FROZEN SPLEEN REIMPLANTED AND CHALLENGED WITH BARTONELLA

WILLIAM H. CROSBY, COLONEL, MC, AND NAOMI R. BENJAMIN, B.S.

From the Department of Hematology, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, D.C.

Following splenectomy in many mammals, bits of splenic tissue or the entire spleen may be reimplanted in the animal's body. Here they undergo almost complete necrosis and then are reconstituted to form splenculi with a microscopic structure almost indistinguishable from that of the original organ.[1,2] After it had been learned that these excised bits of spleen could be frozen and thawed without destroying the ability to become reconstituted,[3] it was desired to pursue the problem further, to learn if the reconstituted spleens possessed any of the functions peculiar to the spleen.

It is known that animals which are susceptible to infection by *Haemobartonella muris* do not usually develop clinical evidence of the disease when the spleen is present.[4] After splenectomy the organism produces a severe hemolytic anemia characterized by the presence of the organism in the circulating red cells. The hemolytic process is acute, severe, and the animals often die. It was the purpose of this experiment to ascertain whether implants which had been frozen and thawed would react to the *H. muris* infection in the same manner as the intact spleen.

MATERIAL AND METHODS

The experiment employed 3 litters of white rats of the WRCF strain. At the age of 2 weeks (weight about 20 gm.) the members of 2 litters had splenectomies, and the spleens were reimplanted. The third litter did not have splenectomy. Splenectomy was performed through a left abdominal incision under ether anesthesia. The wound was closed with silk. After its removal, the spleen was cut into several pieces which were dropped into the peritoneal cavity through a stab wound in the right abdominal wall. Before implantation, some of the splenic fragments were frozen; some were not. Those specimens to be frozen were first placed in a test tube and incubated for 40 minutes in 10 ml. of a mixture of 30 per cent glycerol and 70 per cent normal saline. To freeze the tissue, the test tube (11 by 150 mm.) was placed in acetone and dry ice and left there at least 10 minutes, when it was placed in water at 37° C. The thawed fragments were immediately placed in the peritoneum. The nonfrozen tissue was also incubated in the glycerol solution at room temperature. All implantations were autologous.

Four months later all of the animals (weight 300 to 350 gm.) were inoculated with *H. muris* by intraperitoneal injection of 0.5 ml. of a 2 per cent suspension of red cells from a splenectomized rat known to be infected and with a high rate of visible parasitization of his red cells. Thereafter, blood smears of the inoculated animals were

Accepted for publication, March 1, 1961.

examined at intervals until the animals died. Each animal was examined at necropsy, with particular attention to the site of splenectomy and the site of implantation. The urinary bladder was opened for evidence of hemoglobinuria; black urine indicated the presence of the hemolytic anemia which occurs in bartonellosis. At the time of inoculation a group of control rats of the same strain and of comparable weight and age with intact spleens were also given injections of the infectious material.

Results

Twenty-one rats had splenectomy, and the spleens were reimplanted (Table I). The implanted spleen tissue survived and reconstituted itself in 14 rats. In 5 animals no splenic tissue was found at necropsy. In 15 animals the spleen had been frozen and thawed before reimplantation;

Table I

RESULTS OF INTRAPERITONEAL IMPLANTATION OF SPLENIC TISSUE *

Rat no.		Frozen or not	Death after inoculation (days)	Site of implant	Splenic bed
Litter 12	98	F	5		+
	99	F	5	Abdominal wall	
	100	F	5		
	101	F	5	Intestine	
	102	F	8	Pancreas	
	103	F	8	Omentum	
	104	N	5	Omentum	
	105	N	6	Abdominal wall	
Litter 13	106	N	5	Subcutaneous tissue	
	107	N	5	Mesentery	
	108	N	5	Stomach	
	109	N	5	Abdominal wall	
	110	F	5		+
	111	F	5		
	112	F	5	Mesentery	+
	113	F	6		
	114	F	5	Mesentery	
	115	F	0		
	116	F	5		
	117	F	8	Colon	
	118	F	5	Intestine	

* Implantations were made 4 months earlier. Some implants had been frozen, others not. All animals (except no. 115) died of bartonella anemia.

in 8 of the 15 the implanted spleen survived. In 6 of the animals the spleen had not been frozen; in all of these the spleen survived. Splenculi were found in the splenic bed in 3 rats. These splenculi could have represented remnants of the original spleen broken off during the operation, or accessory spleens, or the implant which migrated from the site of implantation before becoming fixed. In only one of these 3 animals was there a splenculus at the site of implantation in addition to the one in the splenic bed.

