

TRANSPLANTATION OF THE ENTIRE PANCREAS WITH LIGATION OF THE EXOCRINE DUCTS

AN EXPERIMENTAL STUDY IN DOGS

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD
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PROF. DR. P. W. KLEIN
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Introduction

Of the various forms of diabetes mellitus, two types predominate. One of these types is characterized by a later onset and in general has a favorable prognosis when treated with insulin or orally administered anti-diabetic drugs. The other, the juvenile type, usually develops at younger ages and has, according to WHITE (148), a life expectation which on average is 17 years shorter than normal, even under insulin treatment. In addition, juvenile diabetes mellitus often results in severe disability due to vascular, nephrogenic, ophthalmogenic, and neurogenic complications.

The exact number of diabetes mellitus patients is not known. The *Diabetes Fact Book 1962* (34) gave a figure of 2.9 million for the United States, but LILLEHEI *et al.* (95) put the number at 4 to 5 million, 4 to 5 per cent being of the juvenile type. WHITE (148) reported that there were 118,000 juvenile diabetics in the U.S.A. in 1956.

According to the Netherlands Diabetics Association, there are 125,000 known cases of diabetes mellitus in The Netherlands and an equal number of unknown cases may be assumed, which would bring the total to 250,000 cases. The Association estimates that 1,000 of these patients suffer from the juvenile form, but if the American estimate of 4 per cent is applied the number of juvenile diabetics in The Netherlands would be about 10,000.

According to these American sources, diabetes mellitus is among the diseases with the most rapidly increasing incidence. No data are available on this point for The Netherlands, because little epidemiological research has been done in this field (110).

Most endocrinologists (88) are in agreement that the primary cause of diabetes mellitus is a disturbance localized in the endocrine part of the pancreas. Among other considerations, this raised the question of whether pancreas transplantation might be a suitable form of treatment for the group of patients

with the juvenile type of diabetes mellitus in whom insulin therapy is ultimately inadequate. The pancreas is, however, a complex organ, both anatomically and physiologically, and this explains the number of surgical techniques which have been developed and applied for pancreas transplantation. All of these methods represent complicated surgical procedures; the number of successful transplantations in man has been very low and the mortality and morbidity among the recipients quite high (63).

The value of pancreas transplantation would be increased by simplification of the surgery involved, comparable to kidney transplantation. This attracted the interest of experimental surgery. In addition, an experimental model could solve some of the remaining problems related to the pathophysiology of diabetes mellitus. Such problems can, for example, be approached by transplantation of a pancreas in dogs with the juvenile form of diabetes mellitus.

These considerations led us to investigate the possibilities for the development of a simple form of pancreas transplantation.

Problems associated with pancreas transplantation

1. HISTORICAL REVIEW

JOHANN CONRAD VON BRUNNER (1652–1727) (19) was the first to perform resection of the pancreas in the dog. During one of his experiments he noticed that a dog became extremely thirsty after the operation. This was very probably the first case of experimentally induced diabetes mellitus, but it was not until 1890 that the relationship between the pancreas and diabetes mellitus was discovered by MERING & MINKOWSKI (104).

SSOBOW (1901) (131) was the first to describe the ligation of the pancreatic duct *in vivo*, which he performed in dogs, cats, and rabbits. He followed the post-operative course in these animals for 60, 100, and 400 days, respectively, and found no glucose in the urine over these periods. Microscopically, he found atrophy of the acinar cells, whereas the islets of Langerhans remained intact. This led him to conclude that the islets of Langerhans were the most important factor in diabetes mellitus.

In 1922, BANTING *et al.* (7) succeeded in producing an extract of pancreas tissue, which they used to treat animals suffering from diabetes mellitus. Ten weeks after ligation of the pancreatic duct, when acinar atrophy had developed, the favorable effect of the extract could still be demonstrated.

Meanwhile, HÉDON (65) had performed the first pancreas autotransplantation in 1892 by subcutaneous implantation of a pancreatic segment with the blood vessels intact. After new vessels had grown into the glandular tissue, he cut the original vessels and found that the transplant remained viable.

In the following period pancreas autotransplantations were mainly performed by physiologists (33,64,78) to investigate hormonal and enzymatic mechanisms in the organ. These studies showed that the hydrochloric acid in

the duodenum stimulated the exocrine functioning of a pancreas implanted in the neck (64) and that such implants caused a temporary lowering of the glucose level in the urine. The anastomoses established by these authors were primitive, using Payr's cannulas as described by BOTTIN & DELREZ (16), who investigated the early development of thromboses in the anastomoses made for pancreas transplants in the neck. Attempts were also made (18, 73, 128) to retain the function of subcutaneously implanted pancreas tissue without vascular anastomoses, but unsuccessfully.

Among the authors who ligated the pancreatic duct were SWAN & RUNDLES (134), who clearly indicated in 1957 that they saw their work as a continuation of the investigations done by HÉDON (65). In 1957, too, LICHTENSTEIN & BARSCHAK (92) performed a pancreas allotransplantation in a dog specifically to study the therapeutic possibilities of pancreas transplantation for future use in man. Unsuccessful allotransplantations with ligated ducts in animals were described by BROOKS & GIFFORD (17), but they were the first to perform pancreas implantation in man. They transplanted pancreas tissue from a fetus to the mother, implanting it in the m. quadriceps femoris. Of their 6 patients, only one showed a reduced insulin requirement, but even this was not significant.

In 1962, DEJODE & HOWARD (31) were the first to report a series of successful pancreas allotransplantations in dogs. They used the celiac artery as afferent vessel and ligated all of the vessels that did not run to the pancreas. The gastroduodenal vein was used as the efferent vessel, and the portion of the duodenum into which the exocrine ducts emptied was included in the graft. The transplants functioned well but were rejected on average after 6 days. Immunosuppressive drugs were not used. Of the investigators who transplanted pancreas tissue without the use of vascular anastomoses, DUBOIS & GONET (37) and HOUSE *et al.* (68) found a transient effect in the form of a drop in the glucose level in the blood of rats and hamsters, respectively.

The first pancreas allotransplantation in man was performed in December of 1966 by LILLEHEI's group and was described by KELLY *et al.* (81).

2. SPECIFIC PROBLEMS

As organ, the pancreas has an exocrine and an endocrine function. The digestive enzymes are formed in the acinar cells and transported via the pancreatic ducts to the duodenum. The hormones formed in the islets of Langerhans are carried via the portal vein to the liver before they reach the large circulation.

The pancreas has 3 afferent blood vessels: the superior pancreaticoduodenal artery, the inferior pancreaticoduodenal artery, and the splenic artery. The

venous drainage is analogous except that all of the veins converge in the portal vein. The pancreas is also attached to the duodenum and in close contact with the choledochus duct. For further details reference is made to the textbooks on anatomy (58,108), physiology (122,138), and histology (9,88). The described anatomy means that in man an orthotopic allotransplantation involves a major and technically very complicated operation.

As the introduction indicates, the main concern in pancreas transplantation is the endocrine function of the organ. Theoretically, therefore, the best site for a pancreas transplant would not be the original anatomical position and preference would be given to transplantation to another site with only the endocrine function preserved. This raises a number of problems on which extensive research has been done but consensus has not been reached. These problems will be discussed successively.

2.1. The influence of ligation of the exocrine ducts on the pancreas

As already mentioned, SSOBOLW (131) ligated the exocrine ducts of the pancreas in dogs, cats, and rabbits and found atrophy and fibrosis of the acini but no changes in the islets of Langerhans. BANTING *et al.* (7) performed similar experiments and found insulin in the islet tissue 10 weeks after ligation of the duct.

In 1943, DRAGSTEDT (35) was the first to mention deterioration of the endocrine function of the pancreas after ligation of the ducts, leading in some cases to diabetes mellitus. HIEBERT *et al.* (66) ligated the duct but, like ANLYAN *et al.* (4), were unable to demonstrate an influence on the endocrine function.

AMBROMOVAGE *et al.* (2) followed-up 10 dogs for 83 weeks after ligation of the pancreatic duct. During this period they administered glucose orally, which led to an abnormal insulin reaction and a glucose tolerance test (GTT) with a diabetic curve. IDEZUKI *et al.* (74) separated the head and tail of the canine pancreas, ligated the pancreatic duct of the tail, and subsequently removed the head. Two-thirds of the surviving dogs showed similarly diabetic GTT curves. No such curves were obtained for dogs in which these steps were done in one operation.

CHAYA *et al.* (22), who ligated the pancreatic ducts in dogs used as donors several weeks later when transplantation was performed, found no difference in the oral GTT results obtained before and after the ligation. REEMTSMA *et al.* (121) performed similar experiments in dogs and rats and directly after the operation found no difference between the serum glucose levels in transplants with and without ligation of the pancreatic duct. In isologous rat strains the blood-sugar values found 150 days after transplantation of a pancreas with a ligated duct were not diabetic, but when the transplant was extirpated these

values immediately rose. Using a similar technique in dogs, SEDDON & HOWARD (127) saw normal serum glucose levels after transplantation. One of their dogs could be followed for 75 days post-operatively.

2.2. Pancreatitis

All of the authors who ligated the pancreatic duct report that pancreatitis developed, in most cases a mild edematous form. The necrotising form was only seen in a few cases.

LARGIADER *et al.* (82,83) did not observe pancreatitis after orthotopic transplantation of the pancreas with duodenum, i.e., without ligation of the duct and with end-to-side suturing of the donor's portal vein to the recipient's portal vein. Others authors (63,153), however, found elevated amylase levels and sometimes even necrotising pancreatitis in pancreas transplants in which the pancreatic duct had not been ligated.

Lastly, TEIXEIRA & BERGAN (136) demonstrated that the venous anastomosis should be made as ample as possible to avoid pancreatitis. Necrotising pancreatitis was seen significantly more frequently for end-to-end anastomoses (portal vein on iliac vein).

2.3. Insulin and the liver

Insulin is secreted by the beta cells directly into the blood of the portal vein and passes through the liver before reaching the large circulation. KAPLAN & MADISON (80) showed that half of the insulin is withdrawn from the blood in the liver, and BEGER *et al.* (10) even measured an 82 per cent drop in the portal insulin level after intra-abdominal operations. ZIMMERMANN (153) found higher insulin levels in the portal blood than in the blood in the vena cava. When, however, an anastomosis is established between the portal and caval veins, passage through the liver is excluded and higher insulin concentrations would be expected in the peripheral venous blood. Nevertheless, WADDELL & SUSSMAN (143) did not see a significant elevation of the insulin level in the peripheral blood after making a porto-caval shunt, and no difference in effect on the blood-sugar level was found by STARZL *et al.* (133), who administered insulin peripherally and via the portal circulation. LEVEEN *et al.* (91), to the contrary, saw an improvement in experimental canine diabetes when the venous drainage of the pancreas by-passed the liver, and HEARN & PATON (61) saw an improvement of the diabetic state in diabetic patients given a porto-caval shunt.

In heterotopic pancreas transplantations, too, the insulin will not reach the

liver directly, and anomalous insulin patterns would be expected. BERGAN & TEIXEIRA (12) indeed found higher insulin levels in the peripheral blood of dogs after heterotopic than after orthotopic transplantations. This finding could not, however, be confirmed by LARGIADER (85), IDEZUKI *et al.* (75), or LILLEHEI *et al.* (95). SELLS *et al.* (129) found higher insulin levels after heterotopic transplantations in the pig, but ZIMMERMANN *et al.* (152) could not reproduce their results.

2.4. Denervation

Like every transplanted organ, the transplanted pancreas will be completely denervated. The effect of vagotomy on the enzymatic and hormonal function of the pancreas is of interest. HOUSSAY *et al.* (71) found no influence of vagotomy on the GTT in the dog, but LIEDBERG *et al.* (93) showed in rats that the insulin index (Δ insulin/ Δ glucose) is significantly lower after vagotomy.

RUSSEL *et al.* (125) investigated the effect of vagotomy on glucagon production and found a distinctly reduced secretion of the hormone after vagotomy in man. SMITH *et al.* (130) did a study in man by investigating the influence of truncal vagotomy and selective vagotomy on the excretion of pancreatic enzymes, and GOVAERTS & KIEKENS (46) performed truncal vagotomy in dogs. In both studies enzyme production was lower after the operation.

2.5. Ischemia

JOISON (79) avoided an ischemic period during pancreas transplantations in dogs by leading blood from the recipient through the donor's pancreas via the femoral vessels. The animals died immediately after the transplantation; apart from a sudden sharp drop of the calcium level, no reason for the fatal course could be found.

BINDER *et al.* (14) demonstrated that *in situ* the endocrine part of the canine pancreas can survive an hour of ischemia without perfusion and under normothermic conditions. The serum amylase level was elevated after ischemia in all of their experiments.

CERRA *et al.* (21) drew attention to the fact that in pancreas transplantations with ligated ducts and prolonged ischemia, the recipient shows abnormally high serum insulin levels and low glucose values in the period immediately after the operation. According to these authors, the explanation of these findings is that ischemia leads to damage of the beta cells, which releases the active insulin localized in the granules of such cells.

