The Fate of Transplanted Pancreatic Islets in the Rat

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Syngeneic pancreatic islets transplanted into the liver or the spleen reverse streptozotocin-induced diabetes in the rat, but allogeneic islets function only briefly and are rejected. Shortly after transplantation, thrombi often form around transplanted tissue, particularly around nonislet tissue that contaminates islet preparations. These thrombi are a source of transient liver injury in recipients of intrahepatic grafts. A few days after transplantation, syngeneic islets injected into the portal vein are found at the periphery of portal tracts in direct contact with periportal hepatocytes, some of which become hypertrophied. Isografts remain situated in the portal tracts for prolonged periods without adverse effect on the surrounding liver. In contrast, allogeneic islets injected into the portal vein are infiltrated by small lymphocytes within 2 days of transplantation and are rapidly destroyed by the host. Syngeneic islets injected into the splenic pulp localize in the sinusoids and, 1 month or more after transplantation, are often surrounded by connective tissue or local collections of hemosiderin-laden macrophages. Allogeneic islets injected into the spleen are rejected with the same intensity and at approximately the same rate as allogeneic islets injected into the portal vein. Transplant rejection leaves no significant lasting morphologic effect on the host liver or spleen. (Am J Pathol 94:85–96, 1979)

DESPITE THE AVAILABILITY of insulin since 1922, diabetic patients continue to have high rates of vascular, neural, and obstetric complications with correspondingly high rates of disability and mortality.[1] Because of these ongoing problems, new approaches to diabetic therapy have been sought. In 1972, Ballinger and Lacy[2] reported that transplantation of syngeneic pancreatic islets into the peritoneal cavity of rats partially reversed streptozotocin-induced diabetes. Subsequently, complete remission of drug-induced diabetes was achieved by implantation of syngeneic islets into either the liver or the spleen by many investigators.[3–5] In contrast to results obtained with syngeneic islets, islets transplanted across histocompatibility barriers function only briefly and are quickly rejected by the host. Rapid immunologic destruction occurs in every site where islets have been successfully transplanted as isografts.[6–9]

Although there have been many reports concerning physiologic effects of islet transplants on experimental diabetes, less attention has been given

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to morphologic aspects of islet transplantation. This paper describes sequential changes in the microscopic appearance of pancreatic islet isografts and allografts over both brief and longer periods. Liver and spleen were the implantation sites, and the effect of transplantation on the morphology of these organs is described.

Materials and Methods

Wistar Furth (WF) rats weighing 175 to 225 g were both islet donors and isograft recipients; ACI rats weighing 150 to 175 g served as allograft hosts. Diabetes was induced in transplant recipients by an intravenous injection of streptozotocin (65 mg/kg). This dose produced profound polyuria, polydipsia, and marked hyperglycemia within 24 to 48 hours after administration. Only animals with persistent hyperglycemia (400 mg/dl or greater) were transplanted. Four groups were studied: a) WF to WF intraportal isografts; b) WF to ACI intraportal allografts; c) WF to WF intrasplenic isografts; and d) WF to ACI intrasplenic allografts. WF (Ag-B2) to ACI (Ag-B4) grafts cross a major histocompatibility barrier.

Islets were isolated according to previously described methods.[10] Briefly, after distension with cold Hanks' solution, donor pancreata were minced and then digested with collagenase (Worthington Type IV) for approximately 20 minutes. Islets were then partially purified by discontinuous ficoll gradient centrifugation. Batches of six pancreata processed in this way yielded approximately 1200 islets. When examined by phase contrast microscopy, the islet preparations were found to contain varying amounts of nonislet pancreatic tissue including ductal, acinar, and vascular fragments (Figure 1).

