THE EFFECT OF PANCREATIC ISLET CELL ALLOTRANSPLANTATION

ON DIABETIC RETINOPATHY IN RATS


by


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# ACKNOWLEDGEMENTS

# ABSTRACT

The effect of pancreatic islet cell allotransplantation on
the development of microvascular changes in the diabetic
retina was evaluated using light and ultrastructural
qualitative and quantitative morphologic methods and the
trypsin digestion technique in the streptozotocin induced
diabetic rats. The animals were divided into the following
groups:

1) Intraportally transplanted, rejected graft (n=5)

2) Intracerebrally transplanted, rejected graft (n=4)

3) Intraportally transplanted, accepted graft (n=6)

4) Intracerebrally transplanted, accepted graft (n=4)

5) Diabetic non-transplanted (n=5)

6) Non-diabetic controls (n=5)

All animals were sacrificed at the age of 18 months. The
graft accepted and control groups showed euglycaemia. These
groups also showed a normal body weight gain. A significant
increase in the basement membrane thickness, increased
pericyte profile area, diminished lumenal area, increased
number of degenerating pericytes and loss of pericytes in
the retinal capillaries were noted in the diabetic
non-transplanted group. The animals with successful
allotransplantations did not show these changes and were not
different from non-diabetic control animals. On the other
hand, groups with unsuccessful allotransplantation showed
changes similar to the diabetic non-transplanted group.

These results led to the conclusion that pancreatic islet cell allotransplantation has a preventive effect on the development of retinal microvascular changes caused by diabetes in the streptozotocin induced diabetic rat.

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# 1. INTRODUCTION:

## I.Background:

Diabetes mellitus is an ever increasing, common, chronic disease. Statistical evidence shows that the diabetic population doubles every 15 years, (Report of the National Commission of Diabetes to the Congress of United States, 1975). Thanks to the quality of modern medical care, diabetics can look forward to a longer life span. With the increasing longevity of diabetic patients, chronic complications of diabetes mellitus such as retinopathy, neuropathy, nephropathy and atherosclerosis are becoming more common. The eye suffers a number of diabetic complications, diabetic retinopathy being the most important.

Diabetes mellitus is the most important systemic disease causing blindness. The average incidence of retinopathy in diabetics is 52% (L'Esperance and James, 1981). Diabetic retinopathy is the most significant cause of blindness among diabetics accounting for more than 70% (Caird et al., 1969). Retinopathy accounts for a 25-fold increase in the incidence of blindness in patients with type-I diabetes compared with the age-matched non diabetic population (Report of the Second National Diabetic Research Conference, 1984). Diabetic retinopathy is the leading cause of new blindness in the 30 to 60 year age group (Amos, 1974 and Caird et al., 1969).

The rationale for the treatment of diabetic

retinopathy is twofold i) to treat the underlying disease, in order to prevent the development of eye complications and ii) treatment directed to the specific complication when it is established. The former mode of treatment is preferable, since it has as its goal to achieve a physiological insulin regulation. Presumably if a normal glucose homeostasis could be provided, the development or progress of the secondary complications affecting the eye, kidney and the nervous system might be prevented or halted (Sutherland, 1983). The ideal approach for the replacement of insulin may be achieved by successful transplantation of the whole pancreas or pancreatic islet cells, since there is evidence to suggest that good metabolic control acheived by multiple daily doses of insulin reduces the severity of diabetic complications, such as retinopathy (Engerman et al., 1977).

In the introduction to follow pathological features and the present state of the pathogenesis of diabetic retinopathy as well as experimental models for this complication will be reviewed. The retinal structure and ultrastructure and the present state of the role of pancreatic and islet cell transplantation in the prevention of the secondary complications of diabetes will be discussed.

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II.Diabetic Retinopathy in Human:

A. Pathology:

Diabetic retinopathy can be divided into background or non-proliferative and proliferative retinopathy. Background retinopathy consists of capillary microangiopathy, venous abnormalities, intraretinal haemorrhages, hard exudates, nerve fiber layer infarctions (soft exudates) and macular changes. The features of capillary microangiopathy include loss of pericytes, basement membrane thickening, microvascular obstruction and permeability changes, capillary non-perfusion, microaneurysms and intraretinal microvascular abnormalities (IRMA). The venous abnormalities consist of dilatation, duplication and beading of retinal veins and central or branch retinal venous occlusion. The hard exudate is a collection of lipid and lipoprotein from leaking capillaries, usually in the outer plexiform layer of the retina. Focal areas of retinal capillary non perfusion can damage axons in the nerve fiber layer causing interruption of axonal transport and axonal swelling. Histologically, focal areas with infarcted neurones appear as cytoid bodies (Murphy and Patz, 1983). The macular changes consists of macular edema with progression to macular retinoschisis, macular hole or macular ischemia due to retinal vascular occlusion. The IRMAs include the dilated, telangiactatic and leaky capillaries shunting blood from arterioles to venules

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in the areas between perfused and non perfused retina. IRMA, venous occlusion and nerve fiber layer infarction have also been grouped under preproliferative retinopathy (Murphy and Patz, 1983).

Proliferative retinopathy consists of neovascularisation and fibrous tissue proliferation which can be intraretinal, on the retinal surface, on the optic disc or manifested as elevation of the disc or new retinal vessels in the vitreous. Proliferative retinopathy may lead to preretinal or vitreous haemorrhage and tractional retinal detachment.

It has been suggested that increased permeability of the blood-retinal barrier at the level of the endothelial cell of small retinal vessel is an early abnormality of the capillary vasculature. A preceeding alteration of the retinal pigment epithelium or of the retinal neuronal tissue may be responsible for the permeability defect in retinal vessels (Cunha-Vaz, 1983).

## B. Pathogenesis:

A variety of factors compromising blood supply, oxygen transport and delivery are involved in the pathogenesis of diabetic retinopathy (Ashton, 1983). It is generally agreed that hypoxia may be a key factor responsible for diabetic retinopathy (Aston, 1963). Hypoxia in the diabetic retina is produced by a variety of factors (Little, 1981) such as, (i) reduced level of 2,3 diphosphoglycerate in red blood cells,

(ii) an increase in hemiglobin $A_1C$, (iii) changes in the blood elements leading to increased blood viscosity and (iv) increased polyol pathway activity.

(i)  In early and poorly controlled diabetes, a reduction of 2,3 diphosphoglycerate in red blood cells is caused by a decreased level of plasma inorganic phosphate. The oxygen releasing potential of 2,3 diphosphoglycerate deficient red blood cells is reduced and a supra-normal oxygen binding power impairs oxygen release and causes hypoxia in the target tissue.

(ii) There is an increase in hemoglobin $A_1C$ due to non-enzymatic glycation of hemoglobin, in which glucose binds to the terminal valine residue of the $\beta$-chain of hemoglobin and blocks the action of the already decreased 2,3 diphosphoglycerate causing a further impairment of oxygen release.

(iii) The retinal microcirculation may be affected by the aggregation of red blood cells, altered plasma protein content in serum and impaired fibrinolytic activity. An increased aggregability of platelets has been noticed, which may be caused by elevated synthesis of platelet thromboxane $A_2$ (Haluska et al., 1982). Hypercoagulability of diabetic platelets in the presence of ADP and epinephrine is associated with an elevated level of factor VIII-related von Willebrand factor. This factor appears to be influenced by growth hormone (Colwell et al., 1976). The role of an elevated growth hormone level was

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first drawn to attention by Hansen (1971). Since growth hormone increases protein synthesis through a secondary factor named somatomedin, the plasma protein levels, including those of fibrinogen and $\alpha$-2 macroglobulin increase (Hall and Luft, 1974). Increased concentration of plasma proteins raises the blood viscosity and the aggregation of red blood cells, which may result in focal obstruction of the microcirculation leading to retinal hypoxia (Little, 1976).

(iv) Altered glucose metabolism is thought to be an important factor in the development of retinopathy. As a result of insulin deficiency and hyperglycemia, excessive glucose is metabolised via an activated polyol pathway. The rate limiting enzyme in this pathway is aldose reductase. The end products of the polyol pathway, sorbitol and fructose, accumulate intracellularly resulting in increased cellular osmotic pressure and osmotic decompensation of the cell (NIH Conference, 1984; Frank, 1984). This series of events may lead to impaired oxygen diffusion and hypoxia in the target tissue. Aldose reductase has been localised in Muller cells, ganglion cells and in the pericytes of the human retina (Akagi et al., 1983, 1984). The same enzyme has also been demonstrated in Müller cells, ganglion cells (Ludvigson and Sorenson, 1980) and cultured microvessels of the rat (Kennedy et al., 1983). Human retinal endothelial cells cultured in a medium having high glucose level exhibited cytotoxic features with accumulation of

PAS-positive granules, cytoplasmic vacuolisation and cell degeneration (Tripathy and Tripathy, 1982). In order to block this pathway, aldose reductase inhibitors are now being investigated clinically and experimentally as to their potential effect in preventing and treating diabetic retinopathy. In a recent clinical trial by Cunha-Vaz et al. (1985) Sudinlac, an aldose reductase inhibitor as well as a prostaglandin synthetase inhibitor, has been shown to prevent the breakdown of the blood-retinal barrier.

Prior to the detection of clinical changes in diabetic retinopathy, however, there is a stage of increased retinal blood flow and vasodilatation. An autoregulatory response to metabolic abnormalities in diabetic patients may be responsible for this change (Little, 1981).

Thickening of the capillary basement membrane has been accepted as the dominant feature of diabetic microangiopathy. Possible biochemical mechanisms include enzymatic glycation of hydroxylysine residues of collagens by glucose and/or galactose via the -OH groups of the amino acid (Spiro and Spiro, 1971) and non-enzymatic glycation of lysine residues by the same sugars at the exposed e-NH2 groups (Cohen et al., 1980; Urbanowski et al., 1982). Other possibilities that have been suggested include increased synthesis of collagen or other basement membrane proteins such as laminin or fibronectin, either as a primary event or as a compensatory mechanism secondary to decreased production of basement membrane proteoglycan (Szarfman et

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al., 1982). Increased polyol pathway activity may play a role, as it has been shown that galactosemia induced capillary basement membrane thickening can be prevented by aldose reductase inhibitor (Robinson et al., 1983; Frank et al., 1983). It is still not clear how aldose reductase affects the above mentioned activities; one possible explanation is that sorbitol and other sugar alchohols may alter the activity of a critical biochemical step (Frank, 1984).

The thickening of basement membrane is probably associated with endothelial cell damage and plasma leakage (Ashton, 1974). Thickening of capillary basement membranes in diabetes has been considered as a proliferative response of the cellular constituents of the capillary to injury (Williamson and Kilo, 1983). Repeated endothelial cell death and regeneration with retention of old and the addition of new layers of basement membrane may be a factor in the production of basement membrane duplication and thickening (Vracko and Beneditt, 1970). McMillan (1983) suggested that due to modified viscous properties of the red blood cell membrane such as reduced deformiability, the red blood cells may exert a greater mechanical force on the capillary wall which may be responsible for basement membrane thickening. However, it is not clear as to what extent the stiffening of red blood cells play a role in retinopathy (Williamson and Kilo, 1983).

Areas of endothelial cell degeneration and

proliferation have been noted in diabetic retinopathy (Bloodworth, 1982). Endothelial cell damage has been thought to be a factor in the breakdown of blood retinal barrier (Cunha-Vaz, 1983).

The loss of capillary pericytes is one of the early features of the disease (Cogan et al., 1961). The normal ratio of pericyte to endothelial cells is 1:1 in healthy young individuals. Aging causes a decrease in the number of endothelial cells (Apple, 1981). In diabetic retinopathy this ratio is altered with a progressive loss of retinal pericytes. According to Cogan et al. (1961) this change in the ratio is the key pathological feature of diabetic retinopathy. Chuna-Vaz (1983) is of the opinion that the pericyte damage is secondary to endothelial cell damage. While damaged endothelial cells can easily be replaced by the sliding of the neighbouring cells, the pericytes cannot be replaced due to their special location where they remain trapped in the vessel wall. The weakness created in the vessel wall due to the loss of pericytes may explain the formation of microaneurysms and retinal hemorrhages in diabetic retinopathy.

Microaneurysms are seen near areas of capillary non perfusion and pericyte loss, yet no correlation has been established between microaneurysms and pericyte loss (Oliviera, 1966). However microaneurysms are not pathognomonic of diabetic retinopathy as they are also seen in other retinal diseases such as branch retinal vein

occlusion, dysproteinemic retinopathy and Coat's disease.

Neovascularisation is the dreaded problem of diabetic retinopathy. If untreated it causes haemorrhages, glaucoma and traction retinal detachment leading to blindness. It is initiated by capillary non-perfusion and retinal ischemia in an attempt to revascularise the affected areas. The existence of a possible angiogenic factor was first launched by Michelson in 1948, to explain retinal neovascularisation. It has been postulated that areas of non-perfused retina liberate an angiogenic substance (Patz, 1984). Angiogenic activity has been demonstrated from retinal extract of various species including human (Chen and Chen, 1980). These extracts promote thymidine uptake by vascular endothelial cells and initiate vascularisation in cultured chorioallantoic membranes (Glaser et al., 1980). As to the characterisation of the angiogenic factor(s), an active proteinaceous macromolecule has been isolated from bovine retinal extract (D'Amore et al., 1981). A low molecular weight angiogenic factor, which induces neovascularisation in the chick chorioallantoic membrane, has been isolated from healthy adult cats (Kissun et al., 1982). Autologus retinal implant into rabbit corneal pockets has also been shown to stimulate angiogenesis (Felton et al., 1980). Recently the presence of a low molecular weight substance which inhibits aortic endothelial cell proliferation has been shown in normal as well as in diabetic vitreous (Jackobson et al., 1984).

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Possible pathogenetic mechanisms responsible for diabetic retinopathy are summerised in figure 1 (Chakrabarti et al., 1985).

III. Diabetic Retinopathy in Experimental Animals:

An extensive search has been undertaken to find a
suitable animal model in which diabetic retinopathy can be
produced and studied in relation to severity and duration of
diabetes and in which the effect of various treatment
modalities can be evaluated. Several changes have been
demonstrated in various experimental models with either
spontaneous onset of diabetes or chemically induced
diabetes.

The chemically or surgically induced diabetes in dogs
produce lesions which closely resemble the changes seen in
diabetic patients (Engerman, 1975). In this model
microaneurysms, pericyte degenerations, retinal hemorrhages,
shunts and other changes of the microvasculature have been
found (Engerman, 1982). Recently Arnest et al. (1983)
reported increased preretinal oxygen tension in this model
when they were allowed to breath 100% oxygen, which may be
accounted for by hyperglycaemia causing impaired oxygen
autoregulation. The disadvantage with this model is that it
takes several years before retinopathy develops.

The spontaneously diabetic chinese hamster shows a marked
thickening of capillary basement membranes and pericyte
degeneration (Ghosh et al., 1970). A decreased pericyte
endothelial cell ratio has also been seen in this model
(Federman and Gerritsen, 1970).

In the rhesus monkey, polyol pathway activity has been demonstrated in cultured retinal pericytes. Alloxan induced diabetes in monkeys show microaneurysm 6 to 8 years after onset of diabetes (Gibbs et al., 1969). An immunopathogenic mechanism for diabetic retinopathy and other diabetic ocular diseases has been suggested by experiments performed on rhesus monkeys and rabbits (Shabo and Maxwell, 1976, Shabo et al., 1983).

The spontaneously diabetic BB rat appears to be a good model for the study of diabetic retinopathy. Pericyte and endothelial cell degeneration, thickening of capillary basement membrane, microthrombi, decreased pericyte endothelial cell ratio has been found in this model (Sima et al., 1985). A retinal pigment epitheliopathy which may be responsible for the early breakdown of blood retinal barrier has also been shown in this model (Blair et al., 1984).

Alloxan induced diabetic rats were also found to have basement membrane changes (Kojima, 1971).

The streptozotocin induced diabetic rats appears to be the most widely studied model of diabetes. Endothelial cell proliferation, vascular dialatation (Sosula et al., 1972), microaneurysm, basement membrane irregularities, in the retinal capillaries have been produced in this model (Luenberger et al., 1974). Electron dense deposits in the Muller cells, basement membrane thickening, pericyte degeneration, decreased number of pericyte nuclei has been described (Hori and Muhai 1979, Studer et al., 1976). An

early breakdown of the blood-retinal barrier due to retinal pigment epitheliopathy was also demonstrated in the streptozotocin diabetic rats (Grimes and Laties, 1980). Polsum et al. (1983) failed to find any protective effect against the development of diabetic retinopathy by the use of aldose reductase inhibitor but a reversibility of increased vitreous fluorosence has been demonstrated in this model following vigorous insulin therapy (Kernel and Arnqvist, 1983).

Several authors have shown that galactosemia induced basement membrane thickening of the rat retina can be prevented by aldose reductase inhibitor (Robinson et al., 1983; Frank et al., 1983). This is important since the ultrastructural changes in the galactosemic rat retina are similar to those found in diabetic retinopathy. In the diabetic hooded rats, vitreous fluorophotometry shows an increased vascular pemeability, which however does not diminish after treatment with aldose reductase inhibitors (Krupin et al., 1982). Isolated microvessels (primarily capillaries) from bovine retina and cerebral cortex as well as cultured bovine retinal pericytes and porcine and canine endothelial cells do contain aldose reductase; but aldose reductase may not be a pathogenetic mechanism in the production of retinopathy as the vascular changes are only seen in the retina not in the cerebral cortical vessels (Kennedy et al., 1983).

IV. Structure of the Retina:

The retina in man and rat consists of the following layers from without inwards: (Prince, 1956) (Figure 2)

1)  Pigment epithelial layer.

2)  Layer of photoreceptors.

3)  External limiting membrane.

4)  Outer nuclear layer.

5)  Outer plexiform layer.

6)  Inner nuclear layer.

7)  Inner plexiform layer.

8)  Ganglion cell layer.

9)  Nerve fiber layer.

10) Internal limiting membrane.

The pigment epithelium is formed by a single layer of cuboidal cells resting on a basement membrane that forms part of Bruch's membrane. The inner plasmalemma of these cells consists of regular infoldings of about 1 µm in length. Along the inner surface of the cells there are numerous fringe-like cytoplasmic processes which extends between the outer segments of the photoreceptors. The presence of pigment granules is a characteristic feature of the cells in this layer.

The layer of photoreceptors (rods and cones) is made up by the outer segment of the photoreceptors. A connecting

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cilium joins them with the inner segment.

The external limiting membrane appears as a deeply stained punctate line external to the outer nuclear layer. This is not a true membrane but formed by the adjoining ends of the Müller cell processes. The nuclei of the photoreceptors compose the outer nuclear layer; the rod nuclei being in the inner aspect and the cone nuclei in the outer aspect. The area where the photoreceptors are connected with the dendrites of the bipolar cells is called the outer plexiform layer. The fibers of horizontal, amacrine and Müller cells are also present in this layer. The cell bodies of bipolar, horizontal, amacrine and Muller cells lie in the inner nuclear layer. The Müller cell processes extend from the outer limiting membrane to the inner limiting membrane and have supportive and nutritive functions. The inner plexiform layer contains the synapses of the bipolar and amacrine cells with the ganglion cells. The ganglion cell layer is composed of a single layer of cells except at the edge of the area centralis, where two layers can be found. The axons of the ganglion cells make up the nerve fiber layer. Neuroglial cell and Müller cell processes also form part of this layer. The inner limiting membrane is a thin membrane that forms the inner limit of the retina.

The retina in man and most mammals, including the rat, is vascularised in the inner layers, to the junction between the inner nuclear layer and the outer plexiform layer

(Prince, 1956). The choriocapillaries supply the outer layers of the retina. The main arteries and veins are located in the nerve fiber layer and the ganglion cell layer. The arterioles arise from the main branches either by side branching or by dichotomous branching (Hogan et al., 1971). The retinal vessels in the rat are arranged in two capillary beds, superficial and deep (Bernstein and Hollenberg, 1965). The post capillary venules seem more prominent in the deeper layer while the precapillary arterioles are more allied to the superficial layer (Wise et al., 1971).