Following inoculation with *H. muris*, the red cells in blood smears contained no inclusions until the fourth or fifth day. The 11 control animals without splenectomy also developed inclusion bodies on the fifth day, and one of them died on that day. The rest were re-examined on the eighth day and no inclusion bodies were found. After 3 months these animals were still surviving and in evident good health. After the appearance of red-cell inclusion bodies, the splenectomized rats usually died within a day, but several of them survived for 3 days. Death was associated with evidence of hemolytic disease, extreme pallor of tissues and hemoglobinuria manifest in the bladder at necropsy. The splenculi were usually small, 5 to 10 mm., and often lobulated or nodular. None was on a pedicle. The blood supply evidently was provided by multiple small vessels (Fig. 1). The implanted spleens were found embedded on various abdominal organs: stomach, intestine, pancreas, omentum, liver and parietal muscles (Table I).

The microscopic anatomy of the reconstituted spleens closely resembled that of the normal rat spleen, with cords of red pulp between the malpighian corpuscles. Characteristic splenic sinusoids and central arteries were also present, but there appeared to be no trabecular structure. In the animals dead with bartonellosis the red pulp was preponderant and filled with phagocytic cells containing innumerable erythrocytes. The sinusoids were inconspicuous. Except for the trabeculae and the thinness of its capsule, the spleen in the control rat without splenectomy that died with bartonellosis was indistinguishable from the splenculi in the splenectomized animals (Figs. 2 to 4).

In some sections splenic tissue appeared to be growing beyond the splencular capsule and extending into structures upon which the splenculus had implanted itself (Figs. 5 and 6). Several of the rats were pregnant at death, and in two cases the blood of the pups almost at term was examined. In one instance the mother died on the fifth day, and the pups' blood was negative for inclusion bodies; in the other case the mother died on the eighth day, and the pups' blood contained inclusions.

Discussion

Autologous splenic tissue which had been frozen, thawed, and replaced in the peritoneal cavity of rats was viable and capable of implanting and reconstituting itself. It was impossible to distinguish between successful implants which had been frozen and those which had not. They differed from the intact spleen in that the trabecular structure was not apparent and the blood supply was received, not through a hilar ramification but through numerous vessels at the periphery of the implant. The appearance of some implants suggested that they were growing, the splenic

tissue penetrating its capsule and extending itself into structures such as muscle or omentum, upon which engraftment had occurred. It is suspected that these excrescences become nodules and that this pattern of growth could account for the nodular appearance of some of the implants.

In these experiments the splenic tissue was dropped loose into the peritoneal cavity, and the successful implants attached themselves to the peritoneal organs. They were found on the omentum, the mesentery, the pancreas, the liver, the intestine and the abdominal wall. They had penetrated the peritoneum, received blood supply and had become covered by serosa (Figs. 7 and 8). The implants were not blandly symbiotic at the site of attachment but were capable of causing some degree of local accommodation (Fig. 5). In this regard they resembled endometrial implants.

The animals were inoculated with *H. muris* to determine whether the implants were capable of functioning as splenic tissue. In at least one clinical case, splenic implants demonstrated splenic function.[5] During splenectomy for hereditary spherocytosis, a woman's spleen was ruptured and splenic pulp spilled into her abdomen. Immediately after the operation her hemolytic disorder was relieved but later recurred, and abdominal exploration revealed many splenculi implanted in the peritoneal cavity. The implants had resumed the splenic function of destroying prematurely the spherocytic red cells.