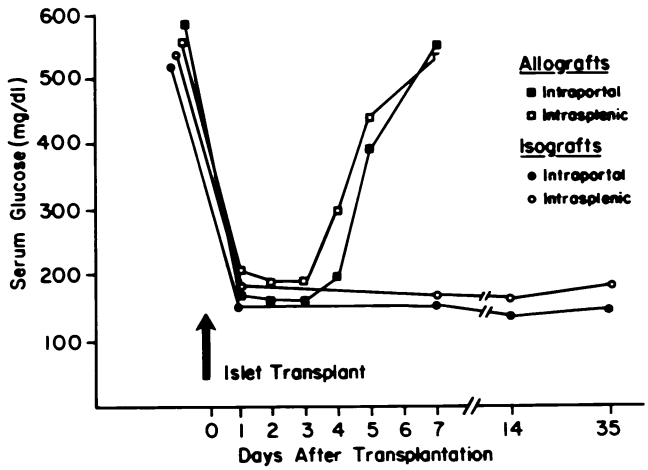
Animals in all groups received between 1200 and 1500 islets, which were injected either into the portal vein or into the splenic pulp. One day after transplantation, serum glucose was less than 200 mg/dl. Thereafter, blood sugar levels were monitored weekly in isograft recipients and daily in allograft hosts until physiologic evidence of rejection was observed. This latter phenomenon was defined as a rise in serum glucose of 100 mg/dl greater than the lowest posttransplant level.

For histologic study, 2 to 3 animals receiving intrahepatic allografts were killed at daily intervals for 1 week after transplantation to observe the earliest manifestation of the rejection reaction. Animals in this group were then killed at weekly intervals for 1 month and at 3 months after transplantation to gauge the subsidence of the rejection reaction and to observe the long-term effects of transplant rejection on liver morphology. Intraportal isograft recipients and intrasplenic transplant recipients were killed at analogous intervals. In addition, five intraportal isograft recipients were killed 7 to 9 months after transplantation. Tissue from these animals was examined to determine the long-term effects of the presence of pancreatic tissue in the liver and the spleen. Tissues from the implantation sites of sacrificed animals were fixed in 10% buffered formalin, embedded in paraffin, sectioned, and subsequently stained with hematoxylin and eosin.

Results

Physiologic Data

Successful islet transplantation produced a marked fall in serum glucose within 24 hours. This was observed in all four groups studied. Recipients of islet isografts remained normoglycemic for long periods. In contrast, functional rejection, indicated by recurrence of hyperglycemia, occurred on the fourth or fifth day after transplantation in both intraportal and intrasplenic islet allograft recipients (Text-figure 1).



TEXT-FIGURE 1—Mean serum glucose values characterizing the response to islet transplantation in the four groups of rats studied. Note that functional rejection manifested by recurrent hyperglycemia occurs by Day 5 in both intraportal and intrasplenic allograft recipients.

Morphologic Data

Immediate Posttransplant Period

Initial morphologic changes were similar in all animals, whether they received syngeneic or allogeneic grafts. In livers of animals receiving either intrahepatic isografts or allografts, islets were found lodged in the lumina of small portal veins 1 day after transplantation (Figure 2). At this time, nonislet tissue in varying quantities was also found in portal veins but in larger branches of these vessels than those in which most of the islets were found (Figure 3). This nonislet tissue was associated with thrombus formation of varying extent. When large thrombi or large amounts of nonislet pancreatic parenchyma were present, necrosis of adjacent hepatocytes was also found. In contrast, uncontaminated islet tissue was often found without associated thrombus formation. One day after transplantation, similar appearing islets and nonislet pancreatic structures were found in the spleens of animals receiving either intrasplenic isografts or allografts. In both cases, islet and nonislet tissues were often surrounded by brightly eosinophilic fibrinous material (Figure 8).

