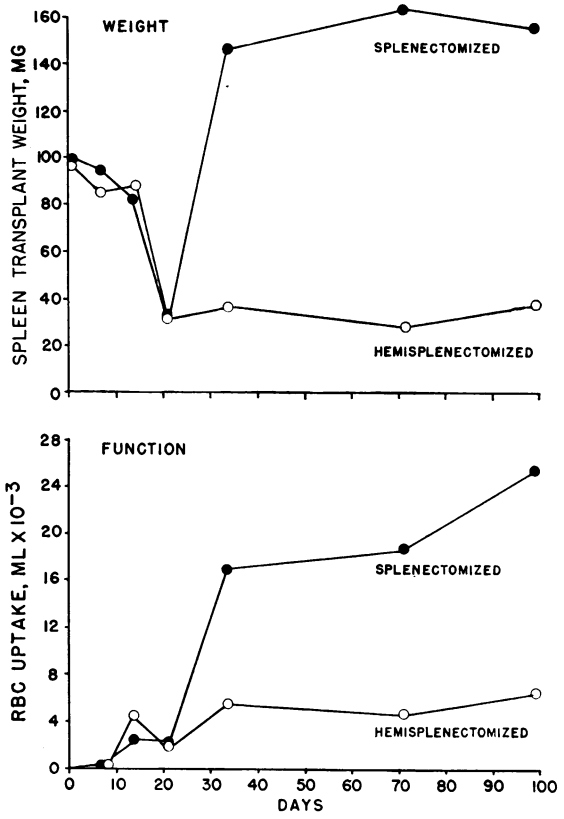

FIG. 3. EFFECT OF SPLENECTOMY AND HEMISPLENECTOMY ON THE GROWTH (UPPER) AND FUNCTION (LOWER) OF RAT SPLEEN AUTOTRANSPLANTS. After an initial loss in weight due to necrosis of the original tissue, spleen transplants in splenectomized rats (solid circles) regenerate rapidly and grow beyond their original weight. Though viable, transplants in animals with half spleens remaining *in situ* (open circles) show far less growth. As shown in the lower portion, transplants begin to sequester Cr^{51}-labeled red cells coated with an incomplete antibody within 2 weeks of surgery to an extent paralleling their size. Each of the points represents the mean value for two animals. The small number of animals sacrificed at each time interval precluded any reasonable statistical comparisons. Individual values deviate from the means portrayed by less than ± 15 mg (top) and by less than $\pm 4 \times 10^{-3}$ ml (bottom).

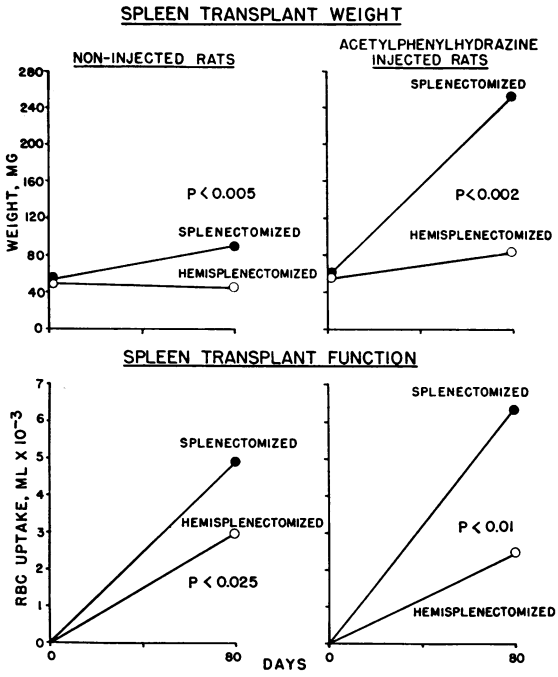

FIG. 4. EFFECT OF β-ACETYLPHENYLHYDRAZINE ADMINISTRATION ON THE WEIGHT (UPPER) AND FUNCTION (LOWER) OF SPLEEN AUTOTRANSPLANTS. In untreated animals (left), the differences between the growth and function of transplants from splenectomized rats (solid circles) and those from hemisplenectomized animals (open circles) are significant. Repeated administration of β-acetylphenylhydrazine (right) accentuates these differences. Note the threefold increase in transplant size in splenectomized rats treated with β-acetylphenylhydrazine (upper right) over that in their noninjected mates (upper left). Each of the points represents the mean value from twelve animals.

Growth and function of spleen autotransplants. Although the histologic appearance of transplants from splenectomized and hemisplenectomized animals was similar, their rate of growth and function differed markedly. As shown in the top portion of Figure 3, transplant weight decreased in both sets of animals for the first 3 weeks; during this time, necrosis and early regeneration took place. Thereafter, particularly from weeks 3 to 5, transplants grew rapidly in splenectomized animals. In contrast, transplants in hemisplenectomized animals grew but slightly, and in vari-

ous experiments attained only $\frac{1}{4}$ to $\frac{1}{2}$ the size of those from their splenectomized mates. Although the percentage "take" was the same in both groups of animals, the final mass of spleen tissue generated in splenectomized animals eventually exceeded the weight of the original transplant by from $1\frac{1}{2}$ to 2 times, while in the hemisplenectomized animals, regeneration ceased before the original weight of the tissue transplant had been restored. Histologically, the transplants of splenectomized animals appeared to contain more spleen cells than did those of hemisplenectomized animals. The lower portion of Figure 3 shows that within 2 weeks after surgery, transplants from both groups began to function, as measured by their uptake of Cr^{51}-labeled red cells that had been coated with incomplete antibody. The ability of transplants to trap altered red cells paralleled their rate of growth. Thus, a rapid increase in sequestering function occurred during the phase of rapid growth of the transplant in splenectomized rats. On a gram-for-gram basis, transplant sequestering function was roughly equal in the two groups, and reached about one-half that of native spleen and 10 to 30 times that of liver.

Results similar to those shown in Figure 3 were obtained when the sequestering function of transplants was measured with red cells that had been treated with *N*-ethylmaleimide. A large number of animals was employed to permit statistical comparisons (Figure 4). As shown in the left upper portion of Figure 4, 80-day-old transplants were twice as large in splenectomized as in hemisplenectomized animals. As with sensitized red cells, this was associated with a significantly greater trapping of the treated red cells, shown in the left bottom portion of the figure.