2.6. Rejection

Allotransplantation of the pancreas leads to rejection of the organ, because foreign proteins activate cellular and humoral defence mechanisms. The first investigators to perform successful experimental pancreas allotransplantations (31, 48, 82, 113, 120) reported an organ survival time of 6 to 11 days. Later, REEMTSMA *et al.* (121) used mercaptopurine and azathioprine as immunosuppressive drugs and obtained longer organ survival in transplants, both with and without ligation of the pancreatic duct. Many authors (51, 62, 76, 94, 152) applied a combination of azathioprine and prednisone. We investigated the influence of tissue typing and anti-lymphocyte serum (ALS) (55).

The parameters reflecting rejection are still uncertain. A number of authors (13, 49, 77, 86, 153) observed elevation of the serum amylase level just before rejection, and GRENIER *et al.* (51) reported the occurrence of hyperinsulism during rejection without a change in the serum glucose level. ROMIEU *et al.* (123) pointed out that the blood-sugar level does not rise until the rejection process is almost completed, and the same conclusion was reached by ZIMMERMANN (153) and OTA *et al.* (113), who are of the opinion that a sudden elevation of the serum glucose level after pancreas allotransplantation is more indicative of acute thrombosis than of rejection. VAN HEE (63) considered the occurrence of lipaemia to be the most constant initial sign of rejection.

3. TECHNICAL POSSIBILITIES

Several surgical transplantation techniques have been developed for pancreatic grafts with vascular anastomoses.

The pancreas can be implanted at the original anatomical position (orthotopic) or at another site (heterotopic transplantation), with or without ligation of the pancreatic ducts.

Orthotopic pancreas allotransplantation was described by LARGIADER *et al.* (82, 83, 84) and later by others (72, 152). With this method the duodenum is included, and a duodenal-intestinal anastomosis is made.

ZIMMERMANN (153) performed porcine orthotopic transplantations without inclusion of the duodenum, but maintained enzyme secretion by a Roux *en Y* anastomosis of the jejunum on the pancreas.

To the best of our knowledge, orthotopic transplantation with ligation of the pancreatic duct have not been performed.

For *heterotopic pancreas allotransplantations* the vascular relations permit three localizations: in the neck, with vascular anastomoses to the carotid artery

and the jugular vein; in the abdomen on the aorta and caval vein below the renal blood vessels, and in the left or right fossa iliaca, the anastomoses being made with the iliac vessels.

Some authors (36,48) implanted the pancreas in the neck region with preservation of the enzyme secretion. Others ligated the pancreatic ducts and implanted the pancreas in the abdomen or a fossa iliaca. The latter method was preferred by LUCAS *et al.* (98), REEMTSMA *et al.* (121), and COUINAUD & MALTA-BEY (30), but they transplanted only part of the organ (the tail). BERGAN *et al.* (11) transplanted the entire organ with ligated ducts, using the celiac artery of the transplant as arterial afferent and as venous efferent the portal vein, which were sutured to the recipient's aorta and caval vein under the renal vessels.

However, most of the experimental pancreas transplantations and all of those in man have been heterotopic, almost always with intact drainage of the pancreatic enzymes. Such heterotopic transplantations of the pancreas with the duodenum have been performed by many investigators (36,63,67,77,96,153). In these pancreas-duodenum (PD) grafts the duodenum was connected to the digestive tract or led out via the skin as a fistula on one side, the other end being closed as described by MURAT *et al.* (111).

MEYER *et al.* (107) did not include the duodenum but insured the transport of the enzymes with a Roux *en Y* loop on the cut pancreas. This method was also used by AQUINA *et al.* (5). GLIEDMAN *et al.* (42) used a pancreatic duct-ureter anastomosis in both dogs and man to obtain outflow of pancreatic enzymes via the bladder.

4. CLINICAL PANCREAS TRANSPLANTATIONS

Up to December 1974, 36 human pancreas transplantations had been reported. In 12 of these cases, some of them very recent, the organs functioned for only a few days or not at all, and therefore no other details are available. The other cases will be briefly discussed here.

4.1. Body and tail transplantations

In these cases the pancreas was transplanted without the head and with ligation of the pancreatic ducts. Five transplantations of this type have been performed.

In a case reported by GLIEDMAN *et al.* (42) a large amount of amylase-rich fluid accumulated around the transplant. The pancreas functioned well, but

not the kidney transplanted at the same time. Five weeks after the operation, when sepsis developed and the patient died, the pancreas was still capable of correcting elevated blood sugar levels without the administration of insulin.

In the other 4 cases, which included the first human pancreas transplantation, performed by LILLEHEI and described by KELLY *et al.* (81) in 1966, the organ functioned for only 5 to 6 days.

4.2. Pancreatico-duodenal transplantations

Sixteen operations of this type have been performed. The longest duration of organ function was 12½ months; at death, due to sepsis, the pancreas transplant was functioning well but the simultaneously transplanted kidney had had to be removed because of rejection (94). CONOLLY *et al.* (28) lost their patient with the longest survival time, who was in good condition 10 months after the operation, in an automobile accident.

The function of the other pancreatico-duodenal transplants varied in duration from nil to about 8 months. Of interest in this connection is the report by LILLEHEI *et al.* (96) of the death of a patient due to perforation of the simultaneously transplanted duodenum as the result of rejection duodenitis.

Of the 16 PD-graft patients, 12 have died. Four are still alive, but without the transplant; in these cases the organ was removed and the patient put back on insulin therapy. CONOLLY *et al.* (28) recommend excision of the transplant at the least sign to rejection.

4.3. Transplantations with pancreatic duct to ureter anastomosis

In 3 cases of body and tail transplantations GLIEDMAN *et al.* (42) also made an end-to-end anastomosis of the pancreatic duct to the ureter in patients whose kidneys had been removed because of diabetic nephropathy. One of these patients still had reasonably good endocrine function after 7 months. The second developed leakage of the pancreas-ureter anastomosis and vascular-suture thrombosis after the transplant had functioned adequately for 33 days; the graft was removed and insulin therapy reinstituted. The third patient had good pancreas function 10 months after the operation and no longer required insulin.

5. OBJECTIVES OF PANCREAS TRANSPLANTATION

It is evident from the foregoing that agreement has not yet been reached on the

ultimate influence of pancreatic duct ligation on the endocrine function. It is, however, clear that a mild form of pancreatitis invariably develops when ligation is performed. Consequently, after performing their first human body-and-tail transplantations with ligated duct both LILLEHEI and GLIEDMAN changed to a method providing for persisting enzyme outflow, the former by including the duodenum in the transplant and the latter by connecting the duct with a ureter. Several serious objections can be raised to these methods:

1. Inclusion of the duodenum means the transplantation of two organs which may not behave in the same way immunologically. This entails the risk of rejection duodenitis, as described by LILLEHEI *et al.* (96) for human recipients, which can lead in turn to perforation of duodenum.

2. Drainage of the pancreatic enzymes into the digestive tract, either via the included duodenum or via a Roux *en Y* shunt between the recipient's jejunum and the transplanted pancreas, involves intra-abdominal intestinal anastomoses that may become dehiscant under rejection processes and thus lead to peritonitis, which can take a serious course due to the use of immunosuppressive drugs and the escape of pancreatic juices into the abdominal cavity.

3. The establishment of an anastomosis between the pancreatic duct and a ureter involves the possibility of local leakage but also a much more serious problem, i.e., the sacrifice of a still functioning kidney, which is certainly contra-indicated in the severe form of diabetes mellitus that can lead to renal insufficiency. As a result, this operation has so far only been applied in patients with terminal kidney failure due to diabetes mellitus, which means that sooner or later a kidney transplantation will have to be performed. The main purpose of pancreas transplantation can, however, be expected to be the prevention of terminal renal insufficiency.

4. All of the described forms of pancreas transplantation involve major, complicated operations for which a high morbidity can be expected, as indicated by the discussion of clinical pancreas transplantation.

These objections could be eliminated by ligation of the exocrine ducts of the pancreas. In that case 2 vascular anastomoses situated extraperitoneally would suffice. Although many authors have pointed to the injurious effect of duct ligation on the endocrine function of the pancreas (2,35,87), other investigators (11,66,121,127) have argued that there is no such effect.

Thus, before pancreas allotransplantations with duct ligation are undertaken it must be known exactly what the final consequences of this ligation are for the endocrine function of the organ and why the above-mentioned authors obtained divergent results. Once the effects of duct ligation are known, separate investigation can be made, as far as possible, of factors originating as a result of allotransplantation that might also influence the endocrine function. The authors who claim that duct ligation has an unfavorable effect on the endocrine

function of the pancreas, observed the reduced function only after several months (74) to 1½ years (2). This makes it necessary to follow up a number of allogenic pancreas transplantations over a long period.

In studying the data in the literature concerning experimental transplantations we found a high degree of variability in the survival of the transplanted organs. This could be due to the use in these investigations of mainly mongrel dogs in which the histocompatibility between donor and recipient was unknown. Studies done by VRIESENDORP *et al.* (141) on the canine histocompatibility system and by him and others on the influence of compatibility for this system (tissue typing) on the survival of heart (15), kidney (147), skin (142), and intestine (146) allotransplants, support our assumptions. The influence of compatibility or incompatibility for the histocompatibility system on the duration of the survival of pancreas allotransplants had not, however, been investigated yet.

If pancreas allotransplants behaved in this respect like other vascularized transplants, this would be an indication that there is no difference in antigenicity between endocrine tissue and, say, kidney tissue, as assumed by LILLEHEI & IDEZUKI (94). It would also mean that on the basis of tissue typing the endocrine function of pancreas allotransplants with ligated duct could be studied for long periods without the use of immunosuppressive drugs which might also have an influence on the endocrine function. For a reliable investigation of the possibilities of allogenic pancreas transplantation with ligated exocrine ducts, therefore, the experimental set-up for the study described here was chosen such that as much information as possible could be obtained about the possible unfavorable influence of several factors: duct ligation, ischemia, denervation, heterotopic localization, and rejection.

In the following chapters a discussion will be given of a number of experimental designs and experiments done in dogs in an attempt to obtain answers to four questions:

1. What is the effect of ligation of the exocrine ducts of the pancreas on the endocrine function of the organ?
- 2a. How does the endocrine function of the pancreas react ultimately after heterotopic pancreas autotransplantation with ligated endocrine ducts?
- 2b. How does the endocrine function of the pancreas react ultimately after heterotopic pancreas allotransplantation with ligated endocrine ducts?
3. What is the influence of tissue typing on the survival time of a heterotopic pancreas allotransplant?
4. What is the influence of immunosuppressive drugs on the survival time of a heterotopic pancreas allotransplant under known tissue incompatibility between donor and recipient?

CHAPTER II

The experimental design and methods

I. THE ANIMALS

The dog was chosen as a suitable animal for this study on the following grounds: its tractability; surgically, it offers no special problems and is comparable with man; the availability of specially bred strains with known parentage (beagles); and the fact that diabetes mellitus occurs in dogs (1:152; ref. 29).

For the experiments in which body weight and histocompatibility would not influence the results, use was made of mongrels not older than 5 years and weighing between 9 and 25 kg. These animals were obtained commercially and were used after a 3-week quarantine period under veterinary supervision (Central Laboratory Animal Service, Faculty of Medicine, Erasmus University, Rotterdam; head: Dr. W. VAN DIJK).

When weight or histocompatibility could have an effect, use was made of beagles weighing between 10 and 15 kg and aged 9 to 18 months. Some of these animals were obtained from the Central Institute for the Breeding of Laboratory Animals T.N.O. (Zeist, The Netherlands; head: Dr. J. VAN VLIET), and some had been bred in our own kennels.

2. THE EXPERIMENTAL GROUPS

In connection with the questions formulated in Chapter I, 3 experimental groups were formed:

Group I: Mongrels in which both pancreatic ducts were ligated to determine the long-term effect of elimination of the exocrine function on the endocrine function of the pancreas.

Group 2: Mongrels given a heterotopic autotransplant with ligated ducts to determine whether ischemia, denervation, or the heterotopic localization would lead to differences in the endocrine function as compared with group 1.

Group 3: Typed beagles given a heterotopic pancreas allotransplant with ligated ducts. The immunological factor was included to permit evaluation of our method of tissue typing; to this end, the group was divided into 3 subgroups as follows:

- 3a. unrelated, non-identical pairs
- 3b. related, non-identical pairs (differing by 2 haplotypes)
- 3c. related, identical pairs (0 haplotype difference)

In addition, the influence of immunosuppressive drugs was studied in 2 subgroups:

- 3d. related, non-identical pairs (differing by 1 haplotype) given ALS
- 3e. related, non-identical pairs (differing by 1 haplotype) given azathioprine and prednisone.

3. SELECTION OF THE BEAGLES

Prospective selection for serologically determined (SD) dog leukocyte antigens (DL-A) was done according to VRIESENDORP *et al.* (141,142) and WESTBROEK *et al.* (146). Combined serological and transplant-survival experiments in small intestine, skin, and heart transplantations made probable the existence of a major histocompatibility locus in the dog (DL-A) in analogy with the HL-A locus demonstrated in man (1,20).