The trypsin digested retinal preparation (Toussaint et al., 1961) permits the identification of two type of cells within the capillaries, pericytes and endothelial cells. The normal ratio of these two cell types in a healthy adult man is 1:1 (Yanoff and Fine, 1975). However, the normal ratio in many laboratory animals appears to be lower than that in man (Engerman et al., 1982). The endothelial cells are oriented along the axis of the capillary and have long pale staining nuclei, whereas the pericytes lie outside the endothelium and have round dark staining nuclei (Hogan et al., 1971). Degenerated capillaries appear as bloodless cords and are called mesodermal bridges.

V. Ultrastructure of the retinal capillaries:


The retinal capillaries are lined by endothelial cells resting on a basement membrane. The basement membrane is split to accomodate pericytes and their processes (Hogan et al., 1971). The endothelial cells lie parallel to the long axis of the vessel. The cell body is non-fenestrated and thin except in places where the nucleus is located. The cell junctions near the vascular lumen is of zonula occludens type. The chromatin is evenly distributed in the nucleus with slight aggregation along the nuclar membrane. Numerous pinocytotic vesicles are present along both borders of the cells. Smooth and rough endoplasmic reticulum and free ribosomes are scattered throughout the cytoplasm. The Golgi apparatus and the centrioles are encountered in the vicinity of the nucleus. The mitochondria are relatively small in size and few in number (Toussaint and Dustin, 1963; Ishikawa 1963; Hogan and Feeny, 1963). Ultrastructurally the presence of Weibel-Palade bodies are considered to be specific of vascular endothelial cells. These are rod-shaped cytoplasmic components, consisting of a bundle of fine tubules and enveloped by a tightly fitted membrane (Weibel and Palade, 1964). Although these bodies were identified in the endothelial cells in various species (Matsuda and Sugiura, 1970), some investigators failed to find these bodies (Buzney and Massicotte, 1979; Frank et al., 1978). However,

the function of these organelles is still obscure.

The pericytes encircle the the vessel wall, and wrap it with their processes. They are seen as profiles of the cytoplasm in most electronmicrographs (Hogan et al., 1971). The cytoplasm contains all the organelles described in endothelial cells, except for the Wiebel-Palade bodies, pinocytotic vesicles are also present (Ishikawa, 1963).

Human capillary basement membranes show cavitations which increase in number and size with age (Hogan and Feeny, 1963; Toussaint and Dustin, 1963). Although ultrastructurally the basement membrane of rat and man are similar, no cavitations are found in the normal rat (Hogan and Feeny, 1963; Kissen and Bloodworth, 1961).

VI. Pancreas and Islet Cell transplantation and the
Complications of Diabetes:


   There are a number of promising approaches in the
treatment of diabetes mellitus, pancreatic or islet cell
transplantation being the most important. The interest in
transplantation of the pancreas as a means of treatment of
juvenile diabetes mellitus and as a method for the
prevention and reversal of diabetic complications began in
1960. Largiader et al. in 1967 were the first to
successfully transplant an entire pancreaticoduodenal
allograft and established exocrine drainage to the bowel of
the recipient dog. Initial reports of clinical
transplantation came from Lillehei et al. (1970). They were
able to achieve a normalisation of plasma glucose level
without exogenous insulin in some of their patients.

   Beta cell replacement therapy appears to be the most
logical and comprehensive approach for the treatment of
diabetes mellitus in patients with insulin deficiency and
who are ketosis prone and highly susceptible to the
development of diabetic microangipathy, ocular disease and
neuropathy (Jonasson, 1980). Whether the pathogenesis of
insulin dependant diabetes mellitus is a viral infection, an
autoimmune destruction of the beta cells, an inactivation of
beta cells or some other factors or combination of factors,
transplantation of normal pancreatic islets should be a

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feasible way to achieve a cure. (Jonasson and Hoverstein, 1978).

Several clinical and experimental observations support the hypothesis that the lesions observed in diabetes are secondary to carbohydrate dysmetabolism (Tchobroutsky, 1978). The best possible way to correct this dysfunction may be by islet cell transplantation. The results of islet cell transplantation appears to have beneficial effect on the course of diabetes mellitus and its complications. Sutherland (1981) reviewed experimental islet cell transplantation and came to the conclusion that it can reverse naturally occuring diabetes in animal models which are characterised by beta cell destruction and insulin defiency. Regression of renal glomerular lesions as evidenced by a decrease in mesangial thickening, mesangial deposition of IgM, IgG and complement and urinary albumin excretion has been shown to occur after islet cell transplantation (Steffes et al., 1980; Mauer et al., 1978). Gray and Watkins (1976) were able to prevent the renal complication of diabetes in transplanted rats. Marked prolongation of the survival period, ameloriation of diabetic symptoms and prevention of diabetic cataract have been found in transplanted streptozotocin diabetic rats (Slijepcevic et al., 1975). Improvement of diabetic neuropathy has been seen following transplantation (Orloff et al., 1975). Recently it has been shown that successful islet cell transplantation normalises hyperglycaemia and

ameloriates diabetic nerve dysfunction (Tze et al., 1985).

There are very few reports on the effect of pancreatic or islet cell transplantation on the course of diabetic retinopathy. Krupin et al. (1979) showed a decrease in the vitreous fluoroscence, which is an indicator of breakdown of the blood  retinal barrier, following pancreatic islet cell isograft. Arrest of new vessel formation  and retinal capillary dilatation in transplanted streptozotocin diabetic rats have been observed (Gray and Watkins, 1976). Alloxan induced diabetic rats undergoing whole pancreas transplantation has shown similar findings along with prevention of corneal and iris neovascularisation and cataract formation (Worthen et al., 1976). Naeser and Anderson (1983) have demonstrated that syngenic pancreatic islet cell transplantation has a protective effect on pericyte loss in alloxan induced diabetic mice. Unfortunately detailed morphometric evaluation of the blood vessels are lacking in these reports.

The result of human pancreatic transplantation is encouraging as the ideal homeostatic control of the carbohydrate metabolism. Although clinical application of pancreas and islet cell transplantation is technically difficult, the prospect is excellent. At present, islet transplantation although less efficient is a safer procedure (Sutherland, 1981). The development of kidney graft nephropathy has been shown to be prevented by pancreas transplantation (Bohman et al., 1985). Gliedman et al.

(1978) studying the long term effect of islet cell transplantation concluded that this procedure, although it does not reverse the complications such as advanced retinopathy or atherosclerosis, it may be valuable in preventing progression of these complications. Goetz in 1980 also reviewed the results of human islet transplantations and came to the conclusion that diabetic vascular disease is the compelling reason for the efforts to achieve effective islet transplantation.

## 2. HYPOTHESIS:

The biochemical sequelae of diabetic dysmetabolism are thought to initiate a sequence of structural events causing diabetic complications. Therefore, one would expect that strict control of insulin deficiency and hyperglycemia would prevent the development of such complications.

To test this hypothesis early microvascular changes in the diabetic retina were investigated following the most physiological way of diabetic control, namely transplantation of insulin producing cells.

## 4. SPECIFIC OBJECTIVES:

To investigate:
1) Morphological changes in the pericytes, endothelial cells and basement membrane.
2) Changes in the number of the pericytes and endothelial cells.
3) Changes in the thickness of the capillary basement membrane.
4) Changes in the capillary lumenal, pericyte profile and endothelial cell profile areas.
5) The presence of microaneurysms.
6) Changes in the retinal vascular density.

## 4. MATERIALS AND METHODS:

### I. Animals:

Twenty-nine inbred male Lewis rats of the ACl (AgB 4/4) strain were used in this study. Diabetes was induced in 24 rats at the age of 4 months by intravenous injection of streptozotocin (50mg/kg of body weight, in 0.1M citrate buffer pH 4.5). The other 5 rats were kept non-diabetic for the observation period and used as age-matched non-diabetic controls. Diabetes was defined as fasting blood glucose levels greater than 400mg/dl on three successive occassions. Pancreatic islet cell transplantations were carried out in 19 rats 12.8$\pm$3.4 days (mean$\pm$SD) following streptozotocin injection.

### II. Transplantation procedure:

The transplantation procedure was carried out by Dr. W. J. Tze in the Departrment of Endocrinology, University of British Columbia, Vancouver, B.C.

A. Donors:

Male inbred Lewis rats (Le;Ag Bl/1) with body weights ranging from 350 g to 500 g were used as donors of pancreatic tissue.

B. Preparation of the donor tissue:

Two types of donor tissues were used:

1) Pancreatic pseudoislet (PI) or pancreatic whole islets (PWI) for intraportal transplantation.

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2) Pancreatic endocrine cells (PEC) for intracerebral transplantation.

The animals were anesthetised with sodium pentobarbital (50mg/kg of body weight). The abdomen was opened and the pancreas was dissected out. Pancreatic tissue was digested with collagenase and islets were hand picked under a dissecting microscope. Contaminating acinar tissue and blood vessels were removed using single layer Hypaque-Ficoll separation technique (Tze and Tai, 1976). Clean islets collected at the interface were either cultured at $26^{O}C$ in 5% $CO_2$ for one week prior to transplantation (PWI) or immediately dissociated into PEC with trypsin-EDTA solution. The preparation of PI was carried out by culturing of PEC at $37^{O}C$ in 5% $CO_2$ for 5 days to allow cellular aggregation (Tze et al 1984).

C. Allotransplantation:

For intraportal allotransplantation, a total of 1,500 PWIs or PIs were injected into the portal vein of each ACI diabetic recipient. For intracerebral transplantation, PEC (2-3X10$^6$ cells) were implanted stereotaxically into the right hemisphere of the brain in each diabetic recipient (Tze and Tai 1983). Blood glucose, body weight, 24-hours urine volume, urine sugar and ketone bodies were assayed daily prior to and for two weeks after allotransplantation and at regular intervals thereafter. The islet allografts of 5 intraportally transplanted animals and 4 intracerebrally transplanted animals were challenged with skin graft from

the same donor strain 7-10 days following transplantation.
Rejection was considered to have occurred when the blood
glucose level exceeded 200mg/dl on two consecutive days.

III. Clinical monitoring:

All the animals were shipped to our laboratory one month
following transplantation. They were then kept individually
in air-filtered metabolic cages and fed with rat-chow (Wyane
Lab Blox F-6, Wayne Feed Division, Winnipeg, Manitoba,
Canada.) and water ad libitum. The diabetic animals were
monitored regularly with respect to blood-sugar and
body-weight.

The age of the animals in all groups at the time of
sacrifice was 18 months. The following groups of animals
were investigated.

| Group | | No. in each group |
|---|---|---|
| | Animals with rejected graft: | |
| I | Intraportal transplant | 5 |
| II | Intracerebral transplant | 4 |
| | Animals with accepted graft: | |
| III | Intraportal transplant | 6 |
| IV | Intracerebral transplant | 4 |
| V | Positive controls: | 5 |
| | (Diabetic non transplanted) | |
| VI | Negative controls: | 5 |
| | (Non-diabetics) | |

The animals were anesthetised with sodium pentobarbital (50mg/kg of body-weight) intraperitonially. The thorax was opened and a canula was introduced into the left ventricle directed towards the aorta. They were perfused with 0.1M cacodylate buffered (pH 7.4) gluteraldehyde (2.5%). The total volume perfused was 2.5ml/g of body-weight.

IV. Morphological Technique:

A. Tissue selection:

In order to perform the study in a double blind fashion, all animals were given a code number and the animal identity was unknown to the investigator.

Both eyes were enucleated and post-fixed in the same fixative for 4 to 6 hours at $4^{\circ}$C. Qualitative and quantitative light and electron microscopical studies were performed using the right eye of each animal. The left eye of each animal was used for trypsin digested preparation.

B. Qualitative Studies:

After fixation 5 random radially oriented retinal segments ($1mm^2$) near the optic nerve head were cut and washed overnight in 0.1M cacodylate buffer (pH 7.4). The tissues were post-fixed in cacodylate buffered 1% osmimum-tetraoxide (pH 7.4) for 2 hours, dehydrated in graded alchol and embedded in Epon. Semithin sections (0.5μm) stained with toluidine blue were used for light

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microscopic orientation and detection of any abnormalities such as exudates and haemorrhages. These sections were also used for measuring retinal vascular density (described later).

Ultrathin sections (50-80nm) were cut with a diamond knife on a ultramicrotome (Reichert Jung, Vienna, Austria). The sections were picked up on copper grids (200 mesh) and stained with aqueous uranyl acetate and lead citrate. The grids were examined on a Philips EM 200 electron microscope.

## C. Trypsin Digestion Preparation:

Following fixation, the retina of the right eye was dissected. It was washed overnight in running water and then incubated with 3% trypsin solution (1:250, Difco Laboratories, Detroit, Michigan, U.S.A.) in 0.1M tris buffer pH 7.8 for 30min-1 hour. The retina was then transfered to clean water. The internal limiting membrane was peeled off. The adherent tissue was removed by gentle shaking. The preparations were mounted on glass slides, allowed to dry and stained with PAS-hematoxylin. The preparations were examined light microscopically for microaneurysms and were also used for the calculation of pericyte-endothelial cell ratio (described later).

## D. Quantitative Structural Studies:

All morphometric studies were done with the aid of a Hewlett-Packard 9874A digitizer connected with a

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Hewlett-Packard 9825A desktop computer (Hewlett-Packard Co., Fort Collins, Colorado, U.S.A.).

a) Vascular Density: Ten random areas were photographed from each toluidine blue stained semithin sections of the retina and enlarged to a final magnification of 800 times. The total number of vessels in all retinal layers were counted and the retinal area ($\mu m^2$) was measured. The vascular density per $mm^2$ was calculated as,

$$\frac{\text{Number of vessels X 1,000,000}}{\text{Total area studied } (\mu m^2)}$$

b) Pericyte, endothelial cell ratio: Five randomly selected fields from the retinal vasculature of the trypsin digested preparations were photographed and enlarged to a final magnification of 1600 times. The vascular area ($\mu m^2$) was measured, the number of pericyte and endothelial cell nuclei were counted and their number per $mm^2$ was calculated and the ratio obtained.

c) Endothelial cell profile area (A), pericyte profile area (P), lumenal area (L) and basement membrane thickness(BMT): Twenty randomly selected and transversely sectioned capillaries were photographed from each animal, 10 in the inner nuclear layer and outer plexiform layer (deep capillary bed) and 10 located in the nerve fiber layer and ganglion cell layer (superficial capillary bed). The electronmicrographs were enlarged to a final magnification of 16,000 times and were examined for structural changes.

The electronmicrographs of the capillaries were used to measure the above mentioned areas ($\mu m^2$) which were expressed as percentage of total capillary area (T). Only transversly sectioned capillaries were used for these mesurements. They were judged as being transversly cut if the perpendicular diameter ratio was less than 2 and if they displayed a sharp border of the basement membrane.

BMT was measured according to a modification of a technique described by Robinson et al (1983). The Hewlett-Packard digitizer fitted with a specially designed program was used inplace of a planimeter. Basement membrane area (BMA) and Basement Membrane length (BML) were determined as,

$$BMA = T - (L + P + E)$$

and
$$BML = \frac{\text{The length of lines delimiting the BM}}{2}$$

The relative thickness of the basement membrane was expressed in terms of area per unit length

$$BMT(nm) = (BMA/BML) \times 1000 .$$

## V. Statistical analysis:

One way analysis of variance, student's two tailed t-test and chi-square test were used to analyse the data. P

value of 0.05 or less was accepted as being significant (Schefler 1984).

## 5. RESULTS:

## I. Clinical observations:

Body weight and blood glucose levels were monitored regularly during the course of the experiments. During the observation period diabetic non-transplanted animals showed hyperglycemia in the range of 350-420 mg/dl and a progressive weight loss to 80% of normal body weight at 18 months of age.

Diabetic rats with accepted grafts showed a complete normalisation of blood sugar levels (Table 1, Figure 3) and their body weights were not significantly different from those of the non-diabetic control rats at the time of sacrifice (Table 2). On the other hand diabetic rats with rejected grafts  showed hyperglycemic blood sugar levels similar to those of the diabetic non-transplanted animals (Table 1, Figure 3). The body weights of rats with rejected grafts were less and were not significantly different from those of diabetic non-transplanted rats. Hence successful islet cell allotransplantation acheived normalisation of hyperglycemia and body weight.

## II. Qualitative structural changes:

Light microscopic examination of the trypsin digested retinal preparations and semithin (0.5 μm) toluidine blue stained sections failed to show microaneurysms, haemorrhages, exudates as well as nerve fiber layer

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infarctions in the diabetic non transplanted animals. These relatively advanced retinopathic changes were not demonstrated in the diabetic animals with accepted or rejected grafts either. Mesodermal bridges were seen in all experimantal groups, whereas pericyte ghost cells were demonstrated predominantly in the diabetic groups.

Ultrastructurally the endothelial cells appeared normal in all groups examined. They contained elongated nuclei with peripherally located chromatin. Normal appearing organelles such as mitochondria, centrioles, smooth and rough endoplasmic reticulum, ribosomes and Golgi complexes were identified. Occasional Weibel-Palade bodies were also observed. Pinocytotic vesicles were seen along the cytoplasmic membrane both on the lumenal and ablumenal side. Tight junctions joining neighbouring endothelial cells appeared normal in all groups.

The most striking qualitative ultrastructural change consisted of degenerative changes in pericytes in experimental groups with hyperglycemia. Although similar changes could also be demonstrated in animals with euglycemia, they were less extensive.

Altered pericytes appeared swollen with watery cytoplasm. There was loss of intracytoplasmic organelles such as mitochondria, rough and smooth endoplasmic reticulum. The nuclei appeared shrunken with increased

electron density of the chromatin and indistinguishable nuclear membranes (Fig. 4). However pericytes were not uniformly affected, within the same specimen some pericytes appeared normal, while others showed degenerative changes. Normal appearing pericytes showed well formed nuclei with the chromatin located along the nuclear membrane. The cytoplasm showed normal organelles and finely dispersed free ribosomes. The number of pericytes with degenerative changes were significantly higher in the groups with rejected grafts (I and II; 24.7% and 26.3% respectively) and in the diabetic non-transplanted group (V; 29.0%) compared with non-diabetic control animals (VI; 11.7%). The number of degenerated pericytes in groups with accepted grafts ( III and IV; 15.1% and 17.1% respectively) were not significantly different from the non-diabetic control group (VI), but tended to be less than the diabetic non-transplanted group (VI) (Table 3, Table 4).

Qualitative basement membrane irregularities were seen in all rats except for the non-diabetic control rats. These changes, although not quantified, appeared to be more common in groups I, II, and V (i.e, groups with rejected grafts and the diabetic non-transplanted group). The irregularities consisted of localised nodular thickenings and the deposition of fibrillar material. Finger like projections of basement membrane material between the processes of the surrounding glial cells were also noted in these groups

(Fig. 5). Such projections were never seen in non-diabetic control rats (Gr. VI).

III. Quantitative structural changes :

Light microscopic evaluation of retinal vascular density, as a mesure of late occuring neovascularisation, revealed no differences between the various groups (Table 5).

Calculation of the pericyte numbers per $mm^2$ of vascular area in trypsin digested preparations showed marked loss of pericytes in all hyperglycemic groups. Rats with accepted intracerebral grafts showed a normal pericyte density, whereas animals with accepted intraportal grafts demonstrated a lower density in keeping with diabetic non-transplanted rats (Table 6). A similar count of endothelial cells showed no significant differences between any of the groups (Table 7). When the pericyte-endothelial cell ratios were calculated from the pericyte and endothelial cell densities, all diabetic groups exibited significant lower ratios compared to that of the non-diabetic control group. However, it is interesting to note, that pericyte endothelial cell ratios in the groups with accepted grafts were significantly higher than that of the diabetic non-transplanted group (Table 8, Figure 6).