Growth and function of spleen autotransplants in diffusion chambers. From the foregoing, it would appear that the absence of parent spleen tissue stimulates the growth of transplants. To elucidate the mechanism of this stimulation, transplants were isolated from circulating particulate matter, but not from humoral factors, by

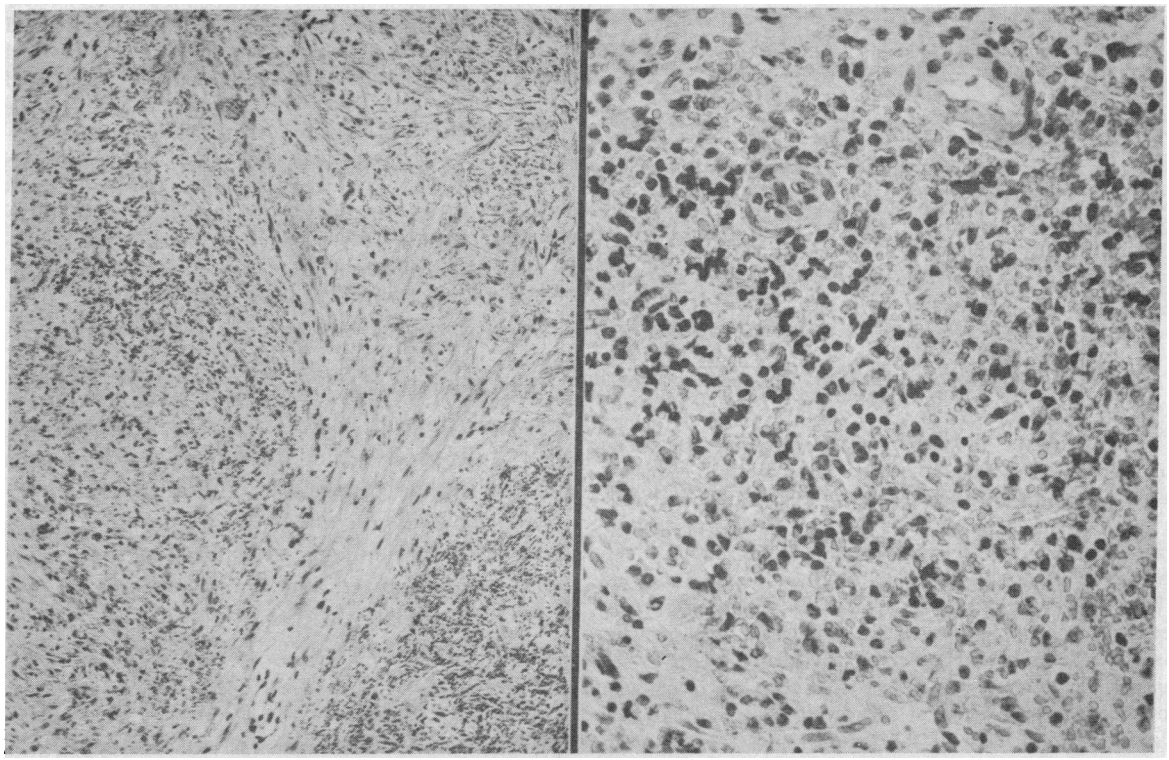

FIG. 5. HISTOLOGY OF SPLEEN TISSUE AFTER AUTOTRANSPLANTATION INTO DIFFUSION CHAMBERS. After several weeks in Millipore chambers, spleen autotransplants are viable, but contain an increased quantity of fibrous tissue as shown under low magnification (200 ×) on the left. On the right (780 ×), lymphocytes (dark nuclei), reticulum cells (light nuclei), intact red cells, and endothelial-lined vascular channels can be seen.

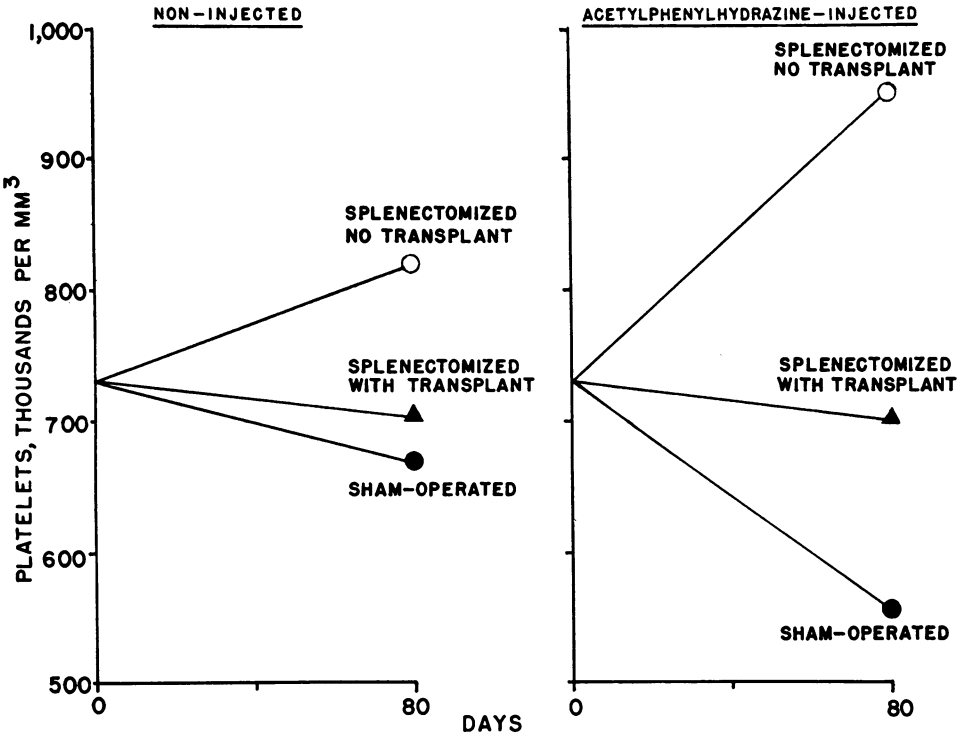

FIG. 6. EFFECT OF SPLEEN TRANSPLANTS ON PLATELET LEVELS. In noninjected animals (left), the usual elevation of platelets after splenectomy (open circles) is suppressed by full-grown spleen transplants (triangles); this difference was statistically significant ($p < 0.025$). Similarly, the exaggerated thrombocytosis after splenectomy in rats given β-acetylphenylhydrazine (right) is inhibited by the presence of transplants ($p < 0.025$). In animals sham-operated on that were injected with β-acetylphenylhydrazine and that possessed hyperplastic spleens (right, solid circles), platelet levels are significantly lower than in their noninjected mates sham-operated on (left, solid circles), $p < 0.001$. Each of the points represents the mean value from twelve animals.

transplantation within Millipore-filter diffusion chambers placed in the peritoneal cavities of a group of 20 animals. Absence of radioactivity in these transplants after the injection of Cr^{51}-labeled red cells into the host animals verified their exclusion from the circulation. This transplanted tissue remained viable histologically (Figure 5), and contained normal splenic cellular elements such as lymphocytes, reticulum cells, red blood cells, fibroblasts, and endothelial-lined vascular spaces (Figure 5, right portion). The splenic architecture was poorly organized, however, most of the tissue consisting of fibroblasts (Figure 5, left portion). Though viable, these transplants failed to grow, whether or not splenectomy had been carried out.