Intraportal Isografts

By Day 4 after transplantation, islet isografts no longer occupied the lumina of portal veins but were situated in the interstitial tissue of the portal tracts in close approximation to periportal hepatocytes. Evidence of periportal necrosis persisted in the form of focal early periportal fibrosis. Small amounts of ductal tissue were found in organizing thrombi in large branches of the portal vein, but no other nonislet pancreatic tissue could be identified in host liver at this time. One to 16 months after trans-

plantation, isografts continued to occupy the same position in the portal tracts, but at this time large hepatocytes were occasionally found adjacent to the islets (Figure 4). No residual evidence of hepatocellular necrosis was present during this interval. Occasional small fibrous nodules containing ductal structures were found in the interstitial tissue of large portal tracts. The location and configuration of the nodules suggested that they represented residual nonislet pancreatic tissue.

Intraportal Allografts

Two days following transplantation, intrahepatic allografts were infiltrated by small lymphocytes (Figure 5). The cells rapidly increased in size and number so that by the time of rejection, 4 to 5 days after transplantation, islets appeared to be compressed by large quantities of mononuclear cells (Figure 6) with relatively abundant cytoplasm and occasional mitotic figures. Shortly after rejection, islet tissue was eliminated from the recipient liver; livers examined 3 days to 1 week after rejection contained no recognizable islet tissue. At this time, however, lymphoid infiltrates were prominent in the portal tracts. These infiltrates gradually subsided, and by 4 weeks after immunologic destruction only small focal mononuclear cell infiltrates and a few fibrous nodules were found in the portal tracts (Figure 7). Three months after rejection, inflammatory changes were absent and only rare fibrous nodules were detectable in the portal tracts. Plasma cells were not observed in the portal tracts at any time during active rejection.

Intrasplenic Isografts

By Day 4 after transplantation, islets were situated in the splenic sinusoids (Figure 9) with little evidence of the fibrinous material which was present around islets examined 1 day after transplantation. One month after transplantation, intact islet tissue remained within splenic sinusoids, often surrounded by a thin lamina of connective tissue (Figure 10). Ductal elements were found in the spleen at this time and were also surrounded by a layer of fibrous tissue. The only discernible morphologic effect of pancreatic tissue on the spleen was the focal presence of small collections of hemosiderin-laden macrophages in the sinusoids adjacent to the transplanted tissue.

Intrasplenic Allografts

Because of the probability that the lymphoid tissue of the spleen would obscure the earliest infiltrates of lymphoid cells responding to the allograft, no attempt was made to detect the initial lymphoid infiltrate of the

rejection reaction. By the time of acute rejection, however, splenic sinusoids contained aggregates of large mononuclear cells in the midst of which ductal tissue could be detected. No islets were seen in the spleen at this time. The mononuclear cells were similar to those found around intraportal allografts at a comparable time. Ductal tissue persisted in the sinusoids for 3 to 4 days after rejection (Figure 11), but by 1 week no pancreatic tissue could be found in the spleen. The only residuum of transplantation at this time was the presence of focal aggregates of large mononuclear cells in the splenic sinusoids (Figure 12). Two weeks after rejection, no morphologic effects of immunologic rejection could be detected.

Discussion

The morphologic changes associated with rejection of transplanted pancreatic islets, their intensity, and the sequence in which they occur are similar regardless of whether the islets are implanted in the liver or in the spleen. The early changes in the livers of intraportal allograft recipients in this study are similar to those recently described by Slater et al [8] and consist of the infiltration of portal tracts by small lymphocytes within 48 hours after transplantation. At the time of acute rejection, both hepatic and splenic sites of implantation are heavily infiltrated by large lymphoid cells. Degenerating islet tissue can be found in the livers of intraportal allograft recipients but is absent from the spleens of intrasplenic allograft recipients. This difference probably does not reflect a significant difference in the rates of rejection at the two sites, since carbohydrate intolerance occurs at approximately the same time in both groups of animals.

Islet rejection does not produce any long-term untoward effects on host organ morphology. In the liver, small aggregates of lymphoid cells can be detected in the portal tracts of some recipients as long as 1 month after rejection and a few fibrous nodules can be detected up to 3 months after rejection. However, these nodules are small and do not appear to have the potential to cause liver malfunction. In the spleen, no residual morphologic effect of islet allotransplantation can be detected 2 weeks after rejection.