In our rats a comparison of intact spleen with frozen-thawed splenic implants, after death of the animals from bartonella anemia, demonstrated a similarity of splenic reaction (Figs. 2 and 3). The pattern of intense erythrophagocytosis indicated that the implanted spleen had reacted to the challenge of Bartonella infection in the same fashion as the intact spleen.

The reaction of the implants and of the host to infection with *H. muris* was surprising. Years ago this sort of experiment was performed by Perla and Marmorston-Gottesman,[6] under somewhat different circumstances and with definitely different results. Their rats were of the Wistar strain, known to be infected with *H. muris*. Splenectomy resulted in a severe, often lethal hemolytic reaction. In one series of experiments these investigators performed a partial splenectomy, cutting off a tip of the spleen and implanting it under the abdominal skin. When, after 4 weeks, the remainder of the spleen was removed, the animals died of Bartonella anemia, but if the second stage of splenectomy was delayed until 7 weeks, the animals survived. The splenic transplant was sufficient to protect them against the disease.

The failure of the implants to protect our rats directed attention to

the several differences between our experiment and that of Perla and Marmorston-Gottesman. (1) The WRCF strain of rats is derived from the Wistar strain, but has been free of *H. muris* infection for many generations. During this time "racial tolerance" to the disease may have disappeared. All of our control animals with intact spleens developed bartonellosis and one of them died; one pregnant rat transmitted the infection to her pups with intact spleens *in utero*. (2) If antibodies against *H. muris* are a part of resistance to the disease (and this point is not clear [4]), our rats were without the benefit of the chronic subclinical infection which was present in Perla's rats. (3) Strains of the *H. muris* organism may vary in virulence. (4) Our inoculum may have been excessive.

It is not possible to say why the splenic implants did not provide protection against Bartonella anemia. It is, however, significant that the implants reacted to the disease in the same manner as the intact spleen of the one animal that died. The defense mechanism was overwhelmed, but the splenic tissue reacted with intense erythrophagocytosis.

Summary

1. In a group of rats, autogenous splenic tissue which had been frozen, thawed, and replaced in the peritoneal cavity remained capable of implanting itself beneath the serosa. The reconstituted splenculi had a microscopic structure closely resembling that of a normal spleen.

2. Splenectomized rats with implanted splenculi were inoculated with *Haemobartonella muris*, and all promptly died of Bartonella anemia. In a control group of animals with intact spleens, all but one survived the infection.

3. The reimplanted spleens reacted to the hemolytic challenge in the same manner as did the intact spleen in one control rat that died. There was engorgement of the red pulp and intense erythrophagocytosis.

4. Some of the implanted spleens appeared to be growing through the capsule and extending splenic tissue into adjacent structures.

References

1. Marine, D., and Manley, O. T. 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. Exper. Med.*, 1920, **32**, 113–133.

2. Perla, D. The regeneration of autoplastic splenic transplants. *Am. J. Path.*, 1936, **12**, 665–676.

3. Rothberg, H., and Crosby, W. H. Preservation of splenic tissue at low temperature. (Abstract) *Clin. Res.*, 1957, **5**, 152.

4. Weinman, D. Infectious anemias due to Bartonella and related red cell parasites (review). *Tr. Am. Philos. Soc.*, 1944, **33**, 243–350.

5. STOBIE, G. H. Splenosis. *Canad. M.A.J.*, 1947, 56, 374–377.
6. PERLA, D., and MARMORSTON-GOTTESMAN, J. 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. Exper. Med.*, 1930, 52, 131–143.

LEGENDS FOR FIGURES

Photomicrographs were prepared from sections stained with hematoxylin and eosin.

