## ABSTRACT

Inconsistent results in diabetes therapy with insulin and other agents have prompted research into the feasibility of pancreatic islet transplantation. Pancreatic primordia from 18-day rat fetuses were cultured individually for ten days on rayon grids in a medium of chicken embryo extract and chicken serum. Explants were transferred to fresh media and culture units every other day. After ten days, cultures on grids were implanted to four sites in Sprague Dawley rats. The presence of the rayon grids greatly facilitated the later recovery of transplants. One half of the hosts were diabetic, having received one 90 mg per kg i.v. injection of streptozotocin. After seven or 30 days as implants, the tissues were recovered, fixed in Bouin's fluid, and stained with aldehyde fuchsin for beta cells. At seven days, implants recovered from control animals had normally staining, granulated beta cells while those from streptozotocin diabetic animals exhibited substantial degranulation. Implant revascularization was observed grossly and microscopically. After 30 days both groups showed lymphocyte pools and extensive vascularity with many large, well developed vessels. Connective tissue covered the rayon grid, replacing implanted pancreatic tissue. Thus, immunological rejection was nearly complete by 30 days. These preliminary data indicate that the morphological integrity of fetal rat pancreatic beta cells is maintained in transplants of cultured material for 7 or more days. Implant site suitability was evaluated on the basis of vascularity and degree of implant integration into host tissue. Accordingly, the kidney capsule and visceral fat made better transplant sites than subcutaneous or intramuscular location. These results are preliminary to studies that could be adapted to human pancreatic culture and implantation.

# EXPLANTED PANCREATIC PRIMORDIUM OF THE RAT AS TRANSPLANT TISSUE TO NORMAL AND DIABETIC HOSTS

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## DEDICATION

This thesis is dedicated to Dr. Jim McDaniel. He was a good friend whose sincere interest and warm concern touched the lives of all students who had the priviledge of studying with him. He is surely missed.

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# INTRODUCTION AND LITERATURE REVIEW

The discovery of insulin as a supplementary treatment for frank diabetes mellitus by Banting and Best in 1922 greatly enlightened the outlook for many clinicians and diabetics. Yet, insulin administration has not proved to be the end-point for diabetes therapy because some other clinical manifestations of diabetes such as peripherial vascular disease, retinopathy and neuropathy often fail to respond to insulin. Furthermore, exogenous insulin treatment only transiently regulates fluctuations in the blood sugar levels in many diabetics. Daily or semi-daily injections of insulin are only crude attempts to control a constantly fluctuating blood glucose concentration and fall short of approximating the *in vivo* fine tuning control that the normal pancreas exerts over insulin synthesis and secretion on a cellular level. By the mid-twentieth century, researchers and clinicians began to seek a mode of treatment which more closely approximated the normal pancreatic function.

Even though the attempts at direct control of blood glucose levels by means other than injectable insulin are relatively recent, several avenues have been explored. Whole organ pancreatic transplantation has been attempted with rather discouraging results (Goetz, 1974). The patients who maintain the transplanted pancreas best are chronic diabetics whose end stage renal disease has been relieved by a kidney transplant. There are serious drawbacks with the involved surgical procedure, especially in attaining vascular anastomosis. Also, the present state of innumological manipulation is somewhat less than adequate for implantation of an organ

with such a vascular dependence. Immunological rejection is inevitable unless high doses of immunosuppressive drugs are administered.

Basic research in subprimate systems minimizes such risks and focuses on processes. Some subprimate transplantations are isografts, allografts, homografts, or xenografts. The rat and mouse models are commonly used.

Josiah Brown (1974) performed isograft experiments with fetal rat pancreas. Whole 18 day postconception pancreases were implanted beneath the kidney capsule of adult artificially-induced diabetic Lewis rats. Transient reversal of diabetic symptoms occurred in these highly inbred rats. Subsequently, it was thought that whole organ transplantation failed because the pancreatic islets were encased by acinar tissue which prevented optimal beta cell function due to limited surface area contact with the host tissue. As a result, an effort was made to isolate the diffusely occurring islets of Langerhans from the surrounding exocrine tissue.

Various techniques have been utilized to isolate islets from the pancreatic stroma and exocrine parenchyma. Collagenase has been used to digest and disperse the pancreatic tissue types. The isolated endocrine tissue was injected intraperitoneally into rats treated with alloxan (Lacy and Kostianovski, 1967). Islet adhesion occurred on both parietal and visceral peritoneum. When this technique was applied on inbred rats, a prolonged normoglycemia was produced. However, when rats of lesser genetic similarity were used, only transient recovery from hyperglycemia was reported.

Partial success has been reported when islets which have been treated with collagenase and subsequently microdissected from acinar tissue were used as an implant source (Kemp et al., 1973). Inconsistent results suggested that damage to the islets occurred during the separation process. With this technique 500-600 islets have been isografted to different sites in inbred Lewis rats through injection (Sharp, 1974). Yet, no effect was seen when these islets were injected into subcutaneous sites in streptozotocin diabetic rats. In contrast to the data of Leonard, et al. (1973), intraperitoneal injections produced only a partial reversal of diabetes. However, hyperglycemic reversal as long as 34 weeks was achieved with intraportal vein injections of the islet isolate. In other isograft experiments performed by Barker (1974), 600 to 1200 islets, partially separated from the acinar tissue, were injected intraperitonally into streptozotocin induced diabetic rats. Two-thirds of an experimental group (n=34) exhibited transitory reversal for at least one month. Some members demonstrated reversal for 15 months, yet late diabetic relapse appeared after this time. The collagenase digestion technique was improved by separating the islet acinar hydrolysate in a ficoll gradient (Kemp, et al, 1973). The ficoll gradient offers a rapid method to harvest large amounts of relatively acinar-free islet tissue which can be used in implant injections or for tissue culture and later implantation.

Of these models, pancreatic isografting in the inbred rat represents a good system to study implanted beta cell function. However, at best, the in vivo insulin response from isolated islet implants has only been maintained for just over one year with this technique.

A method less successful but not unlike the current human implant status is allografting in rats. However, the ultimate problem of immunological rejection in these animals persists.

Xenografts have been performed from the principal islet of sculpin fish into the peritoneal cavity of streptozotocin induced diabetic rats (Weil, 1974). They were rejected 48 hours post-operation. Successful xenografting would provide maximum implantation opportunities for human diabetics since a constant large source of viable islets would be available from lower primate or other lower vertebrate species.

Even though pancreatic allografts offer the most feasible route for control of human insulin levels in the diabetic, the risks generally outweigh the benefits at present. This is obviously due to the immunosuppression necessary to retard the rejection phenomonon. In the late 1960's, tissue culture was considered to be an alternative to extreme immunosuppression for the acceptance of allografts. The possibility that the immunologic character of rat islets could be changed by in vitro culture was tested by renal subcapsular isografting and by allografting 18 day fetal rat pancreas after ten days of organ culture (Goetz, 1974). The isograft transplants functioned normally. Yet, the allograft trials failed. Currently, the pancreas and its islets remain immunogenic.

Even though isografts have met with more success, two main problems exist. Immunological rejection is always a possibility even in the most closely matched systems. In addition, the problem of collecting a sufficient quantity of islets for implantation arises. Schweisthal et al., (1963) have shown that islets undergo hyperplasia as well as

hypertrophy in organ culture. Normal morphogenesis proceeds *in vitro*. This process sometimes involves an increase in the number of islets. The *in vitro* development of islets includes formation of granulated beta cells in many cases. Therefore, the neogenesis of functional tissue occurs *in vitro*. Perhaps sufficient quantities of transplantable material may be obtained through organ culture (Schweisthal *et al.*, 1963, Wells *et al*, 1967a). Hegre *et al.* (1973) have shown by histologic and insulin immunoassay techniques that a difference was found in potential for differentiation of rat islets in organ culture. This difference is related to the developmental state of the pancreas at the time of explantation. Highly differentiated islets of 22 day fetal rat pancreas were simply maintained *in vitro*, while larger increases in the islet mass occurred following organ culture of relatively undifferentiated 18 day fetal rat pancreas. Further, acinar tissue and its exocrine enzymes disappeared from the explants. Organ culture results with human fetal pancreas are remarkably similar to the rat results (Goetz, 1974).

Thus organ culture of pancreatic primordium provides a representative and adequate model of the normal islet cell mass *in vivo*. Since vascularity and innervation are not required for normal development *in vitro*, organ culture offers a method for short term storage, proliferation, and testing of pancreatic primordium survivorship which can be subsequently implanted into a suitable diabetic or nondiabetic host. Thus, another consideration is the viability of isolated rat islets cultured for transplantation. Rayon grids have been used as a convenient matrix for organ culture of pancreas (Schweisthal *et al.* 1963, Wells *et al.* 1967b).

In order for human pancreatic tissue implants to be feasible, a method of carefully harvesting, culturing, and storing the donor tissue is necessary. Since no present method has rendered pancreatic explants immunologically inert, a method for efficiently screening potential donors and recipients on the basis of surface tissue antigens is necessary.

As techniques offer means for mass harvesting of relatively pure rat pancreatic islet preparations without the necessity for microdissection, suitable numbers of immunologically characterized islets will be made available for implantation. A storage method will be needed for dynamic homeostatic maintenance of these islets. Two possibilities are currently conceivable. The traditional tissue and monolayer culture techniques offer feasible short term storage capacities for islets. Yet these methods require considerable manipulation through semi-daily transfers to new culture media, addition of antibiotics and oxygen-carbon dioxide atmospheres. Cryogenic manipulation of short term cultured pancreas offers another plausible technique for long term storage of transplant material. Knight et al. 1973 have accessed the effects of cold storage at $4^{o}C$ on isolated pancreatic islet functioning. Yet, results indicate that these techniques would provide only short term holding prior to transplantation.

Clearly the most desirable prospective substitute for the dysfunctioning pancreatic islet is a feasible mode for implanting living pancreatic tissue which will respond normally when implanted into an in vivo diabetic system. Such a natural system hopefully would treat all diabetic symptoms.

This pretransplant organ culture served at least three purposes. First, the acinar tissue was not maintained *in vitro* (Schweisthal *et al.*, 1963 and Leonard *et al.* 1973) and thus did not adversely effect the implanted endocrine pancreas. This loss of exocrine tissue during organ culture avoided the mechanical and chemical modification associated with collagenase digestion, although isolated islets can be cultured (Scharp in Goetz, 1974). Second, organ culture of fetal rat pancreas for ten days also served as a means of viability screening for the explanted tissue. Possibly the culture period gave the pancreatic primordium ample time to adjust to the non-donor environment which will be further encountered as an implant. Third, culture of the explant allowed time for hyperplasia into the grid fibers and thus anchored the islet-containing explant to a handy maneuverable rayon grid. This latter feature avoided the recovery problems encountered by previous workers.

This study was undertaken to determine whether cultured fetal rat pancreatic tissue could reverse the diabetic symptoms when implanted into diabetic hosts done under these conditions and if and where the use of tissue on rayon acetate grids would serve as a vehicle for ease in implantation to selected recipient sites.

## MATERIALS AND METHODS

Donor Animals- Ten female 200-235 gram Sprague Dawley rats were inbred, and the exact time of conception was determined by witnessed matings. The mating pairs were subsequently separated and the females were maintained for 18 days on Wayne Lab Blox rat chow and water ad libitum. The developing fetal pancreases were removed at 18 days.

Solutions- All solutions used during the experiment were sterile. Tyrode's solution was used as a working solution for fetal washings and for cultures (Appendix A).

Culture media consisted of chicken embryo extract (CEE) and chicken serum. CEE was prepared from fertile white Leghorn chicken embryos purchased from Green Valley Hatchery, Kenly, N. C. Upon purchase, the eggs were placed in a temperature humidity controlled David Bradley egg incubator at 100°F. The eggs were incubated for 12 days at which time they were candled. The 12 day embryos were removed from the eggs under sterile conditions, washed three times with Tyrode's solution, and subsequently minced thoroughly with sterile scissors. The minced chick embryos were collected in centrifuge tubes and diluted one to one with Tyrode's solution. The mixture was centrifuged for three minutes at setting five in an IEC clinical centrifuge and the supernatant was frozen and/or immediately used. The chicken serum was purchased commercially.

Streptozotocin, purchased from the Upjohn Company, was used to induce frank diabetes mellitus. It was reconsituted in fresh citrate buffer (pH 4.5).

Culture Units- Culture units used in this work consisted of a 100 mm diameter petri dish containing a 75 mm concave watch glass surrounded by

cotton according to the watch glass method of organ culture (Schweisthal et al., 1963) (Fig. 1). A stainless steel grid ($1.5cm^2$) was placed on the watch glass forming a concave support for a rayon acetate tissue grid ($7\ mm^2$). The rayon tissue grid was prepared from acetate purchased from Courtaulds Ltd. London, England. Preparation involved removing every other two threads from the weave. This was done as the observer viewed the grid through a ten X dissecting microscope housed in the culture hood.

Culture Oven- During the culture period, culture units are maintained in a culture oven (Electric Heat Control Apparatus Company) at $37^{\circ}C$. The culture oven was equipped with a closed oxygenation system through which the cultures were given an atmosphere of 95% oxygen and 5% carbon dioxide for one hour duration during each day of the culture period.