Of potential clinical importance is our finding that histologic evidence of islet rejection preceded functional islet rejection by several days. It appears that recurrence of hyperglycemia is coincident with the period of most vigorous cellular infiltration and is a manifestation of irreversible damage. In a clinical setting, parameters of islet function more sensitive than glucose levels may be required to detect early rejection so that antirejection therapy can be administered at the most advantageous time.

Islet isografts appear to have minimal effect on the architecture of the host organ, even after prolonged periods. Hypertrophy of hepatocytes adjacent to isogeneic islets is often observed in the livers of intraportal isograft recipients. The reason for this is not clear. Of possible significance is the work of Griffith et al,[11] who have demonstrated by electron microscopy that long-term islet isografts come into direct contact with hepatocytes and that desmosomes bridging the two types of cells can be found within 24 hours of transplantation. Also pertinent is the finding of Starzl et al [12] that hepatic atrophy develops in dogs after portocaval shunts. Perhaps locally high concentrations of insulin or some other, as yet undefined, hepatotrophic factor is the cause of the observed hypertrophy. Hepatocyte hypertrophy is probably of little significance since liver function has been reported to be normal in long-term intraportal isograft recipients.[13]

Intrasplenic isograft recipients sometimes demonstrated accumulations of hemosiderin-laden macrophages adjacent to the transplanted islets. The origin of these cells is uncertain. Their location suggests that they may have resulted from local obstruction of blood flow in the spleen by transplanted pancreatic tissue or from damage to red cells by pancreatic tissue or its secretion products. In addition, syngeneic islets in the spleen are often surrounded by a thin lamina of connective tissue not present in intraportal grafts. Similar fibrosis around the islets has been described by Feldman et al,[14] who suggested that this fibrosis might be the cause of impaired insulin responsiveness which they observed in their transplanted animals. Our finding of hemosiderin-laden macrophages in the spleen suggests that locally sluggish blood flow in the spleen might also contribute to impaired insulin responsiveness.

These experiments indicate that nonislet pancreatic tissues contaminating islet preparations are thrombogenic and transiently injurious to recipient liver. This thrombogenic effect is suggested by the close approximation of thrombi and necrotic liver tissue to embolized acinar, ductal, and vascular fragments. In contrast, pure islet emboli were frequently found in the portal venules without associated thrombus formation and hepatocyte necrosis. These findings are consistent with the recent report of transient elevations of serum transaminase levels observed 24 to 48 hours after intraportal islet embolization.[15] Our observations suggest that these enzyme changes may be the result of a contamination of islet preparations with pancreatic parenchymal elements. Moreover, by phase contrast microscopy, nonislet tissues were found to be surrounded by basement membrane, but this was inapparent around islet tissue. Basement membranes have been shown to consist largely of collagen [16]; col-

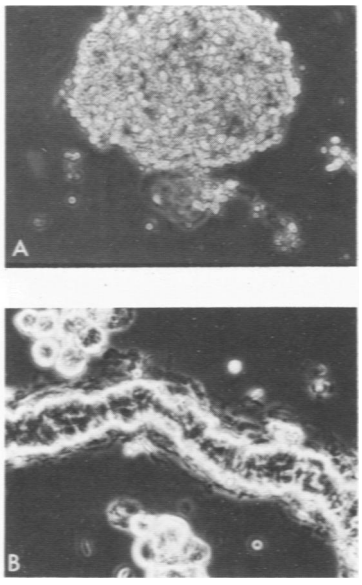
lagenase digestion in the islet isolation procedure may, therefore, have removed the delicate basement membrane surrounding the islets while leaving sturdier nonislet basement membrane substantially intact. Since collagenous tissue may activate the clotting mechanism,[17] it seems likely that exposure of usually extravascular collagenous basement membrane proteins to the intrahepatic bloodstream may be the cause of intraportal thrombosis. Damage to the liver induced by injection of nonislet pancreatic tissue appears to be completely reversible since hepatocellular injury resulting from these thrombotic episodes cannot be detected morphologically 2 weeks after transplantation. Fibrous nodules found in relatively large portal tracts several weeks to months after transplantation probably represent residua of these thrombotic episodes.