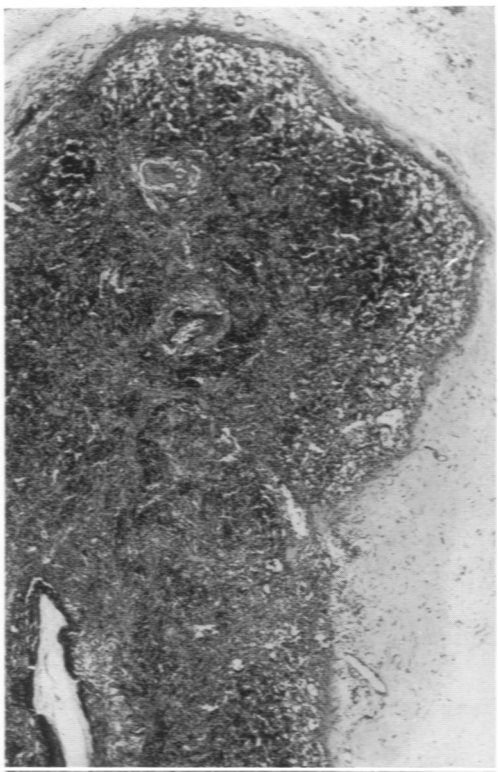
FIG. 1. Rat 10. Frozen-thawed splenic implant, 4 weeks after implantation. Centrally and at bottom left are necrotic remnants of the implanted tissue. The newly reconstituted areas demonstrate characteristic splenic architecture except for the blood supply provided by numerous small vessels which can be seen in the surrounding fibrous tissue. × 25.

FIG. 2. Control rat 1. The intact spleen of a normal rat that died of Bartonella anemia. The malpighian corpuscle stands in dark contrast against the pale, congested red pulp. On the left, perpendicular to the surface, is a splenic trabecula. × 86.

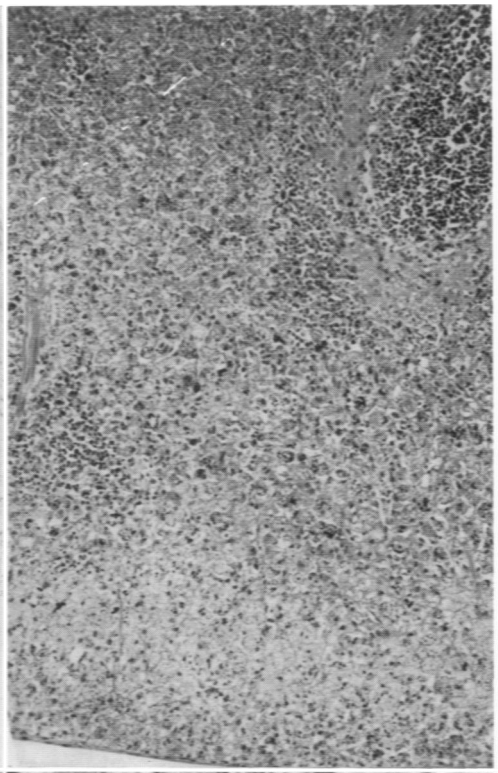
FIG. 3. Rat 114. Implant of frozen splenic tissue in a rat fatally infected with *H. muris*. A similarity to the intact spleen (Fig. 2) is apparent. The sinusoids are easily seen, but there is no trabecular structure manifest. × 86.

FIG. 4. Rat 114. Detail of the red pulp in a splenic implant, showing the reaction to Bartonella. Phagocytic cells packed with erythrocytes have been mistaken for sinusoids.[6] A sinusoid cut longitudinally is in the center of this illustration. × 173.

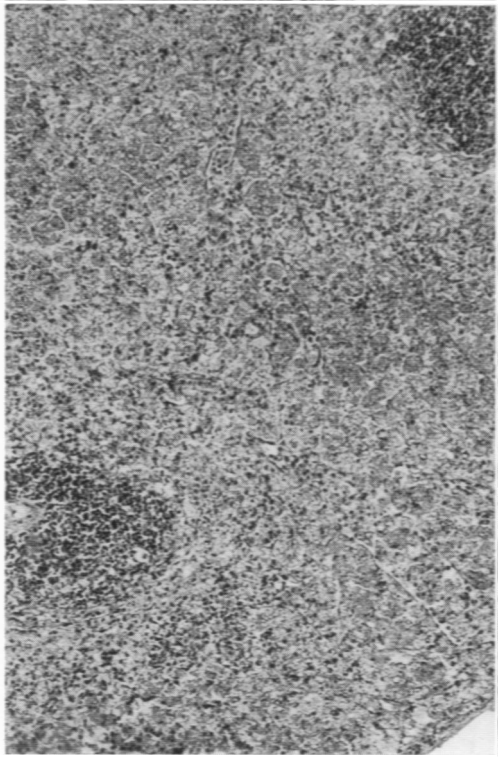
All photomicrographs were made by Miss Sally Craig, Medical Audiovisual Department, Walter Reed Army Institute of Research.