Effect of chronic hemolysis on spleen autotransplant growth and function. To assess further the suggestion that increased "work" stimulates spleen tissue growth and function, experimental groups of rats analogous to those described above were given injections of β-acetylphenylhydrazine 3 times a week, beginning 2 days after surgery, to produce a chronic hemolytic anemia. Giant spleen transplants were induced by this procedure, some nearly as large as the parent spleens. The average weight of such transplants, as shown in the right upper portion of Figure 4, was about 3 times that of transplants in uninjected rats (left upper portion). By extrapolation, it is estimated that such transplants would weigh about 100 g in man. As in the uninjected animals, growth was most pronounced when the transplants represented the sole existing splenic tissue. Although foci of extramedullary hematopoiesis were seen histologically in these enlarged transplants, retic-

uloendothelial hyperplasia was striking, and as shown in the right lower portion of Figure 4, this increase in transplant size in splenectomized animals was accompanied by augmented sequestering function. Transplants within Millipore-filter chambers, thereby denied contact with the circulating red cells injured by β-acetylphenylhydrazine, were not stimulated to greater growth by the hemolytic process.

Effect of spleen autotransplants on circulating platelet and leukocyte levels. The thrombocytosis that usually follows splenectomy was inhibited by the presence of full-grown spleen transplants (left portion, Figure 6). Leukocytosis after splenectomy was similarly suppressed (not shown), confirming the findings of Palmer and associates (27). As shown on the right side of the figure, the repeated administration of β-acetylphenylhydrazine led to an exaggerated thrombocytosis in splenectomized animals lacking transplants; but again, in splenectomized animals possessing transplants, platelets were held to normal levels. By analogy with their uptake of effete red cells, it is probable that these transplants suppress leukocyte and platelet levels by their capacity to sequester blood cells. In favor of this interpretation is the

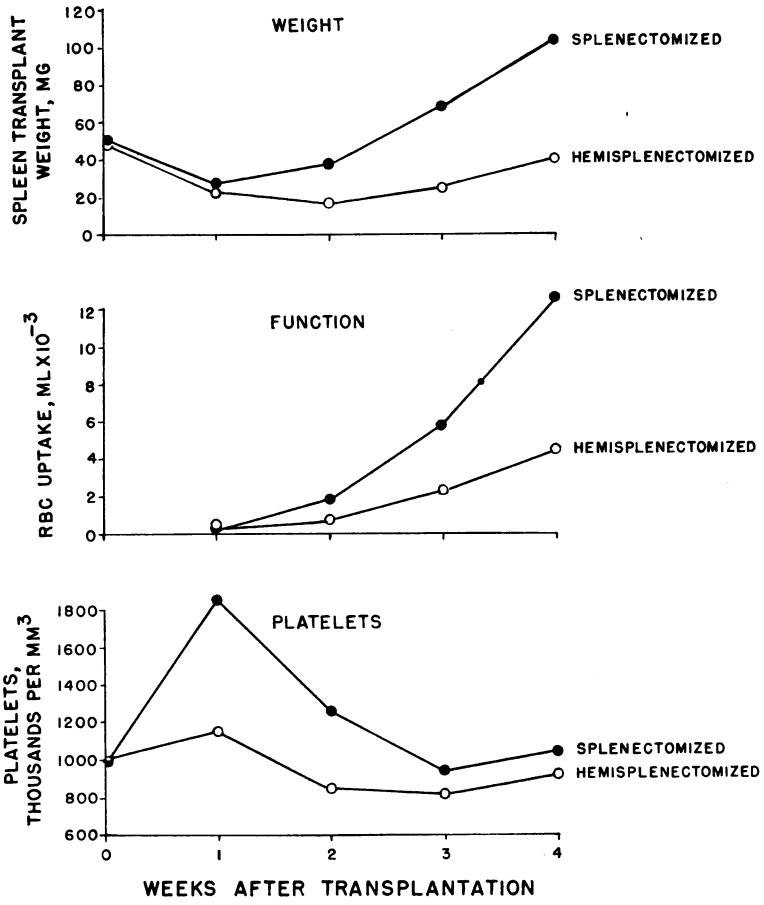

FIG. 7. SEQUENTIAL CHANGES IN PLATELETS IN RELATION TO THE GROWTH AND FUNCTION OF SPLEEN AUTOTRANSPLANTS. In the second week after transplantation, spleen transplants suppress the thrombocytosis (lower) that occurs in splenectomized animals (solid circles). At this time, sequestering function (middle) and growth (upper) have just become manifest. Platelet levels in splenectomized rats without transplants remain elevated throughout (not shown), while in transplant-containing animals with half-spleens (open circles) platelets remain normal. Each point represents the mean value from six animals.