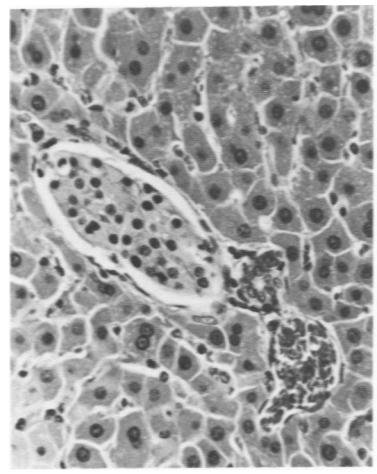
These studies indicate that islet allograft rejection in the immunocompetent host is probably a cell-mediated response that begins soon after transplantation and rapidly destroys the graft. Thrombotic material is observed shortly after transplantation in both recipient liver and recipient spleen; this may be due to contamination of islet preparations with nonislet tissues. Finally, no major adverse effects on host organ morphology are detected in long-term isograft or allograft recipients.

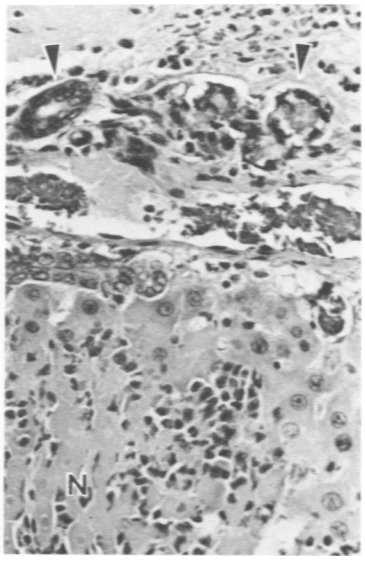
References

1. Knowles HC, Meinert CL, Prout TE: Diabetes mellitus: The overall problem and its impact on the public. Diabetes Mellitus. Edited by SS Fajans. US Department of Health, Education and Welfare Publication No. (NIH) 76–854, pp 11–32
2. Ballinger WF, Lacy PE: Transplantation of intact pancreatic islets in rats. Surgery 72:175–186, 1972
3. Kemp CB, Knight MJ, Scharp DW, Lacy PE, Ballinger WF: Transplantation of isolated pancreatic islets into the portal veins of rats. Nature 244:447, 1973
4. Kemp CB, Knight MJ, Scharp DW, Ballinger WF, Lacy PE: Effect of transplantation site on the results of pancreatic islet isograft in diabetic rats. Diabetologia 9:486–491, 1973
5. Koncz L, Zimmerman CE, DeLellis RA, Davidoff F: Transplantation of pancreatic islets into the spleen of diabetic rats and subsequent splenectomy. Transplantation 21:427–429, 1976
6. Reckard CR, Barker CF: Transplantation of isolated pancreatic islets across strong and weak histocompatibility barriers. Transplant Proc 5:761–763, 1973
7. Reckard CR, Ziegler MM, Barker CF: Physiologic and immunological consequences of transplanting isolated pancreatic islets. Surgery 74:91–99, 1973
8. Slater D, Mangnall Y, Smith C, Fox M: The rejection of the islets of Langerhans following intrahepatic transplantation in the rat. Acta Endocrinol [Suppl] (Kbh) 83(Suppl 205):295–302, 1976
9. Reckard CR, Franklin WA, Schulak JA: Intrasplenic vs. intraportal islet transplants: Quantitative, qualitative and immunologic aspects. Trans Am Soc Artif Intern Organs 24:232, 1978
10. Scharp DW, Kepm CB, Knight MJ, Ballinger WF, Lacy PE: The use of ficoll in the