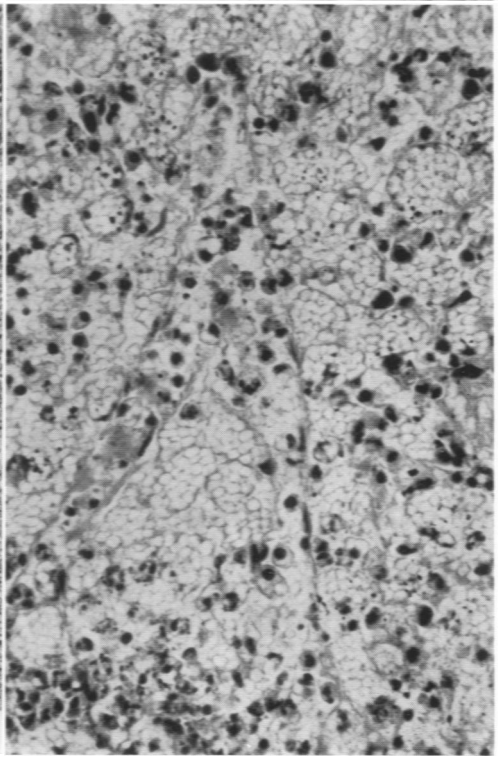
1



2



3



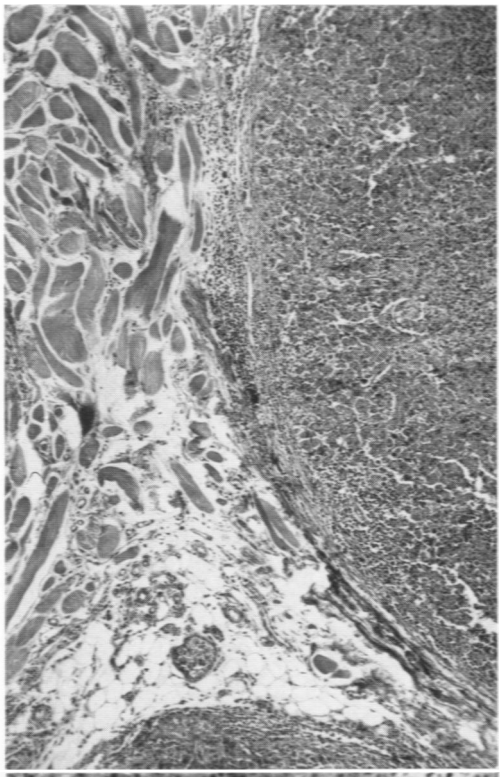
4

Fig. 5. Rat 109. A splenic implant showing its attachment to abdominal wall muscle. Muscle fibers can be seen between the two splenic lobules. The capsule of the spleen is infiltrated with small cells which appear also between muscle fibers adjacent to the spleen. × 45.