Ultrastructurally the basement membrane of the

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superficial vascular bed was found to be thicker than that of the deep vascular bed in all groups (Table 9,Figure 7).

Both in the superficial and in deep vascular beds diabetic non-transplanted animals showed significantly thickened basement membranes (1.4 fold and 1.5 fold respectively) compared to non-diabetic controls. The basement membrane thickness in the groups with successful allotransplantation was not different from that of non-diabetic controls, but were found to be significantly thinner than in diabetic non-transplantated animals. On the other hand the groups with rejected grafts showed a significantly thicker basement membrane compared to the non-diabetic controls (Table 10, Table 11; Figure 8, Figure 9).

Therefore euglycemic control of diabetes by islet cell allotransplantation appears to prevent basement membrane thickening.

In the superficial capillary bed no significant difference were noted among any of the groups with regards to pericyte profile area(Table 12). However, in the deep capillary bed hyperglycemic groups tended to have a larger pericyte profile area than that of non-diabetic control rats; whereas the pericyte profile area in groups with accepted grafts was not significantly different from that in the non-diabetic control group (Table 13).

Measurements of endothelial cell profile area did not reveal any significant differences between any of the

groups, either in the superficial capillary bed (Table 14) or in the deep capillary bed (Table 15).

No significant differences were found in the superficial vascular bed between any of the groups when lumenal areas were compared (Table 16). In the deep capillary bed, however, diabetic non-transplanted group and the groups with rejected grafts exhibited a significantly smaller lumenal area than that of non-diabetic control animals. The groups with successful allotransplantation showed a larger lumenal area which was not significantly different from that in non-diabetic control animals (Table 17).

No significant differences, in any of the parameter analysed, were seen between group III (successful intraportally transplanted) and group IV (successful intracerebrally transplanted) with the exception of the number of pericytes, which was found to be significantly higher in group IV (Table 6).

* The term significant has been used to mean statistically significant.

# 6. DISCUSSION:

Chronic hyperglycemia appears to be the primary pathogenetic factor in diabetic retinopathy as well as in other complications of diabetes (Frank, 1984). Control of blood glucose is an essential part of diabetes therapy (Cahill et al., 1976). Conventional insulin treatment is an effective therapy but it does not cure the disease. Recovery from diabetes and prevention of its complications are only to be expected if the insufficient islets are replaced by the sufficient ones at an early stage of the disease (Bretzel et al., 1983).

The method of allotransplantation used in this study is an accepted method of transplantation of pancreatic islet cells across a major histocompatibility barrier (Tze and Tai, 1983, 1984). The technique of culturing islet cells before transplantation has been shown to increase the graft survival period without resorting to immunosupression (Lacy et al., 1979). Culture in an oxygen rich environment reduces or eliminates the immunogenecity of the graft by removing leukocytes from the transplant prior to grafting (Lafferty et al., 1983). The present study was therefore designed to investigate the effect of pancreatic islet cell allotransplantation on the control of diabetes and on the development of diabetic retinopathy in streptozotocin induced diabetic rats.

Two simple parameters reflecting metabolic control in

the diabetic state are body weight and the blood glucose level. In the present study the characteristic body weight loss and hyperglycemia seen in diabetics were not found in animals with successful allotransplantation. Diabetic non-transplanted rats and the diabetic rats with rejected grafts were found to show hyperglycaemia and lower body weights than the non-diabetic control rats. These findings are in keeping with those of other authors who have noted improvement in body weight gain and acheievment of euglycemia in streptozotocin diabetic rats after successful pancreatic islet cell transplantation (Trimble et al., 1980; Nelken et al., 1977). Following intraportal transplantation blood glucose levels returned to normal within 24 hours (Pipeleers-Marichal et al., 1976) and the body weights normalised within weeks (Bretzel et al., 1983).

The streptozotocin induced diabetic rat has been shown to be a good model of diabetic retinopathy. Several retinopathic changes have been produced in this model; such as basement membrane irregularities, pericyte degenerations, microaneurysms (Luenberger et al., 1974; Fischer and Gartner, 1983), basement membrane thickening and pericyte loss (Hori and Muhai 1979; Studer et al., 1976). The pathological features noted in diabetic non-transplanted animals in the present study confirmed these findings. Although late retinopathic changes, like neovascularisation has not been noted in this model, it is of value for the study of early retinopathic changes and their pathogenesis.

The main findings in the present study were the preventive effect of islet cell allotransplantation on pericyte abnormalities and basement membrane thickning in the diabetic retina.

Trypsin digested, PAS-hematoxylin stained flat preparations of the retina best reveal endothelial cells and pericytes of the retinal capillaries (Wise et al., 1971). In the present study trypsin digested retinal preparations showed a loss of pericytes with reduction of the pericyte-endothelial cell ratio in diabetic non-transplanted animals and in animals with rejected grafts. Functioning allografts achieving a normalisation of the diabetic dysmetabolism prevented pericyte loss, as evidenced by a significantly higher pericyte-endothelial cell ratio in graft accepted groups compared to groups with rejected grafts and the diabetic non-transplanted group. Following syngenic islet cell transplantation Naeser and Andersson (1983) also found a prevention of pericyte loss in alloxan induced diabetic mice. Animals with successful intracerebral transplantation showed a significantly higher number of pericytes than that of the successful intraportally transplantated animals. There is no explanation for this difference. Although one may speculate that the site of transplantation might have different effects on metabolic control.

Loss of capillary pericytes has been regarded as the earliest histological lesion in diabetic retinopathy (Cogan

et al., 1961). This view has later been confirmed by several workers (Yanoff, 1966; Addison et al., 1970). Pericyte loss is not specific for diabetic retinopathy. It is seen in other conditions such as macroglobulinemia, myelomatosis, cyanosis, and polycythemia vera; however the severity and extent of it is nevertheless highly characteristic of diabetic retinopathy (Ashton, 1983).

Although, pericytes are closely associated with capillaries and venules their physiologic functions in different tissues are poorly understood. It has been suggested that the pericytes provide structural support to the vessel wall and synthesize vascular basement membrane. Futhermore they are capable of phagocytosis and may modulate microvascular blood flow (Tilton et al., 1981). In the retina pericyte degeneration has been implicated in the formation of microaneurysms (Kuwabara and Cogan, 1963; NIH conference, 1984). However since the function of retinal pericytes are unknown the pathophysiologic significance of pericyte degeneration in the diabetic retina is unclear (Tilton et al., 1981).

In the deep capillary bed an increase in the pericyte profile area was noted in groups with rejected grafts and in the diabetic non-transplanted group. However, the pericyte profile area of graft accepted groups was not different from that in the non-diabetc control group. Increase in the pericyte profile area may be due to the hydropic swelling of these cells. Increases in the pericyte profile area were

associated with increased pericyte degeneration in these groups.

The cause of pericyte degeneration in the diabetic retina is still not known. It has been found that an increased ambient glucose concentration decreases the proliferation of pericytes in culture (King and Buzney, 1983). Frank (1984) is of the opinion that perhaps insulin is not merely a critical growth factor for the retinal pericytes but also an agent required for their maintainance. Therefore an insufficient concentration of insulin in the blood perfusing the retinal vasculature may induce pericyte degeneration.

Increased polyol pathway activity has also been suggested as a contributing factor causing pericyte degeneration (NIH Conference, 1984). The basis for this hypothesis is that aldose reductase has been localised in the human retinal pericytes (Akagi et al., 1984) and it has been shown that cultured pericytes degenerate and die in high glucose environments (Buzney et al., 1977). In fact, aldose reductase is responsible for the only complication of diabetes, in which the biochemical cause is known, namely diabetic cataract. This enzyme converts aldose sugars to their respective sugar alcohols using NADPH as a cofactor. Together with another enzyme sorbitol dehydrogenase it forms the polyol pathway.

```
            ALDOSE                    SORBITOL
           REDUCTASE              DEHYDROGENASE

GLUCOSE━━━━━━━━━━▷SORBITOL━━━━━━━━━━━▷FRUCTOSE
          NADPH        NADP      NAD        NADH
```
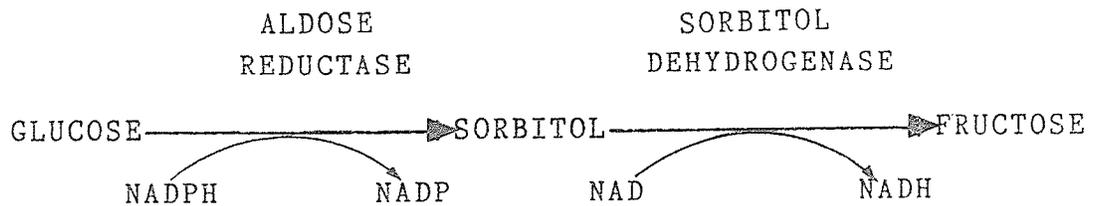
Aldose reductase has a low affinity for glucose. This
pathway therefore only occurs in the presence of excess
intracellular glucose. The sugar alcohols thus formed are
metabolically inert, since they cannot penetrate the cell
membrane. They rapidly accumulate inside the cell leading to
osmotic decompensation (Frank, 1984). These two mechanisms
may be responsible for the increase in the pericyte profile
area and the degenerative changes noted in the groups with
rejected grafts and in the diabetic non-transplanted group.
One interesting point should be noted; although aldose
reductase activity is present in both cerebral and retinal
cultured microvessels, only retinal vessels exhibit diabetic
microangiopathy (Kennedy et al., 1983). This may imply that
factors other than aldose reductase may be associated with
the development of diabetic retinopathy.

Therefore, besides a considerable loss of pericytes in
hyperglycemic rats, the remaining pericytes in these animals
showed in addition a significantly higher incidence of
degeneration.

Thickening of capillary basement is a well documented
phenomenon in diabetic retinopathy. The vascular basement
membrane has been thought to serve as a filtration barrier
and a scaffold for maintaining vascular architecture and as

a retardant to pathologic vasoproliferation (Frank, 1984).

It is not clear how and why the capillary basement membrane is thickened in diabetes. According to Little (1981) it may arise either as a result of overproduction of basement membrane material from increased cellular synthesis, or following increased availability of basement membrane precursors from the blood resulting in accelerated glycoprotein synthesis. Diminished absorption of basement membrane, and augmentation of basement membrane by entrapped plasma protein from vascular leakage or abnormal transport have also been suggested as contributing factors. Studies in human and experimental animals have shown that good diabetic control may prevent capillary basement membrane thickening and other pathological changes in diabetes (Engerman., 1977; Friberg et al., 1985). Williamson et al. (1971) observed that abnormalities of carbohydrate metabolism precedes increased muscle capillary basement membrane thickness in human diabetes.

In the current study, ultrastructurally increased basement membrane thickness (BMT) was seen both in the superficial and deep vascular bed in groups with rejected grafts and in the diabetic non-transplanted group. Increased BMT was found to be prevented in groups with successful transplantation. The BMT in these groups were not significantly different from that in non-diabetic control animals. The BMT in the superficial vascular bed were always found to be thicker than the corresponding BMT in the deep

vascular bed in all groups.

Although no report on retinal capillary BMT following pancreatic or islet transplantation is available, the reduction of glomerular BMT in human and experimental animals has been reported following these procedures (Mandel et al., 1981). The method used for measuring the basement membrane thickness in this study was a modification of the method described by Robinson et al. (1983). The capillary basement membrane is not uniformly thick; this method takes into account these variations and measures the average thickness. This method is also quick and reproducable (Robinson et al., 1983; Shannon, 1982). Previous studies in the spontaneously diabetic BB-rat have shown that aging also causes an increase in the basement membrane thickness (Sima et al., 1985). As the age of all of the rats used in this study were the same, aging as a causative factor of BMT in the diabetic non-transplanted and the graft rejected animals can be eliminated. A thicker basement membrane in the superficial than in the deep vascular bed has been noted in the non-diabetic, streptozotocin diabetic and in the BB rat (Fischer and Gartner, 1983; Sima et al., 1985). Hydrostatic pressure has been thought to be a causative factor in the production of regional variation of basement membrane thickness as it has been shown in man that muscle capillary basement membrane thickness increases from head to foot (Williamson and Kilo, 1971). Sosula et al. (1972) are also

of the opinion that the mean basement membrane thickness in the retina decreases with increased distance from the parent vessel due to decrease in intravascular pressure.

Localised nodular thickening, vacuolation, deposition of fibrillar material and debris in the basement membrane may be caused by degenerated and entrapped pericyte debris, so called ghost cells (Tilton et al., 1981). The finding of projections of basement membrane materials between surrounding glial cells and processes (Müller cells) in diabetes have been noted by several investigators (Bloodworth and Molitor, 1965; Babel and Luenberger, 1974). It has been suggested that this redundant basement membrane is laid down as a result of disturbed Müller cell metabolism, which in itself may be a factor responsible for increased BMT (Fischer and Gartner, 1983).

Ashton (1953) has shown that hyalin degeneration of the retinal arterioles leads to their gradual narrowing and occlusion. Endothelial cell swelling (Sosula et al., 1972; Toussaint and Dustin, 1972), subendothelial deposition of granular and fibrillar material (Ashton, 1983) have also been implicated in the narrowing of the retinal capillary lumen in diabetics. A hypothesised compression from outside due to the edema of the sorrounding retinal tissue has been proposed as another possible factor for the narrowing of retinal capillaries. Decreased capillary lumen may be of significance in producing retinal hypoxia, which is thought to be a key factor in the development of diabetic

retinopathy (Ashton, 1983). Smaller lumenal areas were noted in the deep capillary bed of the diabetic non-transplanted group and in groups with rejected grafts. The lumenal areas of graft accepted groups were not significantly different from the non-diabetic control animals.

It is interesting to note that, a significantly smaller lumenal area and larger pericyte profile area were seen in the deep capillary bed in animals with rejected grafts and diabetic non-transplanted animals. There is no explanation for the occurrence of these tophographical differences. The speculation may be made that, as the capillaries of the deep vascular bed are formed by the branches from the superficial bed (Henkind, 1969; Warwick, 1976) they are perfused by less oxygenated blood and may therefore suffer more in a potentially hypoxic situation. His (1880) felt that the capillaries in the deep vascular bed of retina are mainly venous, compared to arterial capillaries in the superficial bed. Although this view has not been supported by other investigators, precapillary arterioles are more common in the superficial vascular bed whereas the postcapillary venules are more common in deep capillaries (Wise et al., 1971). Previous studies in the BB rat have also revealed that the earliest change in the diabetic retina is a dilatation of the deep capillary bed, which has been thought to be due to defect in autoregulatory response due to hyperglycemia(Sima et al., 1985).

In this study no demonstrable endothelial cell

abnormalities were seen. Although endothelial cell
abnormalities has been found by some workers (Sosula et al.,
1972), Bloodworth and Molitor (1965) are of the opinion that
despite the various abnormalities of the basement membrane
surrounding capillaries in the diabetic retina, each
capillary is usually completely lined by intact endothelial
cells which are connected by normal cell junctions.

In summary this study has demonstrated that pancreatic
islet cell allotransplantation at an early stage of diabetes
mellitus results not only in a good metabolic control and
normal weight gain, but also it prevents in a significant
way the development of microvascular changes in the diabetic
retina.

## 7. CONCLUSION:

The present study has shown that the streptozotocin induced diabetic Lewis rat develops microvascular changes of diabetic retinopathy such as, pericyte degeneration and loss, basement membrane thickening and vascular narrowing. Successful pancreatic islet cell allotransplantation of diabetic animals were found to prevent basement membrane thickening and vascular narrowing. Pericyte loss and degeneration was partially prevented by this transplantation procedure. As would be expected, unsuccessful allotransplantation had no such protective effect.

These findings lead to the conclusion that pancreatic islet cell allotransplantation may be a rational approach for the prevention of the microvascular complications in the diabetic retina.

Table 1

Blood glucose levels at the time of sacrifice

| Groups(n) | Blood Glucose mg/dL mean±s.d. |
|-----------|-------------------------------|
| I    (5)  | 357 ± 60 |
| II   (4)  | 318 ± 15 |
| III  (6)  | 99 ± 8 |
| IV   (4)  | 102 ± 15 |
| V    (5)  | 365 ± 41 |
| VI   (5)  | 90 ± 4 |

n = Number of animals

Gr.I    Intraportally transplanted, graft rejected.

Gr.II   Intracerebrally transplanted, graft rejected.

Gr.III  Intraportally transplanted, graft accepted.

Gr.IV   Intracerebrally transplanted, graft accepted.

Gr.V    Diabetic non-transplanted.

Gr.VI   Non-diabetic control.

Table 2

Body weight.

| Gr. No. | Wt (gms) mean±s.d.(n) | | Gr. vs | Gr. | t-value(d) | probability |
|---------|------------------------|---|--------|-----|------------|-------------|
| I | 273±31 (5) | | I | VI | −3.032 (8) | <0.002 |
| II | 263±14 (4) | | I | V | 0.415 (8) | N.S. |
| III | 309±21 (6) | | II | VI | −4.606 (7) | <0.002 |
| IV | 259±62 (4) | | II | V | −0.065 (7) | N.S. |
| V | 264±36 (5) | | III | VI | 1.207 (9) | N.S. |
| VI | 329±29 (5) | | III | V | 2.467 (9) | <0.005 |
| | | | IV | VI | −2.109 (7) | N.S. |
| | | | IV | V | 0.138 (7) | N.S. |
| | | | V | VI | −3.172 (8) | <0.002 |
| | | | III | IV | 1.567 (8) | N.S. |

N.S.= Non significant, n = Number of animals
d = degrees of freedom, Gr.= Group
Gr.I    Intraportally transplanted, graft rejected.
Gr.II   Intracerebrally transplanted, graft rejected.
Gr.III  Intraportally transplanted, graft accepted.
Gr.IV   Intracerebrally transplanted, graft accepted.
Gr.V    Diabetic non-transplanted.
Gr.VI   Non-diabetic control.

One way analysis of variance yielded a variance ratio of
3.665 (P<0.05).
See Appendix (Table 1) for details of analysis of variance

Table 3

Pericyte Degenerations

Chi-Square test

| | Group I | Group II | Group III | Group IV | Group V | Group VI | |
|---------|-----------|-----------|-------------|-----------|-----------|-----------|-----|
| Present | 24(19.50) | 21(16.08) | 18(23.92) | 13(15.28) | 29(20.10) | 11(21.11) | 116 |
| Absent | 73(77.50) | 59(63.92) | 101(95.08) | 63(60.72) | 71(79.90) | 94(83.89) | 461 |
| Total | 97 | 80 | 119 | 76 | 100 | 105 | 577 |

$X^2 = 16.4358$, $P < 0.001$

Degrees of freedom=5, Expected values are shown inside brackets.

Gr.I    Intraportally transplanted, rejected graft.

Gr.II   Intracerebrally transplanted, rejected graft.

Gr.III  Intraportally transplanted, accepted graft.

Gr.IV   Intracerebrally transplanted, accepted graft.

Gr.V    Diabetic non-transplanted.

Gr.VI   Non-diabetic control.

Table 4

Pericyte degenerations

Chi-Square test

| Group | vs. | Group | $X^2$ value | Probability |
|-------|-----|-------|-------------|-------------|
| I   | | VI | 7.158  | <0.01 |
| I   | | V  | 0.451  | N.S. |
| II  | | VI | 8.114  | <0.01 |
| II  | | V  | 0.222  | N.S. |
| III | | VI | 1.075  | N.S. |
| III | | V  | 6.027  | <0.05 |
| IV  | | VI | 1.681  | N.S. |
| IV  | | V  | 3.367  | N.S. |
| V   | | VI | 11.196 | <0.01 |
| III | | IV | 0.137  | N.S. |

N.S.= Non significant

See table 3 for individual values and definition of groups.