preparation of viable islets of Langerhans from the rat pancreas. Transplantation 16:686–689, 1973
11. Griffith RC, Scharp DW, Hartman BK, Ballinger WF, Lacy PE: A morphologic study of intrahepatic portal-vein islet isografts. Diabetes 26:201–214, 1977
12. Starzl TE, Porter KA, Putnam CW: Intraportal insulin protects from the liver injury of portacaval shunt in dogs. Lancet 2:1241–1242, 1975
13. Amamoo DG, Wood JE, Holley KE: Effect of intrahepatically implanted islets of Langerhans on hepatic function in the rat. Mayo Clin Proc 50:416–419, 1975
14. Feldman SD, Hirshberg GE, Dodi G, Raizman ME, Scharp DW, Ballinger WF, Lacy PE: Intrasplenic islet isografts. Surgery 82:384–394, 1977
15. Oakes DD, Spees EK, Annable CA, Reckard CR: Hepatocellular damage following intraportal transplantation of pancreatic islets in rats. J Surg Res 24:182–187, 1978
16. Kefalidles NA: Basement membranes: Structural and biosynthetic considerations. J Invest Derm 65:85–92, 1975
17. Nossell HL: The early stages of blood coagulation. Recent Advances in Blood Coagulation. Edited by L Poller. London, J. & A. Churchill, Ltd., 1969, pp 39–67







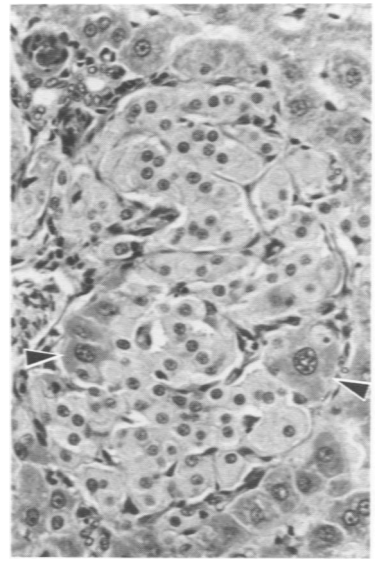
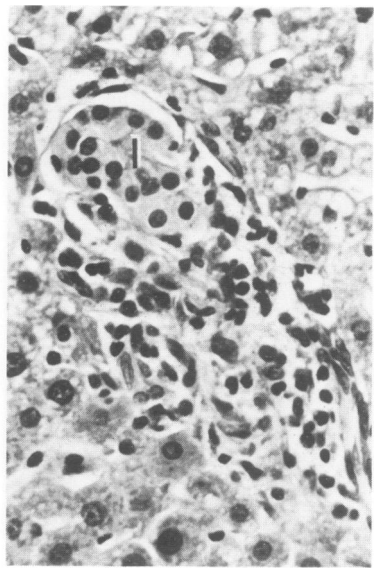


Figure 1—Phase contrast photomicrograph of islet suspension. **A**—Pancreatic islets. **B**—Contaminating acinar and ductal tissue surrounded by translucent basement membrane. (H&E, × 85) **Figure 2**—Allogeneic pancreatic islet in small branch portal vein 1 day after transplantation. (H&E, × 200) **Figure 3**—Intraportal isograft 1 day after transplantation. Ductal and acinar tissue (*arrows*) is surrounded by fibrin in lumen of large branch of portal vein. Necrotic hepatocytes (*N*) with polymorphonuclear leukocyte infiltrate are present in adjacent hepatic lobule. Similar changes were present in allografts at comparable time after transplantation. (H&E, × 200) **Figure 4**—Intraportal isograft 1 month after transplantation. Islet is present in portal tract and appears to be in direct contact with enlarged hepatocytes (*arrows*). (H&E, × 200)