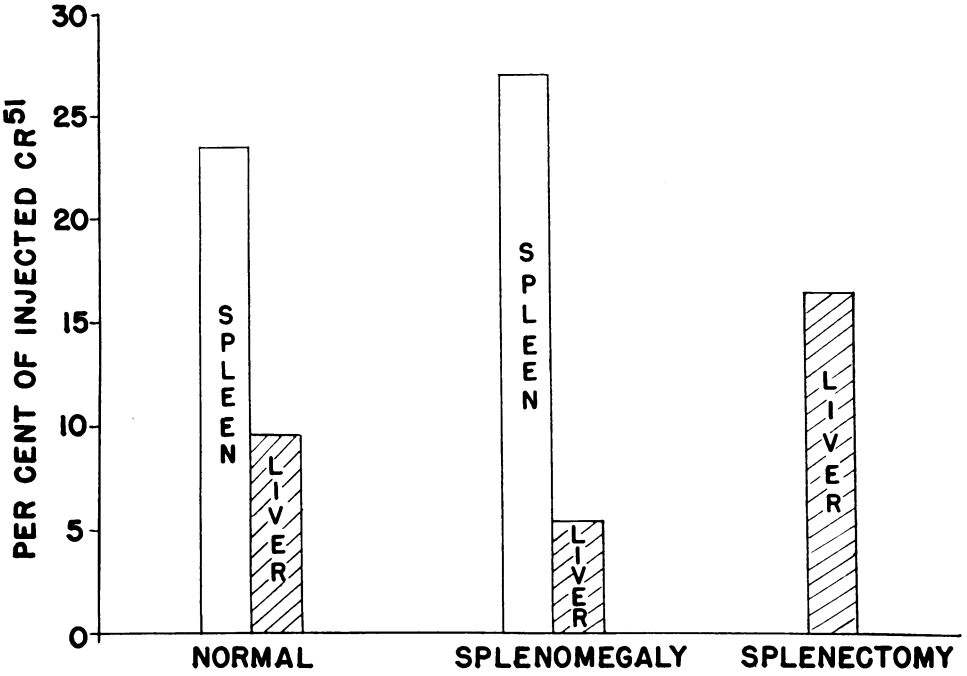

FIG. 8. INTERRELATIONSHIPS BETWEEN HEPATIC AND SPLENIC ERYTHROPLASIA. As compared to normal animals (left), the uptake by the liver of labeled red cells treated with *N*-ethylmaleimide was nearly reduced by half in animals made splenomegalic by β-acetylphenylhydrazine administration (middle), $p < 0.001$. On the other hand, hepatic uptake was increased in animals that had been splenectomized 8 weeks previously (right), $p < 0.001$. Each bar represents the mean value from twelve animals.

finding that the increased platelet and leukocyte levels of splenectomized animals were not suppressed by transplants within Millipore-filter chambers, although it is emphasized that spleen regeneration was poorly organized within these diffusion chambers.

The temporal sequence of thrombocytosis after splenectomy, and its suppression by spleen transplants, is shown in the bottom portion of Figure 7. Significant depression of the thrombocytosis in splenectomized animals is not evident until 2 weeks after transplantation, at a time when the increased weight (Figure 7, upper portion) and function (Figure 7, middle portion) of the transplanted tissue have just become manifest. In contrast, in splenectomized animals without transplants, platelet levels remained high during the period of observation (not shown), while in hemisplenectomized rats with transplants, platelet levels remained normal.[7] In this same study, leukocyte alterations paralleled those observed with platelets.

The administration of β-acetylphenylhydrazine to animals sham-operated on caused hyperplasia of their spleens as it did in those with spleen autotransplants. Such spleens were 4 to 5 times larger than those from noninjected controls. Although, in part, this increment in size reflected an accumulation of red-cell debris and the appearance of extramedullary hematopoiesis, reticulum-cell hyperplasia was evident histologically. In these splenomegalic animals sham-operated on, depicted at the bottom right of Figure 6, platelet levels were depressed significantly below those of noninjected rats (bottom left). Leukocyte levels were similarly affected. Thus, splenic hyperplasia, induced by an abnormality of red cells, led to a reduction of the levels of platelets and leukocytes as well.

Interrelationships between the liver and spleen. As shown in Figure 8, the sequestering function of the liver is affected by that of the spleen. Ap-

[7] In contrast to the other experiments, female rats were employed in this study, which may explain the somewhat higher base-line level of platelets.

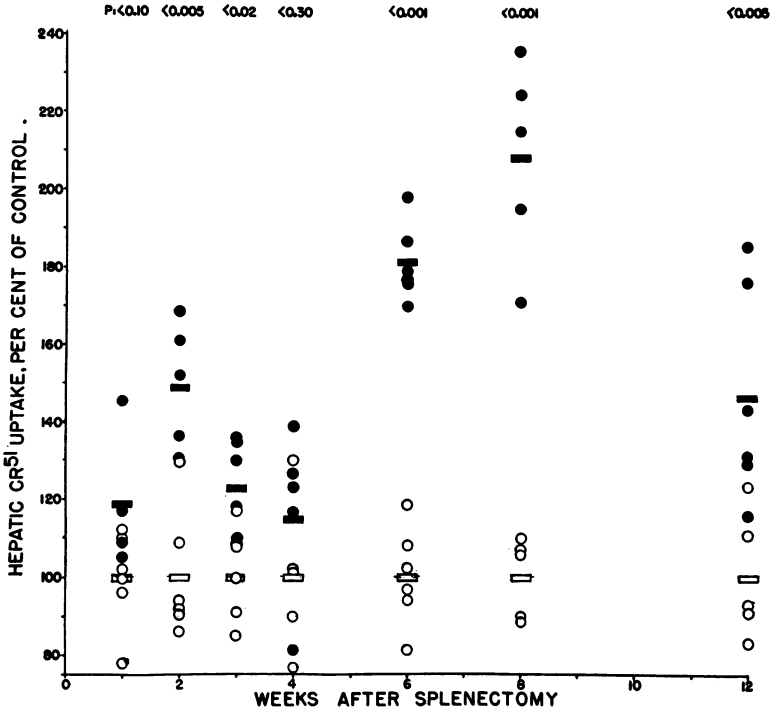

FIG. 9. INCREASE IN HEPATIC SEQUESTRATION OF ABNORMAL RED BLOOD CELLS AFTER SPLENECTOMY. After splenectomy, hepatic sequestration of labeled red cells treated with *N*-ethylmaleimide gradually increased as compared with control animals sham-operated on (represented by 100% on the ordinate). Values from animals sham-operated on are depicted by open symbols; those from splenectomized animals by closed symbols. Mean values are indicated by horizontal bars. Although there was considerable variation in the data, it appeared that sustained increases in hepatic reticuloendothelial function were not attained until about 2 months after splenectomy.

proximately 10% of sulfhydryl-inhibited red cells were sequestered in the livers of normal animals 3 hours after injection (left portion); this hepatic function was nearly reduced by half in animals made splenomegalic by prior β-acetylphenylhydrazine administration in which an increased proportion of cells was trapped in the spleen (middle portion). In contrast, the sequestering ability of the liver was markedly increased in animals that had lacked spleens for several months (right portion). In these studies, over 50% of the labeled cells were still in the circulation at the time of sacrifice. This excess of circulating cells, shown previously to be altered in a uniform manner (12), assures that the liver has a comparable opportunity in each study to sequester labeled cells. Thus, the observed differences in hepatic sequestration reflect variation in its avidity for the abnormal red cells, rather than differences merely in splenic competition for these cells.[8]

The hepatic response to splenectomy was studied further. As shown in Figure 9, hepatic sequestering ability underwent an irregular increase following splenectomy, as compared to that observed in animals sham-operated on and injected with the same injured red cells. The sequestering ability of the livers appeared to reach a maximum some 6 to 8 weeks after splenectomy. Of further interest was the histologic finding that Kupffer cells from animals splenectomized and treated with β-acetylphenylhydrazine for pro-

[8] Previous studies (39) have demonstrated that rat livers removed from animals and assayed for radioactivity as in these studies contain 3.5% of the circulating blood volume. Correction of hepatic radioactivity for this intravascular activity would increase the relative differences depicted in Figure 8.