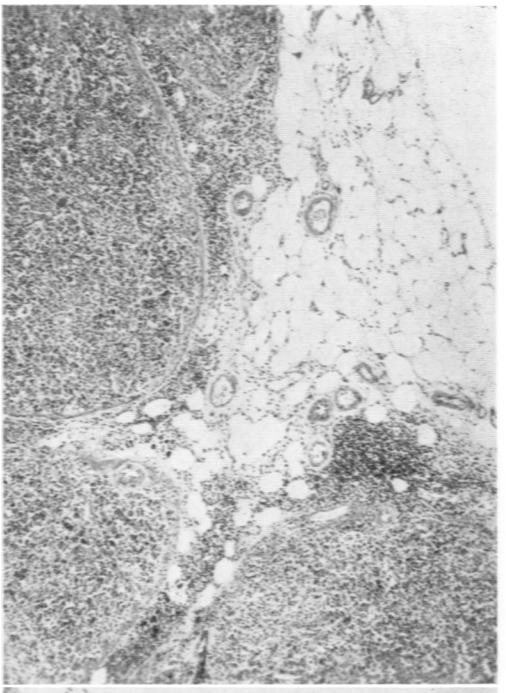
FIG. 6. Rat 117. A frozen implant showing a pseudo-hilus formed by 4 nodules. The splenic capsule at the tips of the 2 nodules on the right has all but disappeared. On the lower nodule the infiltrating cells have concentrated in a pattern resembling a malpighian corpuscle. Above, there appears to be some red pulp outside the infiltrated capsule. This may be the manner by which small splenic implants become larger and nodular. × 52.

FIG. 7. Rat 101. A splenic implant on the small intestine. The serosa is reflected over the implant. × 86.

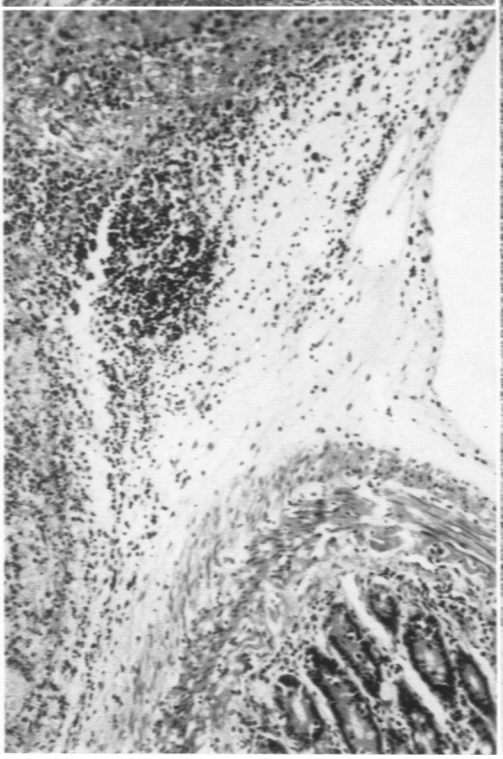
FIG. 8. Rat 117. A splenic implant on the surface of the colon. The implant was evidently embedded in the omentum. × 45.



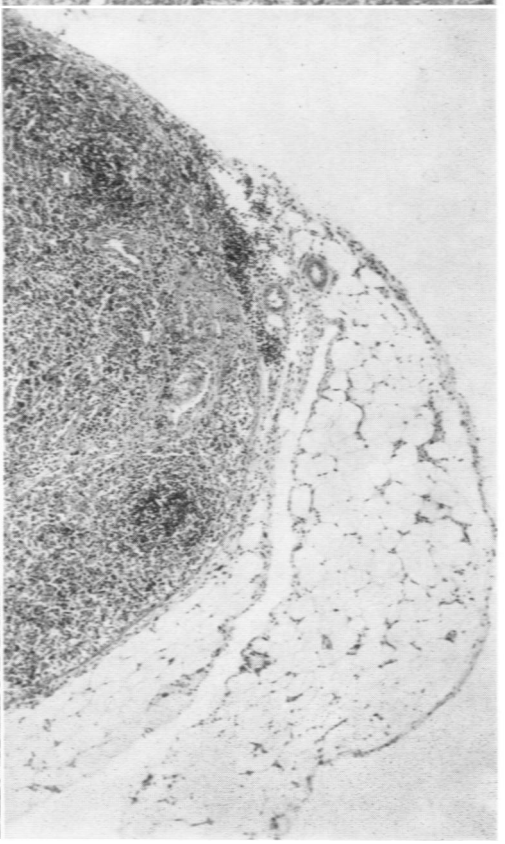
5



6



7



8