## Table 5

### Vascular density

#### One way analysis of variance.

| Gr. No. | Vessels/mm² mean± s.d.(n) |
|---------|----------------------------|
| I. | 299.3 ± 8.3 (5) |
| II. | 323.5 ± 54.6(4) |
| III. | 311.3 ± 43.5(6) |
| IV. | 244.4 ± 34.1(4) |
| V. | 278.8 ± 61.7(5) |
| VI. | 307.0 ± 45.2(5) |

| Source of variation | Sum of squares | D.F. | Mean square | Variance ratio |
|---------------------|----------------|------|-------------|----------------|
| Between treatments | 17197.850 | 5 | 3439.57 | 1.734* |
| Within Treatments | 45610.625 | 23 | 1983.071 | --- |
| Total | 62808.475 | 28 | | |

* Non significant, D.F. = Degrees of freedom, Gr.= Group
Gr.I   Intraportally transplanted, rejected graft.
Gr.II  Intracerebrally transplanted, rejected graft.
Gr.III Intraportally transplanted, accepted graft.
Gr.IV  Intracerebrally transplanted, accepted graft.
Gr.V   Diabetic non-transplanted.
Gr.VI  Non-diabetic control.

Table 6

Pericyte number (Trypsin digested preparation)

| Gr. No. | Cells/mm$^2$ mean±s.d.(n) | | Gr. vs | Gr. | t-value(d) | probability |
|---------|---------------------------|---|--------|-----|------------|-------------|
| I | 838.0± 298.8 (5) | | I | VI | −4.044 (8) | <0.005 |
| II | 725.6± 135.4 (4) | | I | V | −1.345 (8) | N.S. |
| III | 1110.4±183.0 (6) | | II | VI | −5.549 (7) | <0.001 |
| IV | 1666.1±146.4 (4) | | II | V | −3.272 (7) | <0.02 |
| V | 1065.0±175.7 (5) | | III | VI | −3.228 (9) | <0.02 |
| VI | 1667.7±348.1 (5) | | III | V | 0.4195 (9) | N.S. |
| | | | IV | VI | 0.0090 (7) | N.S. |
| | | | IV | V | 5.5976 (7) | <0.001 |
| | | | V | VI | −3.458 (8) | <0.01 |
| | | | III | IV | −5.319 (8) | <0.002 |

N.S.= Non significant, n = Number of animals
d = Degrees of freedom, Gr.= Group
Gr.I    Intraportally transplanted, graft rejected.
Gr.II   Intracerebrally transplanted, graft rejected.
Gr.III  Intraportally transplanted, graft accepted.
Gr.IV   Intracerebrally transplanted, graft accepted.
Gr.V    Diabetic non-transplanted.
Gr.VI   Non-diabetic control.

One way analysis of variance yielded a variance ratio of
13.37 (P<0.005).
See Appendix (Table 3) for details of analysis of variance.

## Table 7

### Endothelial cell number (Trypsin digested preparation)

### One way analysis of variance.

| Source of variation | | Sum of squares | D.F. | Mean square | Variance ratio |
|---|---|---|---|---|---|
| Between | Treatments | 1289150.20 | 5 | 257830.04 | 2.56 * |
| Within | Treatments | 2312008.88 | 23 | 100522.13 | --- |
| Total | | 3601159.08 | 28 | | |

| Gr. No. | Cells/mm$^2$ mean ± s.d. (n) |
|---|---|
| I. | 2561.0 ± 304.1 (5) |
| II. | 2022.0 ± 106.9 (4) |
| III. | 2058.4 ± 321.4 (6) |
| IV. | 2510.3 ± 342.5 (4) |
| V. | 2418.7 ± 438.1 (5) |
| VI. | 2205.4 ± 260.5 (5) |

* Non significant, D.F. = Degrees of freedom, Gr. = Group

Gr.I    Intraportally transplanted, graft rejected.
Gr.II   Intracerebrally transplanted, graft rejected.
Gr.III  Intraportally transplanted, graft accepted.
Gr.IV   Intracerebrally transplanted, graft accepted.
Gr.V    Diabetic non-transplanted.
Gr.VI   Non-diabetic control.

Table 8

Pericyte-Endothelial cell ratio

| Gr. No. | P/E Ratio mean±s.d.(n) | Gr. vs | Gr. | t-value(d) | probability |
|---|---|---|---|---|---|
| | | I | VI | -12.589(8) | <0.001 |
| I | 0.32±0.04(5) | I | V | -6.425 (8) | <0.001 |
| II | 0.36±0.07(4) | II | VI | -8.299 (7) | <0.001 |
| III | 0.54±0.04(6) | II | V | -2.259 (7) | N.S. |
| IV | 0.56±0.05(4) | III | VI | -5.921 (9) | <0.001 |
| V | 0.44±0.01(5) | III | V | 5.818 (9) | <0.001 |
| VI | 0.73±0.06(5) | IV | VI | -4.526 (7) | <0.005 |
| | | IV | V | 4.693 (7) | <0.005 |
| | | V | VI | -10.45 (8) | <0.001 |
| | | III | IV | -0.669 (8) | N.S. |

N.S.= Non significant, n = Number of animals
d = Degrees of freedom, Gr.= Group
Gr.I    Intraportally transplanted, rejected graft.
Gr.II   Intracerebrally transplanted, rejected graft.
Gr.III Intraportally transplanted, accepted graft.
Gr.IV   Intracerebrally transplanted, accepted graft.
Gr.V    Diabetic non-transplanted.
Gr.VI   Non-diabetic control.

One way analysis of variance yielded a variance ratio of
47.829 (P<0.001).
See Appendix (Table 2) for details of analysis of variance.

Table 9

Basement membrane thickness.

Comparison between superficial and deep capillary bed.

| GR. No. | BMT(Superficial) mean±s.d.(n)nm | BMT (deep)nm mean±s.d.(n) | t-value(d) | probability |
|---------|-------------------------------|---------------------------|------------|-------------|
| I   | 189.8 ± 13.5(5) | 134.2 ± 7.6 (5) | 8.805 (8)  | <0.001 |
| II  | 194.5 ± 14.7(4) | 135.3 ± 8.3 (4) | 7.005 (6)  | <0.001 |
| III | 159.7 ± 12.9(6) | 108.7 ± 10.3(6) | 7.588 (10) | <0.001 |
| IV  | 157.3 ± 24.1(4) | 110.9 ± 14.4(4) | 3.297 (6)  | <0.02  |
| V   | 210.7 ± 6.6 (5) | 149.1 ± 3.8 (5) | 18.04 (8)  | <0.001 |
| VI  | 146.2 ± 6.7 (5) | 97.4 ± 7.4 (5)  | 10.87 (8)  | <0.001 |

n = Number of animals in each group.

d = Degrees of freedom.

Gr.= Group

Gr.I    Intraportally transplanted, rejected graft.

Gr.II   Intracerebrally transplanted, rejected graft.

Gr.III  Intraportally transplanted, accepted graft.

Gr.IV   Intracerebrally transplanted, accepted graft.

Gr.V    Diabetic non-transplanted.

Gr.VI   Non-diabetic control.

## Table 10

### Basement membrane thickness (superficial capillary bed)

| Gr. No. | BMT nm mean±s.d.(n) | Gr. vs | Gr. | t-value(d) | probability |
|---------|---------------------|--------|-----|------------|-------------|
| I       | 189.8±13.5 (5)      | I      | VI  | 6.485 (8)  | <0.001      |
| II      | 194.5±14.7 (4)      | I      | V   | −3.118 (8) | <0.02       |
| III     | 159.7±12.9 (6)      | II     | VI  | 6.089 (7)  | <0.001      |
| IV      | 157.3±24.1 (4)      | II     | V   | −2.037 (7) | N.S.        |
| V       | 210.7±6.6 (5)       | III    | VI  | 2.234 (9)  | N.S.        |
| VI      | 146.2±6.7 (5)       | III    | V   | −8.439 (9) | <0.001      |
|         |                     | IV     | VI  | 0.0892 (7) | N.S.        |
|         |                     | IV     | V   | −4.301 (7) | <0.001      |
|         |                     | V      | VI  | −15.36 (8) | <0.001      |
|         |                     | III    | IV  | 0.186 (8)  | N.S.        |

N.S.= Non significant, n = Number of animals
d = Degrees of freedom, Gr.= Group
Gr.I    Intraportally transplanted, rejected graft.
Gr.II   Intracerebrally transplanted, rejected graft.
Gr.III  Intraportally transplanted, accepted graft.
Gr.IV   Intracerabrally transplanted, accepted graft.
Gr.V    Diabetic non-transplanted.
Gr.VI   Non-diabetic control.

One way analysis of variance yielded a variance ratio of
16.194 (P<0.001).
See Appendix (Table 1) for details of analysis of variance.

Table 11

Basement membrane thickness (deep capillary bed)

| Gr. No. | BMT nm mean±s.d.(n) | | Gr. vs | Gr. | t-value(d) | probability |
|---|---|---|---|---|---|---|
| I | 134.2±7.6 | (5) | I | VI | 7.709 (8) | <0.001 |
| II | 135.3±8.3 | (4) | I | V | −3.934 (8) | <0.01 |
| III | 108.7±10.3 | (6) | II | VI | 7.088 (7) | <0.001 |
| IV | 110.9±14.4 | (4) | II | V | −3.062 (7) | <0.02 |
| V | 149.1±3.8 | (5) | III | VI | 2.102 (9) | N.S. |
| VI | 97.4±7.5 | (5) | III | V | −8.945 (9) | <0.001 |
| | | | IV | VI | 1.704 (7) | N.S. |
| | | | IV | V | −5.149 (7) | <0.002 |
| | | | V | VI | −13.76 (8) | <0.001 |
| | | | III | IV | 0.274 (8) | N.S. |

N.S.= Non significant, n = Number of animals
d = Degrees of freedom, Gr.= Group
Gr.I    Intraportally transplanted, graft rejected.
Gr.II   Intracerebrally transplanted, graft rejected.
Gr.III  Intraportally transplanted, graft accepted.
Gr.IV   Intracerebrally transplanted, graft accepted.
Gr.V    Diabetic non-transplanted.
Gr.VI   Non-diabetic control.

One way analysis of variance yielded a variance ratio of
22.972 (P<0.001).
See Appendix (Table 5) for details of analysis of variance.

## Table 12

### Pericyte profile area (superfpcial capillary bed)

### One way anslysis of variance.

| Source of variation | Sum of squares | D.F. | Mean square | Variance ratio |
|---|---|---|---|---|
| Between treatments | 35.281 | 5 | 7.056 | 0.857* |
| Within Treatments | 189.271 | 23 | 8.229 | -- |
| Total | 224.552 | 28 | | |

| Gr. No. | Area** mean±s.d.(n) |
|---|---|
| I. | 11.1±2.3 (5) |
| II. | 9.3±2.9 (4) |
| III. | 7.9±2.5 (6) |
| IV. | 7.9±1.8 (4) |
| V. | 8.7±3.8 (5) |
| VI. | 9.7±3.3 (5) |

* Non significant, D.F. = Degrees of freedom, Gr. = Group
Gr.I Intraportally transplanted, rejected graft.
Gr.II Intracerebrally transplanted, rejected graft.
Gr.III Intraportally transplanted, accepted graft.
Gr.IV Intracerebrally transplanted, accepted graft.
Gr.V Diabetic non-transplanted.
Gr.VI Non-diabetic control.

** Expressed as percentage of total capillary area

Table 13

Pericyte profile area (deep capillary bed)

| Gr. No. | Area* mean±s.d. (n) | | Gr. vs | Gr. | t-value(d) | probability |
|---------|---------------------|---|--------|-----|-----------|-------------|
| | | | I | VI | 4.359 (8) | <0.005 |
| I | 8.9±1.4 | (5) | I | V | -0.557 (8) | N.S. |
| II | 7.4±1.8 | (4) | II | VI | 1.962 (7) | N.S. |
| III | 5.8±2.2 | (6) | II | V | -1.461 (7) | N.S. |
| IV | 9.9±4.3 | (4) | III | VI | 0.377 (9) | N.S. |
| V | 9.8±3.1 | (5) | III | V | -2.472 (9) | <0.05 |
| VI | 5.4±1.3 | (5) | IV | VI | 2.039 (7) | N.S. |
| | | | IV | V | 0.016 (7) | N.S. |
| | | | V | VI | 3.007 (8) | <0.02 |
| | | | III | IV | -1.609 (8) | N.S. |

N.S.= Non significant, n = Number of animals
d = Degrees of freedom, * Expressed as percentage of total
capillary area, Gr.= Group
Gr.I    Intraportally transplanted, graft rejected.
Gr.II   Intracerebrally transplanted, graft rejected.
Gr.III Intraportally transplanted, graft accepted.
Gr.IV   Intracerebrally transplanted, graft accepted.
Gr.V   Diabetic non-transplanted. Gr.VI Non-diabetic control.

One way analysis of variance yielded a variance ratio of
3.263 (P<0.025).
See Appendix (Table 6) for details of analysis of variance.

## Table 14

### Endothelial cell profile area (superficial capillary bed)

### One way analysis of variance.

| Gr. No. | Area ** mean±s.d. (n) |
|---------|------------------------|
| I. | 16.4±2.3 (5) |
| II. | 12.9±3.0 (4) |
| III. | 13.5±2.5 (6) |
| IV. | 14.5±3.3 (4) |
| V. | 12.4±1.7 (5) |
| VI. | 15.2±4.9 (5) |

| Source of variation | Sum of squares | D.F. | Mean square | Variance ratio |
|---------------------|----------------|------|-------------|----------------|
| Between Treatments | 54.768 | 5 | 10.954 | 1.155* |
| Within Treatments | 218.041 | 23 | 9.480 | -- |
| Total | 272.810 | 28 | | |

\* Non significant, D.F. = Degrees of freedom, Gr. = Group
Gr.I   Intraportally transplanted, graft rejected.
Gr.II   Intracerebrally transplanted, graft rejected.
Gr.III   Intraportally transplanted, graft accepted.
Gr.IV   Intracerebrally transplanted, graft accepted.
Gr.V   Diabetic non-transplanted.
Gr.VI   Non-diabetic control.

\** Expressed as percentage of total capillary area

Table 15

Endothelial cell profile area (deep capillary bed)

One way analysis of variance

| Gr. No. | Area** mean±s.d.(n) |
|---|---|
| I. | 16.6±2.1 (5) |
| II. | 13.8±2.4 (4) |
| III. | 15.1±3.6 (6) |
| IV. | 17.8±5.8 (4) |
| V. | 16.2±5.3 (5) |
| VI. | 15.4±1.7 (5) |

| Source of variation | Sum of squares | D.F. | Mean square | Variance ratio |
|---|---|---|---|---|
| Between Treatments | 39.764 | 5 | 7.953 | 0.565* |
| Within Treatments | 323.958 | 23 | 14.085 | -- |
| Total | 363.722 | 28 | | |

\* Non significant, D.F. = Degrees of freedom, Gr. = Group
Gr.I    Intraportally transplanted, graft rejected.
Gr.II   Intracerebrally transplanted, graft rejected.
Gr.III  Intraportally transplanted, graft accepted.
Gr.IV   Intracerebrally transplanted, graft accepted.
Gr.V    Diabetic non-transplanted.
Gr.VI   Non-diabetic control.

\*\* Expressed as percentage of total capillary area

## Table 16

### Lumenal area (superficial capillary bed)

### One Way analysis of variance

| Gr. No. | Area** mean±s.d. (n) |
|---------|----------------------|
| I.      | 57.0±7.2 (5)         |
| II.     | 62.4±3.2 (4)         |
| III.    | 64.3±4.9 (6)         |
| IV.     | 58.8±0.9 (4)         |
| V.      | 62.7±7.0 (5)         |
| VI.     | 59.8±5.9 (5)         |

| Source of variation | Sum of squares | D.F. | Mean square | Variance ratio |
|---------------------|----------------|------|-------------|----------------|
| Between             |                |      |             |                |
| Treatments          | 192.579        | 5    | 38.516      | 1.262*         |
| Within              |                |      |             |                |
| Treatments          | 702.248        | 23   | 30.524      | --             |
| Total               | 894.627        | 28   |             |                |

\* Non significant, D.F. = Degrees of freedom, Gr.= Group
Gr.I    Intraportally transplanted, graft rejected.
Gr.II   Intracerebrally transplanted, graft rejected.
Gr.III  Intraportally transplanted, graft accepted.
Gr.IV   Intracerebrally transplanted, graft accepted.
Gr.V    Diabetic non-transplanted.
Gr.VI   Non-diabetic control.

** Expressed as percentage of total capillary area

Table 17

Lumenal area (deep capillary bed)

| Gr. No. | Area ** mean±s.d.(n) | Gr. vs | Gr. | t-value(d) | probability |
|---------|----------------------|--------|-----|------------|-------------|
| I | 61.3±3.6 (5) | I | VI | -4.662 (8) | <0.002 |
| II | 65.2±1.9 (4) | I | V | 0.979 (8) | N.S. |
| III | 68.9±1.9 (6) | II | VI | -3.512 (7) | <0.01 |
| IV | 67.6±3.4 (4) | II | V | 1.621 (7) | N.S. |
| V | 59.5±7.6 (5) | III | VI | -0.363 (9) | N.S. |
| VI | 70.2±2.4 (5) | III | V | 2.739 (9) | <0.05 |
| | | IV | VI | -1.406 (7) | N.S. |
| | | IV | V | 1.966 (7) | N.S. |
| | | V | VI | -3.043 (8) | <0.02 |
| | | III | IV | 2.163 (8) | N.S. |

N.S.= Non significant, n = Number of animals
d = Degrees of freedom, ** Expressed as percentage of total
area, Gr.= Group
GR.I    Intraportally transplanted, graft rejected.
Gr.II   Intracerebrally transplanted, graft rejected.
Gr.III  Intraportally transplanted, graft accepted.
Gr.IV   Intracerebrally transplanted, graft accepted.
Gr.V   Diabetic non-transplanted.,Gr.VI Non-diabetic control.

One way analysis of variance yielded a variance ratio of
5.904 (P<0.005).
See Appendix (Table 7) for details of analysis of variance.

67

Figure 1 -- Schematic outline of proposed pathogenetic
mechanism of diabetic retinopathy.
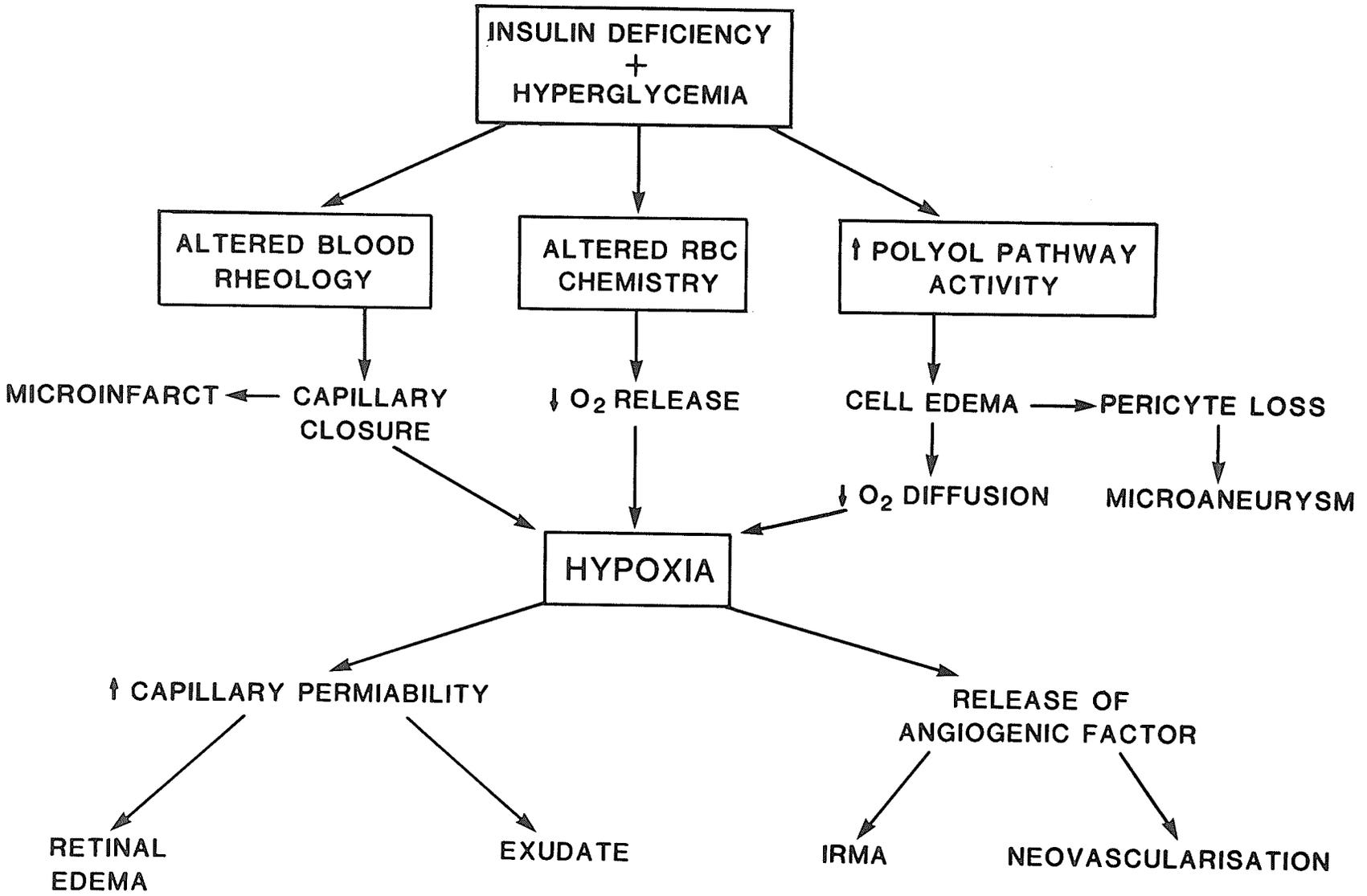
Figure 2 -- Cross section of the rat retina showing its different layers.