TABLE I

*Effect of splenectomy on liver and femur sequestration of altered red cells**

Days splenectomized before sacrifice	No. of animals	RBC uptake, ml $\times 10^{-3}$	
		Liver†	Femurs‡
2	6	63.5 ± 4.5 SD	2.3 ± 1.4
60	7	77.5 ± 11.5	4.2 ± 1.1

* Incubated with 30 μmoles *N*-ethylmaleimide per ml red blood cells.
† Liver: $p < 0.05$.
‡ Femurs: $p < 0.01$.

longed periods of time showed much more erythrophagocytosis than did similarly treated animals with intact, hyperplastic spleens. In ancillary studies, it was found that erythrophagocytosis by Kupffer cells occurred in all animals, splenectomized or not, early in the course of β-acetylphenylhydrazine-induced hemolysis. However, after several weeks of hemolysis, erythrophagocytosis by Kupffer cells diminished in animals with spleens (these having become greatly enlarged), but persisted in animals without spleens.

Another experiment was performed to verify the effect of splenectomy on hepatic sequestering function. Two days before sacrifice, one group of rats that had been splenectomized for about 8 weeks was sham-operated on, while another, previously sham-operated on group was splenectomized. Forty-eight hours later, all of the animals were injected with Cr^{51}-labeled, altered red cells, and organ radioactivity was measured as usual. The hepatic uptake in animals without spleens for the longer period exceeded that by the newly splenectomized rats; the difference was relatively small but significant (Table I). In addition to the spleen and liver, reticuloendothelial tissue of the bone marrow participated to a small extent in the trapping of abnormal red cells. Such bone marrow activity was also found to be regulated in relation to the activity of the spleen. As seen in Table I, femurs from animals without spleens for 8 weeks trapped nearly twice the number of altered red cells as did those from animals splenectomized 2 days before sacrifice.

DISCUSSION

Effect of "work load" on reticuloendothelial growth and function. These studies suggest that the size of the reticuloendothelial system and its functional capacity with respect to cell sequestration are governed by the work required of it. The finding that transplanted splenic tissue is stimulated to greater size after splenectomy supports this suggestion and confirms the observation of Marine and Manley (25). In our studies, this stimulation was associated with an increase in transplant sequestering function and was further intensified by the added imposition of a hemolytic process. Although not completely excluded by these studies, it seems unlikely that humoral factors are involved in this stimulation. Thus, viable spleen autotransplants, which normally grow well in the peritoneal cavity of rats (38, 39), did not appreciably increase in size, even in splenectomized animals, when isolated from circulating particulate matter in diffusion chambers. Furthermore, the presence of hyperplastic parent spleens produced by injections of β-acetylphenylhydrazine did not inhibit transplant growth; indeed, such transplants were larger than those of uninjected animals. Enlargement of spleens and spleen transplants in these studies was due to an increase in their number of cells as evidenced by: *1*) the general cellular hyperplasia seen histologically and *2*) the close parallelism between function (as measured by blood-cell sequestration) and size. A possibility not excluded by the present studies is that much of the growth increment in spleen transplants reflects an immigration of cells from other sites, a mechanism that would account for a failure of "growth" in diffusion chambers. Presumably the sources of such a possible cellular influx would be nonsplenic, since existing spleen tissue inhibits rather than abets spleen transplant growth.

Although in these experiments splenic hyperplasia was induced by sequestration of β-acetylphenylhydrazine-injured red cells, other kinds of particles presumably may also stimulate spleen growth and hyperfunction. For example, splenomegaly with some features of "hypersplenism" has been described in lipidoses such as Gaucher's disease (20, 40), in clinical (41) as well as experimental (42) hyperlipemia, and after the experimental injection of macromolecules such as bacterial endotoxins (43), gelatin (17), shellac (44), zymosan (21), polyvinylpyrrolidone (45), and methylcellulose (18, 19). The view that par-

ticulate matter may stimulate the growth of reticuloendothelial cells by purely physicochemical rather than immunologic mechanisms is consistent with the deductions of Bílek (45) and of Thorbecke and Benacerraf (46). Regardless of mechanism, the consequence of this stimulation in patients with certain chronic hematologic disorders is that a vicious cycle may ensue. Thus, red-cell sequestration would stimulate reticuloendothelial hyperplasia, which in turn would exaggerate further sequestration. Indeed, in many such cases, interruption of this cycle by removal of the progressively enlarging spleen has been of marked clinical benefit (10, 14, 20, 47, 48). It may be noted, however, that such a cyclic process, while often dangerous, appears to be self-limited. Thus, splenic growth secondary to hemolytic processes tends to level off, which is presumably the reason that spleen enlargement of itself rarely produces marked anemia (20). This in turn may indicate that the capacity of the spleen to increase its rate of cell generation has a limit, as is the case with the bone marrow (49). This limit may be determined by the supply of stem cells.

The behavior of experimentally produced spleen transplants in these studies is analogous to that of the splenules in the clinical entity splenosis. This condition, manifested by a disseminated growth of splenules within the peritoneal cavity, is associated almost exclusively with previous splenectomy (50). Although splenic trauma before or during surgery originally seeds this tissue, it would appear that its growth is stimulated in a manner analogous to that of spleen transplants. Indeed, one or more unusually large and probably functional splenules have been reported (51, 52) in the peritoneal cavities of two patients with hereditary spherocytosis who relapsed after initially beneficial splenectomies. The gradual emergence postoperatively of "accessory spleens" may often reflect such a process.

Reticuloendothelial tissue, when stimulated as in these studies to become hyperplastic, was not discriminating in its hyperfunction. Overgrowth of the spleen, induced by its sequestration of abnormal red cells, significantly depressed the levels of platelets and leukocytes as well. It seems probable that depression of these elements was due to their heightened sequestration in hyperplastic spleens. This mechanism might explain the thrombocytopenia often seen in chronic hemolytic states (53, 54), and probably accounts for the pancytopenias associated with administration of materials such as methylcellulose (18, 19) and zymosan (21).