In these experiments use was made of the microcytotoxicity test after KISSMEYER-NIELSEN as modified by VAN ROOD *et al.* (124). Thus, in our experimental design donor-recipient combinations were prospectively selected on the basis of serological tests. In this study donor-recipient pairs were not tested for LD determinants (MLC test).

4. TREATMENT

Each of the 3 main groups will be discussed in a separate chapter, except for standardized techniques or methods used in all 3 groups, which will be dealt with here. For example, the surgical technique, which was different for each group, will be discussed in the relevant chapter.

4.1. Preparation for surgery

The endocrine and exocrine function of the pancreas was checked pre-operatively in all animals. In addition, the kidney function, liver function, and blood picture were evaluated. The dogs were not fed for 16 hours before the operation. On the day of the operation the administration of antibiotics was started pre-operatively.

4.2. Antibiotics

Na-benzylpenicillin (Specia, Paris, France) and streptomycin sulfate (Mycofarm, Delft, The Netherlands) were given intramuscularly in combination in a dosage of 2 million U penicillin and 0.5 g streptomycin twice daily. For a second operation, ampicillin (Penbriten, Beecham, United Kingdom) was given intramuscularly in a dosage of 500 mg twice daily.

4.3. Anesthesia

Endotracheal inhalation anesthesia was used throughout, without pre-medication. Sodium pentothal® (Abbot N.V., Amsterdam, The Netherlands) was administered as preliminary anesthetic in a dose of 30 mg per kg body weight intravenously together with 0.5 mg atropine. Anesthesia was maintained with 0.05 mg Fentanyl® (Janssen Farmaca, Beerse, Belgium), 6 litres N₂O-O₂ in a ratio of 2:4, and fluothane (Halothane, ICI, United Kingdom). Under anesthesia, the following fluids were administered parentally: dextran 40 10%, canine plasma, glucose 5%, 0.9% NaCl, 5% NaHCO₃, and when necessary blood. The amount varied appreciably for the various groups, and will be discussed separately. Dextran 40 was given in connection with the favorable effect on the course of acute pancreatiti demonstrated by GOODHEAD (43). For the same reason, Trasylol® (Bayer) was also given (50,000 KIU intravenously) during the operation, because of the reported (57,103,140) inhibitory effect on the liberated pancreatic enzymes kallikrein and trypsin. During the course of the study doubts arose concerning the value of this drug, which were reinforced by the results of new studies on its use in this situation (126). However, we continued to use it rather than change the experimental conditions during the study.

During anesthesia, the pulse and an ECG were registered on an Elema Schönander minograph 81 and visualized on an Elema Schönander oscilloscope EM 530. In a number of the experiments the arterial and venous pressures were

measured intravascularly at the same time and observed with the same equipment.

4.4. Post-operative treatment

Antibiotics were administered during the first 5 days after the operation. In general, water was available *ad lib* on the first post-operative day and food on the second, except for pancreatectomized dogs, which were not given solid food until the third day.

4.5. Diet

Because the exocrine function of the pancreas was eliminated in these experiments, special attention was given to the feeding and enzyme supplementation of the animals. Food was given *ad lib*, and dogs showing reduced appetite were put on a different diet. Dependent on the appearance and consistency of the feces and the course of the body weight, the following food was given:

Hope Farm pellets (Hope Farms N.V., Woerden, The Netherlands)

Liquid diet: Wessanen* (Wessanens Koninklijke Fabrieken N.V., Wormerveer, The Netherlands)

Pelsifood (Trouw N.V., Ermelo, The Netherlands)

Bonzo Dinner (Bogena N.V., Rotterdam, The Netherlands)

Blokvlees (Bogena N.V., Rotterdam, The Netherlands)

Protifar (Nutricia N.V., Zoetermeer, The Netherlands)

Liga biscuits (Liga Fabrieken N.V., Roosendaal, The Netherlands)

For enzyme supplementation, a choice was made for combination among:

Combizym compositum (Luitpold, obtained from Will Pharma, Amsterdam, The Netherlands)

Cotazym (Organon, Oss, The Netherlands)

Pancreatin powders (5 g)

Lipase capsules (25 mg)

* This food was specially composed for our experiments by Wessanens Koninklijke Fabrieken (J. N. VAN HAASTER) and contained:

Protein	20%	Ash	3%
Fat	30%	lactose	trace
Moisture	5%	Cal./gram	5

Minerals and vitamins were added to an average level for feeds for omnivorous species.

5. DETERMINATION OF PANCREAS FUNCTION

5.1. Endocrine function

a. Serum glucose level

The serum glucose level was determined according to the enzymatic glucose-oxidase method with O-dianisidine as chromogen, as described in *Standard Methods of Clinical Chemistry* (132a). In addition to serum glucose determinations made at arbitrary times, glucose tolerance tests (GTT) were performed at regular intervals. For these tests a loading dose of 1 g glucose per kg body weight was injected intravenously after a fasting period of at least 16 hours. After a fasting value had been obtained, blood samples were taken 5, 10, 20, and 30 minutes after the glucose injection. From the GTT values, the K value was calculated according to LUNDBAEK (99), this value giving the serum glucose disappearance rate as percentage per minute. Plotting of the GTT-derived values on semi-logarithmic paper gives a linear curve from which the K values can be calculated according to HAMILTON & STEIN (59) (see Fig. 1a,b for example).

The K values made it possible to detect small differences in non-pathological blood sugar curves. LUNDBAEK found that the mean K value for normal human subjects amounted to 1.72%/min, and CONARD (25,26,27) obtained a value of 1.74%/min. In patients, both authors found decreasing values with increasing age. The highest human K values lie between 3 and 3.5%/min, the lowest approach zero. On this basis, CONARD concluded that in man diabetes mellitus can be considered to be present at K values lying below 0.95 and can be excluded at values above 1.05%/min. OLEFSKY *et al.* (112) put the borderline between normal and abnormal K values in man at 1.2%/min.

To obtain an impression of these values in dogs in our laboratory, we performed the intravenous glucose tolerance test and calculated the K values for 3 groups of dogs. The first group comprised 20 healthy mongrels not older than 5 years, the second 25 healthy beagles aged 9 to 18 months, and the third 50 beagles and mongrels after total pancreatectomy. The mean GTT values and standard deviations were calculated for various time points. The following values and standard deviations were obtained (see Fig. 1a,b): for the first group the mean K value was 4.1%/min \pm 0.8 and for the second group 4.1%/min \pm 1.3; K = 0 was obtained for all of the dogs of the third group. Since there was no difference between the mean K values for the first and second groups, they were combined for the determination of the distribution shown in Fig. 2.

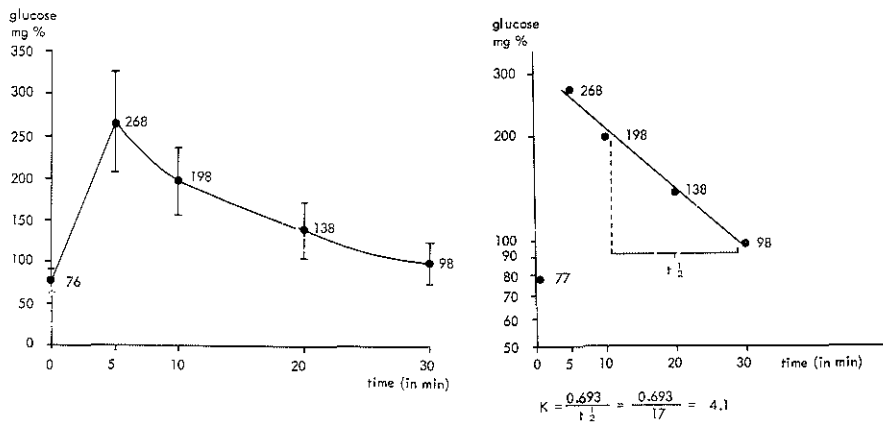


Fig. 1 a and b. Glucose tolerance test and K value.

a. Mean GTT value of 20 dogs, with standard deviation. $K = 4.1\%/min$.

b. Example of calculation of the K value from the GTT results (see a).

nr of dogs

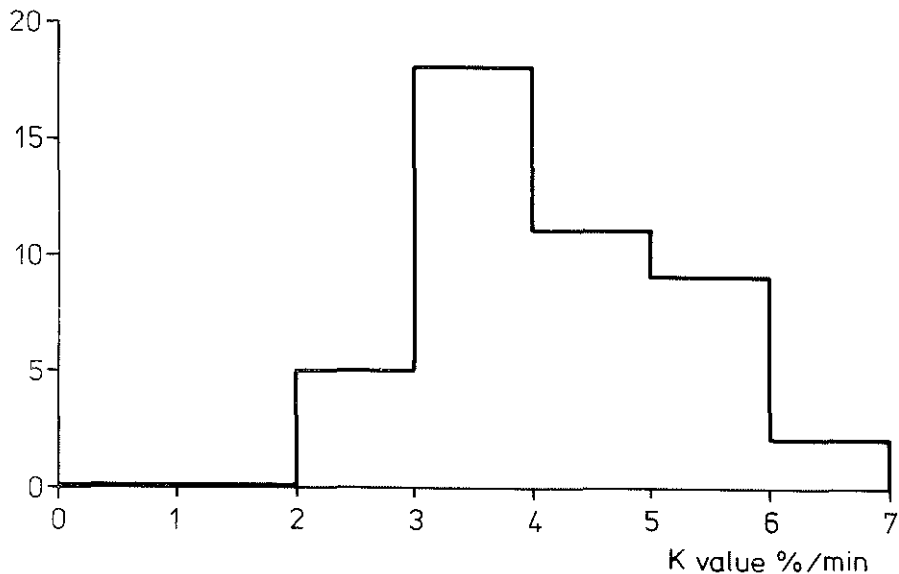


Fig. 2. Distribution of the K values of 45 normal dogs.

b. Serum insulin level

The serum insulin level was determined by the radio-immunological method of YALOW & BERSON (150) with one modification, i.e., the substitution of charcoal dextran for free and bound insulin in paper-electrophoretic separation. The determinations were done in the laboratory of the Department of Internal

Medicine III of the University Hospital Dijkzigt in Rotterdam. The peak insulin value was taken as the measure for pancreas function, i.e., the highest serum insulin value measured after glucose loading, which in this investigation was observed 5 or 10 minutes after the injection of 1 g glucose per kg body weight.

The blood samples for the insulin determinations were taken at the same time as those for the GTT, so that the insulin secretion pattern could be evaluated if necessary. To obtain an impression of the normal peak insulin values in the dog, determinations were made in 60 healthy mongrels and beagles, chosen at random. The frequency distribution of these peak values proved to deviate distinctly from a normal (Gaussian) distribution. However, the distribution of the logarithmics of the peak values closely approached a normal distribution curve. On the basis of these data, a normal-value range of 29-199 $\mu\text{U/ml}$ was found (95% variation limits). On the logarithmic scale a mean of 1.88 with a standard deviation of 0.21 was found.

5.2. Exocrine function

To evaluate the exocrine function, the fecal levels of 2 proteolytic enzymes, alpha-chymotrypsin and trypsin, were determined according to AMMANN (3) and others (52,54) by titration. These determinations were done in the laboratory of the Department of Internal Medicine II (Dr. P. HÖRCHNER). Trypsin was determined at pH 8.2 at 25°C, with Na benzoyl-L-arginine-ethylester (BAEE) as substrate, alpha-chymotrypsin at pH 7.8 at 25°C with N-acetyl-L-tyrosine-ethylester (ATEE). The feces were diluted 1:10 with a 0.9% saline solution and filtered. To 1 ml of the filtrate, 3 ml 0.05 N Tris buffer (pH 9.5) and 2.5 ml substrate were added. The liberated acid was titrated under N_2 with 0.01 N NaOH, and the results expressed in international units (an international enzyme unit is defined as the amount of enzyme converted by a micro-mol substrate per minute at 25°C).

Here, too, the normal values and reliability were evaluated by prior determination of the enzymes in the feces of 45 healthy dogs (mongrels and beagles) and of 14 dogs after total pancreatectomy. The results are shown in Figs. 3 and 4.

In dogs with a normal exocrine pancreas function the trypsin content of the feces was higher than 1 IU, with a mean value of 16 IU; the alpha-chymotrypsin was above 1 IU, with a mean of 64 IU. After total pancreatectomy both levels lay below 1 IU, and in 6 of the 14 dogs the alpha-chymotrypsin content was even nil. When a total absence of the exocrine function had to be demonstrated, a fat balance according to KAMER, TEN BOKKEL HUININK & WEYERS (45) was performed as well.