Ilm - Internal limiting membrane

Nvl - Nerve fiber layer

Gcl - Ganglion cell layer

Ipl - Inner plexiform layer

Inl - Inner nuclear layer

Opl - Outer plexiform layer

Onl - Outer nuclear layer

Elm - External limiting membrane

Phl - Photoreceptor layer

Rpe - Retinal pigment epithelium

Scb - Superficial capillary bed

Dcb - Deep capillary bed

Ch  - Choroid

Scl - Sclera

Toluidine blue stain 340X

Scb

Dcb

Ch

Scl

Ilm

Nvl
Gcl

Ipl

Inl
Opl
Onl
Elm
Phl
Rpe
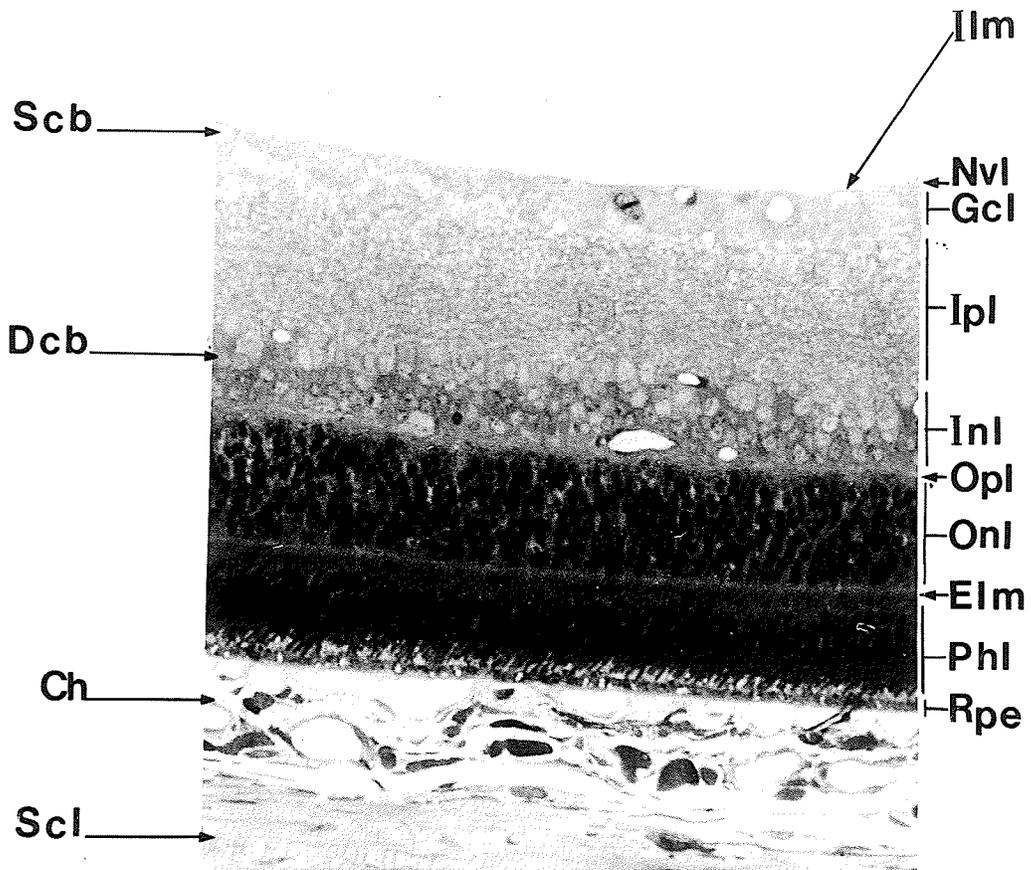
Figure 3 --    Post transplantation plasma glucose levels

mg/dl (mean±s.d.) of

△—△ Intraportally transplanted, rejected

graft. (n=5)

◆—◆ Intracerebrally transplanted, rejected

graft. (n=4)

▨—▨ Intraportally transplanted, functional

graft. (n=6)

O—O Intracerebrally transplanted, functional

graft. (n=4)

▲—▲ Diabetic non-transplanted. (n=5)

Shaded area -- Non-diabetic controls. (n=5)

Day 0= day of transplantation.

Blood glucose level mg/dl

Post transplantation days
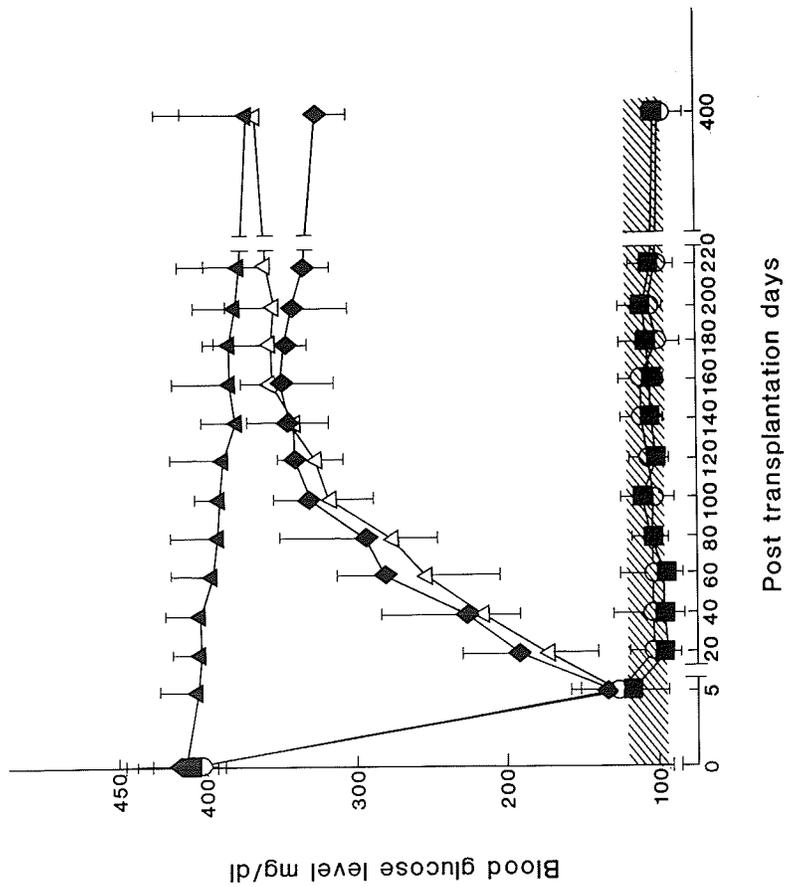
Figure 4 --   (A) Cross section of a retinal capillary from a

diabetic rat with rejected pancreatic islet

allograft.

(B) Enlargement of the part marked in (A)

showing a pericyte in a degenerated state with

a pyknotic nucleus (PN).

See fig.7 for explanation of the labels.

A

mv

BM

E

P

*

1.0μm

B

PN

*

E

P

1.0 μm
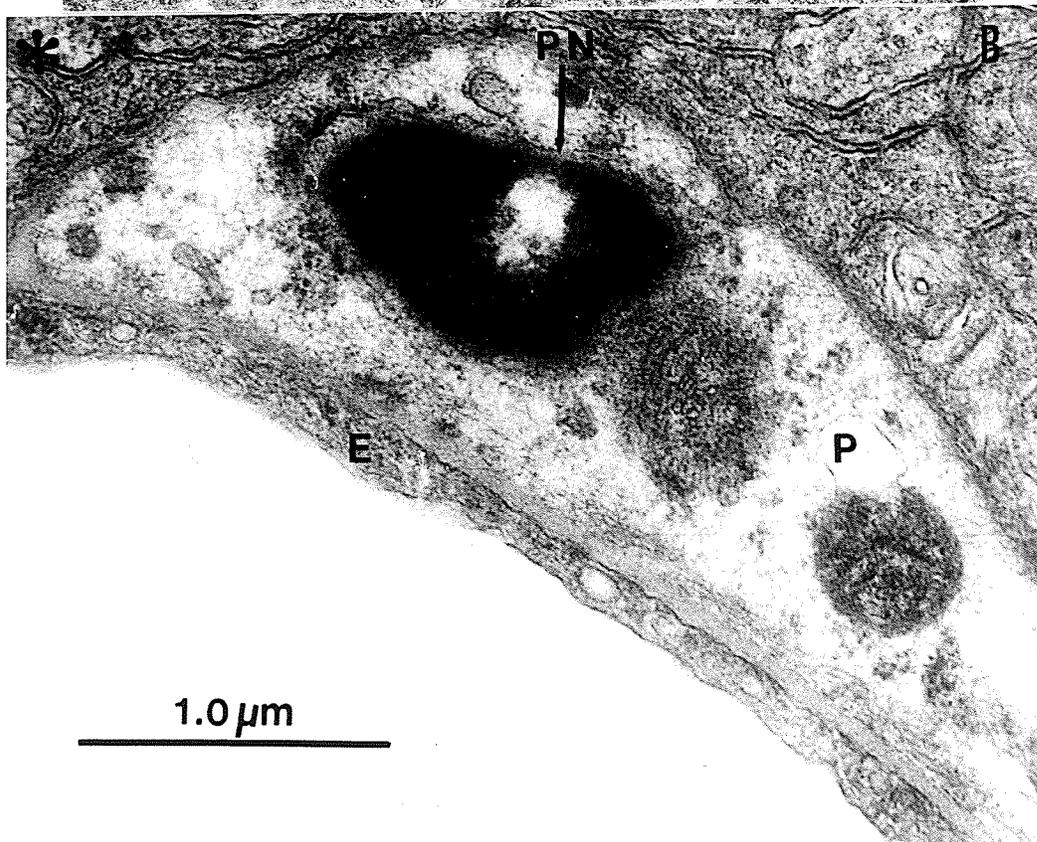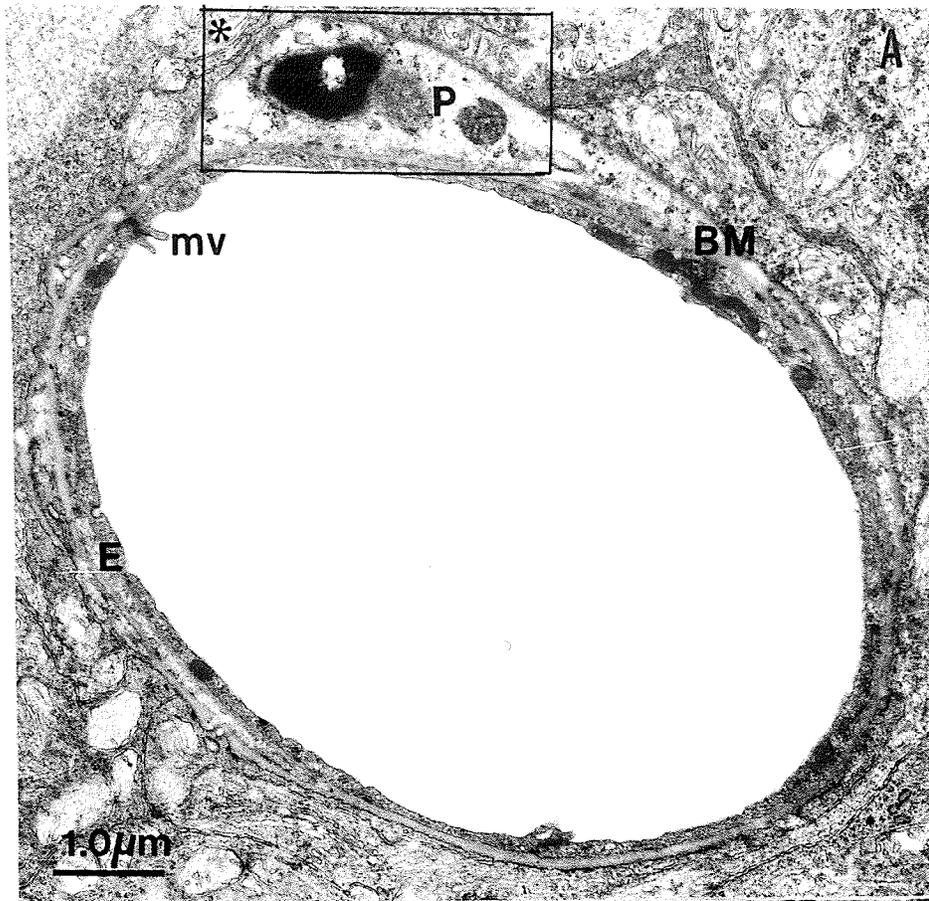
Figure 5 -- (A) Cross section of a retinal capillary of a

diabetic non-transplanted rat.

(B) Enlargement of part marked in (A) showing

basement membrane irregularities,

nodular thickening(arrow) and

projection of basement membrane material

towards the surrounding glia (arrowhead).

See fig.7 for explanation of labels.

A

BM

E

1.0μm

*

B

E

BM

1.0 μm
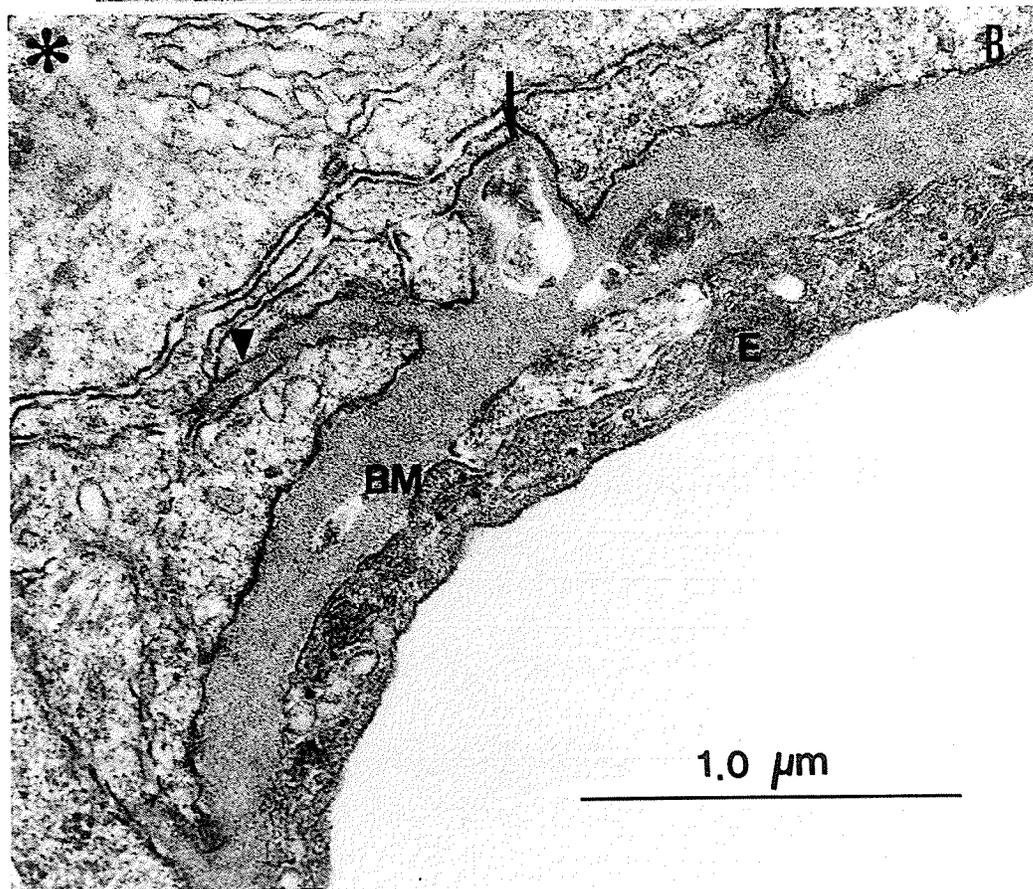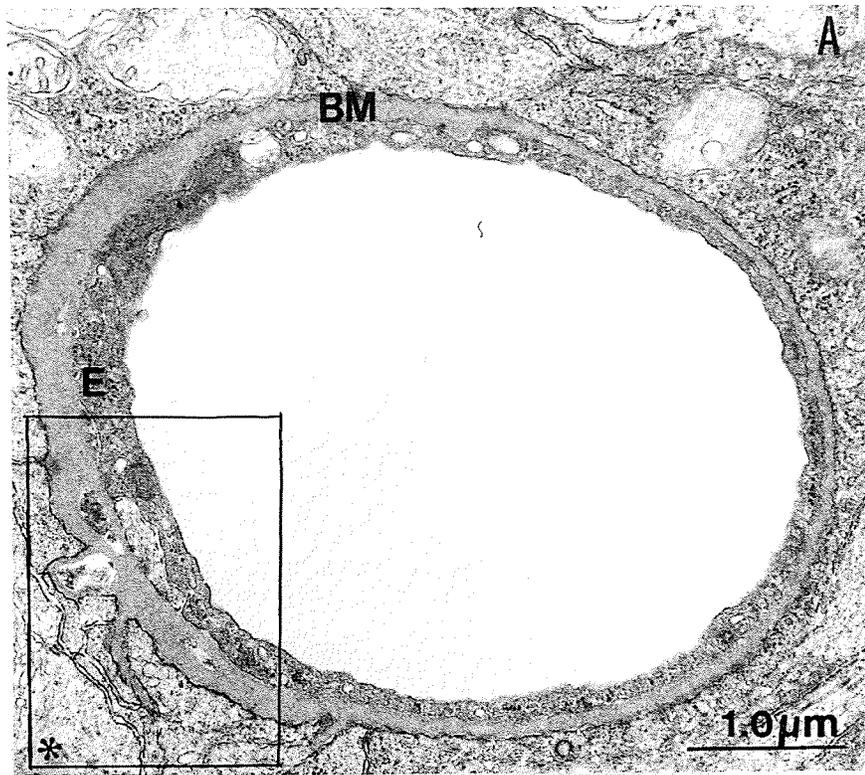
Figure 6 --    Trypsin digested preparations of rat retinal

capillaries showing the effect of pancreatic

islet cell allotransplantation on Pericyte-

endothelial cell ratio.

(A) Non-diabetic control

(B) Diabetic non-transplanted

(C) Diabetic with rejected graft

(D) Diabetic with accepted graft

B,C and D show fewer pericyte nuclei than A. C

and D show fewer pericyte nuclei than B.