Experimental Animals- Group I, Grid Implant Controls: Three rats received only rayon acetate grids to the chosen implant sites. These animals were used to test acceptance by a recepient of the rayon as a vehicle for implant mobility. Four sites were chosen in the recipient animals to receive implants. Sites chosen were (1) beneath the capsule of the kidney, (2) an artifical pocket produced in visceral mesenteric fat, (3) a subcutaneous pocket created between the dermis and musculature lateral to the abdominal incision, and (4) between fibers of the rectus abdominus muscle, just lateral to the original abdominal incision.

Group II, Beginning Controls: Two animals received only implants, one to each of the four chosen sites. Animals in this group were not diabetic and thus served as controls for those diabetic groups.

Group III, Diabetic Controls: Two rats served as diabetic controls. A 90 mg per kg body weight dose of streptozotocin was administered intravenously to these animals. These rats served as a comparison with the diabetic rats that received implants.

Group IVa, Diabetic Implant Recipients: Three rats received streptozotocin (90 mg/kg). Three days later, cultured pancreatic implants were inserted in the four chosen sites. These animals served as experimental recipient rats.

Group IVb, Diabetic Implant Recipient: One rat received streptozotocin (90 mg/kg). Three days later, it received eight cultured pancreatic implants to the same four implant sites in a bilateral fashion.

Group V, Long Term Cultures: Ten explants remained in culture for thirty days.

During the culture period, blood samples (3µl) from the lateral tail vein were taken from the rats in the Group I-IV. These samples were to be used in subsequent glucose tolerance tests (GTT) and in a radio-immunoassay (RIA) for insulin. These samples were collected in a calibrated 100 µl, heparinized capillary tubes, sealed with a clay-wax material and placed in a freezer. Subsequent centrifugation indicated that the blood samples had partially freeze-dried, thus prohibiting GTT's or RIA's.

Procedures

Fetal Pancreatic Removal and Culture- Eighteen days post-coitum, gravid rat uteri from female donors were removed *in toto* through a ventromedial abdominal incision. The fetuses were immediately placed in a covered petri dish containing Tyrode's solution to prevent desiccation. At this point, pancreatic primordium was visualized and dissected with

the aid of a binocular dissecting microscope, cataract knives, iridectomy scissors, and micro-forceps. The initial cut was made inferior to the transverse axial line of the forelimbs by drawing two cataract knives across each other removing the head, neck, and forelimbs. The remaining caudal portion was washed in fresh Tyrode's solution and a cataract knife was then inserted just ventral to the spinal column as a second knife was drawn along the first knife, thus removing the vertebral column. The pancreas and adnexa were then divided from the fetus. Further micro-dissection and washings in Tyrode's solution resulted in an intact dorsoventral pancreatic anlagen which has not yet developed into the diffuse structure found in the adult. The pancreatic primordium was immediately placed on a sterile grid of rayon acetate which was resting on a stainless steel metal grid. Cotton in the culture unit was then saturated with sterile glass distilled water, which prevented dessiccation. A working solution of equal parts of chicken serum and CEE was added to the space beneath the convex metal grid to a point at which the metal grid began to float. The culture unit containing the freshly explanted pancreas was labeled and immediately placed inside a culture oven at 37°C. The culture units were contained in modified glass desiccators (Fig. 2). The pancreatic explants were cultured for ten or 30 days and transferred to new culture units and fresh media every other day, repeating the same steps described for preparing the original culture unit. At each transfer, each culture was evaluated for viability by grossly observing the tissue and culture media. Healthy tissue was pink-cream in color and elaborated a thin spreading margin. The normal culture medium was light amber and transparent. Contaminated cultures

were white in color and exhibited distinct borders with no indication of spreading. Contaminated media were cloudy to cream in color and usually contained white bacterial colonies and was covered with a lipid-like film.

Pancreatic Implantation- After ten culture days, implants were administered to the four chosen sites in animals of Groups II, IVa, and IVb. The animal was initially anesthesized with an intraperitoneal injection of chloral hydrate (35 mg/100 gm body weight). Supplemental anesthesia was administered with a nose cone saturated with ethyl either. The rat was placed in a supine position and the abdomen was opened by a ventral incision. The kidney was located and the renal capsule was perforated on the ventral surface. The rayon grid containing the pancreatic culture was trimmed to the size of the explant and inserted beneath the capsule. The renal surface was slightly traumatized to stimulate subsequent vascular supply. The capsule was released and a natural membraneous seal was subsequently formed over the explant. The renal artery was noted near the implantation site. A pocket was also formed in pink mesenteric fat by blunt dissection and an implant was inserted. The pocket was closed with surgical silk to help mark grid location as well as to secure the implant. The walls of the peritoneum and overlying musculature were reapproximated and closed with silk sutures. The rectus abdominus muscle was divided by sharp dissection and an implant was inserted. The muscle pocket was closed by silk sutures. The subcutaneous pocket was formed lateral to the abdominal incision by blunt dissection. An implant was inserted and the pocket was closed similarly. The skin was reapproximated and closed with wound clips.

Recipient Sacrifice and Tissue Preparation- After 7 or 30 days of implantation, the rats were sacrificed by an overdose of sodium pentobarbital, the implants removed, fixed in Bouin's fluid for 24 hours, embedded in 57°C paraffin, serially sectioned on an American Optical paraffin microtome at four micra thickness, and mounted on plain glass microscope slides. The tissue was stained for the presence of insulin granules with Gomori's aldehyde fuchsin-ponceau de xylidine stain (1950). Random slides from each tissue were stained with hematoxylin and eosin Y for evaluation of fibroblastosis. All slides were evaluated relative to previous work, both from the literature and from control slides included from our laboratory collection. Tissue viability and implant success were evaluated on the basis of grid vascularization, host-implant integration, necrosis or rejection, staining characteristics, and tissue organization.

Photography- After slide evaluations were made, color and black and white photographs were taken of representative sections from each group with a Carl Zeiss Photomicroscope II loaded with Kodachrome II and H. S. Ektachrome color film and Panatonic X black and white film. Plates used in this paper were compiled from the black and white photos printed on Kodak Kodabromide single weight paper.

# RESULTS

Ten Day Cultures- Although no photographs were made of these cultures, it was microscopically confirmed that explants were viable in organ culture for ten days. This pilot study was done to verify the necessary procedures of subsequent study. Gross observations indicated that the ten day cultures survived *in vitro* with only minimal contamination occurring on a few cultures. The majority of the cultures exhibited no abnormal change in tissue color, consistency, shape, or media. The explants adhered well to the rayon grids and in all cases grew into the grid pores. Aldehyde fuchsin-stained paraffin sections of the ten day cultures demonstrated tissue grid integration (Figure 3).

In these culture controls, numerous round or oval highly granulated islets were seen interspersed between ductular exocrine tissue. During the ten days in culture, the exocrine tissue underwent a modification which appeared as a size reduction. The B-cells stained strongly with aldehyde fuchsin to produce deep purple granules. These granules were believed to represent condensed insulin or proinsulin molecules. Numerous blood vessels were seen in close association with the islets of these ten day cultures. Erythrocytes were often seen in the vessel lumen. Interlobular ducts course through the acinar tissue. The islets were separated from the surrounding acinar tissue by a layer of reticular fibers. Little connective tissue was seen within the islet. Isolated islet cell groups were occasionally seen among acinar cells or closely associated with the ducts.

Group I, Grid Implant Controls: Rayon grids retrieved from the host rats after seven days showed no evidence of implant incompatibility in any of the four sites (Figures 3 and 4). The surrounding host tissue permeated the grid pores without demonstration of inflammation or other pathological change. Blood vessels were numerous. The consistent integration noted for the grid implant controls afforded a representative model for comparison with the culture implant groups.

Group II, Beginning Controls: Tissue recovered from the four implant sites was evaluated on the basis of host-implant integration in terms of host tissue intimacy with the rayon grid and with the pancreatic implant. Transplant site suitability was also evaluated relative to degree of new vascularity of the implant (Table 1). Thus, cyto-differentation and histogenesis appeared to continue in the transplant environment.

Implants removed from these beginning controls exhibited granulated islets of Langerhans (Figures 5, 6, and 7). Cultured implants faired best in the kidney capsule site. Large well-granulated islets were seen near glomeruli (Figures 5A-5C). Some islets were surrounded by acini which seemed to indicate excellent host-implant integration (Figure 6A). Numerous, large blood pools were seen adjacent to and in the implant which is suggestive of revascularization (Figures 6C and 6D). Grossly, revascularization was observed (Figures 7A and 7B).

Granulated islets were also seen in implants placed in the intra-muscular site (Figure 5D).

Group III, Diabetic Controls: The rats that received streptozotocin and subsequent grids but no explants served as diabetic grid implant

controls. These animals developed diabetic symptoms of polyphagia, polyuria, polydypsia, ketonuria, and hemoconcentration three to five days following the single injection (90 mg/kg bw, iv) of streptozotocin. Although no photos were taken, microscopic examination of pancreatic tissue from these induced diabetic rats revealed disrupted B cells within otherwise normal islets and normal acinar tissue. As expected, severe islet degranulation was noted in these sections. The islet cells were atrophic and appeared shrunken from their limiting connective tissue. This selective destruction was indicative of the high specificity for B cells by which streptozotocin exerts its diabetogenic properties.

Group IVa, Diabetic Implant Recipients: Cultured implants were removed from the chosen sites in the streptozotocin-induced diabetic adult rats. Grids were most easily transplanted beneath the kidney capsule. This membrane forms an ideal container for implants without disruption of renal integrity. Implants to this serous but nonvascular capsule became vascularized by new vessel growth from the kidney proper and the renal artery in all groups (Figure 7).

Microscopically, the implant became well vascularized within a seven day period. When stained with aldehyde fuchsin, partially degranulated islets were seen near ducts (Figures 8A-8E). Numerous lymphocytes, macrophages, and monocytes were seen near the implanted pancreatic tissue (Figures 8A-8E). Cultures implanted into Group IVa animals for one week demonstrated normal islet morphological integrity. Significant B-cell granulation was seen in Group IVa (Figures 8-10). This staining suggested that the islets were functional and were actively synthesizing

insulin in response to the artificially induced hyperglycemia. Small granulated cells were seen adjacent to ducts and larger islets which indicated continued development of the primordium in vitro and also during the implantation period. This ability to continue cytodifferentiation in the implant was an indication of implant viability. The new vascularity noted in the implant would lend support to the continued development.

Implants to the kidney capsule site (KC) were easily visualized through the transparent capsule. This availibility greatly facilitated implant removal. Also, implants placed into KC sites exhibited the best host-implant tissue integration. Grossly, blood vessels, presumably originating from branches of the renal arteries, were seen transversing the implant, subcapsularly.

Grid recovery from the adipose site was more difficult than from the kidney site due to the amorphous nature of fatty tissue. Pink fat is richly vascularized. Implants placed in the fatty sites became re-vascularized and thus faired well in vivo. Blood vessels and sinusoids were seen in the grid tissue (Figure 9B). The implanted fetal tissue integrated well with the fat. Group IVa implants in the adipose site were well granulated.

Implants placed subcutaneously appeared less well adapted to the host than did the kidney site implants as was evidenced by less revascularization from the subcutaneous tissues (Figures 9A and 9C). Blood vessels were not as intimately associated with the implant-grid area as seen in the kidney capsule or fat sites. Since Group II grids in the SC site demonstrated little pre-implant vascularity, the lack of vascular reconnection was not surprising in the Group IVa tissues.

Host-implant integration was not as great as that seen in the kidney capsule or fat sites, although noticable integration did occur with the subcutaneous fascia and rectus abdominus muscle.

Implants placed intramuscularly into Group IVa animals maintained their islet integrity during the implant period. Richly granulated islets were observed microscopically (Figure 10). Little in vivo change was noted grossly upon implant removal after seven days which may indicate endocrine survival in the diabetically stressed animal. Good host-implant integration was seen at grid level (Figure 10).

Group IVb Diabetic Implant Recipients: Implants retrieved after thirty days from the four sites were hypotrophic and recognized as implants only by the presence of the rayon grids. Implants from control and diabetic hosts demonstrated no recognizible endocrine tissue (Figures 12 and 13). Tissue recovered from diabetic hosts appeared less homogeneous than tissue from implant controls which may be a result of the added stress of the diabetes. Numerous lymphocytes were seen as signs of immunological rejection in implants which were placed into these moderately inbred animals. Histologically, the thirty day implants at grid level demonstrated implant tissue replacement by connective tissue in most cases (Figures 12 and 13). Implant-host integration was consistantly good as was revascularity in these thirty day implants. Yet, in all cultures allografted into recipient rats for thirty days, evidence of end state immunological rejection was seen. Hydropic degeneration, lymphocyte and giant cell infiltration was noted in most sections. This pathologic change was reflected in a general deteriorated appearance of the tissue.

Long term implants placed into adipose tissue of Group IVb animals exhibited good vascularity with many large arteries (Figure 12D).

Implants placed into muscle sites showed no culture cells after thirty days. Only grid remained and was separated from muscle fibers by a light purple connective tissue. Portions of the grid were completely enclosed by muscle fibers and large vessels were closely associated with the grid in this site.

The Group IVb host animals exhibited a biphasic pattern of diabetic response to the implants. Three days after the recepient animals were rendered diabetic, an overt diabetes was noted. Seven days subsequent to implantation of the cultures there appeared to be a stasis in the increasing diabetic state as evidenced by less polydypsia and polyuria. This period ends between days 14 and 20 when a severe increase in the diabetic state occurred. This final state persisted until sacrifice at day 30. The animals existing in this last state were extremently debilitated, exhibited severe polyuria, polydipsia, polyphagia, ketonuria, and hemoconcentration. Lateral tail vein blood samples were difficult to obtain from these animals due to acute hemoconcentration. Group IVb animals became lethargic.