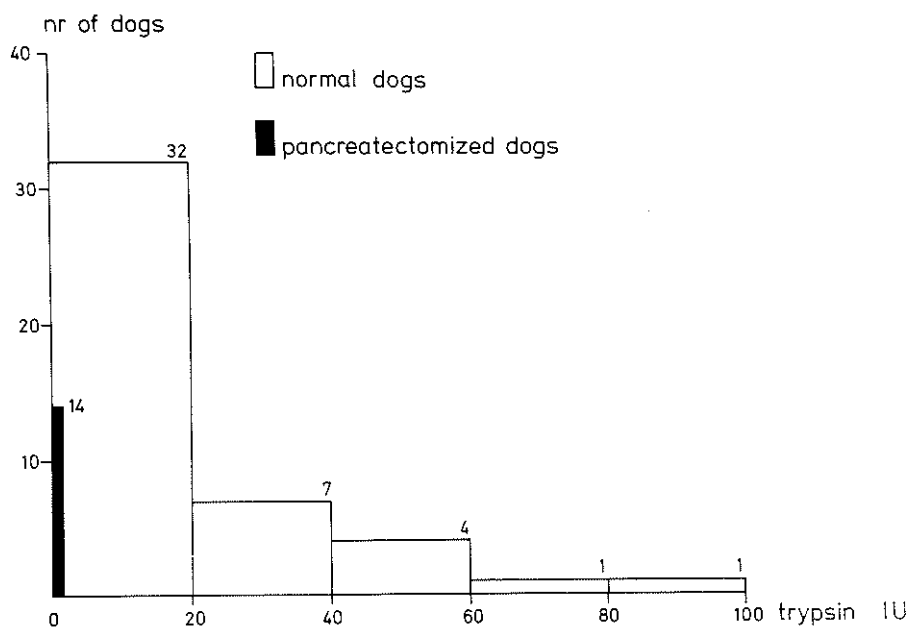


Fig. 3. Fecal trypsin in normal dogs and after total pancreatectomy.

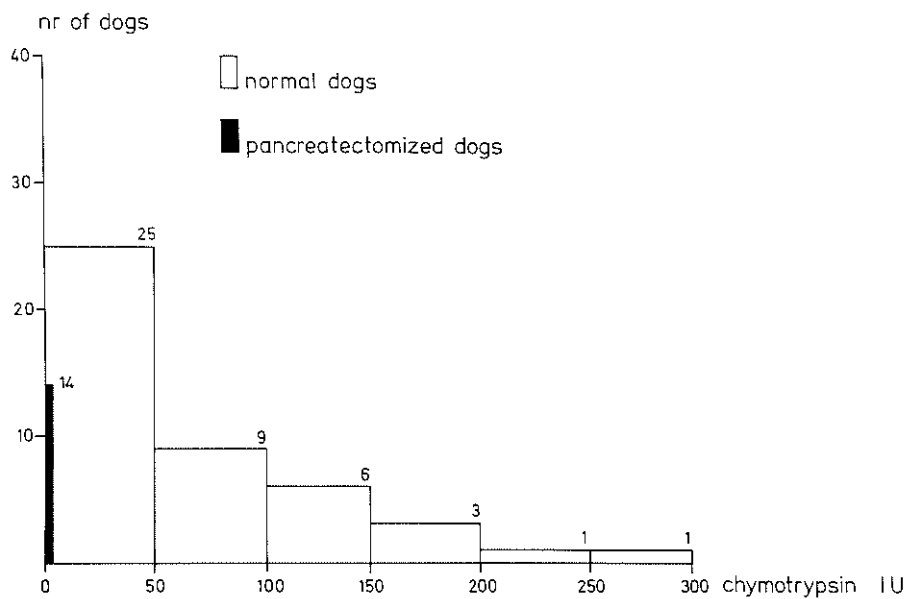


Fig. 4. Fecal alpha-chymotrypsin in normal dogs and after total pancreatectomy.

5.3. Other determinations

The amylase activity of the serum was determined according to WOHLGEMUTH (149). To estimate the normal values given by this method under our experimental conditions, the serum amylase level was determined in 30 normal mongrels and beagles and in 30 dogs which had undergone a total pancreatectomy 3 days before. The value usually obtained was either 64 or 128 U, and in a few cases 256 U. Since with this method the values are indicated as a geometric progression, there is little point in showing them graphically. To obtain a general impression and to permit comparison of the 2 groups, the frequency distributions for both are shown in Fig. 5.

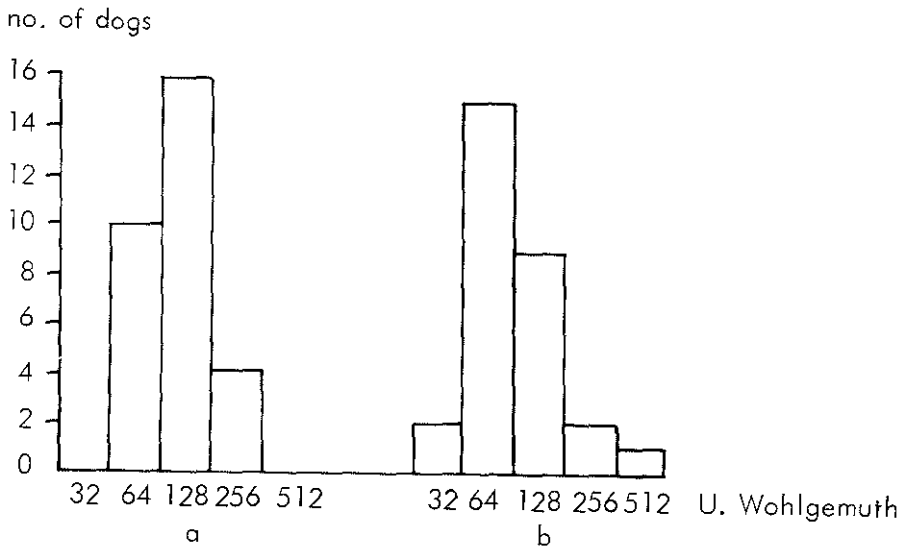


Fig. 5. Serum amylase levels in 30 dogs before (a) and after (b) pancreatectomy.

It is evident from Fig. 5 that the values lie much higher than those obtained in man with the same method. There is also no difference in the amylase activity before and after pancreatectomy. According to ETO *et al.* (40), in the dog iso-enzymes are measured that do not occur in man, which explains the higher levels as well as the absence of a change after pancreatectomy.

The serum sodium level was determined flamphotometrically with a Beckman flamphotometer, which showed normal values of 3.3-5.8 mmol/litre, with a coefficient of variation of $\pm 4\%$.

The serum bilirubin level determined according to JENDRASSOK-GRAFT (132c) gave normal values of 1.7-9.5 mmol/litre, coefficient of variation $\pm 5\%$.

Serum alkaline phosphatase was measured according to BESSEY *et al.* (132b) with dinatrium-*p*-nitrophenyl-phosphate as substrate, the glycerine buffer at pH 10.5, and the reaction temperature 37°C. Normal values 8-50 IU/litre (BESSEY), coefficient of variation $\pm 5\%$.

Serum calcium was determined by titration with EDTA and Murexide® as indicator. Normal values: 2.30-3.00 mmol/litre, coefficient of variation: $\pm 3\%$.

Serum magnesium was determined in the same way as serum calcium but with Ericom T as indicator. Normal values: 0.50-1.32 mmol/litres, coefficient of variation: $\pm 5\%$.

Serum creatinine was determined by the Jaffé reaction after adsorption to frankonite according to GORTER & DE GRAAF (44). Normal values: 40-90 mmol/litre, coefficient of variation: $\pm 5\%$.

The hemoglobin content was measured according to the HiCN method of the Netherlands Institute for Public Health. Normal values: 13.8-14.7 g %, coefficient of variation: $\pm 3\%$.

The blood gas values were determined with an Astrüp micro instrument type A.M.E.I.c. (Radiometer, Copenhagen, Denmark).

6. PATHOLOGICAL ANATOMY

An autopsy was performed on all dogs which died or were killed. The microscopical investigation was performed by Dr. I. MACDICKEN (Laboratory for Experimental Surgery of Erasmus University). Tissue was fixed in buffered neutral formalin 4% (pH 6.8) for at least 24 hours, embedded in paraffin, cut into 4-5 μ sections, and stained with hematoxylin-azophloxine-saffron (HAS). When the presence of beta-cell granules remained uncertain after this staining, use was made of aldehyde fuchsin staining according to GOMORI (42a).

Ligation of the pancreatic duct

I. INTRODUCTION

The divergence of the reported results on the effect of ligation of the exocrine outflow of the pancreas on the endocrine function of the organ has already been mentioned in Chapter I. Because of these differences and the fact that some of the results are not convincing, it was considered necessary to determine the influence of ligation on the function of the pancreas before performing pancreas transplantations with ligation of the exocrine ducts. It seemed possible, for one thing, that the gradual deterioration of the endocrine function after the heterotopic allotransplantation of a pancreas with ligated duct is due to beta cell degeneration rather than to chronic rejection phenomena.

On this basis it seemed worthwhile to start by ligating the exocrine ducts in a number of animals and following the endocrine function for an extended period. For a study of this kind, the following criteria had to be satisfied:

1. Use of the same species as for pancreas transplantation.
2. Evaluation of the exocrine function, which should be completely abolished after ligation.
3. Periodic evaluation of the endocrine function.
4. Histological investigation at various intervals after ligation.
5. Prolonged follow-up.

2. MATERIAL AND METHODS

Sixteen mongrel dogs were operated on via an incision in the upper abdomen

under endotracheal anesthesia and aseptic conditions. The pancreas was dissected from the duodenum as described MARKOWITZ *et al.* (102), leaving the vascularization intact. Both pancreatic ducts were identified, cut, and both ends tied off. The duodenum was wrapped in a flap of omentum such that the pancreas and duodenum were securely separated, after which the abdomen was closed in 2 layers with tied silk sutures.

In connection with the reduced vascularization of the duodenum, the animals were not allowed to drink *ad lib* until the third day; until then, fluid was supplied by hypodermoclysis in the form of a 0.9% saline solution. On the third day, solid food supplemented with pancreatic enzymes was given; thereafter, the diet was adapted to the needs of the individual animals. The administration of pancreatic enzymes was based on the consistency of the feces and the body weight.

The animals were evaluated pre-operatively and a week, a month, 5 months, a year, 1½ years, and 2 years post-operatively. The evaluation consisted of an intravenous glucose tolerance test, from which the K value was calculated, and determination of the insulin peak values (IPV). Furthermore, at each check the liver and kidney functions, the serum Ca and Mg levels, and the amylase content of the serum were determined as well as, at entirely random times, the non-fasting serum glucose level. The checks at the above-mentioned intervals included determination of the proteolytic enzymes trypsin and alpha-chymotrypsin in the feces, which were only sampled after the supplementary pancreatic enzymes had been withheld for at least 5 days. At regular intervals, an animal was killed for histological investigation of the pancreas.

The techniques, materials, and laboratory determinations have been described in Chapter II.

3. RESULTS

Of the 16 dogs, 3 died shortly after the operation. One of these died on the first post-operative day; the autopsy results indicated only the onset of pancreatitis. The second animal died on the 12th post-operative day of pneumonia, and the third on the 17th day of a duodenal perforation.

The remaining 13 dogs could be followed for 2 years except those used for the histological studies. The dogs maintained a satisfactory weight on a daily dose of 1 tablet Combizym and ½ tablet Cotazym per kg body weight.

3.1. Exocrine function

The exocrine function was supposed to be nil, in other words equal to that of totally pancreatectomized animals. Therefore, as mentioned in Chapter II, the proteolytic enzymes in the feces were determined and compared with the norms after total pancreatectomy. The results are shown in Table I, from which it is

TABLE I

Nr.	Trypsin (IU)			α chymotrypsin (IU)		
	pre-op.	4 weeks post-op.	24 weeks post-op.	pre-op.	4 weeks post-op.	24 weeks post-op.
452	—	0.8	0.2	—	0.4	0.5
481	15.1	0.8	0.3	12.4	0.4	0
477	8.2	0.5	0.6	0	0.3	0
465	10.3	0.4	0.8	12	0.4	0.4
476	8.8	2.5	<i>1.9</i>	224	<i>11.3</i>	<i>3.9</i>
502	6.8	0.3	1.2	19	0	0.4
493	9.7	0.4	0.5	89	0.1	0.6
583	6.0	0.7	0.5	43	0	0
588	4.5	<i>1.9</i>	<i>1.2</i>	88	<i>20.0</i>	<i>13</i>
613	30.3	9	<i>1.8</i>	145	<i>51</i>	<i>8.9</i>
766	43	0.5	0.4	77	0.5	0.7
892	7.8	0	1.0	9	0.3	0
899	7.5	0.5	0	68	0	0

evident that 3 dogs (nrs. 476, 588, and 613) did not satisfy the norms, because some endocrine function persisted. The enzyme levels of these 3 dogs are shown in italics. The endocrine function of these dogs was not followed further.

Thus, 10 dogs remained available for the study. Of these, 6 could be followed for 2 years. At the end of this time the exocrine function, as determined on the basis of the 2 proteolytic enzymes in the feces, was still nil.

3.2. Endocrine function

Table II shows the K value and insulin peak values for the 10 dogs followed post-operatively for 2 years or until killed. The first post-operative K value of dog nr. 493, as well as the K and insulin peak values of nr. 583 after 1½ years, are lacking because the blood samples were lost.

Among the first post-operative K values, the abnormally high 6.5%/min for dog 583 is striking. Nevertheless, this value corresponds well with the insulin peak value of 180 μ U/ml.