P = Pericyte nuclei

E = Endothelial cell nuclei

PAS stain 825X.
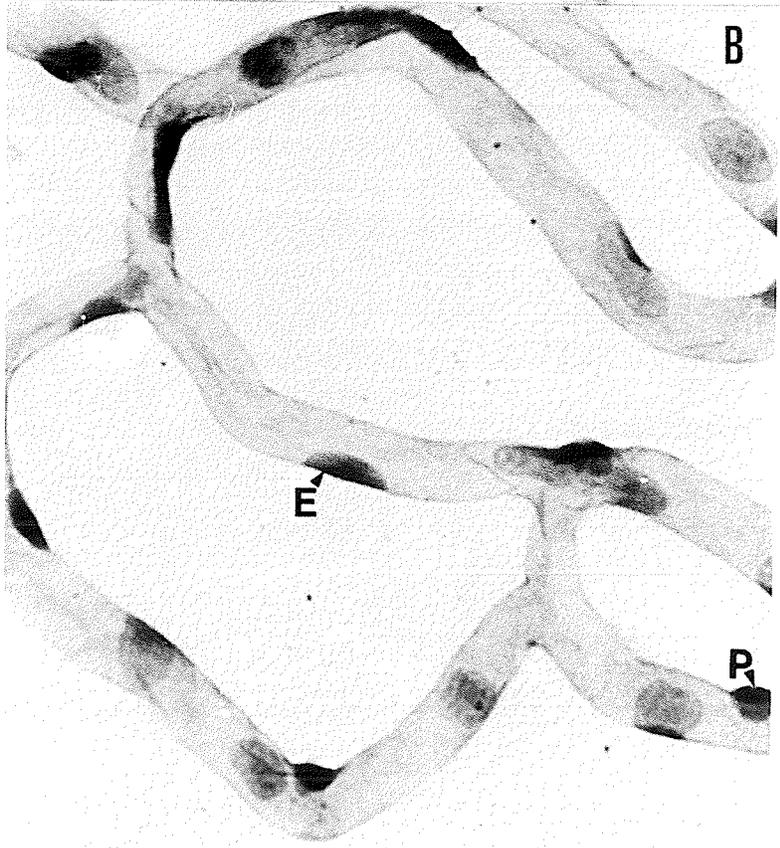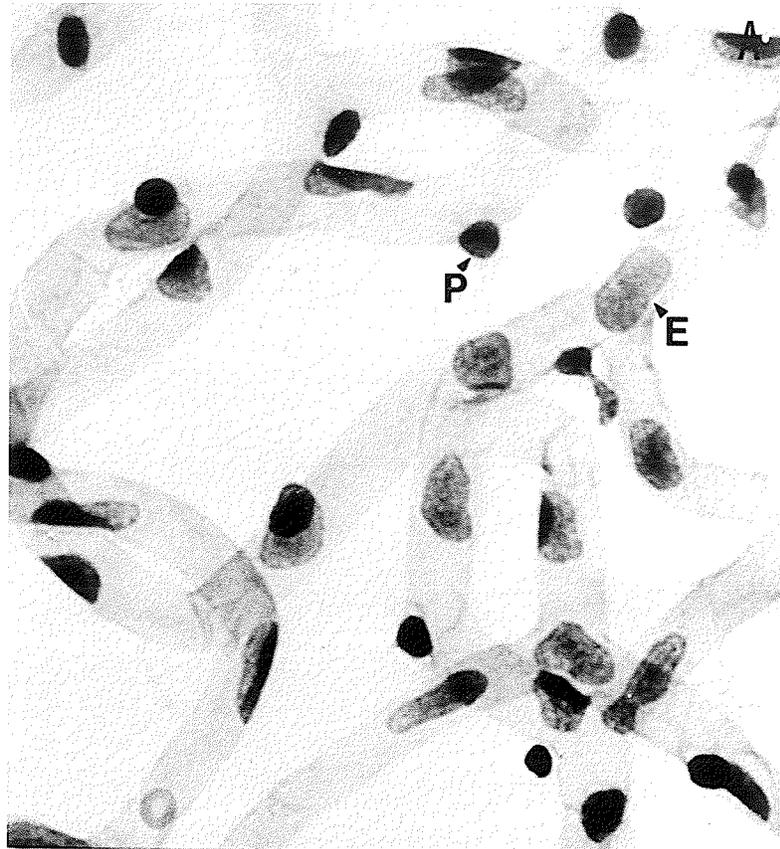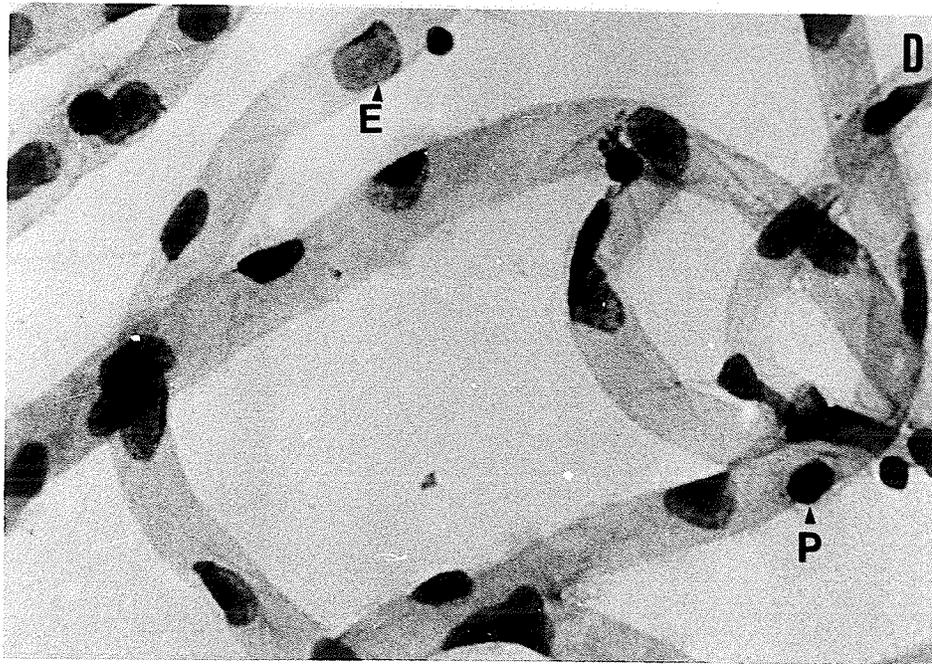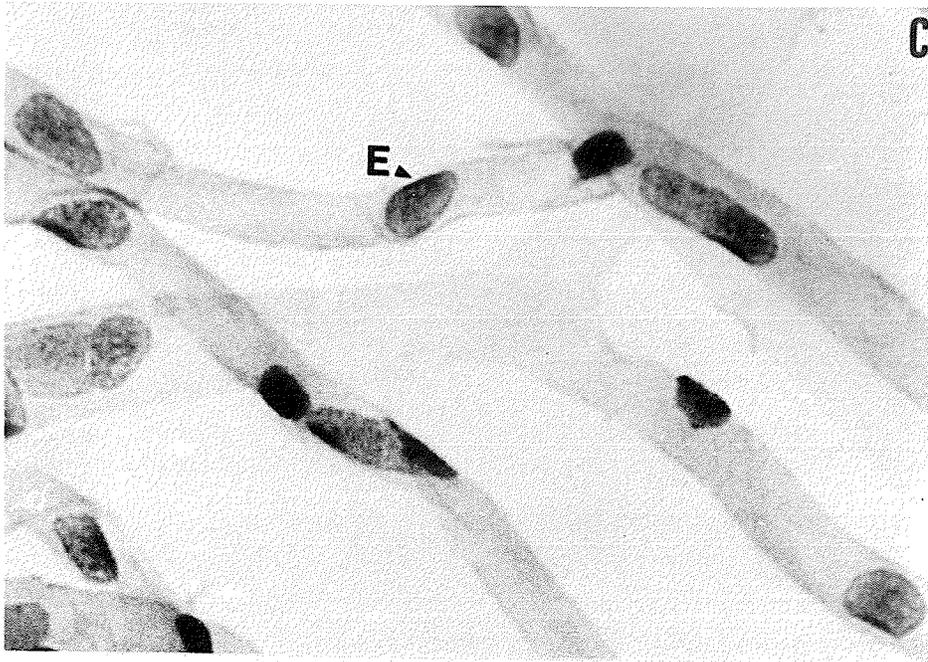
Figure 7 -- Cross sections of retinal capillaries from a
normal rat showing difference of basement
membrane thickness (BMT) in superficial (A)
and in deep capillary bed (B).

BMT of A= 112.448nm.

BMT of B= 80.843nm.

BM = Basement membrane

P = Pericyte

E = Endothelial cell

EN = Endothelial cell nucleus

j = Endothelial cell junction

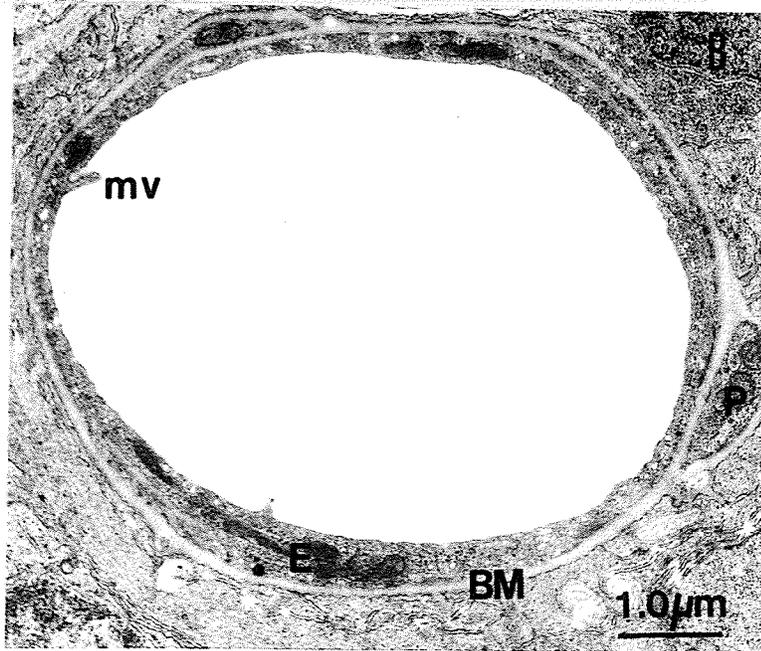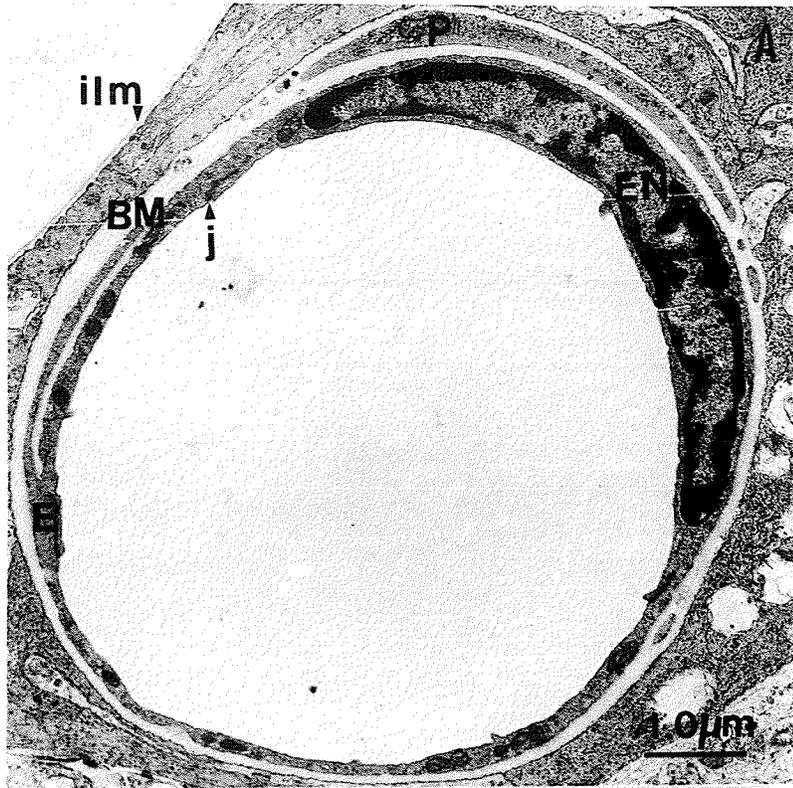mv = Microvilli

ilm = Internal limiting membrane

Figure 8 --  Cross sections of rat retinal capillaries from
            superficial capillary bed showing the effect of
            pancreatic islet cell allotransplantation on
            basement membrane thickness (BMT).

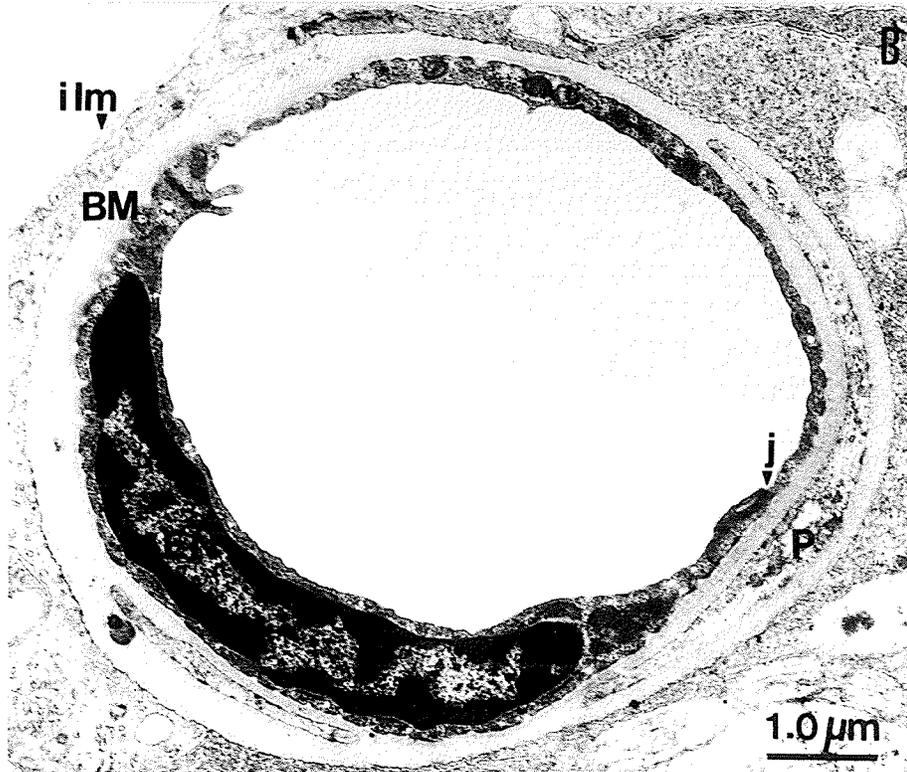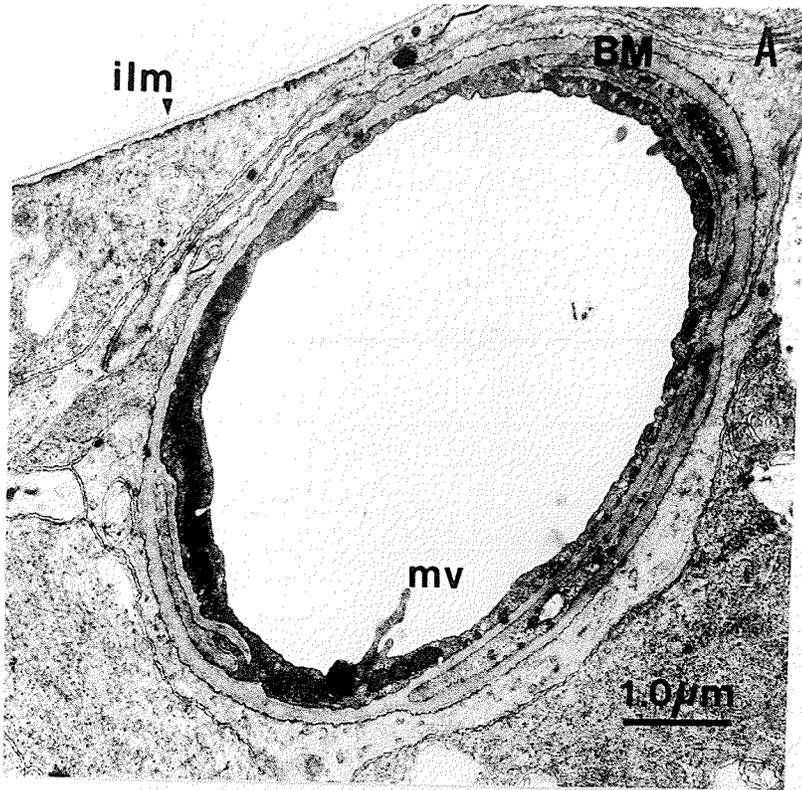            (A) non-diabetic control, BMT = 117.80nm

            (B) Diabetic non-transplanted, BMT = 213.437nm

            (C) Graft rejected animal, BMT = 211.437nm

            (D) Graft accepted animal, BMT = 131.713nm

            See fig.7 for explanation of labels.