Interrelations of the reticuloendothelial activity of different organs. The present studies have demonstrated that the spleen, liver, and to a lesser extent, the bone marrow share the sequestration of effete red cells in such a way that the activity of one organ is affected by that of the others. Thus, the reticuloendothelial activity of the liver and marrow is stimulated by the extra work load imposed on them after splenectomy. This compensatory response occurs gradually. Its development may explain the inappreciable effect of splenectomy on red-cell life-span in otherwise normal individuals and may also, in part, account for late relapses following splenectomy in various hemolytic anemias. That hepatic sequestration of vulnerable red cells does indeed become prominent in such relapses after surgical splenectomy has been documented by radioisotopic techniques (2).

The compensatory response of distant reticuloendothelial cells after splenectomy would seem to be imperfect in many situations, possibly because of an inability to duplicate certain anatomic features peculiar to the spleen. Thus, splenectomy in hereditary spherocytosis leads to permanent remission in virtually all cases, although it is not certain that red-cell survival remains perfectly normal thereafter (55). In addition, the capacity to remove pathogens from the circulation would not appear to have been efficiently transferred to other reticuloendothelial tissues in those patients developing severe infections after splenectomy (56–60), although increased propensity to infection in these patients is denied by some (61, 62). On the other hand, Finland (63) has suggested that increased susceptibility to fulminant infection may be manifest for only a short period after splenectomy. Thus, with bacteria, the compensatory response of the liver may be gradual, as was observed in these studies with effete red cells.

Conversely, a depression of hepatic reticuloendothelial activity was observed in animals with hyperplastic spleens. In rats with β-acetylphenylhydrazine-induced splenomegaly, a diminished

hepatic uptake of labeled, effete red cells and an inhibition of erythrophagocytosis by Kupffer cells was noted. By analogy to these findings, it would seem reasonable to suspect that removal of a hyperplastic spleen in humans might entail a more dangerous reduction of net reticuloendothelial activity, at least temporarily, than removal of a normal spleen. Indeed, removal of enlarged spleens from individuals with underlying hematological disease has been most commonly associated with fulminant infection after splenectomy (58, 63), whereas no increased propensity to infection could be demonstrated in individuals with normal-sized spleens after splenectomy for traumatic rupture (61).

That the growth and function of reticuloendothelial tissues are interdependent is further supported by reports that previous or coincident splenectomy stimulates hepatic regeneration following partial hepatectomy (64, 65). Histologically, this enhancement of growth entails mainly hyperplasia of Kupffer cells, which are observed to be actively erythrophagocytic during this stage of regeneration (64). Conversely, Stern (66) has recently shown that spleens significantly increase their size and uptake of particulate matter following partial hepatectomy; this augmentation continues until full hepatic regeneration has occurred.

Mechanism of work-load stimulation of reticuloendothelial growth and function. It seems reasonable to conclude that reticuloendothelial cells multiply in direct proportion to the quantity of unwanted particles reaching them. The mechanism of this stimulatory response remains obscure. Evidence reviewed recently by Karnovsky (67, 68) establishes that phagocytosis of itself accelerates metabolic processes within leukocytes *in vitro*. By analogy, it may be that a similar stimulation of metabolism in reticuloendothelial cells *in vivo* might provide the energy for their mitoses. In this regard, it is of interest that following a short "blockade" period, a single injection of particulate matter into animals increases their "phagocytic mass," at least as measured by the subsequent increased removal rate of circulating particles (69, 70). This rebound phenomenon has been shown by Kelly and her co-workers (71, 72), using various organic particles, to be associated with an increased rate of cellular division in the littoral cells of the liver. It is difficult in such studies to exclude the possibility that immunologic mechanisms are responsible for the cellular proliferation observed. Nevertheless, the proliferative response of Kupffer cells to foreign particles is similar to the splenic response to the sequestration of autologous blood cells in these studies.

Accordingly, it is postulated that abnormal cells or particles on contact with reticulum cells stimulate a biochemical response that engenders mitosis. Such stimulation to cellular division is reminiscent of that occurring in parthenogenesis whereby physical, chemical, or pathological (73) wounding induces cell division.

Cellular proliferation induced by such agents, including effete red cells, may be essentially reparative in nature and involve a nonspecific response to injury, rather than being a specific response to humoral or immunologic stimulation. Recent studies (74) indicate that the splenic response to hemolysis involves the generation of all cellular elements and more nearly resembles wound healing than it does a response to a specific regulator. In any case, the result of this stimulation, when applied to reticuloendothelial tissue, is that the population of active phagocytes is temporarily and usefully increased. When such stimulation is excessive, marked reticuloendothelial hyperplasia ensues with possible pathologic sequelae.

SUMMARY

Studies were made in rats of the growth, histology, and function of reticuloendothelial tissue. It was shown that the growth and function of spleens, livers, and spleen autotransplants, as measured by their size and by their capacity to sequester Cr^{51}-labeled altered red cells, were regulated in proportion to the "work" required of them. Thus, spleen autotransplants, in animals lacking other spleen tissue, grew larger and functioned more actively than did such transplants in animals possessing spleens. Increasing the "work load" by imposing a hemolytic process produced a hyperplastic response both in spleens and in spleen transplants. When spleen tissue *in vivo* was isolated from particulate matter of the circulation by transplantation within diffusion chambers, it remained viable, but did not grow

or detectably function despite the stimulus of hemolysis. Hyperplasia of splenic tissue induced by chronic hemolysis depressed the levels of all blood cell elements, probably through increased sequestration.

The induction of hyperplasia in the spleen inhibited reticuloendothelial function in the liver. Conversely, splenectomy led to a compensatory increase in hepatic and marrow sequestering function.

These findings seem best explained by the following proposals: *1*) particulate matter directly and locally stimulates the division of reticuloendothelial cells and *2*) the co-ordinated homeostatic regulation of the various reticuloendothelial organs is governed simply by the total particulate work load.

Evidence is discussed suggesting that the proliferative response of reticuloendothelial cells to particulate matter is basically reparative. It is postulated that the energy for cell division is derived from the biochemical excitation that attends phagocytosis.