In Figs. 6 and 7 these values and the means are shown graphically, and Fig. 8

TABLE II

K values and insulin peak values before and at various times after pancreatic duct ligation.

Dog nr.	Pre-op.		1 week post-op.		5 weeks post-op.		20 weeks post-op.		1 yr. post-op.		1½ yr. post-op.		2 yr. post-op.	
	K %/min	insulin μ U/ml	K %/min	insulin μ U/ml	K %/min	insulin μ U/ml	K %/min	insulin μ U/ml	K %/min	insulin μ U/ml	K %/min	insulin μ U/ml	K %/min	insulin μ U/ml
452	3.5	73	3.5	108	2.2	15	4.1	24						
477	3.9	49	4.6	26	2.5	8	3.9	16						
481	4.6	180	2.0	18	2.8	20	1.9	34	4.6	48	3.0	51	3.9	67
465	5.3	57	2.0	30	2.7	29	2.0	14	2.4	9	3.8	26		
502	4.6	91	2.3	56	2.3	34	2.0	49	3.5	58	3.2	39	2.3	103
493	5.8	83	—	66	4.6	115	2.1	10	5.3	55				
583	4.6	—	6.5	180	3.8	97	2.8	73	2.3	46	—	—	2.0	33
766	3.8	140	3.8	130	3.8	80	2.3	58	3.3	91	3.8	240	2.2	115
892	4.6	240	3.9	126	2.0	64	3.0	57	2.2	104	3.9	56	2.3	39
899	3.0	100	2.0	77	2.5	59	2.0	30	2.8	75	2.0	37	4.1	64

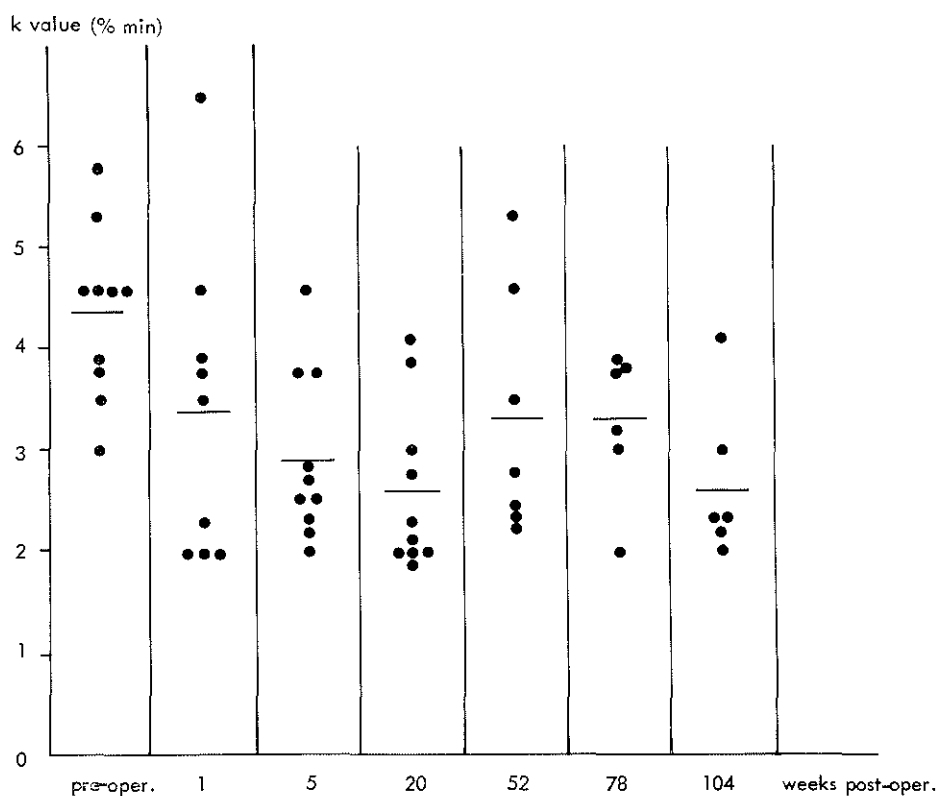


Fig. 6. K values before and after pancreatic duct ligation.

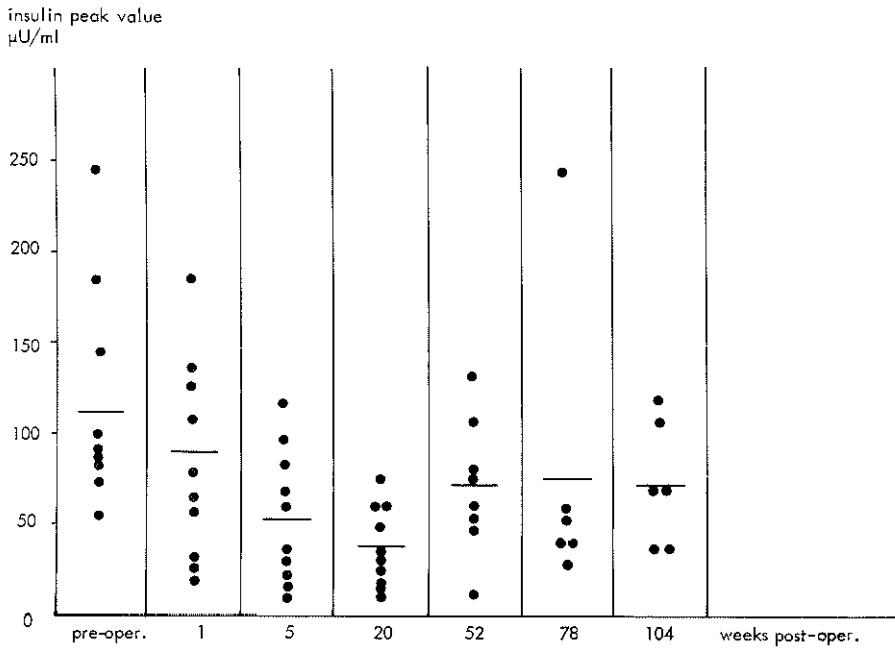


Fig. 7. Insulin peak values before and after pancreatic duct ligation.

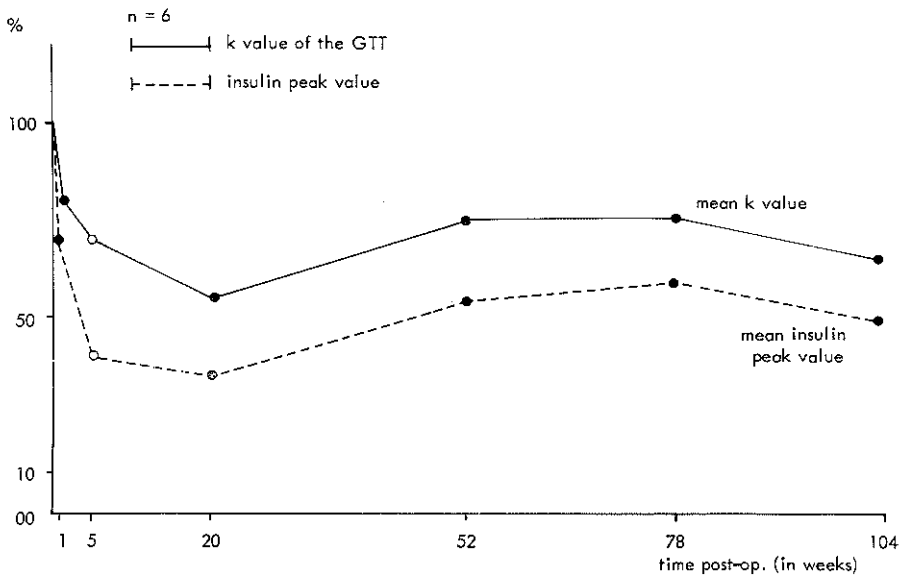


Fig. 8. The course of endocrine function after duct ligation in 6 dogs over a period of 2 years.

gives the average course in 6 dogs followed for 2 full years. Here, the pre-operative values were taken as 100 per cent. The values for the non-fasting blood sugar level did not exceed 100 mg% at any time.

3.3. Other determinations

A slight elevation of the alkaline phosphatase level (90 U/litre Bessey) was observed only once, in the first week after the operation. Throughout the entire period of observation none of the dogs showed any other anomalous changes in either the bilirubin or alkaline phosphatase levels. All of the animals showed significant increases in the amylase content during the first post-operative week, the highest values occurring on the third day and amounting on average to 2048 U. After that, the amylase values returned to normal.

There were wide variations within dogs, but elevated values were not seen after the 13th post-operative day. The creatinine, Ca, and Mg levels remained normal throughout, and the same holds for the hemoglobin level and the morphological blood picture.

3.4. Pathological anatomy

Table III shows the data for the autopsied animals. Macroscopically, little of the original pancreas could be found, usually not more than some pinkish fibrotic strands (Plate 1). The presence of adhesions made these strands difficult to find, however, and in a few cases (e.g. dog nr. 493) no pancreas tissue at all could be demonstrated in the biopsy samples.

Microscopical examination revealed disappearance of acinar tissue, which had been replaced by connective tissue in which nests of islet tissue could be found (Plate 3). This change could be observed as early as the 12th and 16th days when, remarkably enough, there had been hardly any signs of pancreatitis. In the dogs (nrs. 588, 476, 613) which still showed enzyme activity, distinct acinar tissue could be seen.

4. DISCUSSION

The body weight of the 10 dogs increased by 2 to 3 kg in the 2 years during which they were observed. The pancreatic enzyme preparations Combizym and Cotazym (1 and $\frac{1}{2}$ tablet per kg body weight, respectively) were given to maintain a good general condition.

TABLE III

Data on dogs with ligated pancreatic ducts (available for histological investigation), in order of duration of survival.

Dog nr.	Trypsin & α -chymotrypsin*	Cause of death	Time post-op. (in days)	Histological findings
529	†	Pancreatitis	1	Early stage hemorrhagic pancreatitis
850	†	Pneumonia	12	Extensive fibrosis with loss of acinar tissue. Islets distinctly present. No signs of pancreatitis.
978	†	Duodenal perforation	16	Fibrosis. Few signs of pancreatitis. Marked loss of acinar tissue. Capsular peritonitis. Islets easily recognizable.
588	+	Killed	167	Some sections show normal pancreas tissue, others atrophic acinar tissue with dilated ducts. No signs of pancreatitis. Extensive areas of fibrosis containing islet tissue with beta cell granules.
452	—	Killed	181	In all sections extensive fibrotic areas with only islet tissue remaining. No signs of pancreatitis.
477	—	Killed	218**	Marked fibrosis with islet tissue and remnants of atrophied acini. Periductal fibrosis. No signs of pancreatitis.
476	+	Killed	245	Marked fibrosis; islet tissue present. Some lobe sections show only partially atrophied acinar tissue. No signs of pancreatitis.
493	—	Killed	329	No pancreas tissue demonstrable in any of the sections.
613	+	Killed	716	Total fibroid degeneration; some acinar tissue intact but islet tissue predominating. No signs of pancreatitis.
465	—	Killed	716	Total fibrotic degeneration of acinar tissue; islet tissue distinct and showing beta cell granules. No signs of pancreatitis.
418	—	Killed	884	Total fibroid degeneration of the acinar tissue, in which islet cells and beta cell granules can be distinguished. No signs of pancreatitis.

* Symbols: † = died before enzyme determinations could be made.

+ = persisting enzyme activity.

— = no enzyme activity.

** See Plates 1 and 3.

It is clear that the surgical technique applied did not lead to complete abolition of the exocrine function of the pancreas in all cases. Thus, a reliable check on this point is indispensable; for this, the determination of the proteolytic enzymes trypsin and alpha-chymotrypsin in the feces proved to be an adequate method. In the cases in which enzyme activity could still be demonstrated in the feces, the histological investigation showed the presence of intact acinar tissue.

The persistence of exocrine pancreas function despite ligation of both ducts could be due to one of four possibilities: a) regeneration of exocrine tissue (LEHV & FITZGERALD, ref. 89); b) perforation of a pancreacyst in the duodenum; c) existence of aberrant pancreatic ducts; d) existence of aberrant pancreatic tissue elsewhere (84,94).

In the dogs with a limited persisting exocrine function we invariably found intact acinar tissue near the duodenum, just distal to the pylorus. This would argue for point c, the presence of aberrant ducts. Regeneration (point a) is unlikely, because none of the dogs lacking the exocrine function immediately after the operation regained this function during the 2-year observation period. Duodenal perforation (point b) seems excluded, because no signs were found histologically. Point d is in conflict with the consistent observation of acinar cells in, and not outside, the pancreas in the dogs with persisting exocrine function, whereas no acinar tissue was demonstrated in the dogs lacking this function.

The course of the endocrine function of the other animals showed an initial gradual decrease to 50 per cent of the original K value of the intravenous GTT and to 35 per cent of the original insulin peak value. After the 20th week, however, both values began to rise, the K value reaching 75 per cent of the original value and the IPV 60 per cent. But it must be kept in mind here that one dog showed an abnormally high value for the first post-operative determination, i.e., a K value of 6.5 with an IPV of 180 μ U/ml. If this had not occurred, the total picture would be quite different: a drop in the endocrine function immediately after the operation and partial recovery starting after the 20th week. Nevertheless, even in the period of poor endocrine function, no pathological blood sugar values were found, even directly after a meal.