A

ilm

BM

mv

1.0 μm

B

ilm

BM

j

P

1.0 μm

C

P

ilm
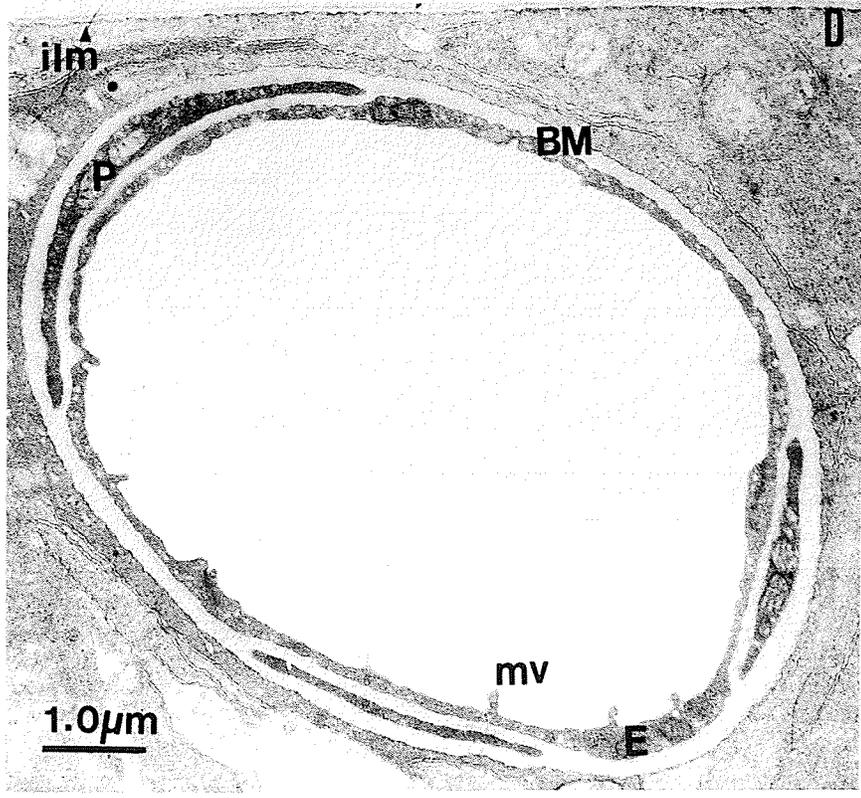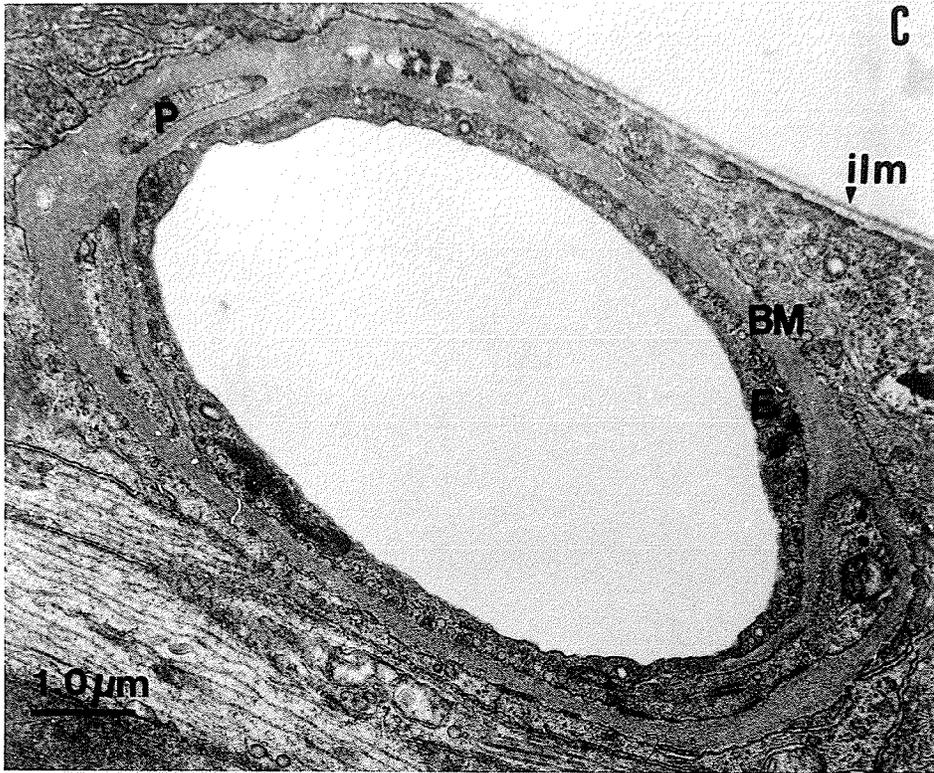
BM

E

1.0μm



D

ilm

P

BM

mv

E

1.0μm

Figure 9 -- Cross sections of rat retinal capillaries from

deep capillary bed showing the effect of

pancreatic islet cell allotransplantation on
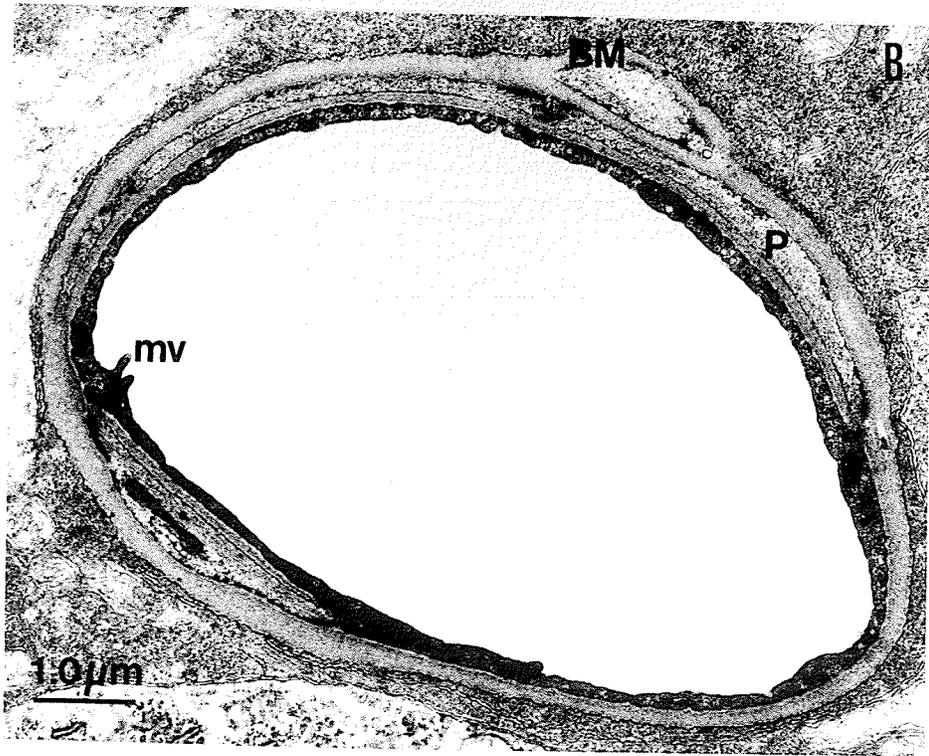
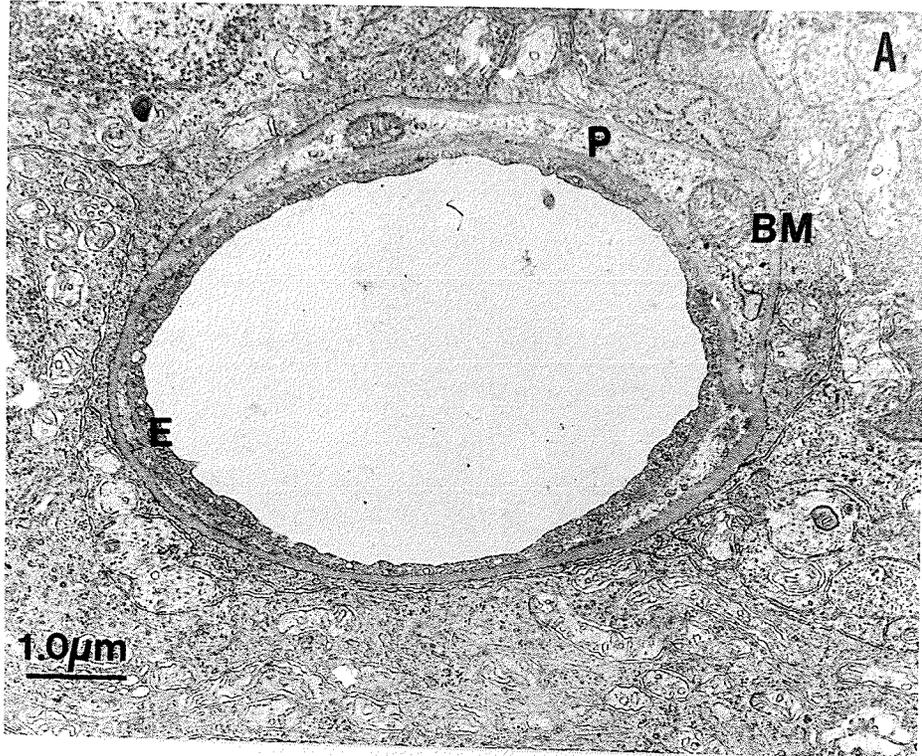basement membrane thickness (BMT).

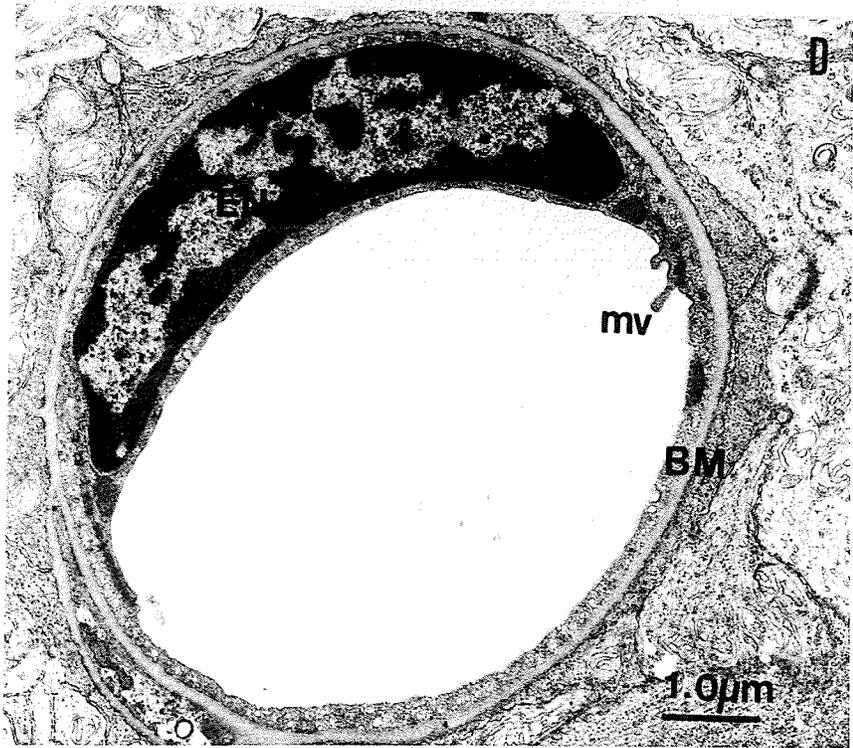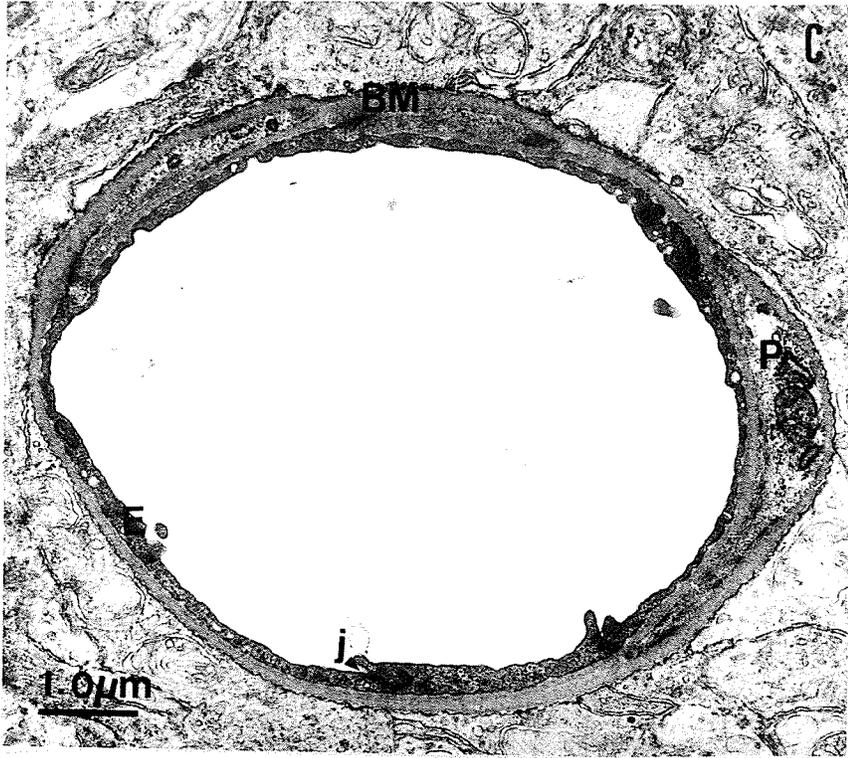(A) Non-diabetic control, BMT = 100.118nm

(B) Diabetic non-transplanted, BMT = 177.518nm

(C) Graft rejected animal, BMT = 148.536nm

(D) Graft accepted animal, BMT = 113.513nm.

See fig.7 for explanation of labels.

A

P

BM

E

1.0μm

B

BM

P

mv

1.0μm

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# THÈSES CANADIENNES

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THE EFFECT OF PANCREATIC ISLET CELL ALLOTRANSPLANTATION ON

DIABETIC RETINOPATHY IN RATS.

by

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A thesis
presented to the University of Manitoba
in partial fulfillment of the
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in

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# 8. REFERENCES:

Addison DJ, Garner A and Ashton N. Degeneration of intramural pericytes in diabetic retinopathy. Brit. Med. J. 1: 264-266, 1970.

Akagi Y, Kador PF, Kuwabara T, and Kinoshita JH. Aldose reductase localisation in human retinal mural cells. Invest. Ophthalmol. Vis. Sci.24: 1516-1519, 1983.

Akagi Y, Yajima Y, Kador PF, Kuwabara T, and Kinoshita JH. Localisation of aldose reductase in the human eye. Diabetes 33: 562-566,1984.

Amos JF. The epidemiology of diabetes mellitus and blindness due to diabetes. Am. J. Optom. Physiol. Optics. 51: 676, 1974.

Apple DJ. Pathology. In: Diabetic retinopathy clinical evaluation and management. L'Esperance FA Jr. and James WA Jr. (eds.). The C.V.Mosby Co. St. Louis, Toronto, London. Chapter 3, pp 38-57, 1981.

Arnest TJ, Goldstick TK and Engerman RC. Hyperglycaemia impairs retinal oxygen autoregulation in normal and diabetic dogs. Invest. Ophthalmol. Vis. Sci. 24:985-989, 1983.

Ashton N. Arteriolar involvement in diabetic retinopathy. Brit. J. Ophthalmol. 37: 282, 1953.

Ashton N. Studies in the retinal capillaries in relation to diabetic and other retinopathies. Brit. J. Ophthalmol. 47: 521, 1963.

Ashton N. Vascular basement membrane changes in diabetic retinopathy. Brit. J. Ophthalmol. 58: 344, 1974.

Ashton N. Pathogenesis. In: Diabetic retinopathy. Little HL, Jack RL, Patz A and Forsham PH (eds.) Thieme-Straton Inc, New York, Chap 7, pp 85-106, 1983.

Babel J and Luenberger P. A long term study on the ocular lesions in the streptozotocin. Albrecht. Von. Grafes. Arch. Klin. Exp. Ophthalmol. 189: 191-209, 1974.

Bernstein MH and Hollenberg MJ. Fine structure of choriocapillaries and retinal capillaries. Invest. Ophthalmol. 4:1016-1025, 1965.

Blair NP, Tso MOM and Dodge JT. Pathologic studies of the blood retinal barrier of the spontaneously diabetic BB-rat. Invest. Ophthalmol. Vis. Sci. 25:302-311, 1984.

Bloodworth JMB. In: Endocrine pathology general and

surgical. Williams and Willkins. New York. pp 643-721, 1982.

Bloodworth JMB and Molitor DL. Ultrastructural aspects of human and canine diabetic retinopathy. Invest. Ophthalmol. 1: 1037-1048, 1965.

Bohman SO, Tyden G, Wilzek H, Lundgren G, Jarenko G, Gunnarson R, Ostman J and Groth CG. Prevention of kidney graft nephropathy by pancreas transplantation in man. Diabetes. 34: 306-308, 1985.

Bretzel RG, Dobroschke J and Federlin K. Current state of experimental islet transplantation. Horm. and Metabol. Res. 13(suppl): 20-27, 1983.

Buzney SM, Frank RN, Varma SD, Tanashima T and Gabbay KH. Aldose reductase in retinal mural cells. Invest. Ophthalmol. Vis. Sci. 16(5): 392-396, 1977.

Buzney SM and Massicotte SJ. Retinal vessels: proliferation of endothelium in vitro. Invest. Ophthalmol. Vis. Sc. 18: 1191-1195, 1979.

Cahill GF. Jr, Etzwiler DD and Frenkel N. "Control" and diabetes. N. Eng. J. Med. 294: 1004-1005, 1976.

Caird FI, Pirie A and Ramsell TG. Diabetes and the eye.

Blackwell Scientific Publications. Oxford & Edinburg, pp 37-58, 1969.

Chakrabarti S, Basu PK and Sima AAF. Pathogenesis of diabetic retinopathy- a review of present research. A. A. J. Ophthalmol. June 1985.

Chen CH and Chen SC. Angiogenic activity of vitreous and retinal extracts. Invest. Ophthalmol. Vis. Sci. 19: 596-602, 1980

Cogan D, Toussaint D and Kuwabara T. retinal vascular patterns. IV.Diabetic retinopathy. A.M.A. Arch. Ophthalmol. 66: 366-378, 1961.

Cohen MP, Urdanivia E, Surima M and Wu VY. Increased glycosylation of glomerular basement membrane collagen in diabetes. Biochem. Biophys. Res. Comun.95: 765-769, 1980.

Colwell JA, Halushka PV, Sarji K, Levine J, Sagel J and Raghavan MGM. Altered platelet function in diabetes mellitus. Diabetes 25 (Suppl. 2): 826-831, 1976.

Cunha-vaz JG. Studies on the pathophysiology of diabetic retinopathy. The blood retinal barrier in diabetes. Diabetes 32 (Suppl. 2):20-27, 1983.

Cunha-vaz JG, Mota CC, Leite EC, Abren JR, Ruas MA. Effects of Sulindac on the permiability of the blood retinal barrier in early diabetic retinopathy. Arch. Ophthalmol. 103: 1307-1311, 1985.

D'Amore P, Glaser BM, Brunson SK and Fenselau A. Angiogenic activity from bovine retina, partial purification and characterisation. Proc. Nat. Acad. Sci. 78(5): 3068-3072, 1981.

Engerman RL. Animal models of diabetic retinopathy. Trans. Am. Acad. Ophthalmol. Otolaryngol. 81: 710-715, 1975.

Engerman RL, Bloodworth JMB, and Nelson S. Relationship of microvascular disease in diabetes to metabolic control. Diabetes 26:760-769, 1977.

Engerman RL, Finkelstein D, Agurrie G, Diddie KR, Fox RR, Frank RN and Varma SD. Ocular complications. Diabetes 31 (Suppl. 1):82-88, 1982.

Federman JF and Gerritsen GC. The retinal vasculature of chinese hamster, A preliminary study. Diabetologia. 6: 186-191, 1970.

Felton SM, Brown GL, Felberg LT and Federman JL. Neovascularisation induced by intracorneal retina implants.

Invest. Ophthalmol. Vis. Sci. 19 (Suppl. ARVO): 137, 1980.

Fischer F and Gartner J. Morphometric analysis of the basal laminae of the rats with streptozotocin diabetes L.II. Retinal capillaries. Exp. Eye. Res. 37: 55-64, 1983.

Frank RN. On the pathogenesis of diabetic retinopathy. Ophthalmology 91: 626-634, 1984.

Frank RN, Keirn RJ, Kennedy A and Frank KW. Galactose induced retinal capillary basement membrane thickening: prevention by Sorbinil. Invest. Ophthalmol. Vis. Sci. 24: 1519-1524, 1983.

Frank RN, Kisney VE, Frank KW, Mikus KP and Randolph A. In vitro proliferation of endothelial cells from kitten retinal capillaries. Invest. Ophthalmol. Vis. Sci. 18: 1195-1200, 1978.

Friberg TR, Rosenstock J and Raskun P. Effect of intensive diabetes management on early diabetic retinopathy and capillary basement membrane thickening. Invest. Ophthalmol. Vis. Sci. 26(3)(Suppl. ARVO): 27, 1985.

Ghosh M, Hausler HR, Basu PK and Stachowska B. An electronmicroscopic study of retinal capillaries of normal and sponteneously diabetic chinese hamsters. Canad. J.

Ophthalmol. 5: 187-193, 1970.

Gibbs G, Wilson R and Ho CK. Experimental diabetes in the monkey. Proceedings of the 2nd international congress of primates. 3: 169-173, 1969.

Glaser B, D'Amore P, Michels RG, Patz A and Fenselau A. Demonstration of vasoproliferative activity from mamalian retina. J. Cell.Biol. 84: 298-304, 1980.

Gliedman ML, Tellis VA, Soberman R, Rifkin H and Veith FJ. Long term effect of pancreatic transplantation function in patients with advanced juvenile onset diabetes. Diabetes Care. 1: 1-9, 1978.

Gray BN and Watkins E Jr. Isolated islet cell transplantation in experimental diabetes. Austral. J. Exp. Biol. Med. Sci. 54(1): 57-70, 1976.

Grimes PA and Laties AM. Early morphological alteration of the pigment epithelium in the streptozotocin induced diabetes: increased surface area of basal cell membrane. Exp. Eye. Res. 30: 631-639, 1980.

Goetz FC. Evaluating the benefits of human islet transplantation. Diabetes 29 (Suppl 1): 52-55, 1980.

Hall K and Luft R. Growth hormone and somatomedin. Adv. Metabol. Disorders. 7: 1 , 1974.