Group V, Long Term Cultures: (See Table 1). Of the fifteen explants maintained in organ culture past the pre-implant ten day culture period, four became contaminated with bacteria. Eleven cultures were uncontaminated. Groups of mildly granulated cells which resemble islets of Langerhans were frequently seen and indicated that cultures can survive and proliferate for a long term. However, these islets appeared morphologically abnormal

and their functional integrity were questioned. The islet cells were retracted from their basement membrances and appeared dystrophic. No recognizable exocrine acini were noted (Figures 14A-14D).

Metabolic Analysis: Changes in the metabolic state of the Group IV and Group II animals were reflected in weight changes over a two week period (Figures 15 and 16). All animals plotted demonstrated some weight loss during the study. Group IVa animals showed the largest weight change (34 gms). Most of the weight loss was reflected during the first seven days after streptozotocin administration. Group II and Group IVb animals demonstrated nonsignificant weight loss (17 gms).

Figure 15 shows that of those animals plotted, all experienced a weight loss. Those streptozotocin diabetic animals with eight implants had weight change results comparable with the controls. In addition, all animals had a significantly lower weight loss than the streptozotocin diabetic animals with four implants. This result was remarkable and illustrated that, even though four implants were effective in reducing the hyperglycemia, this number of implants was not adequate to produce a normoglycemia.

Eight grids represented a more significant mass of implanted pancreas which produced an endocrine replacement for a subimmunological period of seven days. After two weeks, the pallative effects of the implants were reduced as immunological rejection probably destroyed the pancreatic tissue. This result is represented in figure 16 by the stippled area over the VV116 and VV120 histogram. After the two week period, the diabetic animal with eight grids continued to exhibit much reduced weight loss relative to the animal with only four implants.

TABLE 1

Evaluation of 18 day explants grown in culture for ten days and transplanted to Sprague-Dawley rats for seven or thirty days.

| Culture No. | Group | Implant Site | Implanted (Weeks) |
|---|---|---|---|
| 637 | II | KC | 1 |
| 642 | III | F | 1 |
| 633 | III | SC | 1 |
| 640 | III | M | 1 |
| 635 | II | KC | 4 |
| 639 | IV | KC | 4 |
| 638 | IV | F | 4 |
| 623 | IV | F | 4 |
| 641 | IV | SC | 4 |
| 624 | IV | M | 4 |
| 643 | IV | M | 4 |
| 636 | IV | SC | 4 |
| 628 | II | KC | 4 |
| 625 | II | F | 4 |
| 627 | II | SC | 4 |
| 626 | II | M | 4 |
| 629 | II | KC | 1 |
| 631 | II | SC | 1 |
| 632 | II | M | 1 |

Table 1 Continued

| Culture No. | Vascularity | Size & No. | N or A | Organization | N or A | Necrosis Inflammation Rejection |
|---|---|---|---|---|---|---|
| 623 SF 28 | 4+ Large arteries | None | | Only grid remaining. No recognizable culture cells of any kind. Closely associated fatty tissue & blood vessels. | | R++++ |
| 624SM 28 | 4+Large arteries | None | | Only grid remaining. Separated from muscle by light purple connective tissue and a few other cells. No recognizable culture cells. Portions of grid completely encased in muscle fibers. Large vessels. | | R++++ |
| 626CM 28 | 4+ | Possible Islets | A | Some culture tissue remaining, large arteries associated. Islets may resist rejection longer than other tissue doe to their basement membranes. | A | R+++ |
| 628CC | 4+ | Possible | A | Arteries all similar to 626. | A | R+++ |
| 627 CC | 4+ | Possible | A | Arteries all similar to 626. | A | R+++ |
| 635 SC | 4+ | None | | Extensive light purple connective tissue. All culture cells replaced by connective tissue. | | R++++ |

Table 1 Continued

| Culture No. | Vascularity | Size & No. | N or A | Organization | N or A | Necrosis Inflammation Rejection |
|---|---|---|---|---|---|---|
| 636 SC 28 | 3+ | Possible | A | Grid associated somewhat poorly with muscle fascia. Inflammation may be caused by many hair fragments. | | R+++<br>I++ |
| 638 SF 27 | 4+ | Possible | A | Numerous large plant-like structures embedded in tissue. Rejection still going on. Associated fat very highly vascular. Tissue wall enclosed by host tissue. A few isolated light fuchsinophilic cells. | A | R+++<br>I++ |
| 639 SKC 28 | 4+ | Possible | A | Some possible culture material remaining. Most replaced by connective tissue. | A | R+++ |
| 629 GKC 7 | 2+ | +++ | N | Non vascular lymphocyte invasion just beginning-extensive into culture in places. May not be immunologic as some kidney also being destroyed. | N | R+<br>I++ |
| 632 GM 7 | 2+ | +++ | N | Tissues from VV121 Control KC-M are about the same but muscle not entirely surrounded by host tissue. | N | R+ |

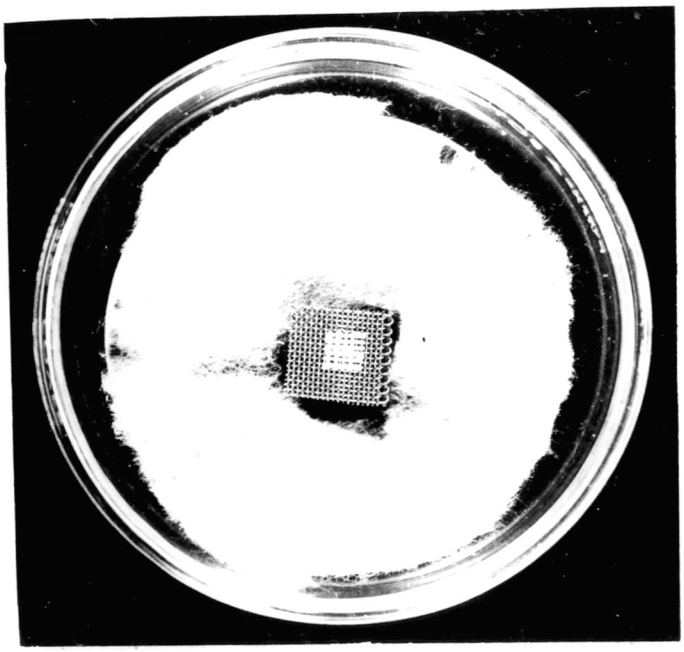
Table 1 Continued

| Culture No. | Vascularity | Size & No. | N or A | Organization | N or A | Necrosis Inflammation Rejection |
|---|---|---|---|---|---|---|
| 633 $\frac{S}{SC}$ | 4+ | +++ | N | Numerous blood vessels in fascia and sub-cutaneous fat. | N | |
| 640 $\frac{S}{M}$ 7 | 2+ | +++ | N | Islets degranulated partially; lymphocyte pools seen. | | I+++<br>R+ |
| 637 $\frac{S}{KC}$ 7 | 4+ | +++ | N | Degranulated islets. | N | I++<br>R+ |

Figure 1: Watch glass organ culture unit. Shown is the absorbant cotton for water, the stainless steel grid, and the rayon grid.

Figure 2: Dessicator culture unit. Shown are representative culture plates and the rubber tube for administration of the $O_2$-$CO_2$ atmosphere.

1



2

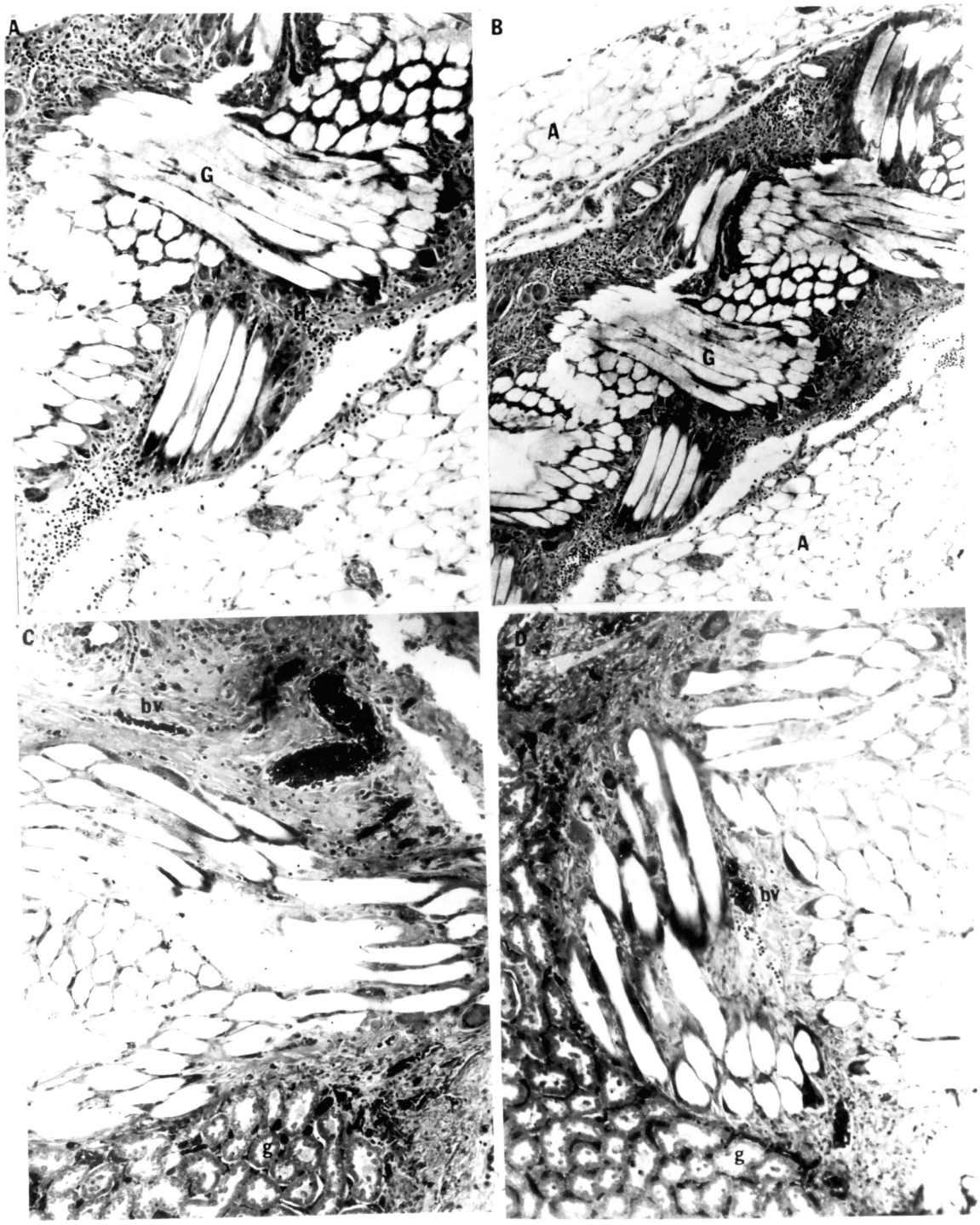
**Figures 3A-3D:** Ten day grid implant controls (Group I). Rayon grids retrieved from host rats after seven days *in vivo*. Haematoxylin and Eosin Y stain.

**Figure 3A:** Tissue from mesenteric fat site. Numerous woven grid fibers (G) are seen in close integration with the host tissue (H). x146.

**Figure 3B:** Same as Figure 4A. The grid (G) is seen to be surrounded by the adipose tissue (A). x89.

**Figure 3C:** Tissue from kidney capsule site. Numerous renal tubules (g) are seen in the kidney proper. Grid material is present as are numerous blood vessels (bv). x146.

**Figure 3D:** Same as Figure 4C. x146.


A
G
H
B
A
G
A
C
bv
g
D
bv
g

Figures 4A-4D: Grid Implant Controls. (Group I) Rayon grids retrieved from host rats after seven days *in vivo*. Hematoxylin and Eosin stain.

Figure 4A: Tissue from subcutaneous site. Shows subcutaneous tissue (SC) and Rayon grid (G). x128.

Figure 4B: Same as 4A. x148.

Figure 4C: Same as 4B. x148.

Figure 4D: Tissue from intramuscular site. Muscle tissue (M) is seen in close association with grid (G). x148.