These findings are in agreement with those of authors (4,22,121,127) who did not see diabetes mellitus after duct ligation. HIEBERT *et al.* (66) and MACK *et al.* (100) even found no reduction of the endocrine function. We saw an initial diminishment of this function, like those authors (2,35,74) who found lower insulin excretion and lower K values after ligation, but we did not see a single case of diabetes mellitus. On the contrary, the endocrine function improved after some time in our series. An explanation for these conflicting findings must be sought, in our opinion, in the possibility of hypertrophy and

regeneration of islet cells, as described by LOGOTHETOPOULOUS (97), which indicates that in cases of pancreas damage the diet and the serum glucose level can influence beta cell degeneration and regeneration. Furthermore, inadequate supplementation of pancreatic enzymes after abolition of the exocrine function leads to a reduction of glucose resorption from the digestive tract (138), which in turn can lead to beta cell degeneration. Thus, the proper supplementation of pancreatic enzymes is of great importance.

The persistence of normal serum Ca and Mg levels and liver function confirms the findings of ANLYAN *et al.* (4). The pancreatitis was not sufficiently severe to depress the calcium level in the serum as described by EDMONDSON *et al.* (38,39). Pancreatitis can be demonstrated both histologically and biochemically. The important factor here is that histological investigation shows that the acinar tissue disappears and is replaced by connective tissue, whereas the islets remain completely intact.

5. CONCLUSIONS

From the foregoing, it is evident that in experimental auto- or allotransplantations of a pancreas with ligated ducts in dogs, the following aspects must be taken into account:

1. Aberrant pancreatic tissue may be present. This must be checked in the pancreatectomized recipient, because otherwise the hormone determinations may be incorrectly interpreted. A reliable method is provided by determination of the proteolytic enzymes in the feces.
2. A recipient lacking exocrine pancreas function should receive at least 1 tablet Combizym and $\frac{1}{2}$ tablet Cotazym per kg body weight, or the equivalent, to maintain metabolic balance.
3. During the first 2 weeks after transplantation a mild pancreatitis develops which is most severe during the first 5 post-operative days.
4. Immediately after the operation a sharp drop in the endocrine function can occur without the development of diabetes mellitus. This endocrine function gradually improves and can possibly be influenced by a carbohydrate-rich diet combined with an excess of pancreatic enzymes.

Autotransplantation of the pancreas with ligated ducts

1. INTRODUCTION

Once the effect of ligation of the exocrine outflow on the endocrine function of the pancreas was known, other potentially negative factors such as ischemia, denervation, heterotopic localization, and immunological rejection phenomena (in combination with duct ligation) remained to be investigated. Heterotopic autotransplantation of the pancreas offered a good model for the study of the first 3 of these factors, since immunological effects are excluded. If the results proved to differ from those obtained with duct ligation alone (see Chapter III), an attempt could be made to determine which of the three factors was responsible and to what degree.

Unfortunately, autotransplantation of the whole pancreas involves difficult technical problems for the surgeon, because the organ is supplied with blood by 3 arteries (see Fig. 9): the splenic artery, the inferior pancreaticoduodenal artery, which arises from the superior mesenteric artery, and the superior pancreaticoduodenal artery, which arises from the gastroduodenal artery. The venous vascularization is analogous to the arterial as far as the junction with the portal vein. Because the donor is also the recipient, the pancreas must be removed without damage to other organs. This means that the blood vessels must be cut close to the pancreas, which in principle would require 6 small anastomoses. In some cases, however, the entire pancreas is supplied via the central vessel, the superior pancreaticoduodenal artery. A case of this kind is shown in Plate 23, a radiogram made after the injection of a contrast medium consisting of a barium suspension (Micropaque®, Nicholas B.V., Amsterdam, The Netherlands). In such cases the transplantation requires only one arterial anastomosis (for the superior pancreaticoduodenal artery) and one venous anastomosis (for the gastroduodenal vein).

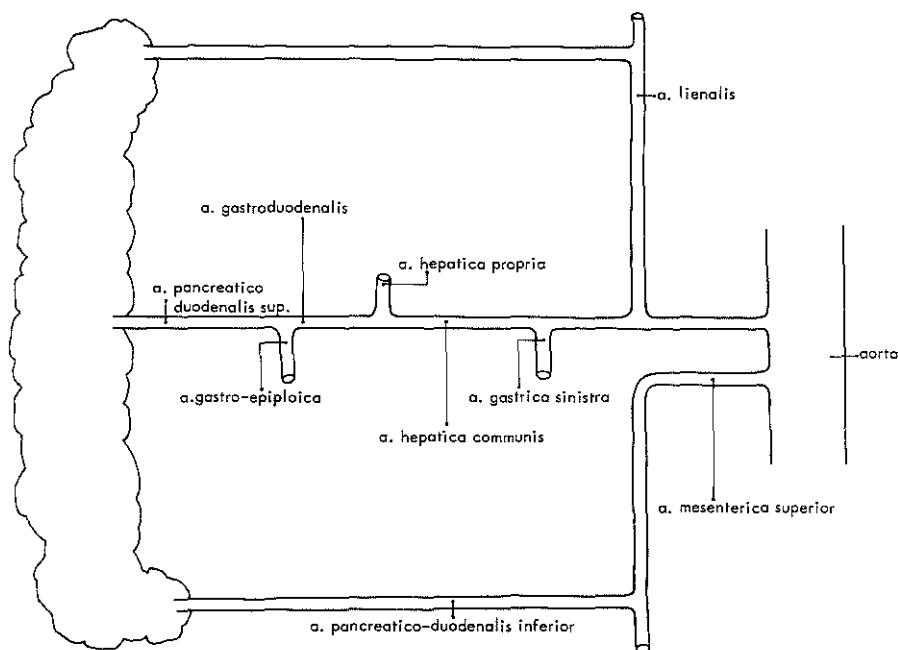


Fig. 9. Schematic representation of the arterial blood vessels supplying the canine pancreas.

UCHIDA *et al.* (139) performed this kind of autotransplantation in dogs, but with inclusion of the duodenum. In a number of cases small areas at both ends of the pancreas became necrotic. Five of the 15 animals survived for 4 to 12 months. The blood glucose values differed little from the pre-operative levels. Nevertheless, pancreatitis developed even though the exocrine ducts had not been ligated.

GRENIER *et al.* (48) performed autotransplantations of the tail of the pancreas to the neck in 13 dogs. The transplant functioned in only 6 of the dogs, the longest duration being 26 days. With the technique applied, the pancreatic duct was cannulated.

MITCHELL & DAVIDSON (109) described canine autotransplantations of the tail of the pancreas, with ligated duct, to the groin. Two of the 20 transplants continued to function until the fifth and sixth months.

MURAT *et al.* (111) performed autologous transplantation of the entire pancreas, with ligated ducts, to the fossa iliaca. One of the 12 dogs did not require insulin over a period of 13 months.

DREILING & ASHIKARI (36) performed auto- and allotransplantations to the neck with sacrifice of the uncinate process. The exocrine function was maintained intact by inclusion of the duodenum or cannulation of the pancreatic duct. For these 40 dogs the authors reported a transplant survival time of

1 to 18 months, the most important observation being marked deterioration of the exocrine function while the endocrine function remained good.

BEAVEN *et al.* (8) used merino sheep for pancreas-tail autotransplantations to the neck 2 to 3 months after ligation of the pancreatic duct. Four of the animals could be followed for 9 months. After loading with glucose and tolbutamide, insulin production was good, and histological investigation after 9 months showed that functioning islets were still present.

It is therefore evident from the literature that regardless of the technique applied, the percentage of successful pancreas autotransplantations, whether with or without ligation of the duct, has been low. When technical complications did not occur and the transplant functioned well, however, the GTT values and the serum insulin levels measured in the peripheral blood were roughly the same as those found pre-operatively. In general, adequate endocrine function was observed after autotransplantation of part of the pancreas, but this function gradually dwindled and eventually disappeared. Only with transplantation of the whole organ (111,139) was prolonged endocrine function obtained.

In view of the important information that a pancreas autotransplantation can provide, we thought that it would be useful for the present study to attempt transplantation of the whole organ with ligation of the exocrine ducts in a number of dogs.

2. MATERIAL AND METHODS

Under the conditions described in Chapter II, the operation was performed in 17 dogs (mongrels). The pancreas was dissected from the duodenum according to MARKOWITZ *et al.* (102), and both ducts were found, cut, and ligated. The inferior pancreaticoduodenal artery and vein were ligated, as well as the branch or branches of the splenic artery and vein. The superior pancreaticoduodenal artery was tied off at the origin from the gastroduodenal and was cannulated distally to permit flushing of the pancreas with about 200 ml of a 500 ml physiological saline solution (4-10°C) containing 25 mg heparin 2% and 50 ml procaine HCl 2%. The gastroduodenal vein was detached from the portal vein. After completion of the flushing, the blood vessels were sutured end-to-side with 6 × 0 silk to the right iliac vessels or to the aorta and caval vein below the renal arteries. After revascularization, any non-vital parts of the pancreas were removed.

During the operation, 25,000 KIU Trasylol was administered, and post-operatively 50,000 KIU was given daily for 5 days. Nothing was given orally

on the first 2 post-operative days, 1500-2000 ml fluid being administered parentally. If the dogs drank adequately on the third post-operative day, solid food was given on the fourth day, supplemented with pancreatic enzymes. Insulin was not given at any time.

All function tests and routine determination were performed as described in Chapter II. Body weight was measured regularly. All dogs which died or were killed were autopsied and the pancreas investigated histologically.

3. RESULTS

All but one dog (nr. 16) required resection of part of the pancreas (tail or uncinate process or both) due to an inadequate blood supply. The other parts of the pancreas acquired a good pink color immediately after revascularization. Extensive intracapsular edema developed rather quickly in all cases. As could be expected, the results were poor. Most of the animals died shortly after the operation.

To facilitate discussion of the results, the animals can be divided into 2 groups, one comprising the dogs which died during the first 5 post-operative days and the other those which survived longer.

3.1. Dogs with a survival time of less than six days

Ten dogs died before the sixth post-operative day. The relevant data are shown in Table IVa.

The only pancreas which functioned demonstrably was the autotransplant of nr. 238. On the first 2 post-operative days the blood glucose values fluctuated between 30 and 40 mg %. Our later experience with allotransplantations (see below) showed that hypoglycemia occurs after transplantation, and although objective evidence is lacking it seems certain that this animal died of hypoglycemia.

The cause of death in the other animals requires no discussion. In 6, thrombosis of the anastomoses was found. All but one dog had severe pancreatitis, the exception being nr. 238, in which the transplant functioned. It is also noteworthy that in nr. 83, in which thrombosis did not develop, signs of the onset of fibrosis with disappearance of acinar tissue were present after 5 days.

TABLE IVa

Results in dogs with a survival time of less than 6 days.

Dog nr.	Survival (days)	Function transplant*	Clinical cause of death	Post-mortem findings
83	5	—	Perf. duodenum	Perforation peritonitis. Slight fibrosis of acinar tissue. Blood vessels normal.
181	2	—	Hypercalcemia assoc. with peritonitis	Arterial thrombosis in anastomosis accompanied by extensive ischemic necrosis.
238	2	+	Pancreatitis, possible hypoglycemia	Pancreas almost normal with some signs of peripheral inflammation.
283	2	—	Unknown	Autolytic pancreatitis with total thrombotic occlusion of the anastomoses.
300	1	—	Ventricular fibrillation	Onset of sludging in anastomoses. Mild ischemic pancreatitis.
316	3	—	Pancreatitis	Extensive hemorrhagic pancreatitis. No thrombosis.
646	5	—	Hyperglycemia	Extensive pancreatitis with thrombosis in anastomoses.
627	3	—	Hyperglycemia	Advanced autodigestion of the pancreas. No signs of thrombosis.
188	1	—	Unknown	Thrombosis in all vessels with total autolysis of the pancreas.
608	4	—	Pneumonia	<i>Pancreas:</i> autodigestion, necrosis, mononuclear cell infiltration, venous congestion. Thrombosis in venous anastomosis. <i>Lungs:</i> pneumonia.

* + = present.

— = absent

3.2. Dogs with a survival time longer than six days

The data for these dogs is shown in Table IVb. In this group several of the transplants functioned. The criterion for function was an upper limit of 80 mg % for blood sugars on the first 2 days after the operation despite the parenteral administration of glucose.

In the dogs which lived longer than 10 days, the proteolytic enzymes in the feces were determined. All values lay below 1 U.

Histologically, this material too showed extensive signs of pancreatitis, numerous venous thromboses, and complete fibrosis of the acinar tissue.

TABLE IVb

Results in dogs with a survival time of more than 6 days.