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REFERENCES

1. Jandl, J. H. Sequestration by the spleen of red cells sensitized with incomplete antibody and with metallo-protein complexes (abstract). J. clin. Invest. 1955, **34**, 912.
2. Jandl, J. H., A. R. Jones, and W. B. Castle. The destruction of red cells by antibodies in man. I. Observations on the sequestration and lysis of red cells altered by immune mechanisms. J. clin. Invest. 1957, **36**, 1428.
3. Jandl, J. H., and R. L. Simmons. The agglutination and sensitization of red cells by metallic cations: interactions between multivalent metals and the red-cell membrane. Brit. J. Haemat. 1957, **3**, 19.
4. Jandl, J. H., and A. S. Tomlinson. The destruction of red cells by antibodies in man. II. Pyrogenic, leukocytic and dermal responses to immune hemolysis. J. clin. Invest. 1958, **37**, 1202.
5. Harris, I. M., J. M. McAllister, and T. A. J. Prankerd. The relationship of abnormal red cells to the normal spleen. Clin. Sci. 1957, **16**, 223.
6. Benacerraf, B., G. Biozzi, B. N. Halpern, and C. Stiffel. Physiology of phagocytosis of particles by the R. E. S. *in* Physiopathology of the Reticuloendothelial System, B. N. Halpern, B. Benacerraf, and J. F. Delafresnaye, Eds. Springfield, Ill., Charles C Thomas, 1957, p. 52.
7. Dobson, E. L. Factors controlling phagocytosis *in* Physiopathology of the Reticulo-endothelial System, B. N. Halpern, B. Benacerraf, and J. F. Delafresnaye, Eds. Springfield, Ill., Charles C Thomas, 1957, p. 80.
8. Young, L. E., R. F. Platzer, D. M. Ervin, and M. J. Izzo. Hereditary spherocytosis. II. Observations on the role of the spleen. Blood 1951, **6**, 1099.
9. Jandl, J. H., M. S. Greenberg, R. H. Yonemoto, and W. B. Castle. Clinical determination of the sites of red cell sequestration in hemolytic anemias. J. clin. Invest. 1956, **35**, 842.
10. Watson, R. J., H. C. Lichtman, and H. D. Shapiro. Splenomegaly in sickle cell anemia. Amer. J. Med. 1956, **20**, 196.
11. Baker, S. J., E. Jacob, K. T. Rajan, and E. W. Gault. Hereditary haemolytic anaemia associated with elliptocytosis: a study of three families. Brit. J. Haemat. 1961, **7**, 210.
12. Jacob, H. S., and J. H. Jandl. Effects of sulfhydryl inhibition on red blood cells. II. Studies *in vivo*. J. clin. Invest. 1962, **41**, 1514.
13. Jandl, J. H., and M. E. Kaplan. The destruction of red cells by antibodies in man. III. Quantitative factors influencing the patterns of hemolysis *in vivo*. J. clin. Invest. 1960, **39**, 1145.
14. Smith, C. H., I. Schulman, R. E. Ando, and G. Stern. Studies in Mediterranean (Cooley's) anemia. I. Clinical and hematologic aspects of splenectomy, with special reference to fetal hemoglobin synthesis. Blood 1955, **10**, 582.
15. Jandl, J. H., H. S. Jacob, and G. A. Daland. Hypersplenism due to infection: a study of five cases manifesting hemolytic anemia. New Engl. J. Med. 1961, **264**, 1063.
16. Hueper, W. C. Macromolecular substances as pathogenic agents. Arch. Path. 1942, **33**, 267.
17. Hueper, W. C. Experimental studies in cardiovascular pathology. V. Effects of intravenous injections of solutions of gum arabic, egg albumin and gelatin upon the blood and organs of dogs and rabbits. Amer. J. Path. 1942, **18**, 895.
18. Hueper, W. C. Reactions of the blood and organs of dogs after intravenous injections of solutions of methyl celluloses of graded molecular weights. Amer. J. Path. 1944, **20**, 737.
19. Palmer, J. G., E. J. Eichwald, G. E. Cartwright, and M. M. Wintrobe. The experimental production of splenomegaly, anemia, and leucopenia in albino rats. Blood 1953, **8**, 72.
20. Motulsky, A. G., F. Casserd, E. R. Giblett, G. O. Broun, Jr., and C. A. Finch. Anemia and the spleen. New Engl. J. Med. 1958, **259**, 1164 and 1215.
21. Gorstein, F., and B. Benacerraf. Hyperactivity of the reticuloendothelial system and experimental anemia in mice. Amer. J. Path. 1960, **37**, 569.