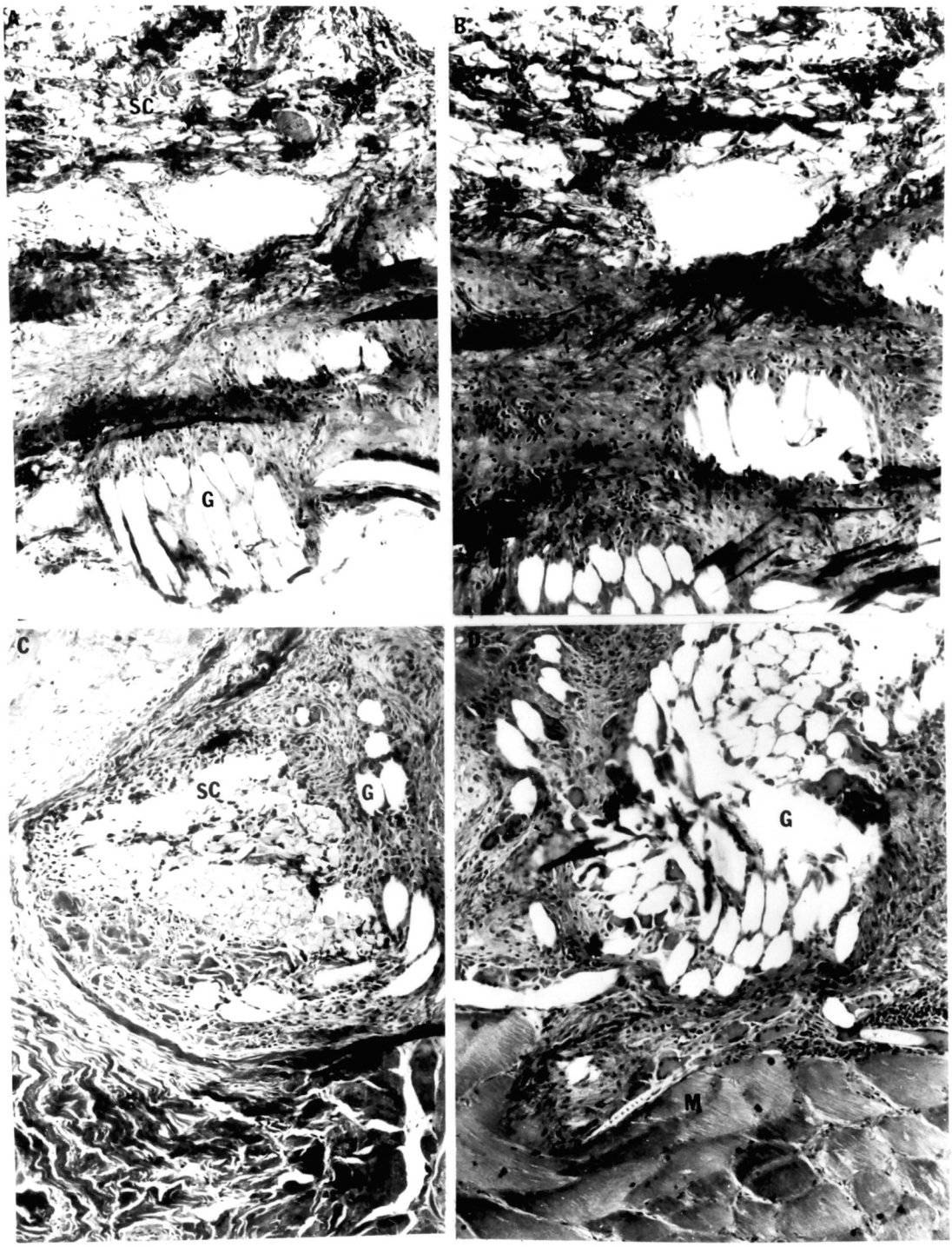

A
SC
G
B
C
SC
G
D
G
M

Figure 5A-5D: Beginning Controls (Group II). Tissue recovered from this group consists of ten day cultures on rayon grids implanted in the normal control animal for one week.

Figure 5A-5C: Shows well differentiated islets of Langerhans (I) seen near ducts (D) in the kidney capsule site. Aldehyde fuchsin Figs. 5A-5B, x328; 5C, x364.

Figure 5D: Shows intramuscular site implant tissue with several islets (I). x147.

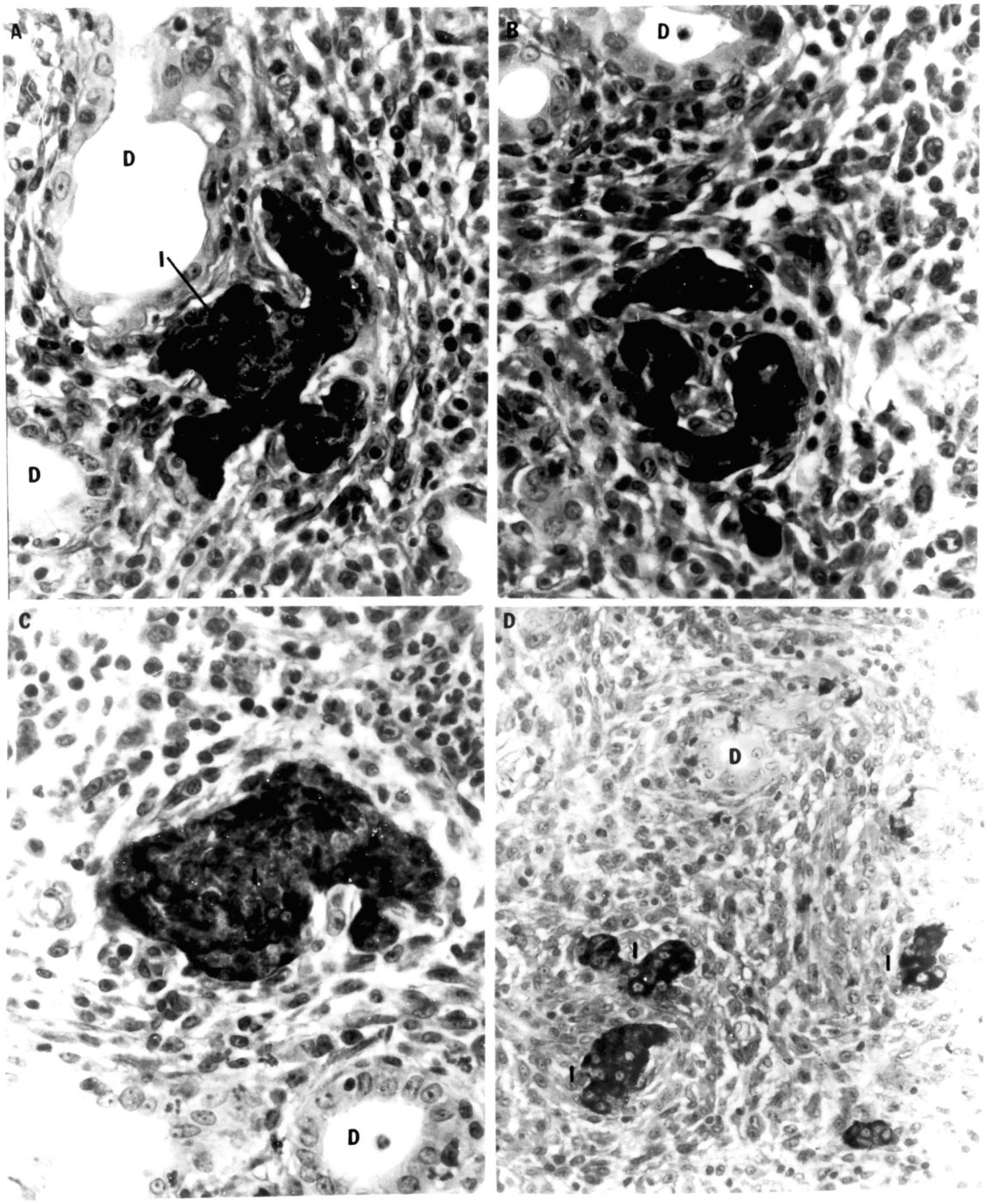

A
D
I
D
B
D
C
I
D
D
I
I
I

Figure 6A-6D: Beginning Controls (Group II). Tissue recovered is from ten day cultures implanted in control animals for seven days.

Figure 6A: Shows excellent host (H) implant (I) integration in the kidney capsule site. x147.

Figure 6B: Shows granulated islet of Langerhan (L) in implanted tissue. Acini (A) border the islet from kidney capsule site. x364.

Figure 6C-6D: Kidney capsule site. Shows excellent integration and abundant vascularity (bv) at grid level (G). x183, x262.

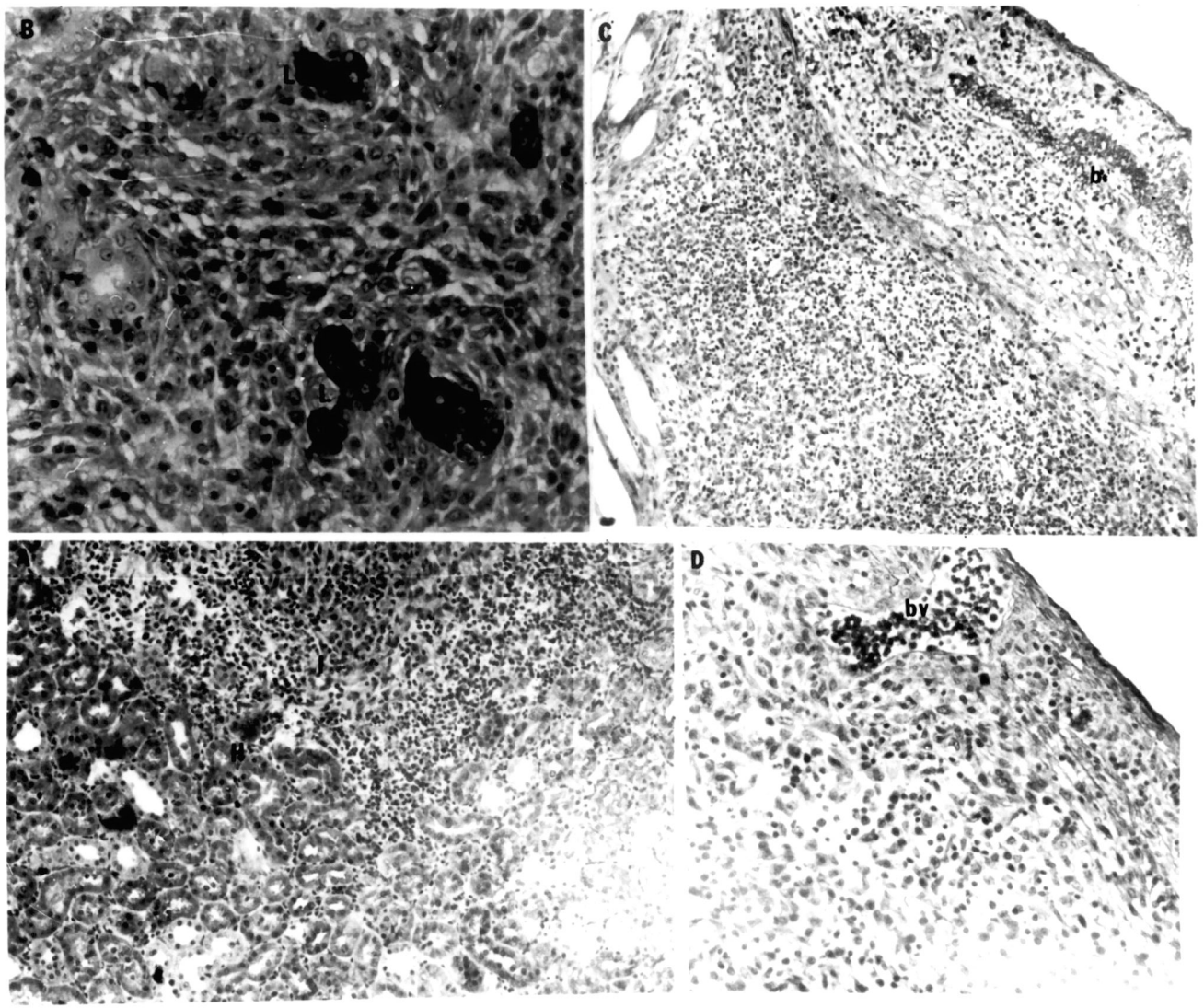

B
L
L
C
bv
H
D
by

Figure 7A-7B: Beginning Controls (Group II). Implants cultured for ten days and implanted in normal host in kidney capsule location for seven days. Shows implant (I) *in situ* under renal capsule. New blood vessels (bv) can be seen grossly x2.


A
B

Figure 8A-8E: Diabetic Implant Recipients (Group IVa). Tissue from diabetic animals in which 10 day cultures were implanted for seven days. All are from the kidney capsular site. Grid spaces are seen (G) which indicates presence of implants. Numerous islets (I) can be seen to contain granulated beta cells (b) near ductular elements (d). Lymphocytes (L) are seen in the implant tissue. Aldehyde fuchsin stain Figure 8Ax458, Figure 8Bx147, Figure 8Cx63, Figure 8Dx462, Figure 8Ex235.