Dog nr.	Survival (days)	Function transplant*	Clinical cause of death	Post-mortem findings
16	1.825	+	Killed	<i>Pancreas:</i> Complete disappearance of acinar tissue. Extensive areas of fibrosis containing islets of Langerhans. B cell granules recognizable. Aldehyde fuchsin staining: alpha and beta cells and B cell granules distinguishable.
31	55	+	Jaundice	Distinct biliary congestion of extra-hepatic origin. Fibrosis of hepatic portal. <i>Pancreas:</i> completely fibrotic; no islet tissue found.
99	10	—	Ligated choledochus duct	Extensive venous thrombosis with edematous pancreatitis. <i>Liver:</i> Many polynuclear cells; biliary stasis. Mild cholangitis.
229	135	+	Killed	<i>Pancreas:</i> Complete fibrotic replacement of acinar tissue, in which normal islets present.
263	7	—	Killed	Severe destructive pancreatitis; autodigestion. No signs of thrombosis.
474	50	+	Killed	<i>Pancreas:</i> fibroid replacement of all acinar tissue. No islet tissue recognizable.
536	16	+	Killed	Advanced digestion of pancreas and mononuclear cell infiltration. Fibrosis of acinar tissue. Hemorrhagic pancreatitis. No signs of thrombosis.

* + = present.

— = absent.

3.3. Endocrine function

The data on the 6 dogs with post-operative pancreas function are shown in Table V (these are the dogs indicated by a plus sign in the third column of Tables IVa and IVb). The second column of Table V shows the length of time that the transplant functioned, not the survival time of the animal.

In the 3 dogs in which the organ functioned for only 1 or 2 days the K value and IPV could not be determined, and therefore the highest blood glucose value measured on the first post-operative day is given in the third column of Table V. The fourth column shows the first post-operative K value and the fifth the corresponding insulin peak value, from which it is evident that only two of the transplants functioned for more than a very brief period.

TABLE V

Endocrine function in autotransplants.

Dog nr.	Duration of transplant function (days)	Highest blood glucose level on 1st post-op. day (mg %)	K value %/min.	IPV (μ U/ml)
16	1,825	50	1.7	65
31	1	71	—	—
229	57	50	2.4	20
238	2	40	—	—
474	1	76	—	—
536	5	60	2.6	39

The endocrine function of dog nr. 229 decreased gradually; 57 days after the transplantation the blood sugar values rose to more than 120 mg % and the K value approached zero. The animal began to lose weight and to drink copiously, suggesting diabetes mellitus. This dog could be kept in good condition for some time by the administration of tolbutamide, and was finally killed on the 133th post-operative day for histological investigation.

Dog nr. 16 showed a very different picture, since the endocrine function improved. After exactly 5 years, this dog was killed for histological investigation. The post-operative course in this animal requires separate discussion.

3.4. Dog nr. 16

This is the only dog in which it did not prove necessary to excise a non-vital part of the pancreas after revascularization. This pancreas, which was anastomosed to the aorta and the caval vein below the renal vessels, was completely viable. The endocrine function was poor immediately after the operation but gradually improved. Fig. 10 shows the 5-year course of the K and IP values graphically; the former are normal and the latter just barely normal.

Because in this case it was important to know whether aberrant pancreatic tissue was present or regeneration had occurred, the proteolytic enzymes in the feces were determined twice in succession a year after the operation. The first determination showed 0.4 IU trypsin and 0.0 alpha-chymotrypsin, the second gave 0.3 IU and 0.0, respectively. In addition, a fat balance was performed. The resorption coefficient was zero, and the same result was obtained 6 months later.

After 3 years a laparotomy was performed to explore for aberrant pancreas

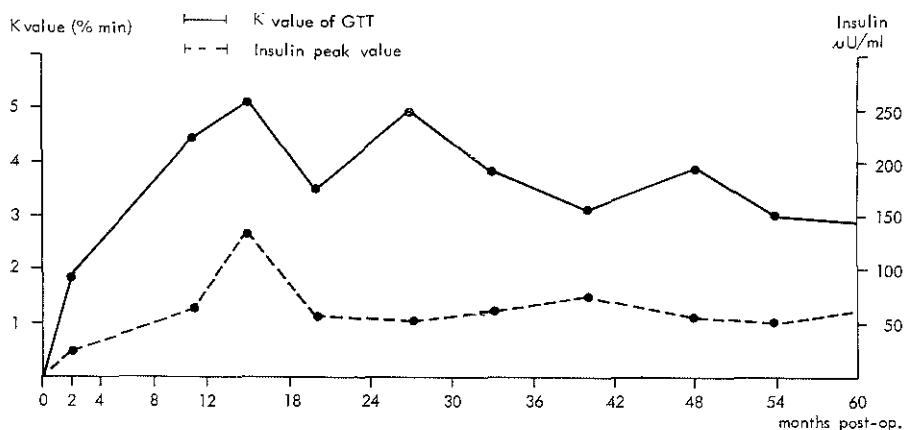


Fig. 10. Endocrine function of a pancreas autotransplant with ligated ducts over a period of 5 years (dog nr. 16).

tissue, and samples of the connective tissue around the duodenum were taken. No pancreas tissue was found.

The exocrine function could be adequately supplemented with 1 tablet Combizym and $\frac{1}{2}$ tablet Cotazym per kg body weight daily, and the animal remained in good condition. The stool was voluminous. Over the 5-year period the body weight increased by 4 kg. Determination of the fecal proteolytic enzymes after 4 and 5 years showed no signs of exocrine activity.

Five years after the autotransplantation the organ was removed per laparotomy (Plate 4) and a thorough search for aberrant pancreas tissue was made (Plate 4a), but none was found.

Histological findings: The entire pancreas showed fibroid degeneration. No acinar tissue could be distinguished. Islets lay in the connective tissue (see Plate 5). With aldehyde fuchsin staining the alpha and beta cells could be distinguished and beta cell granules identified (Plate 6). The biopsy samples from the duodenum showed no pancreas tissue. The liver tissue was normal.

It may also be mentioned for this dog that the amylase values were strongly elevated during the first 14 post-operative days but after that never rose above 128 U. The liver function and serum Ca and Mg values remained normal throughout the 5-year period.

4. DISCUSSION

When a total pancreatectomy after MARKOWITZ *et al.* (102) is performed in the

dog, the duodenum is disturbed as little as possible. Since the pancreas is to be removed, it need not be handled with special care. The reverse holds for an allotransplantation, in which the donor is sacrificed: in the course of the dissection the serosa of the duodenum is often severely damaged but the capsule of the pancreas is left completely intact. For an autotransplantation, both organs must remain in good condition. This is difficult to achieve, however, and both the pancreas and the duodenum are sometimes damaged. The reaction of the pancreas to such lesions may lead to pancreatitis, and duodenal perforation can result from local disturbance of the vascularization of the duodenum.

Another source of pancreatic lesions is related to the fact that the blood supply to the pancreas is provided by 3 arteries, only one of which can be used at transplantation, i.e., the gastroduodenal artery. As a consequence of this, after revascularization part of the tail and the uncinate process of the pancreas may no longer be viable and must be excised. In our series the vascularization after re-implantation was only sufficient to supply the entire pancreas in one case (nr. 16). The fact that this dog also had the longest survival time cannot be taken as an indication of a causal relationship in this limited material, but it is in agreement with the reports in the literature (see page 43).

Furthermore, with the autotransplantation technique the anastomoses of the gastroduodenal artery and vein are narrow, which can promote the development of thromboses. In addition, the pancreatitis leads to a considerable rise in the hematocrit value. In one of the dogs the hemoglobin level rose from 14 g % to 22 g % despite the administration of 1500 ml fluid. This means a change in the viscosity of the blood, which also promotes thrombosis. All these factors may explain the finding of thromboses at one of the 2 sutures in 7 of the dogs.

In a number of cases the lack of, or very poor, transplant function must be assumed to have been caused by the very severe pancreatitis in the absence of thrombotic phenomena.

Two dogs died of a hepatic anomaly due to obstructive jaundice. Nr. 99 had an obstruction of the choledochus duct due to a hemostatic ligature, and nr. 31 proved to have a histologically normal liver with swollen biliary ducts. Although the choledochus duct could be catheterized easily from the duodenum and proved to be patent, the extensive fibrosis in the area of the hepatic portal could have been responsible for the congestion seen in the liver.

With respect to the transplants that functioned, it is noteworthy that the endocrine function was low post-operatively, although the blood sugar values were normal in this period.

The K and insulin peak values of dogs 229 and 16 were determined on the 42nd post-operative day, those of nr. 536 on the 3rd day after the operation. No explanation can be offered for the functional failure in nr. 229, but the

gradual deterioration resembles the findings of MITCHELL & DAVIDSON (109) and GRENIER *et al.* (48).

In comparison with the group of dogs discussed in Chapter III, the signs of pancreatitis were more pronounced in this group, both clinically and morphologically. The loss of endocrine function was appreciably greater, certainly with respect to insulin secretion. It is conceivable that this loss of function was due to the severe pancreatitis caused not only by traumatic damage but probably also by an inadequate blood supply and venous congestion, as described by TEIXEIRA & BERGAN (136).

In the only dog with a long survival time, however, we saw within a year a considerable recovery of the endocrine function, the K value reaching 4.2 %/min. The insulin peak value lagged slightly, as was also the case for the ligated group. The possibility of a vagotomy effect must be taken into consideration here (see Chapter I, section B.4). Another factor which might explain the slightly lowered insulin values is the absence of the exocrine function of the pancreas, since insufficient glucose resorption from the digestive tract can lead to hypofunction of the endocrine system of the pancreas due to a deficiency of alpha-amylase.

5. CONCLUSIONS

In the dog, heterotopic autotransplantation of the entire pancreas or of a large part of the organ with ligated ducts, is usually accompanied by severe lesions incurred by both the pancreas and duodenum during dissection. This damage and the poor blood supply (see above) after autotransplantation can result in severe pancreatitis and thromboses in the vascular anastomoses. These factors can lead to total elimination of the endocrine function of the pancreas.

When the lesions incurred by the pancreas are not severe, the endocrine function can be recovered. The fact that this happened in one case shows that the method itself is adequate if good vascularization of the pancreas can be guaranteed and severe traumas and thrombosis of the anastomoses can be avoided.

The effects of heterotopic localization and of denervation are not clear, but in any case these factors do not have such a strong influence that they can lead to abnormality of the ultimate endocrine function of the pancreas.

Allotransplantations

I. INTRODUCTION

The experiments reported in the foregoing indicated that the negative influence on the endocrine function of the pancreas after ligation of the pancreatic duct did not lead to diabetes mellitus and that this function improved in the course of time. These findings led us to apply allotransplantation of the pancreas with ligated ducts, which offers several advantages compared with techniques in which the ducts are not ligated in order to maintain the exocrine function of the pancreas. These advantages, already discussed in Chapter I, are:

1. The duodenum does not have to be transplanted with the pancreas.
2. Intestinal anastomoses or even pancreas fistulas are not required.
3. An anastomosis with the ureter is avoided, and thus the loss of a kidney.
4. Transplantation of the pancreas with ligated duct is a much simpler operation.

Our investigation of the possibilities for combination of the technique of pancreas transplantation with a study on the influence of tissue typing for the DL-A system enabled us to approach the following problems:

- a. The influence and value of tissue typing in pancreas allotransplantation.
- b. Prolonged survival of some transplants without the administration of immunosuppressive drugs, if achieved, would give a clearer picture of the endocrine function of the pancreas, since the influence of such drugs would be excluded.
- c. The presence of known tissue incompatibility between donor and recipient would make it possible to evaluate the effect of various immunosuppressive drugs on allotransplantations.

For such studies, the endocrine pancreas function of the recipient should be abolished before the transplantation. The best way to achieve this seemed to us to be the performance of a total pancreatectomy in the recipient some time before the transplantation. Conservative depression of the endocrine function, for instance with alloxan, was not adopted, because the associated mortality is reported to be appreciable and in many cases stable diabetic conditions are not obtained (116).

A number of findings from the preceding experiments were applied to these transplantation experiments. Since it had been found (page 35) that functioning exocrine pancreatic tissue could still be present after dissection of the pancreas from the duodenum, a number of pancreas function tests had to be performed after total pancreatectomy to demonstrate the abolition of both the exocrine and endocrine functions. Furthermore, the pancreatitis which invariably accompanied duct ligation (page 38) led to a rise in the hematocrit value with sludging of erythrocytes, increasing the risk of intravascular coagulation. This made it advisable to supply fluids abundantly after pancreas transplantations; on the basis of GOODHEAD's (43) findings, part of this fluid should consist of Rheomacrodex® (dextran 40,000 Mw). Lastly, in connection with the auto-transplantations (page 45) it was found that hypoglycemia could develop after the operation, and we therefore administered glucose intravenously in the post-operative period.