22. De Langen, C. D. Function of the spleen and blood. Acta med. scand. 1943, **115**, 271.
23. Crosby, W. H. Normal functions of the spleen relative to red blood cells: a review. Blood 1959, **14**, 399.
24. Manley, O. T., and D. Marine. The transplantation of splenic tissue into the subcutaneous fascia of the abdomen in rabbits. J. exp. Med. 1917, **25**, 619.
25. Marine, D., and O. T. Manley. Homeotransplantation and autotransplantation of the spleen in rabbits. III. Further data on growth, permanence, effect of age, and partial or complete removal of the spleen. J. exp. Med. 1920, **32**, 113.
26. Perla, D., and J. Marmorston-Gottesman. Studies on Bartonella muris anemia of albino rats. III. The protective effect of autoplastic splenic transplants on the Bartonella muris anemia of splenectomized rats. J. exp. Med. 1930, **52**, 131.
27. Palmer, J. G., I. Kemp, G. E. Cartwright, and M. M. Wintrobe. Studies on the effect of splenectomy on the total leukocyte count in the albino rat. Blood 1951, **6**, 3.
28. Jandl, J. H. Sequestration of sensitized red cells by splenic autotransplants. Clin. Res. 1960, **8**, 210.
29. Jacob, H. S., R. A. MacDonald, and J. H. Jandl. The regulation of spleen growth and sequestering function. J. clin. Invest. 1962, **41**, 1367.
30. Eagle, H. The growth requirements of two mammalian cell lines in tissue culture. Trans. Ass. Amer. Phycns 1955, **68**, 78.
31. Jacob, H. S., and J. H. Jandl. Effects of sulfhydryl inhibition on red blood cells. I. Mechanism of hemolysis. J. clin. Invest. 1962, **41**, 779.
32. Jandl, J. H. The agglutination and sequestration of immature red cells. J. Lab. clin. Med. 1960, **55**, 663.
33. Ham, T. H. A Syllabus of Laboratory Examinations in Clinical Diagnosis. Critical Evaluation of Laboratory Proceedures in the Study of the Patient, revised edition, L. B. Page and P. J. Culver, Eds. Cambridge, Mass., Harvard University Press, 1960.
34. Pohle, F. J. The blood platelet count in relation to the menstrual cycle in normal women. Amer. J. med. Sci. 1939, **197**, 40.
35. Perla, D. The regeneration of autoplastic splenic transplants. Amer. J. Path. 1936, **12**, 665.
36. Calder, R. M. Autoplastic spleen grafts: their use in the study of the growth of splenic tissue. J. Path. Bact. 1939, **49**, 351.
37. Williams, R. G. The microscopic structure and behavior of spleen autografts in rabbits. Amer. J. Anat. 1950, **87**, 459.
38. Cameron, G. R., and K. S. Rhee. Compensatory hypertrophy of the spleen: a study of splenic growth. J. Path. Bact. 1959, **78**, 335.
39. Crosby, W. H., and N. R. Benjamin. Frozen spleen reimplanted and challenged with Bartonella. Amer. J. Path. 1961, **39**, 119.
40. Mandelbaum, H., L. Berger, and M. Lederer. Gaucher's disease. I. A case with hemolytic anemia and marked thrombopenia: improvement after removal of spleen weighing 6822 grams. II. Lipid analysis of the Gaucher's spleen. Ann. intern. Med. 1942, **16**, 438.
41. Berk, M. Secondary hypersplenism with recurrent gastrointestinal bleeding. Calif. Med. 1953, **78**, 518.
42. Stuart, A. E., G. Biozzi, C. Stiffel, B. N. Halpern, and D. Mouton. The stimulation and depression of reticulo-endothelial phagocytic function by simple lipids. Brit. J. exp. Path. 1960, **41**, 599.
43. Ho, M., and E. H. Kass. Hemolytic anemia in rabbits following injection of bacterial endotoxin. Proc. Soc. exp. Biol. (N. Y.) 1958, **97**, 505.
44. Shen, S. C. Unpublished studies.
45. Bílek, O. Experimental production of proliferative lesions of the reticulo-histiocytic system in rats (so-called experimental reticulosis). II. Proliferation of the RHS in rats after polyvinylpyrrolidone. Vnitřni lékařství 1961, **7**, 1324.
46. Thorbecke, G. J., and B. Benacerraf. The reticuloendothelial system and immunological phenomena. Progr. Allergy 1962, **6**, 559.
47. Doan, C. A. Hypersplenism. Bull. N. Y. Acad. Med. 1949, **25**, 625.
48. Sprague, C. C., and J. C. S. Paterson. Role of the spleen and effect of splenectomy in sickle cell disease. Blood 1958, **13**, 569.
49. Crosby, W. H. The metabolism of hemoglobin and bile pigment in hemolytic disease. Amer. J. Med. 1955, **18**, 112.
50. Cotlar, A. M., and E. J. Cerise. Splenosis: the autotransplantation of splenic tissue following injury to the spleen. Report of two cases and review of the literature. Ann. Surg. 1959, **149**, 402.
51. Stobie, G. H. Splenosis. Canad. med. Ass. J. 1947, **56**, 374.
52. Mackenzie, F. A. F., D. H. Elliot, H. H. G. Eastcott, N. C. Hughes-Jones, P. Barkhan, and P. L. Mollison. Relapse in hereditary spherocytosis with proven splenunculus. Lancet 1962, **1**, 1102.
53. Wintrobe, M. M. Clinical Hematology, 5th ed. Philadelphia, Lea and Febiger, 1961, p. 650.
54. River, G. L., A. B. Robbins, and S. O. Schwartz. S-C hemoglobin: a clinical study. Blood 1961, **18**, 385.
55. Jandl, J. H. Hereditary spherocytosis *in* The Metabolic Basis of Inherited Disease, J. B. Stanbury, J. B. Wyngaarden, and D. S. Fredrickson, Eds. New York, McGraw-Hill, 1960, p. 1018.
56. King, H., and H. B. Shumacker, Jr. Splenic studies: I. Susceptibility to infection after splenectomy performed in infancy. Ann. Surg. 1952, **136**, 239.
57. Gofstein, R., and S. Gellis. Splenectomy in infancy and childhood: the question of overwhelming infection following operation. J. Dis. Child. 1956, **91**, 566.

58. Smith, C. H., M. Erlandson, I. Schulman, and G. Stern. Hazard of severe infections in splenectomized infants and children. Amer. J. Med. 1957, **22**, 390.
59. Broberger, O., F. Gyulai, and J. Hirschfeldt. Splenectomy in childhood. A clinical and immunological study of forty-two children splenectomized in the years 1951–1958. Acta paediat. (Uppsala) 1960, **49**, 679.
60. Lucas, R. V., Jr., and W. Krivit. Overwhelming infection in children following splenectomy. J. Pediat. 1960, **57**, 185.
61. McKinnon, W. M. P., S. J. Boley, and J. Manpel. Infection in children following splenectomy for traumatic rupture. J. Dis. Child. 1959, **98**, 710.
62. Laski, B., and A. MacMillan. Incidence of infection in children after splenectomy. Pediatrics 1959, **24**, 523.
63. Finland, M. Serious infections in splenectomized children. Pediatrics 1961, **27**, 689.
64. Higgins, G. M., and J. T. Priestley. Experimental pathology of the liver. VI. Restoration of the liver in white rats after partial removal and splenectomy. Arch. Path. 1932, **13**, 573.
65. Perez-Tamayo, R., and R. Romero. Role of the spleen in regeneration of the liver. An experimental study. Lab. Invest. 1958, **7**, 248.
66. Stern, K. Studies on reticuloendothelial function in relation to the growth processes. Ann. N. Y. Acad. Sci. 1960, **88**, 252.
67. Karnovsky, M. L. Metabolic basis of phagocytic activity. Physiol. Rev. 1962, **42**, 143.
68. Karnovsky, M. L. Metabolic shifts in leucocytes during the phagocytic event *in* Biological Activity of the Leucocyte, Ciba Foundation Study Group No. 10, G. E. W. Wolstenholme and M. O'Connor, Eds. Boston, Little, Brown, 1961, p. 60.
69. Biozzi, G., B. Benacerraf, and B. N. Halpern. The effect of Salm. typhi and its endotoxin on the phagocytic activity of the reticulo-endothelial system in mice. Brit. J. exp. Path. 1955, **36**, 226.
70. Benacerraf, B., and M. M. Sebestyen. Effect of bacterial endotoxins on the reticuloendothelial system. Fed. Proc. 1957, **16**, 860.
71. Kelly, L. S., E. L. Dobson, C. R. Finney, and J. D. Hirsch. Proliferation of the reticuloendothelial system in the liver. Amer. J. Physiol. 1960, **198**, 1134.
72. Kelly, L. S., B. A. Brown, and E. L. Dobson. Cell division and phagocytic activity in liver reticuloendothelial cells. Proc. Soc. exp. Biol. (N. Y.) 1962, **110**, 555.
73. Stolk, A. Pathological parthenogenesis in viviparous toothcarps. Nature (Lond.) 1958, **181**, 1660.
74. Jandl, J. H., H. S. Jacob, and R. A. MacDonald. Reticuloendothelial proliferation stimulated by injured, autologous red cells. Proc. 3rd int. Symposium on Immunopathology. Basel, Benno Schwabe, in press.