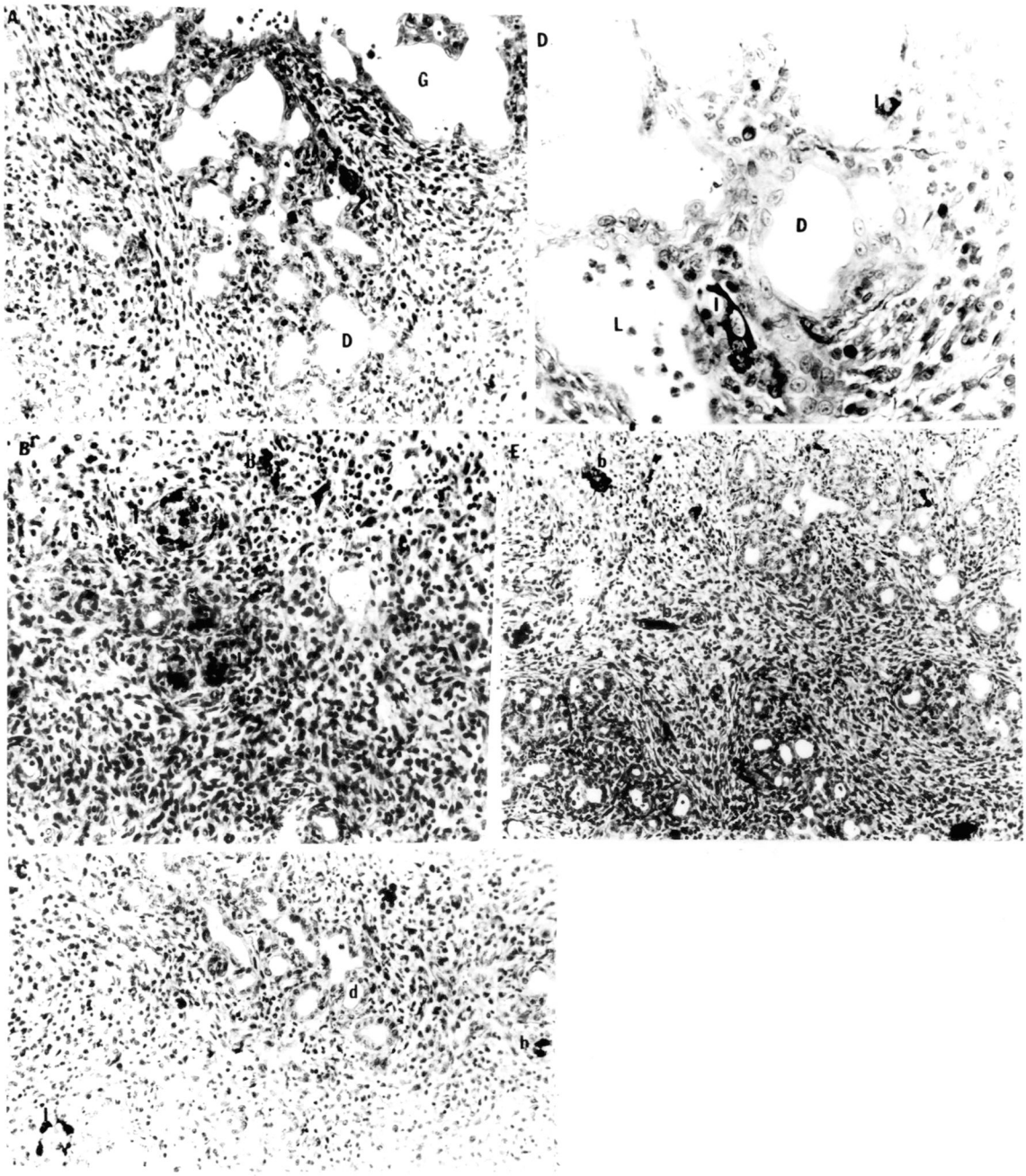
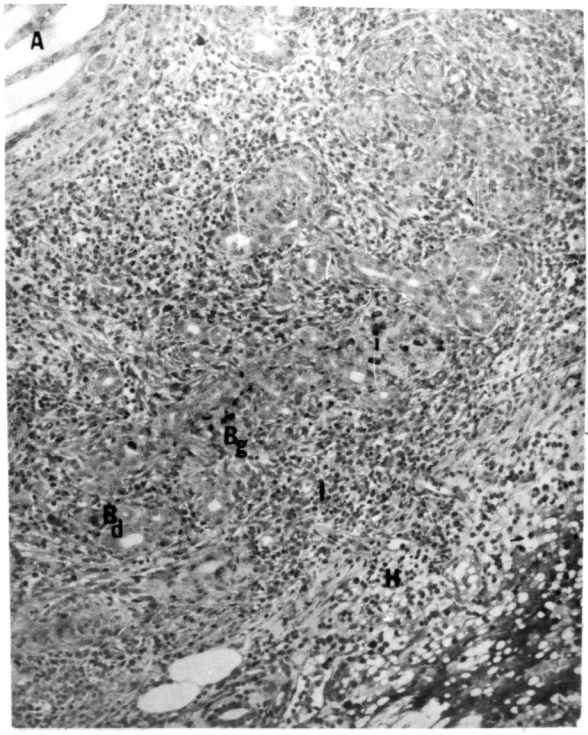

A
G
D
D
D
L
L
B
B
E
b
C
d
b

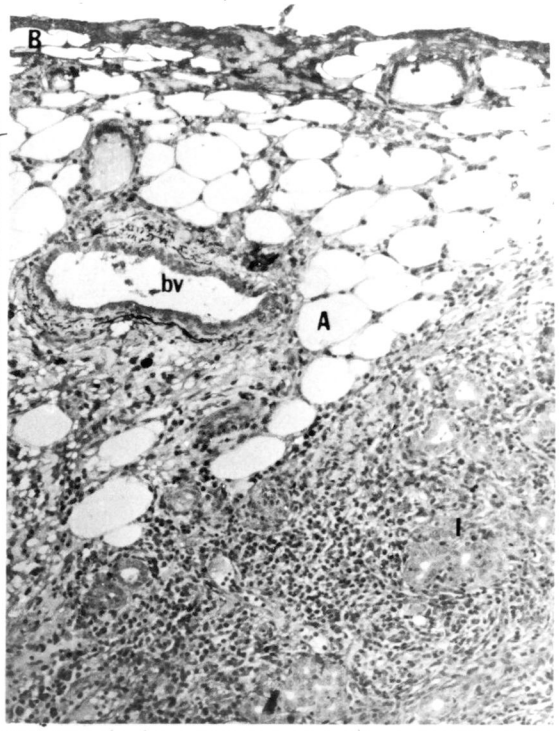
**Figure 9A-9C:** Diabetic Implant Recipients (Group IVa) Tissue recovered after one week of implantation with ten day cultures. Aldehyde-Fuchsin.

Figure 9A: Subcutaneous site showing good host (H) implant (I) integration. Morphologically intact islets can be seen (i). Some granulated (Bg) and some degranulated beta cells (Bd) are noted. x147.

Figure 9B: Adipose site showing adipose cells (A) and their blood supply (bv). Degranulated islets (i) are noted. x147.

Figure 9C: Same as Figure 9A. Blood vessels (bv) are noted in the adipose component of the SC tissue. Grid spaces (G) are seen. x147.


A
I
Bg
I
Bd
H


B
bv
A
I

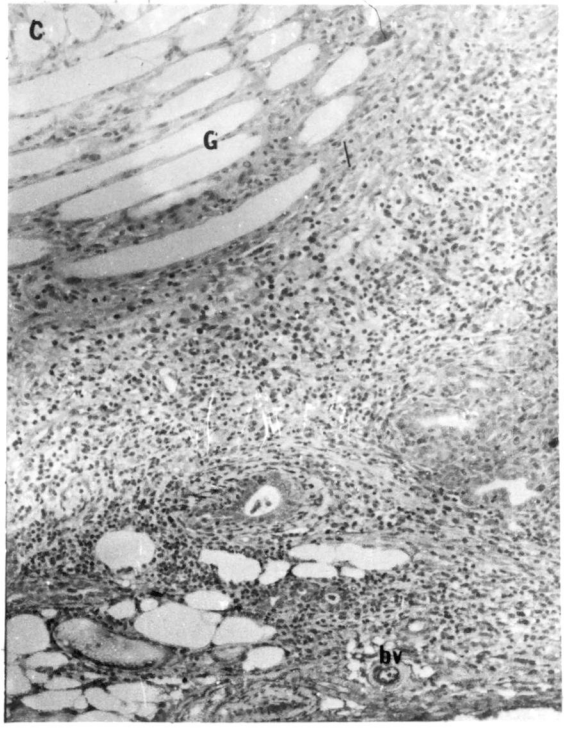

C
G
bv

**Figure 10A-10F:** Diabetic Implant Recipients (Group IVa). Implants recovered from diabetic hosts after one week *in vivo*. Aldehyde Fuchsin stain. Intramuscular site.

**Figure 10A:** Shows grid (G) level implants with good host (H) implant (I) integration. A well granulated islet (i) is seen. Arterioles (bv) and ducts (d) are noted. x147.

**Figure 10B:** Same as 10A. Rich blood pools, (bv) are noted. x147.

**Figure 10C:** Shows large blood vessel (bv) in longitudinal section interposed between implant (I) and host muscle (M) tissue. x243.

**Figure 10D:** Same as 10C. Also seen are mildly granulated beta cells (B). x142.

**Figure 10E:** Shows several richly granulated islets (i) at grid level (G). Numerous ductular elements (D) are seen. x143.

**Figure 10F:** Shows normal appearing islet of Langerhan (i) in the implant parenchyma (I). Ductular elements (D) are noted. x233.

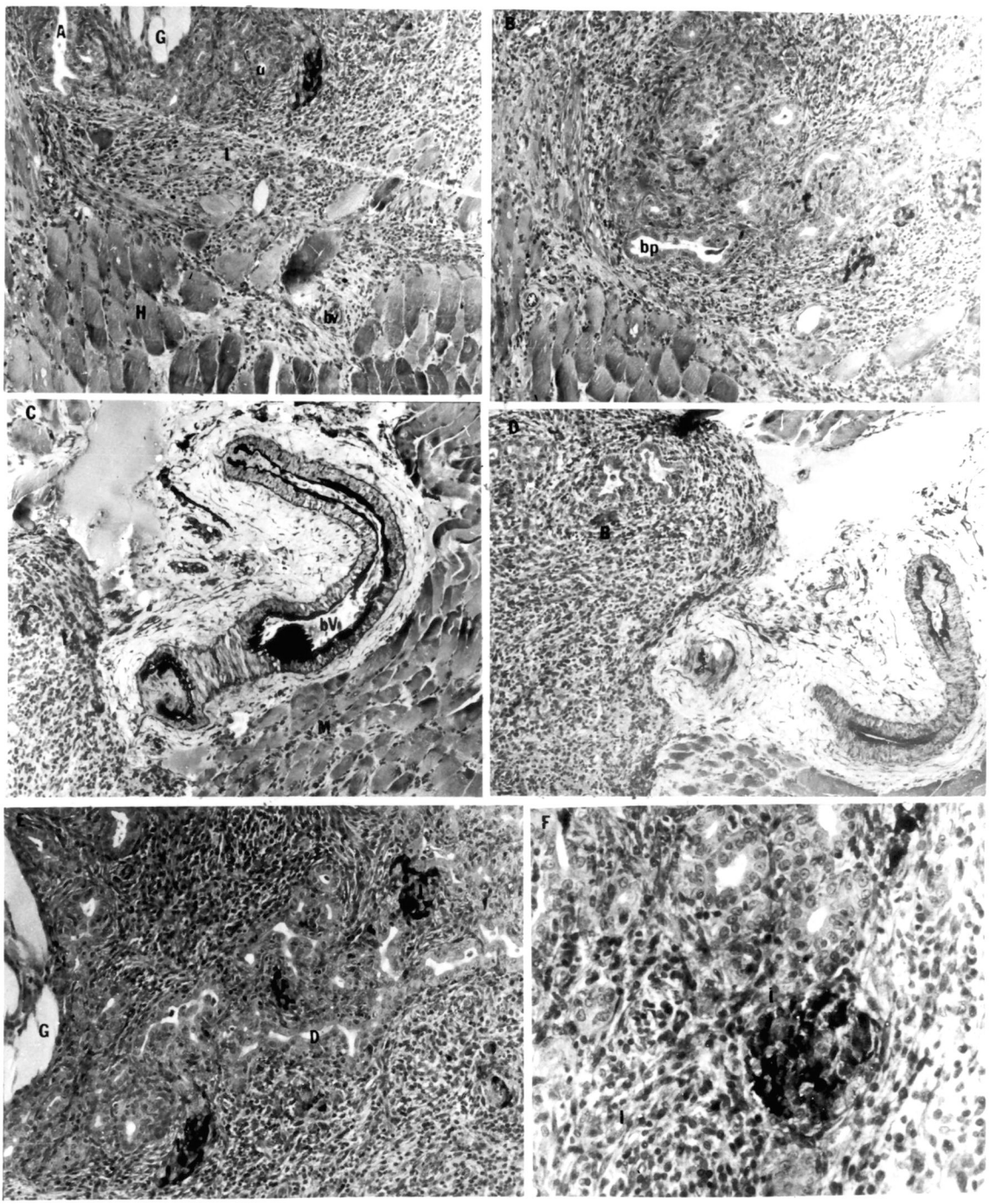

A
G
a
t
H
bv
B
bp
C
bV1
M
D
B
G
D
F
I
I

**Figure 11A-11E:** Diabetic Implant Recipients. Ten day cultured implants taken from diabetic recipients after seven or thirty days. Aldehyde-Fuchsin staining.

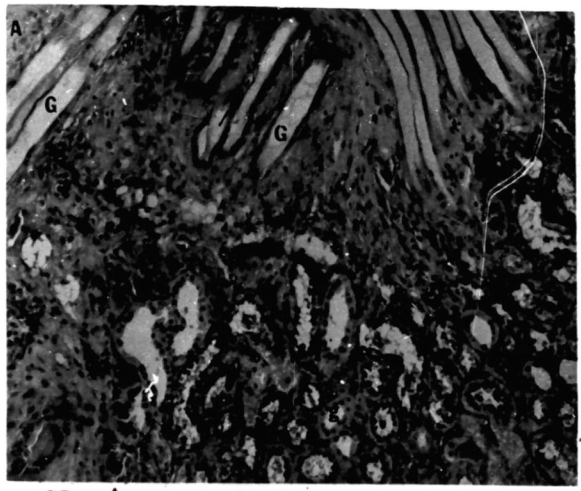
**Figure 11A:** Shows tissue from diabetic animal which had implant to kidney capsule site for thirty days. Grid (G) spaces are seen surrounded by implant (I) tissue which has no obvious pancreatic cellular morphology. Nephritic proximal tubules (g) are seen. x148.