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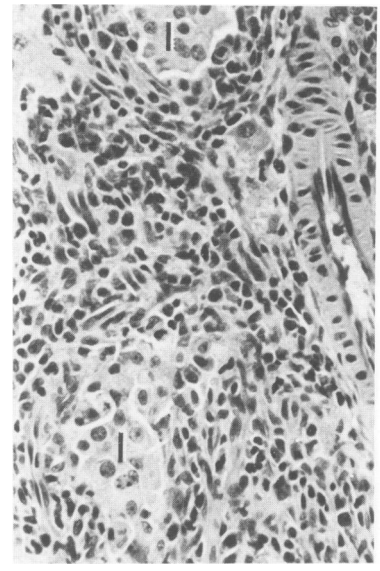

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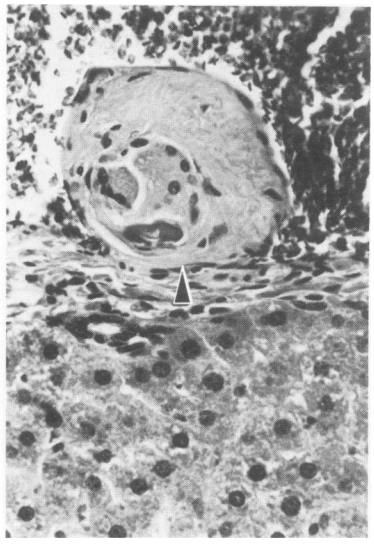

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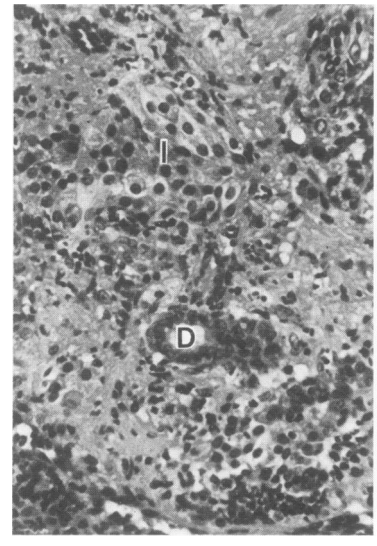

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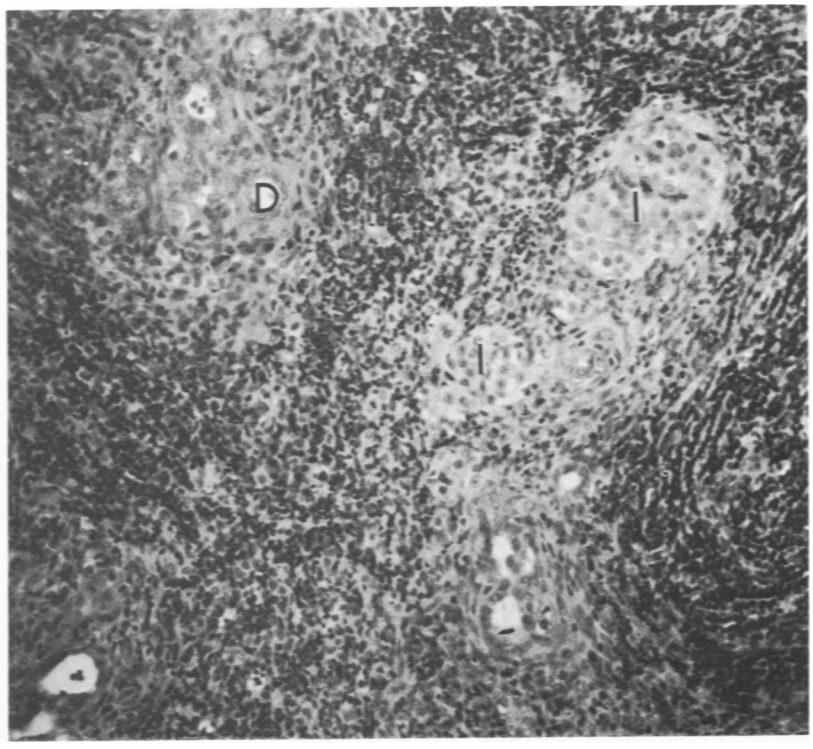
Figure 5—Intraportal allograft 2 days after transplantation. Lymphocytic infiltrate is present in portal tract adjacent to islet (*I*) within portal vein. (H&E, × 320) **Figure 6**—Intraportal allograft at the time of acute rejection. Degenerating islets (*I*) are surrounded by a heavy infiltrate of large lymphoid cells. (H&E, × 200) **Figure 7**—Fibrous nodule in large branch of portal vein 1 month after acute rejection of islet allograft by recipient of intraportal transplant. Crystalline, probably calcified, material (*arrow*) with adjacent foreign body giant cells is present in the nodule. (H&E, × 200) **Figure 8**—Intrasplenic isograft 1 day after rejection. Pancreatic islet (*I*) and duct (*D*) are surrounded by fibrinous material. Allografts examined 1 day after injection had similar appearance. (H&E, × 200)