Halushka PV, Rogers RC, Loadhold CA, Wohtman H, Mayfield R, McCoy S and Colwell JA. Increased prostaglandin and thromboxane synthesis in diabetes mellitus. Horm. Metabol. Res. 11(Suppl.): 7-11, 1982.

Hansen AP. Normalisation of growth hormone hyperresponse to exercise in juvenile diabetes after normalisation of blood sugar. J. Clin. Invest. 50: 1806-1811,1971.

Henkind P. Microcirculation of the peripapillary retina. Trans. Am. Acad. Ophthalmol. Otolaryngol. 73: 890, 1969.

His W. Abbildungen über das Gefasssystem der menschlichen Netzhaut und derjenigen des Kaninchens. Arch. Anat. Entwickleungsg. 5: 224, 1880.

Hogan MJ and Feeny L. The ultrastructure of retinal vessels.. II. The small Vessels. Ultrastruc. Res. 9: 29-46, 1963.

Hogan MJ, Alvarado JA and Weddell JE. Histlogy of the human eye. W. B. Saunders. Co. Publishers, Philadelphia. pp 393-522, 1971.

Hori S and Muhai N. Ultrastructural vascular lessions in diabetic rats. Invest. Ophthalmol. Vis. Sci. 18(Suppl. ARVO.): 17, 1979.

Ishikawa T. Fine structure of retinal vessels in man and macaque monkey. Invest. Ophthalmol. 2: 1-15, 1963.

Jackobson B, Basu PK and Hasany SM. Vascular endothelial cell growth inhibitor of normal and pathologic human vitreous. Arch. Ophthalmol. 102: 1543-1545, 1984.

Jonasson O. Transplantation of the pancreas. In: Management of juvenile diabetes mellitus. Traisman HS (ed.). The C. V. Mosby. Co. St. Louis, London, Toronto. 3rd edition, Chap 30, pp 312-321, 1980.

Jonasson O and Hoverstein GH. Replacement of pancreatic B cells as a treatment for diabetes mellitus. Surg. Annu. 10: 1, 1978.

Kennedy A, Frank RN and Varma S. Aldose reductase activity in retinal and cerebral microvessels and cultured vascular cells. Invest. Ophthalmol. Vis. Sci. 24:1250-1258, 1983.

Kernel A and Arnqvist H. Effect of insulin treatment on the blood retinal barrier in the rats with streptozotocin induced diabetes. Arch. Ophthalmol. 101: 968-970, 1983.

King GL and Buzney SM. Modulation of growth in retinal capillary cells by insulin and hyperglycaemia.: possible role in the development of early diabetic retinopathy. Invest. Ophthalmol. Vis. Sci. 24(Suppl. ARVO): 14, 1983.

Kissen AT and Bloodworth JMB. Ultrastructure of retinal capillaries of the rat. Exp. Eye. Res. 1: 1-4, 1961.

Kissun RD, Hill CR, Garner A, Philips P, Kumar S and Jacqueline BW. A low molecular-weight angiogenic factor in cat retina. Brit. J. Ophthalmol. 66:165-169, 1982.

Kojima K. Electronmicroscopic studies on the retina and the choroid in alloxan diabetic rats. Acta. Soc. Ophthalmol. Jap. 75: 14-25, 1971.

Krupin T, Waltman SR, Scharp DW, Oestrich C, Fieldman SD, Becker B, Ballinger, WF and Lacy PE. Ocular fluorophotometry in the streptozotocin diabetes mellitus in the rat: effect of pancreatic islet isograft. Invest. Ophthalmol. Vis. Sci. 18: 1185-1190, 1979.

Krupin T, Waltman SR, Szewczyk P, Kolomas B, Farber M, Silverstein B and Becker B. Flurophotometric studies on the blood retinal barrier in the experimental animals. Arch. Ophthalmol. 100: 631-634, 1982.

Kuwabara T and Cogan DG. Retinal vascular patterns. I. Normal architecture. A.M.A. Arch. Ophthalmol. 64: 904-911, 1960.

Kuwabara T and Cogan DG. Retinal vascular patterns. VI. Mural cells of the retinal capillaries. Arch. Ophthalmol. 69: 492-502, 1963.

Lacy PE, Davie JM and Finke EH. Prolongation of islet allograft survival following in vitro culture ($24^{o}$C) and a single injection of ALS. Science. 204: 312-313, 1979.

Lafferty KJ, Prowse SJ, Simeonovic CJ and Warren HS. Immunobiology of tissue transplantation: A return to the passanger leukocyte concept. Ann. Rev. Immunol. 1: 143-173, 1983.

Largiader F, Lyons GW, Hidelgo F, Dietzman RH and Lillehi RC. Orthotopic allotransplantation of the pancreas. Am. J. Surg. 113: 70-76, 1967.

L'Esperance FA Jr. and James WA Jr. In: Diabetic retinopathy clinical evaluation and management. The C. V. Mosby. Co. St. Louis, Miss., Chap 2: pp 20-37, 1981.

Lillehei RC, Simons RL, Najarian JS, Weil R III, Uchida H,

Ruiz JO, Kjellstrand CM and Goetz FC.Pancreaticoduodenal allotransplantation; experimental and clinical experience. Ann. Surg. 172: 405-463, 1970.

Little HL. The role of abnormal haemorrheodynamics in the pathogenesis of diabetic retionopathy. Trans. Am. Ophthalmol. Soc. 74: 573-636, 1976.

Little HL. Pathogenesis. In: Diabetic retinopathy clinical evaluation and management. L'Esperance J Jr. and James WA Jr. (eds.). The C. V. Mosby. Co. St. Louis, Miss., Chap 58: pp 58-88, 1981.

Ludvigson MA and Sorenson RL. Immunohistochemical localisation of aldose reductase. II. Rat eye and kidney. Diabetes 29: 450-459, 1980.

Luenberger P, Beauchemin ML and Babel J. Experimental diabetic retinopathy. Arch. Ophthalmol.(Paris) 34: 289-304, 1974.

Mandel TE, Hoffman L and Carter WM. Long term isograft of cultured fetal mouse pancreatic islets. The oncogenic effects of streptozocin and the prevention of diabetic renal complications. Am. J. Pathol. 104: 227-236, 1981.

Matsuda H and Sugiura S. Ultrastructure of the "tubular

body" in the endothelial cells of the occular blood vessels. Invest. Ophthalmol. 9: 919-925, 1970.

Mauer SM, Brown DM, Matas AJ and Steffes MW. Effects of pancreatic islet transplantation upon the increased albumin excretion rates in intact and uninephrectomised rats with diabetes mellitus. Diabetes 27: 959, 1978.

Mauer SM, Steffes MW, Sutherland DER, Najarian JS, Michel AF and Brown DM. Studies of the rate of regression of the glomerular lesion in the diabetic rats treated with pancreatic islet transplantation. Diabetes 24: 280, 1975.

McMillan DE. Alteration of capillary basement membrane in diabetes. In: Diabetic retinopathy. Little Hl, Jack RL, Patz A and Forsham PH (eds.). Thieme- Straton Inc, New York, Chap 8, pp 107-111, 1983.

Michelson IC. The mode of development of the vascular system of the retina with some observation of its significance for certain retinal disease. Part I. Trans. Ophthalmol. Soc. U. K. 68: 42-46, 1948.

Murphy RP and Patz A. The natural history and management of nonproliferative diabetic retinopathy. In: Diabetic retinopathy. Little HL, Jack RL, Patz A and Forsham PH (eds.). Thieme-Straton Inc., New york, Chap 19, pp 225-241,

1983.

Naeser P and Anderson A. Effect of pancreatic islet
transplantation on the morphology of retinal capillaries in
alloxan diabetes mice. Acta. Ophthalmologica (Copenhag). 61:
38-44, 1983.

Nelken D, Friedman EA, Morse SI and Beyer WM. Islet of
Langerhans allotransplantation in the rats. Transplantation.
Proc. 9: 333-336, 1977.

NIH Conference. Aldose reductase and complications of
diabetes. Annals. Int. Med. 101: 82-91, 1984.

Orloff MJ, Lee S, Charters AC, Granbort DE, Storock G and
Knox D. Long term studies of pancreatic islet
transplantation in experimental diabetes mellitus. Ann.
Surg. 182: 198-206, 1975.

Oliveira F de. Pericytes in diabetic retinopathy. Brit. J.
Ophthalmol. 50: 134-143, 1966.

Patz A. Retinal neovascularisation: early contribution by
Prof. Michelson and recent observations. Brit. J.
Ophthalmol. 68: 42-46, 1984.

Pipeleers-Marichal MA, Pipeleers DG, Cutler J, Lacy PE and

Kipnis DM. Metabolic and morphologic studies in the intraportal islet transplanted rats. Diabetes. 25: 1041, 1976.

Polsum R, Boot-Handford RP and Heath H. The effects of long term treatment of streptozotocin diabetic rats with an aldose reductase inhibitor. Exp. Eye. Res. 37: 507-515, 1983.

Prince JH. Comparative anatomy of the eye. Charles. C. Thomas. Publisher. Springfield, Illinois. pp32-54, 1956.

Report of the National Commission of Diabetes to the Congress of U.S. Vol I: The long range plan to combat diabetes. DHEW publication no. 76-1018, pp-1, 1975.

Report on the Second National Diabetes Research Conference.: Progress and promise in diabetic research. Eye complications. Public Health Service, NIH Publication No. 84-661, 1984.

Robinson WG Jr, Kador PF and Kinoshita JH. Retinal capillaries: basement membrane thickening by galactosemia prevented with aldose reductase inhibitor. Science 221: 1177-1179, 1983.

Schefler WC. Statistics for health professionals.

Addison-Wesley publishing Co. Reading, Menlo Park, London, Amsterdam, Don Mills, Sydney. pp 1-240, 1984.

Shabo AL and Maxwell DS. Insulin induced immunogenic retinopathy resembling retinitis proliferans of diabetes. Trans. Am. Acad. Ophthalmol. 81: 497, 1976.

Shabo AL, Maxwell DS and Shintaku P. Immunogenic vasculitis and its role in diabetic ocular disease. In. Diabetic retinopathy. Little Hl, Jack RL, Patz A and Foresham PH (eds.). Thieme-Straton Inc, New York, chap 15, pp 150-177, 1983.

Shanon WA Jr, Rockholt DL and Bates SB. Computer-assisted measurement of biological structures. Comput. Biol. Med. 12(2): 149-155, 1982.

Sima AAF, Chakrabarti S, Garcia-Salinas R and Basu PK. The BBrat – an authentic model of human diabetic retinopathy. Current. Eye. Res. 4(10): 1087-1092, 1985.

Slater DM, Mangall Y, Smythe A, Ward AM and Fox M. Neonatal islet cell transplantation in the diabetic rat: Effect on renal complications. J. Path. 124: 117-124, 1978.

Slijepcevic M, Helmke K and Federlin K. Islet transplantation in experimental diabetes in the rat. III.

Studies in allogenic streptozotocin treated rat. Horm.
Metab. Res. 7: 456-461, 1975.


Sosula L, Beaumont P, Jonson KM and Hollows FC. Quantitative
ultrastructure of capillaries in the rat retina. Invest.
Ophthalmol. 11(11): 916-925, 1972


Sosula L, Beaumont P, Hollows FC and Jonson KM. Dilation and
endothelial cell proliferation of retinal capillaries in
streptozotocin diabetic rats: Quantitative electron
microscopy. Invest. Ophthalmol. 11: 926-935, 1972.


Spiro RG and Spiro MJ. Studies on the biosynthesis of the
hydroxylysine linked disaccharide unit of basement membrane
and collagens. III. Tissue and subcellular distribution of
glycosyltransferase and the effect of various conditions on
the enzyme levels. J. Biol. Chem. 246: 4919-4925, 1971.


Steffes MW, Brown DM, Basgen JM and Mauer SM. Ameloriation
of mesangial volume and surface alteration following islet
transplantation in rats. Diabetes 29: 509-515, 1980.


Studer PP, Muller WA and Reynold AE. In: Current topics in
diabetes research. Int. Diabetes. Fed. Abst. Excerpta
Medica. Amsterdam. pp-125, 1976.


Sutherland DER, Goetz FC, Ramsay RC,Fryd DS and Najarian JS.

clinical and experimental kidney and pancreas transplantation. In: Diabetic retinopathy. Little HL, Jack RL, Patz A and Forsham PH (eds.). Thieme-Straton Inc, New York, chap 18. pp 199-221, 1983.

Sutherland DER. Pancreas and islet transplantation. I. Experimental studies. Diabetologia. 20: 161-185, 1981.

Sutherland DER. Pancreas and islet transplantation. II Clinical trials. Diabetologia. 20: 435-450, 1981.

Szarfman A, Hassel JR, Rohrbach DH et al . Components of basement membranes: their properties, functions and alteration in the disease state.In: New trends in basement membrane research. Kuehn K, Schoene HH and Timple R eds. Raven Press, New York, pp 265-275, 1982.

Tchobroutsky G. Relation of diabetic control to the development of microvascular complication. Diabetologia. 15: 143-152, 1978.

Tilton RG, Hoffman PL, Kilo C and Williamson JR. Pericyte degeneration and basement membrane thickening in skeletal muscle capillaries of human diabetes. Diabetes. 30: 326-334, 1981.

Toussaint D, Kuwabara T and Cogan D. Retinal vascular

patterns. Part II. Human retinal vessel studies in three
dimension. A.M.A. Arch. Ophthalmol. 65: 575-581, 1961.

Toussaint D and Dustin P. Electron microscopy of normal and
diabetic retinal capillaries. A.M.A. Arch. Ophthalmol. 70:
96-108, 1963.

Trimble ER, Karakash C, Malaisse-Lagae F, Vissatine I, Orci
L and Renold AE. Effect of intraportal islet transplantation
on the transplanted tissue and the recipient pancreas. I.
Functional studies. Diabetologia. 19: 341-347, 1980.

Tripathy BJ and Tripathy RC. Human retinal vessels in tissue
culture, A priliminary report on the effect of acute glucose
poisoning on cultured vascular cells. Ophthalmology. 89:
858-864, 1982.

Tze WJ, Sima AAF and Tai J.Effect of pancreas
allotransplantation on diabetic nerve dysfunction.
Metabolism. 34(8): 721-725, 1985.

Tze WJ and Tai J. Successful allotransplantation of purified
pancreatic endocrine cells in diabetic rats. Diabetes 32:
1185-1187, 1983.

Tze WJ and Tai J. Intracerebral allotransplantation of
purified pancreatic endocrine cells and pancreatic islets in

diabetic rats. Transplantation. 38: 107-111, 1984.

Tze WJ and Tai J. Allotransplantation and xenotransplantation of "Pseudoislets" in diabetic rats and mice. Transplantation. 38: 438-440, 1984.

Tze WJ, Wong FC and Tingle AJ. The use of hypaque-ficoll in the isolation of pancreatic islets in rats. Transplantation 22(2): 201-205, 1976.

Urbanowski JC, Cohenford MA et al. Non enzymatically glycosylated serum albumin in a galactosaemic infant. New. Eng. J. Med. 306: 84-86, 1982.

Vracko R and Beneditt EP. capillary basal lamina thickening. Its relationship to endothelial cell death and replacement. J. Cell. Biol. 47: 281-285, 1970.

Warwick R. Eugene Wolfe's anatomy of the eye and orbit. W. B. Saunders Company. Philadelphia and Toronto. chap 2. pp 30-180, 1976.

Weibel ER and Palade GE. New cytoplasmic components in the arterial endothelia. J. Cell. Biol. 23: 101-112, 1964.

Williamson JR. Vogler NJ and Kilo C. Microvascular disease in diabetes. Med. Clin. North. Am. 55(4): 847-876, 1971.

Willamson JR. Vogler NJ and Kilo C. Regional variation in width of the basement membrane of muscle capillaries in man and giraffe. Am. J. Pathol. 63: 359, 1971.

Williamson JR and Kilo C. Capillary basement membrane in diabetes. Diabetes 32(Suppl. 2): 96-100, 1983.

Wise GN, Dollery CT and Henkind P. In. The retinal circulation. Harper and Row publishers. New York, Evanston, San Francisco, London. pp 21-54, 1971.

Worthen DM, Lee S, Nakaji NJ, Sayers HJ and Orloff MJ. Effect of whole pancreas transplantation on eye lession of alloxan diabetes. Surg. Forum. 27: 544-546, 1976.

Yanoff M. Diabetic retinopathy. N. Engl. J. Med. 274: 1344-1349, 1966.

Yanoff M and Fine B. Ocular Pathology. Harper and Row Publisher. New York, pp 575-581, 1975.

APPENDIX

Table 1

Body Weight.

One way analysis of variance.

| Source of variation | S.S. | D.F. | M.S. | Variance ratio |
|---|---|---|---|---|
| Between Treatments | 21105.105 | 5 | 4221.021 | 3.665[*] |
| Within Treatments | 26489.033 | 23 | 1151.697 | -- |
| Total | 47594.138 | 28 | | |

* $P < 0.05$

S.S. = Sum of squares

D.F. = Degrees of freedom

M.S. = Mean square

## Table 2

### Pericyte-endothelial cell ratio.

### One way analysis of variance.

| Source of variation | S.S. | D.F. | M.S. | Variance ratio |
|---|---|---|---|---|
| Between Treatments | 0.541 | 5 | 0.108 | 47.829[*] |
| Within Treatments | 0.052 | 23 | 0.002 | -- |
| Total | 0.593 | 28 | | |

* P<0.001

S.S. = Sum of squares

D.F. = Degrees of freedom

M.S. = Mean square

# Table 3

## Pericyte number (Trypsin digested preparation)

### One Way analysis of variance

| Source of variation | S.S. | D.F. | M.S. | Variance ratio |
|---|---|---|---|---|
| Between Treatments | 3640050.13 | 5 | 728010.03 | $13.37^{*}$ |
| Within Treatments | 1252079.32 | 23 | 54438.23 | -- |
| Total | 4892129.45 | 28 | | |

* $P < 0.005$

S.S. = Sum of squares

D.F. = Degrees of freedom

M.S. = Mean square

Table 4

Basement membrane thickness (superficial capillary bed).

One way analysis of variance.

| Source of variation | S.S. | D.F. | M.S. | Variance ratio |
|---|---|---|---|---|
| Between Treatments | 15800.617 | 5 | 3160.123 | 16.914[*] |
| Within Treatments | 4297.152 | 23 | 186.833 | -- |
| Total | 20097.769 | 28 | | |

* $P < 0.001$

S.S. = Sum of squares

D.F. = Degrees of freedom

M.S. = Mean square

## Table 5

### Basement membrane thickness (deep capillary bed).

### One way analysis of variance.

| Source of variation | S.S. | D.F. | M.S. | Variance ratio |
|---|---|---|---|---|
| Between Treatments | 9854.376 | 5 | 1970.875 | 22.972[*] |
| Within Treatments | 1973.286 | 23 | 85.795 | -- |
| Total | 11827.662 | 28 | | |

* P<0.001

S.S. = Sum of squares

D.F. = Degrees of freedom

M.S. = Mean square

Table 6

One way analysis of variance.

Pericyte profile area (deep capillary bed).

| Source of variation | S.S. | D.F. | M.S. | Variance ratio |
|---|---|---|---|---|
| Between Treatments | 99.926 | 5 | 19.985 | 3.263[*] |
| Within Treatments | 140.857 | 23 | 6.124 | -- |
| Total | 240.783 | 28 | | |

* $P < 0.025$

S.S. = Sum of squares

D.F. = Degrees of freedom

M.S. = Mean square

## Table 7

### lumenal area (deep capillary bed).

### One way analysis of variance.

| Source of variation | S.S. | D.F. | M.S. | Variance ratio |
|---|---|---|---|---|
| Between Treatments | 470.156 | 5 | 94.031 | 5.904[*] |
| Within Treatments | 366.318 | 23 | 15.927 | -- |
| Total | 836.474 | 28 | | |

\* $P < 0.005$

S.S. = Sum of squares

D.F. = Degrees of freedom

M.S. = Mean square