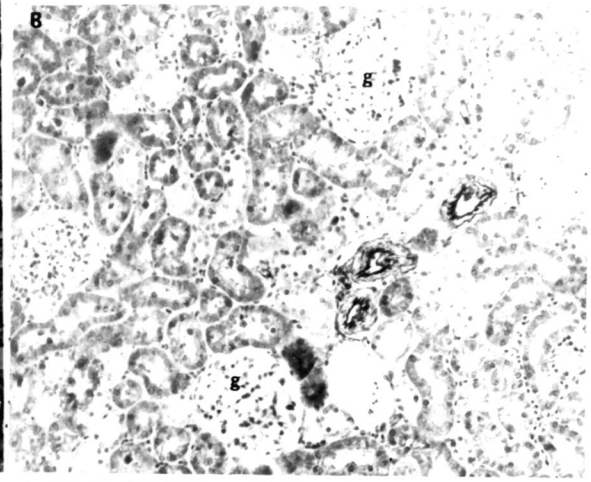
**Figure 11B:** Tissue from diabetic host after seven days in vivo in the kidney capsule site. Nephritic glomeruli (g) noted which indicate the brittle diabetic state. x183.

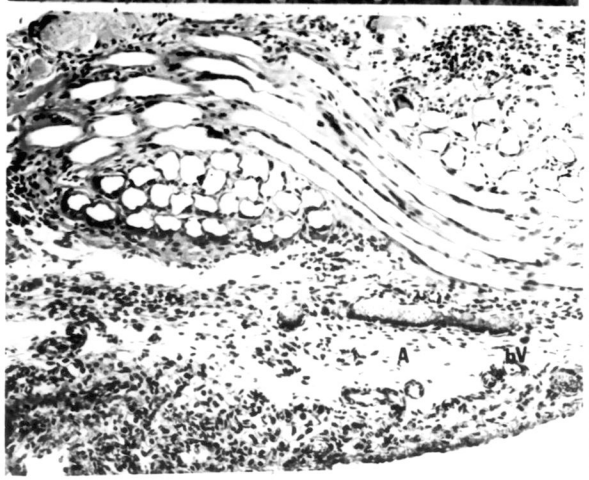
**Figure 11C:** Tissue from thirty day implant to diabetic host in the adipose site. Adipose cells are noted (A) below grid spaces (G). Implant tissue containing blood vessels (bv) contains no recognizible islets. x148.

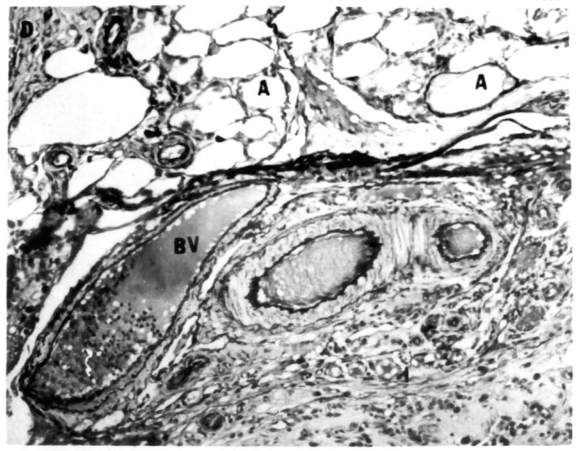
**Figure 11D:** Tissue from thirty day implant to diabetic host to adipose site. Fat cells (A) are seen next to implant tissue (I) which is richly vascularized (bv). x131.

**Figure 11E:** Tissue from one week implant to diabetic host in subcutaneous site. Subcutaneous tissue containing adipose tissue (A) and connective tissue (CT) is noted integrated with implant tissue (I). The implant contains acinar ducts (D). No granulated islets are seen. x147.


A
G
G


B
g
g


A
BV


D
A
A
BV

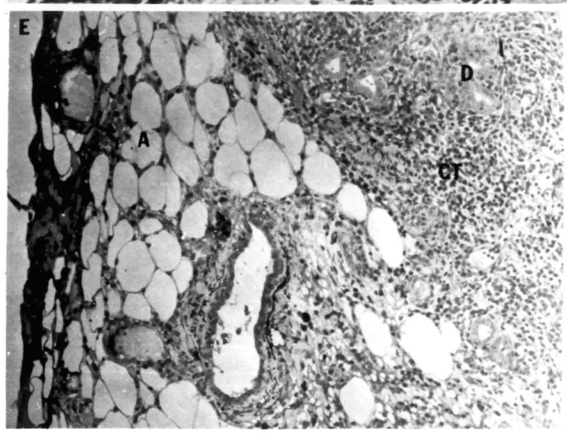

E
A
D
CT

**Figure 12A-12D:** Seven day cultured implants retrieved from diabetic or control hosts after thirty days *in vivo*. (Group II, IVa).

Figures 12A-12B: Tissue at grid level showing grid spaces (G) and homogeneous connective tissue (CT) replacement of implant tissue. No endocrine tissue noted in this control animal. x137. Aldehyde Fuchsin; x148 Haematoxylin Eosin Y staining. Kidney capsule site. (Group II).

Figure 12C: Tissue from subcutaneous site at grid level in this control animal. No recognizible implant endocrine tissue seen, x137, Haematoxylin and Eosin Y stain. (Group II).

Figure 12D: Same as 12C except adipose site in diabetic host. Numerous vascular structures noted (bv) in the adipose tissue (A). x147, Haematoxylin and Eosin Y staining. (Group IVa).

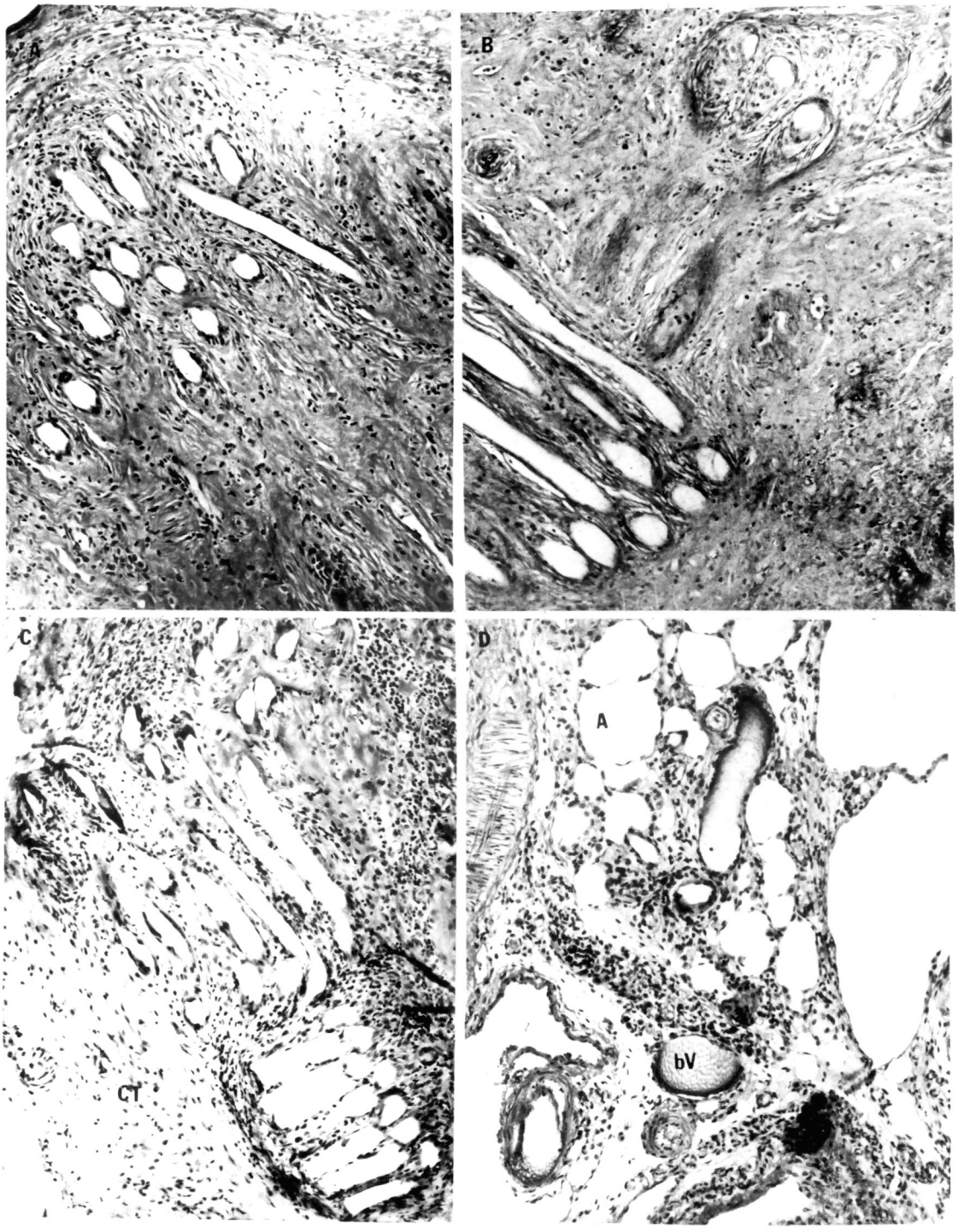
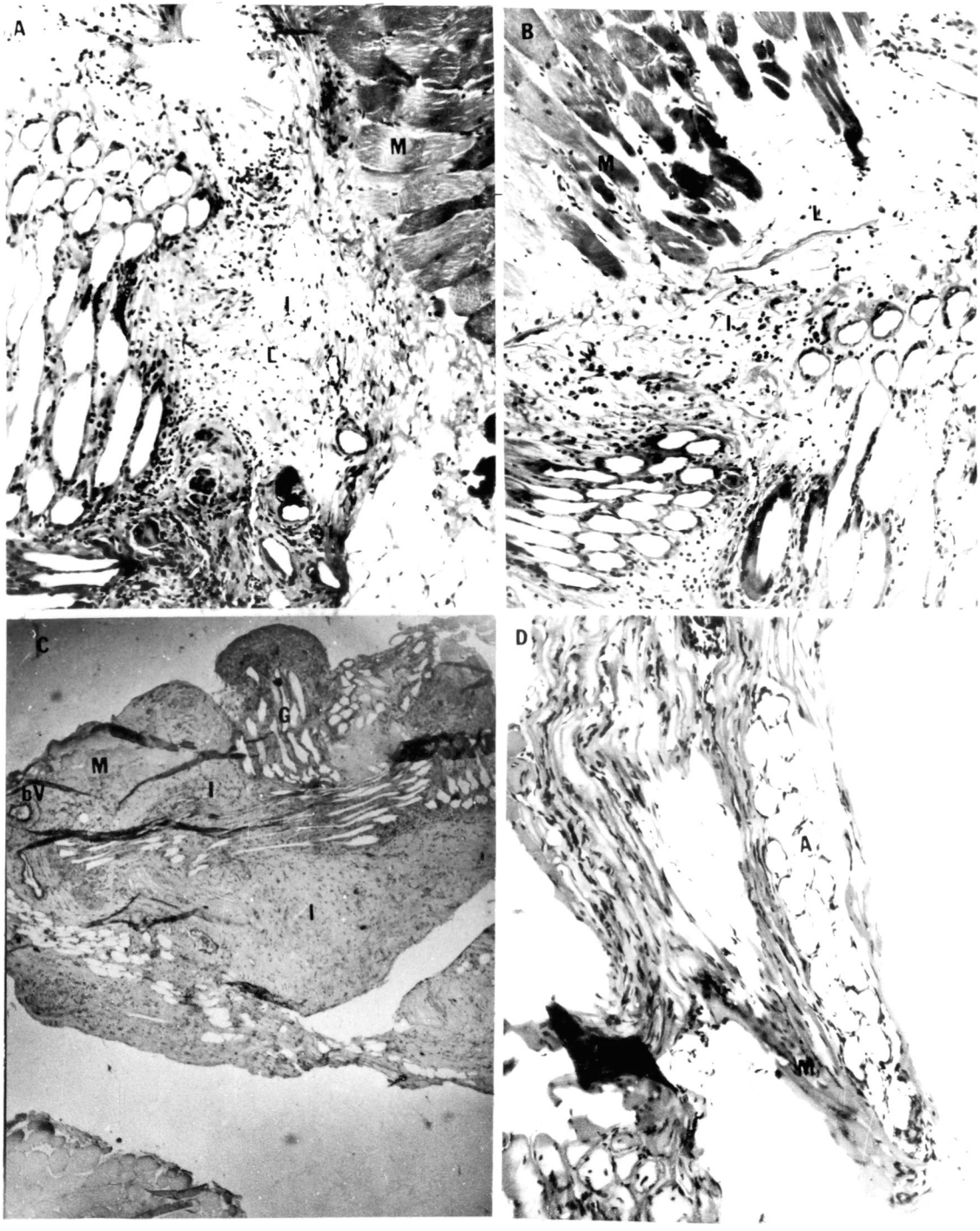

A
B
C
CT
D
A
bV

Figure 13A-13D: Tissue from thirty day implants to diabetic and normal hosts (Group IVa and IVb). Intramuscular site.