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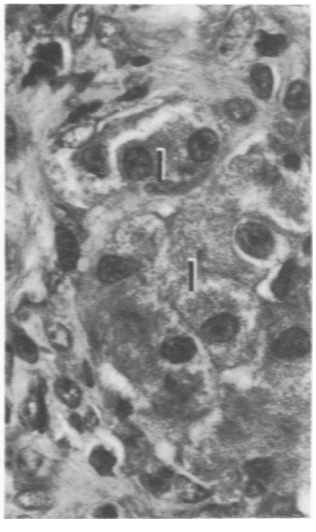

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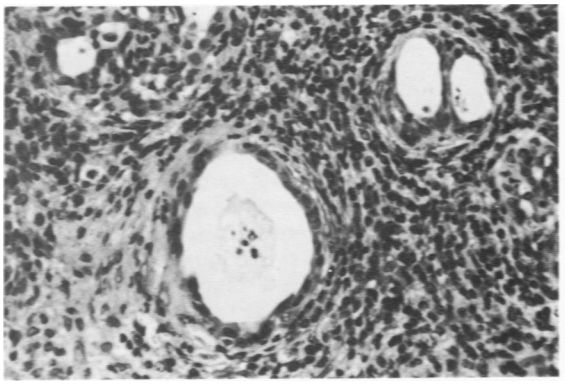

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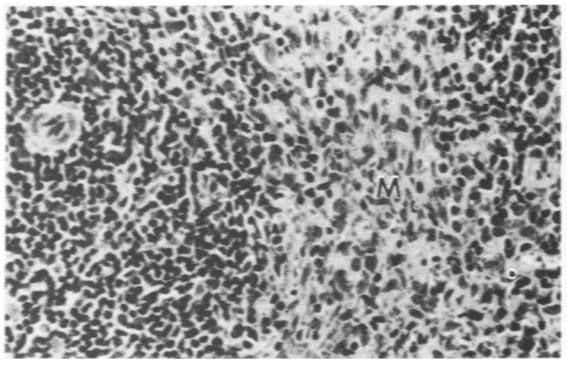

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Figure 9—Intrasplenic isograft 4 days after transplantation. Intact islet (*I*) and ductal tissue (*D*) is present in the sinusoids. (H&E, × 160) **Figure 10**—Intrasplenic isograft 1 month after transplantation. Nests of islet cells (*I*) are surrounded by rim of connective tissue. (Trichrome, × 640) **Figure 11**—Intrasplenic allograft 8 days after transplantation and 4 days after acute islet rejection. Ductal tissue is present in the sinusoids, but islet tissue is absent. (H&E, × 200) **Figure 12**—Sinusoidal accumulation of large mononuclear cells (*M*) in the spleen of an intrasplenic islet allograft recipient 1 week after acute rejection. (H&E, × 200)