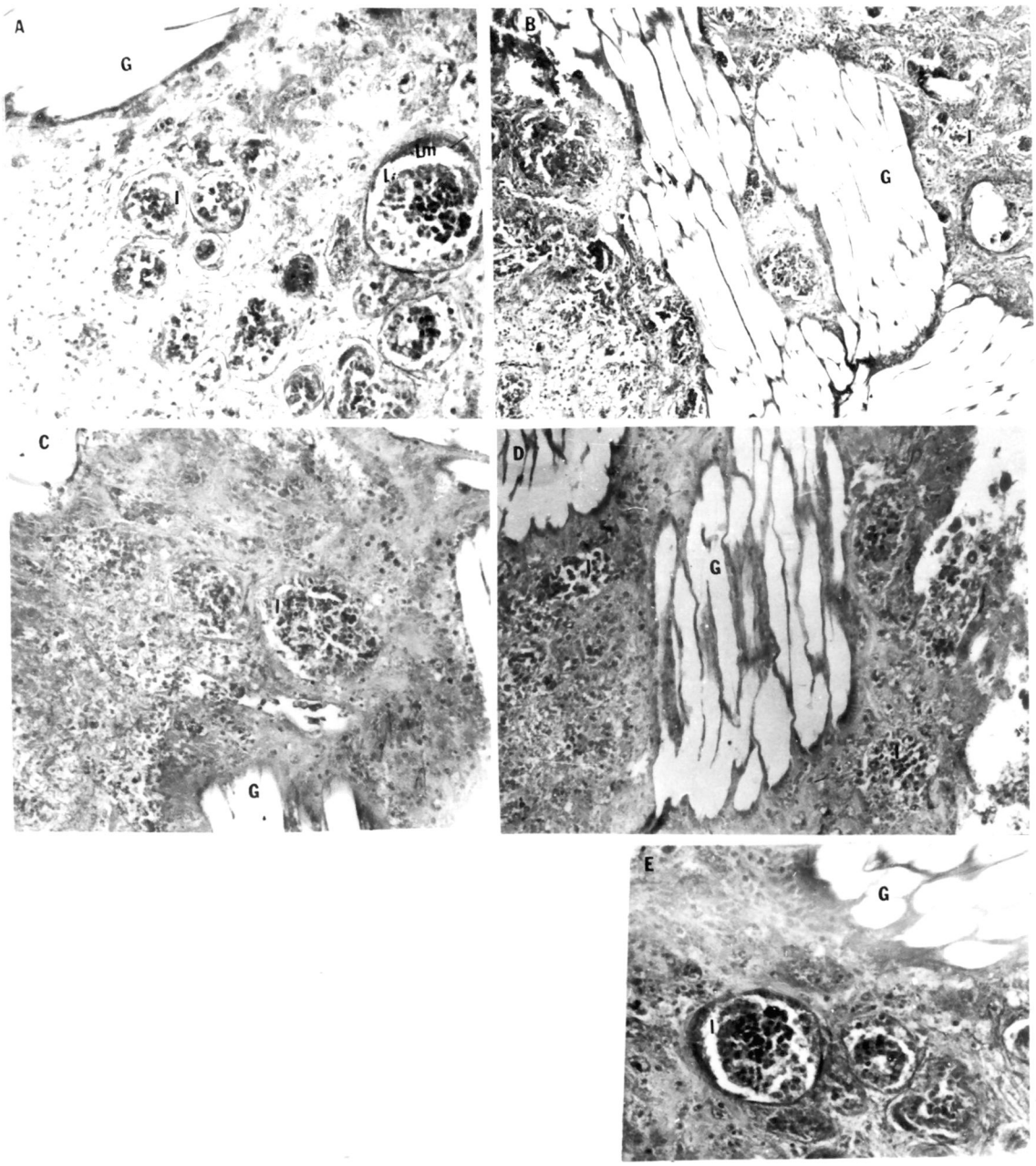
Figure 13A-13B: Showing good integration of implant (I) with muscle (M) at grid level (G). Hypertrophic islets were seen. Numerous lymphocytes (L) are present. x148 Haematoxylin Eosin Y staining.

Figure 13C: Same as 13A and 13B except Aldehyde-Fuchsin staining. Blood vessels (bv) are noted in implant (I) at grid level (G). Host muscle tissue is noted (M). Structures are present which may represent islets of Langerhans (I above grid spaces). x59.

Figure 13D: Same as 13A-13B. Intramuscular site. Some fat cells are noted (A). Host muscle cells are present (M). x192.


A
M
I
C
B
M
L
I
C
G
M
dV
I
I
D
A
M

Figures 14A-14E: Thirty day cultured explants. Numerous moderately granulated islets are seen (I). No typical exocrine acinar tissue is noted. Islets appear to have retracted from limiting membrane (LM). Grid spaces (G) are seen. x226, x143, x240, x224, x152. Aldehyde Fuchsin staining.


A
G
I
Lm
B
G
I
C
I
G
D
G
E
G
I

# GRAPH OF BODY WEIGHT CHANGES OVER TIME

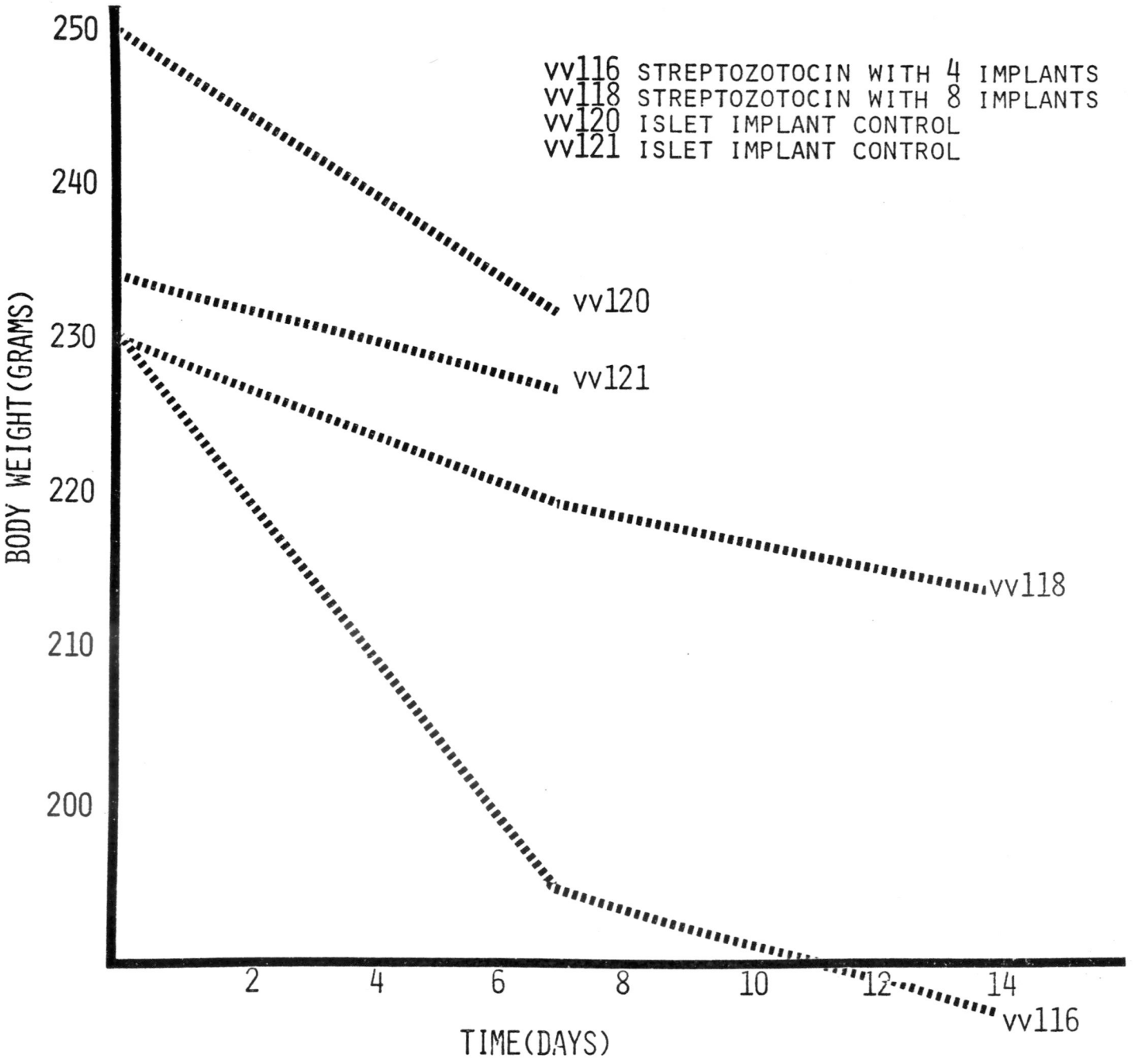



FIGURE 15

# HISTOGRAM OF BODY WEIGHT CHANGES BY GROUP

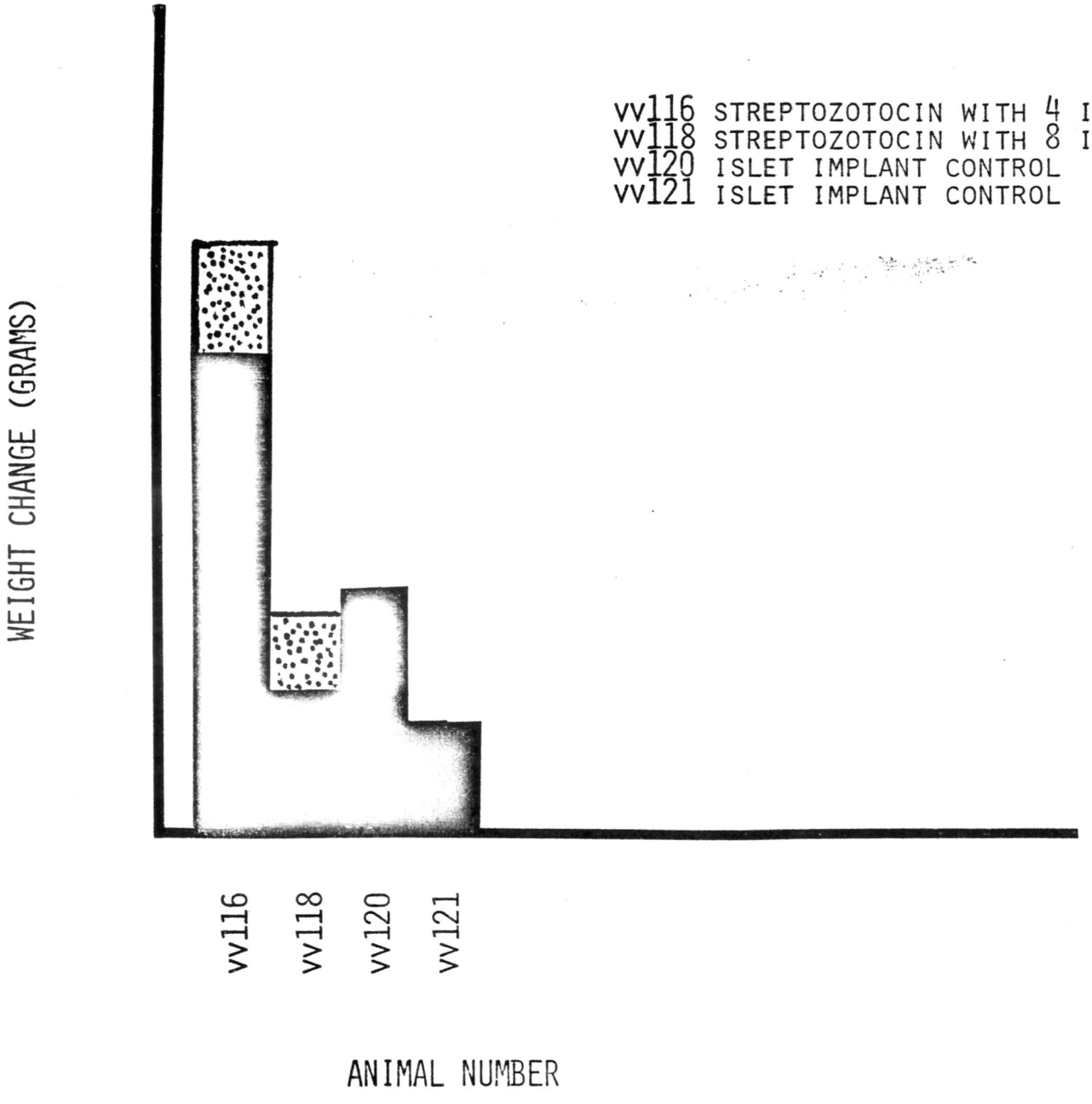



FIGURE 16

## DISCUSSION

Previous transplantation trials with whole pancreases have failed due to immunological rejection (Gliedman in Goetz, 1974) and to surgical anastomosis problems. An approach other than radical whole organ transplantation was indicated and a trend toward more basic research developed. Thus, various techniques and modifications have been attempted in an effort to regulate blood glucose levels and to curtail the diabetic syndrome. Inconsistent results in diabetes therapy with insulin and other agents have prompted research into the feasibility of pancreatic islet transplantation.

Anatomically, the fetal rat or adult rat pancreas is so diffuse that whole organ explantation and transplantation is difficult at best. Further, transplantation of almost any tissue presents an immunological problem. Leonard et al. (1973) and others (Goetz, 1974) have shown that implant rejection occurs in histoincompatible strains of rats. The administration of antilymphocyte serum (ALS) decreases rejection in partially incompatible strains. Yet, when histoincompatibility is great, the injection of pure islets leads to accelerated rejection of a prior allograft (Goetz, 1974). Therefore, islets appear to be both vunerable to immunologic rejection and to tissue death themselves. No apparent success has been demonstrated in graft acceptance by previous tissue culture of the pancreatic explant.

Although organ culture of implanted tissue does not remove surface antigens, organ culture techniques do offer methods of proliferation and storage of fetal and adult pancreatic tissue. Also organ culture may provide the possibility for physical and chemical communication

between endocrine and exocrine portions and various other organ components which would not be available through tissue culture or noncultured material.

The length of culture time for pancreatic tissue varies depending on experimental design. It has been determined that the minimal length of time for explant adhesion to grid and explant acclimation to the unnatural in vitro environment is two to four days. Fibroblastosis begins on day one and proceeds through the ten day culture period as the culture continues to grow over the grid (Schweisthal, personal communication). During this time, the morphological integrity of the acinar tissue is lost, yet islet morphology is maintained. Results from this study agree with this observation. This phenomenon seems to be related to the age of the pancreas at explantation (Schweisthal et al., 1965 and Hegre et al., 1973).

Several investigators have shown that normal endocrine pancreatic ontogeny and morphogenesis occur in organ culture (Schweisthal et al., 1963 and 1965); (Brown et al., 1974); Hegre et al., 1973) and (Picket et al., 1972). The potential for pancreatic endocrine primordium to undergo differentiation is age dependent (Hegre et al., 1973).

Fetal rats of 18 days post-conception were chosen for explantation in this study since fetal pancreas at this age contains all the pancreatic cell types, blood vessels, and nerves found in the adult pancreas and is known to show developing ducts, acini, and islets (Schweisthal et al., 1965).

By explanting 18 day fetal rat pancreas and culturing it for ten days, the explant was approximately equilivalent developmentally to a

22.5 day post-partum rat pancreas (Schweisthal et al., 1963). This fact suggested that neonatal pancreas or cultured fetal pancreas of this age might provide a suitable starting material for transplantation into diabetic and non-diabetic hosts.

The watch glass method of organ culture described previously by Schweisthal et al. (1965) and Wells et al. (1967) was used in this study since consistantly successful culture trials have been previously reported. This technique offers a dynamic culture system through transfers to fresh medium every other day which parallels research with human pancreatic tissue (Goetz, 1974).

Ten Day Cultures: Normal histological development of the endocrine component of the explant continued during the ten day culture period. This was evidenced by granulated islets, by islets in various stages of development. Schweisthal (1963 and 1965) and Hegre et al. (1973) have shown that acinar tissue is not maintained well in culture as is substantiated by the present results. Apparently no acinar-endocrine interaction was necessary for endocrine function.

Group I, Grid Implant Controls: These controls were made to determine if the rayon acetate grid would alter or affect the host. Apparently grid-tissue compatibility was good since no evidence of inflammation or pathological change was observed. Therefore, the rayon was an innoquous carrier for the cultures. In addition, this grid material served an an excellent matrix for physical manipulation of the cultures. Previous reports of intraportal injections of isolated islets and subsequent recovery difficulties illustrated a problem which has been solved by use of rayon acetate grids. By implantation of pancreatic

primordium on grids, the problem of vessel occlusion caused by adhering islets was avoided. After implant recovery, the grids were placed in acetone and the rayon acetate was dissolved. This is a mild step in the histological preparation which does not damage the tissue and leaves spaces in the tissue in place of the grid. Thus, there was no difficulty during sectioning. Also, grid level location of pancreas for positive identification of culture was facilitated by presence of of the grid spaces.

Grid II, Beginning Controls: Since the host animals in this group were not diabetic, no unusual stress was applied to the implants. These animals were included to provide a base line for the implant environment. Normal cytodifferentiation and histogenesis in these implants were indications that this system was adequate to study implanted endocrine pancreas and its effect on diabetes mellitus.

Group III, Diabetic Controls: Since these animals received streptozotocin (90 mg/kg) and no grids or implants, they served to test the effectiveness and time course of action of the diabetogenic agent. Streptozotocin is a relatively fast acting drug that causes destruction of beta cells within the first three days of injection. This condition is seen as a model similar to human juvenile diabetes.

The use and action of streptozotocin has been well documented (Junod et al., 1969 and Golden et al., 1971). The gross appearance of severe diabetic symptoms within 72 hours in all animals injected indicated that the streptozotocin was effective. The rats in this group were difficult to bleed due to hemoconcentration. The odor of ketones in the urine was also present.

Group IVa, Diabetic Implant Recepients: The few previous attempts to implant cultured and uncultured pancreas and isolated islets have brought transient results toward symptomatic reversal of diabetes. Kemp et al. (1974) performed pancreatic isografts, using isolated islets, into three sites in recepient streptozotocin induced (65 mg/kg) diabetic Lewis rats. A subcutaneous injection of islet isolate did not modify the diabetic state over the 80 day period studied. Intraperitoneal injections failed to alleviate abnormal urine volumes, blood glucose, and urine glucose values in contrast to the experience of Leonard, Hegre, and Lazarow (1973). Intraportal injections proved best when judged on the basis of lowered blood glucose levels from 500 mg% in controls to 100mg% in the intraportal group (Kemp et al., 1973). Islet recovery was difficult in all sites. Ballenger and Lacy (1972) also report islet recovery problems in their isograft experiments. Diffusion of islets throughout the abdomen, when injected intraperitoneally, prevented islet tissue recovery for histological examination (Ballenger and Lacy, 1972) and caused death (Chick in Goetz, 1974), presumably due to the large number of cells injected. Thus, although partially effective toward normoglycemia in rat models, the intraportal route is dangerous not only from a surgical viewpoint but also from mass-volume-ratio standpoint.

The problem of islet placement and subsequent recovery was avoided in this study by culturing the fetal rat pancreas on rayon acetate grids.

As seen from previous implantation work (Ballenger and Lacy 1972 and others) the ideal implantation site would be one offering maximal survival of the implant, least surgical trauma at implantation, and ready monitoring of the implant for tissue necrosis or atrophy.

It was found in this study that cultured rat pancreatic primordium can be affixed to a rayon grid and implanted to one or more of four sites with adequate host-implant tissue integration. The sites used in this study were chosen on the basis of the above information and were evaluated for success on the basis of revascularization, host-implant integration, rejection or inflammation, and necrosis. Other criteria used in evaluation were implant site protection by host anatomy, and ease of implant recovery. All these criteria are of important consideration in terms of human pancreatic transplantation trials.

The kidney subcapsular site proved to be a highly feasible implantation site since the kidney offered a natural physiological niche and it provides excellent revascularization and provided easy visualization. This serous membrane protected and secured the implant to the kidney while it provided a mode to facilitate implant issertion and removal without damage to the kidney proper.

Microscopically, the rich blood pools illustrated this good vascularity. Further, the lymphocytes and macrophages were indicative of early immunological rejection. Tissue infiltration by these cell types indicated established rejection. The rejection phenomena could be virtually eliminated in experimental animals by use of more homozygous genotypes for the cell surface antigens. Highly inbred animals are available commercially. However, the animals used in this study were not inbred.

The significant B-cell granular staining seen in Group IVa implants after recovery demonstrated that the islets were functional and were

actively synthesizing insulin in response to the artificially induced excessive hyperglycemia. Further indication of transplant viability was the ability of islets in this group of implants to continue cyt-differentiation in vivo as was evidenced by the groups of small islets near ducts and spatially separated from the larger islets.

Although grid recovery was more difficult from the AP than from the KC site due to its amorphous nature, the pink fat is richly vascularized and affords a good transplant environment. The difference in revascularization and tissue integration was not significantly great from the KC site. Thus, the adipose implant site also offers strong potential for human clinical applications. Histologically, the high degree of B-cell granulation was indicative of optimal islet surviorship in these Group IVa animals.

The less revascularization noted in implants placed in subcutaneous sites (SC) was indicative of less implant site suitability. The subcutaneous tissues were more dense than pink fat and less integration of these tissues with the implant was noted. The yellow fat found in the SC site lacked a high degree of vascularity. In addition, a subcutaneous site would be more easily traumatized by accidental blows in the case of human transplants. These factors made the SC site less desirable as an implant recepient environment.

The fourth implant site was the intramuscular site (IM). Since only moderately granulated islets and little revascularization was noticed, the IM site would be ranked fourth in degree of implant preference. Good host-implant integration was noted at grid level which would indicate that, before immunological rejection was manifest, the

implant survived well in the IM site. This site would perhaps not be attractive for human use as muscular contraction would be chronically traumatic. Thus, from the histological data presented in this study and implications relative to future human implantation trials, it appears that the kidney capsular and visceral fat sites would more closely approximate the normal *in vivo* anatomical and physiological environment for the implanted pancreas.

Group IVb, Diabetic Implant Recipients: Thirty day implants. Those implants recovered from diabetic and normoglycemic animals after thirty days *in vivo* had degenerated. This necrosis, exhibited by the presence of tissue replacement by fibrous connective tissue and of lymphocytes and macrophages, was indicative of an immunological rejection process.

Considering the data from Group IVa and IVb, for a subimmunological period, the allografts were maintained histologically. Presumably the beta cells were challenged to synthesize and secrete insulin in response to elevated blood sugar levels in the diabetic and non-diabetic hosts.

Group V, Long Term Cultures: The fact that the explants maintained for thirty days in organ culture demonstrate intact islets is indicative that organ culture is a workable technique which can be used to preserve and manipulate fetal pancreatic tissue for implantation.

Other workers have shown a decline in A cells and an increase in B cells throughout long term culture. This would be logical since the *in vivo* functioning of the A and B cells is antagonistic. D cells in long term culture (30 days) tend to be maintained (Cecil Frost, 1975, oral communication).

Thirty day cultures demonstrated distended ductular elements similar to those others have observed in the seven day explants. During normal pancreatic development the duct epithelium has the potentiality to differentiate into acinar cells and into islet cells in proportionate ratios. Thus, the ductular element becomes acutely distended and apparently crowds out the acinar-islet tissue mass, which apparently becomes necrotic and is lost. This ductular distension was perhaps caused by a hydraulic effect due to fluid collection within these ducts which causes the ductular epithelial cells to stretch, become thin, and thus no longer form islets or acinar tissue. Further, there could be a loss of a particular ingredient in the media or a biochemical maturation could occur such that a need for an additional deficient metabolite would arise. At this point, differentiation would cease. There could be a loss of communication between ductular elements and islet cells. Evidence for this communication was seen as smaller new islet cells arose in small strings of cells near ducts in the seven day implants. Obviously, differentiation of the implanted fetal rat pancreas continued when the culture was implanted into the diabetic and non-diabetic hosts. These cells were seen more readily in the implant control animals presumably since no extraneous diabetic stress was present.

There was an increased risk of contamination for the extended culture period, however these hazards were reduced by careful handling.

From this study it can be assumed that transplanted pancreas retards weight loss by controlling blood glucose levels. The increased weight loss after one week can be attributed initially to over

degranulation of beta cells and secondarily to immunological rejection of the implant.

The Sprague Dawley rats used in this study were litter mates with similar but not identical genotypes. These implantations were thus allografts. Unfortunately, human isografts are rare and the human pancreatic allograft is likely the working model for future human implantation.

With future technical improvements diabetic patients could be tissue typed and receive highly specific allografts of stored islets. These islets could be frozen for long term storage or could be placed in tissue culture for short term storage. Short term culture has been shown to be feasible with islet cell mass increases of 12% in culture (Hegre et al., 1973). Significant refinement of islet isolation techniques has been done by Lacy et al. (1968) and others to make culture of significant numbers of relatively pure islet preparations possible. Implantations with these preparations could be done simply and would require little immunosuppression. Tissue mobility would be facilitated by post-cryogenic culture on rayon grids for at least two days.

Such a system would mimick the in situ minute-by-minute fine tuning control of blood glucose levels by the normal pancreas. Thus, the long term complications of retinopathy, neuropathy, and peripherial vascular disease would be perhaps alleviated as well as the more symptomatic hyperglycemia.

At best, future clinical trials toward the treatment of diabetes mellitus must involve prior development of tissue typing, mass islet

harvesting and storage techniques, and implantation into suitable diabetic models. The present study was performed as a part of this needed basic research.

# APPENDIX A: TYRODE'S SOLUTION

1. Preparation
   a. Measure 250 ml of glass distilled water into a flask large enough to hold over 1000 ml. Add each following compound individually and dissolve completely. Add more water as needed to totally dissolve chemicals. Total amount of water must equal 1000 ml. Measurements of water and chemicals must be accurate.

   b. Chemicals

| | | |
|---|---|---|
| i. | NaCl | 8.0 gm. |
| ii. | KCl | 0.2 gm. |
| iii. | $CaCl_2$ | 0.2 gm. |
| iv. | $MgCl_2$ | 0.1 gm. |
| v. | $NaH_2PO_4$ | 0.05 gm. |
| vi. | glucose | 1.0 gm. |
| vii. | Dist $H_2O$ | 1000 ml. |

   c. Place 100 ml amounts into 125 ml Erlenmeyer flasks (10), stopper with gauze plugs, cover with metal cap and autoclave for 30 minutes. At end of this time, push metal caps down to secure seal, allow to cool and place in refrigerator for storage.

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