2. MATERIAL AND METHODS

This series of experiments was done exclusively in beagles. The donor-recipient pairs were formed on the basis of a prospective serological selection according to the DL-A system, as described in Chapter II. The following groups were formed:

- Group 3a: Unrelated non-identical beagle pairs
- Group 3b: Related pairs differing by 2 haplotypes
- Group 3c: Related pairs differing by 0 haplotypes
- Group 3d: Related pairs differing by 1 haplotype and treated with azathioprine and prednisone
- Group 3e: Related pairs differing by 1 haplotype treated with ALS

2.1. Pancreatectomy in the recipient

In 77 beagles, total pancreatectomy was performed according to MARKOWITZ

et al. (102). During the operation the animals received 0.9% saline and 10 ml Rheomacrodex 10% per kg body weight, and during the 24 hours after the operation 1 litre 0.9% saline and 300 ml 10% glucose solution with 16 U insulin (Organon) and 1 g KCl per 500 cc parenterally. On the second post-operative day 500 ml 0.9% saline was given once, water *ad lib*, and, at the beginning of the day, 4 U insulin subcutaneously. On the next day the animal was allowed to eat *ad lib* and pancreatic enzymes were given orally. The insulin dosage was based on the blood sugar levels.

2.2. Preparation of pancreatectomized dogs for pancreas allotransplantation

In the first week after total pancreatectomy, an intravenous GTT was performed and the insulin peak value determined. In the second post-operative week the supplementary enzymes were withheld for 5 days, after which the feces were investigated for the presence of trypsin and alpha-chymotrypsin. In the third week the clinical condition of the dog was evaluated on the basis of the body weight, general condition, and liver function. After the third week the dog was considered for a transplant on the basis of the following criteria: a) a K value of 0 for the GTT; b) no insulin demonstrable in the serum after glucose loading; c) proteolytic enzyme activity in the feces less than 1 U; d) metabolic equilibrium, i.e., the dog had to have a good appetite, not show marked loss of weight, and be clinically healthy.

2.3. Preparation of the donor and donor-pancreatectomy

The same endocrine and exocrine function tests were done in the donor as in the recipient. A dog was considered as donor if the function test results were normal and no anomalies were revealed by the clinical, chemical, and hematological routine investigations.

Under aseptic conditions and endotracheal anesthesia (page 25), the operation was performed via a long median abdominal incision. The spleen was removed and the tail of the pancreas mobilized. The mesenteric vessels were ligated distally from their origin from the inferior pancreaticoduodenal artery and vein, after which the gut below the duodenum was excised. The aorta was then dissected over a distance of about 6 cm proximally and distally from the origin of the celiac and mesenteric arteries (Fig. 11). Next, the choledochus duct was cut, the portal vein dissected, and all branches of the common hepatic artery were ligated at the hepatic porta. This left only the gastroduodenal artery, whose branch, the right gastroepiploic artery, was ligated, so that blood

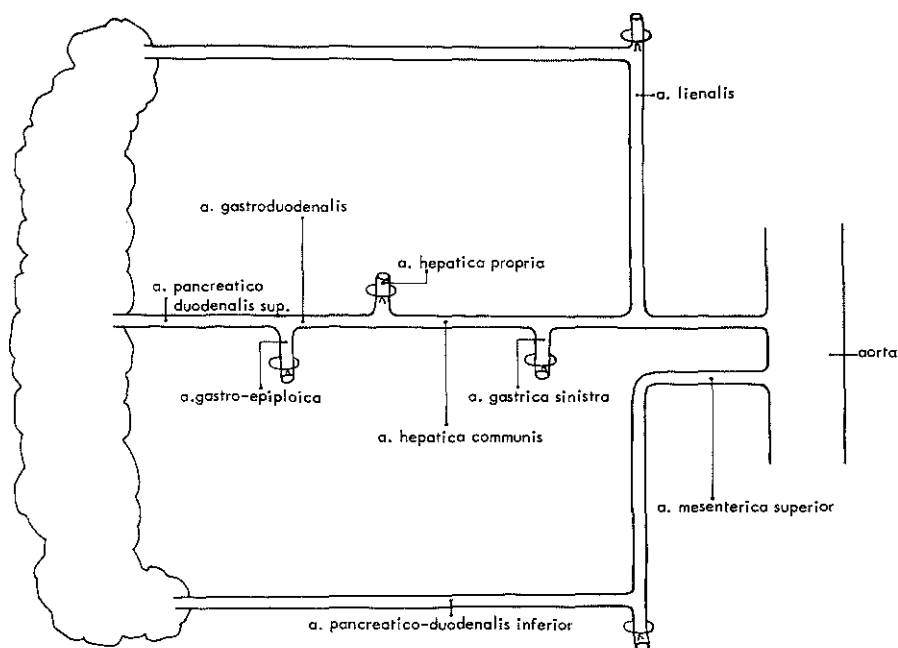


Fig. 11. Schematic representation of the arterial supply to a pancreas allotransplant.

reached the pancreas via the superior pancreaticoduodenal artery. The pancreas was then dissected from the duodenum, and both pancreatic ducts were isolated, cut, and tied off, leaving the organ connected only with the arterial circulation, via the celiac and mesenteric arteries, and with the venous circulation via the portal vein.

The donor was then given 3 mg heparin per kg body weight, and 3 minutes later the ischemic time was started by clamping of the aorta. The portal vein was severed in the hilus of the liver and the aorta segment isolated and cannulated, after which the pancreas was flushed with about 200 ml physiological saline containing 25 mg heparin 2% and 50 ml procaine HCl 2% per 500 ml, and held at 4°C. (See Plate 7.)

During the operation the donor received 0.9% saline, Rheomacrodex 10% in glucose 5% (about 10 ml per kg body weight), and 25,000 KIU Trasylol. After removal of the pancreas, the donor was killed by the administration of an overdose of Pentothal®.

2.4. Implantation

Before the recipient was anesthetized, the hemoglobin, blood sugar, and serum

potassium levels were determined. During the beginning of the anesthesia, glucose 5% and 8 U insulin were administered by infusion, if necessary with the addition of potassium. As an extra measure, a catheter was introduced into the carotid artery on the right side to permit continuous recording of the arterial blood pressure as well as blood sampling for pH and pO_2 determinations. ECG registration was also continuous. If necessary, acidosis was corrected with $NaHCO_3$ 5%. The basic infusion consisted of a 5% glucose solution, to which insulin and KCl were added when required. Via a venous cannula, Rheomacrodex 10% was given in a 0.9% saline solution in a dose of 10 ml per kg body weight as well as plasma and, if necessary, blood. Just before revascularization, 50,000 KIU Trasylol was administered. All this was supplied via two infusions systems.

After the abdomen had been opened with a median incision, all of the organs were inspected before the aorta and caval vein below the renal vessels were dissected. Meanwhile, on another operating table, the donor pancreatotomy had been completed. For the transplantation, a side-to-side venous anastomosis

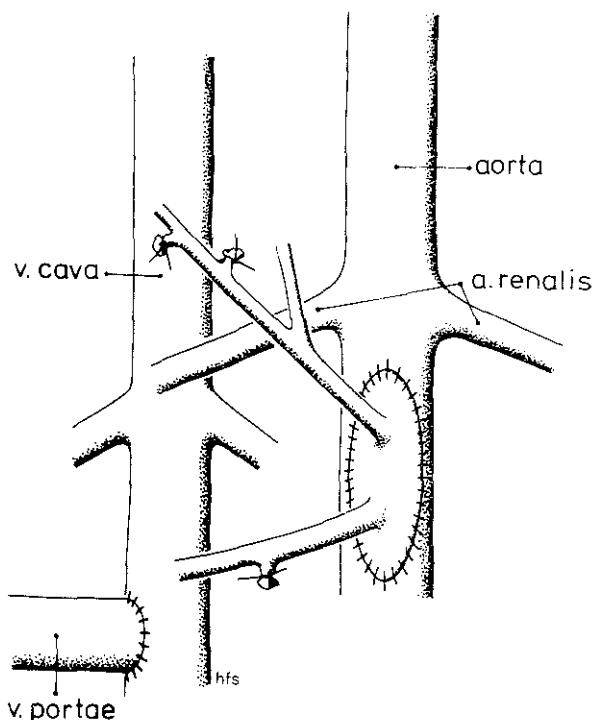


Fig. 12. Drawing showing the vessel-bearing patch implanted in the aorta below the recipient's renal arteries.

was made with 5×0 silk between the portal vein of the transplant and the inferior caval vein of the recipient, and after that the flushing of the pancreas with cold saline was terminated. A Carrel patch cut around the origin of the celiac and superior mesenteric arteries (Plate 8) was sutured with 5×0 silk to the recipient's aorta (Fig. 12). No further fixation of the pancreas was performed. After the anastomoses and the pancreas had been checked for leakage of blood, the abdomen was closed in two layers with tied silk sutures.

2.5. Post-operative management

In connection with the problem of pancreatitis, an essential part of the post-operative management was the supply of abundant fluid in the form of such colloidal liquids as plasma and Rheomacrodex. A 5% glucose solution was given to compensate for the post-operative hypoglycemia. The infusion scheme was as follows:

Amounts and types of fluid given per 10 kg body weight post-operatively

4th hour	100 ml Rheomacrodex 10% in glucose 5% + 1 g KCl/500 ml 100 ml glucose 5% + 1 g KCl/500 ml 100-150 ml plasma
12th hour	100 ml Rheomacrodex 10% in glucose 5% + 1 g KCl/500 ml 100 ml glucose 5% + 1 g KCl 100 ml plasma
20th hour	100 ml Rheomacrodex 10% in glucose 5% + 1 g KCl/500 ml 100 glucose 5% + 1 g KCl 75 ml plasma
28th hour	75 ml Rheomacrodex 10% in glucose 5% + 1 g KCl/500 ml 75 ml glucose 5% + 1 g KCl/500 ml

In the following phase, 75 ml Rheomacrodex 10% in glucose 5% was given parenterally twice daily, with gradual reduction of the amount until on the 5th post-operative day 50 ml was given once. In some cases it was necessary to diverge from this scheme: for instance, when the hemoglobin level became too low, plasma was replaced by blood. During the first 5 days, ampicillin (page 25) and 50,000 KIU Trasylol were given daily.

As early as the first post-operative day the dogs were allowed to drink *ad lib*, and on the second day solid food was given together with pancreatic enzymes. No additional insulin was given.

Whenever possible, a GTT was performed and the insulin peak value, liver function, and Ca, Mg, and amylase levels were determined once during the first 5 days after the operation. When the animal's condition did not permit this, only

the essential determinations were made, e.g. the hemoglobin, blood sugar, and serum potassium levels. The amount of fluid taken orally was recorded. The body weight was recorded once a week except in dogs with poor appetite, which were weighed every other day. Food was given *ad lib*. Throughout the post-operative period, up to rejection, the following determinations were performed. The serum glucose level was measured daily in the first post-operative week and thereafter 3 times a week, the blood being sampled at arbitrary times, thus generally not when the stomach was empty. An intravenous GTT and an insulin determination were done weekly, and more frequently when signs of rejection were found. Hemoglobin and serum amylase were determined daily during the first 5 post-operative days. Liver function was checked once a week. In dogs treated with immunosuppressive drugs the blood picture and the number of thrombocytes present were checked twice weekly.

2.6. Criteria for rejection

Under rejection of an organ transplant we understand the progressive deterioration of organ function due to destruction of the organ by the host. As pointed out in Chapter I, in pancreas transplantations other factors can be responsible for the loss of the endocrine function, and we therefore applied the following criteria for the diagnosis rejection of the pancreas transplant:

- a. Total loss of the endocrine function of the pancreas as demonstrated by:
 1. K value of the GTT approaching nil, and
 2. IPV of less than 20 μ U/ml after glucose loading.

If signs of diabetes mellitus appeared (e.g. polydisia, high blood sugar values) an intravenous GTT was performed and the IPV determined.

- b. The presence of the histological signs characteristic for rejection known from the literature (22,94,113,153).

Some of the animals were not in very good condition during this period, so that it was not always possible to collect the amount of blood required for a good GTT and IPV determination at the usual time. In these cases, two arbitrary blood sugar determinations on day X (day X being the day on which an elevated blood sugar level was first found) and the fasting blood sugar level on day X+1 were considered to suffice. All 3 of these values had to lie higher than 200 mg %. The day on which function disappeared was then called day X-1. As soon as loss of function was observed, the dog was killed.

2.7. The immunosuppressive drugs

In the last 2 experimental groups, 3d and 3e, immunosuppressive drugs were used over a period equalling the mean transplant survival time of group 3c. The 3c dogs were identical for the DL-A system and could be expected to have the longest survival time. It was not necessary to administer the drugs over a longer period, because a large number of dogs with a very long survival time was not required.

Group 3d was given a combination of azathioprine and prednisone, the former in the form of azathioprine sodium (Burroughs Wellcome & Co, London, United Kingdom) in a daily dose of 4 mg per kg body weight, the latter as prednisolonacetate (Di-adreson-F®, Organon) given intramuscularly in a daily dose of 1 mg per kg body weight for the first 2 days, followed by prednisone given orally in the same dose. Administration was started immediately after the operation.

Group 3e received an anti-lymphocyte serum provided by the Radiobiological Institute-T.N.O. (Rijswijk, The Netherlands). This horse anti-dog anti-lymphocyte serum (batch nr. P.M.D. 3-5B) was produced according to GRAY *et al.* (47). A mixture of 10^9 thymus cells and lymph node cells in Freund's adjuvant was injected subcutaneously, and this was repeated 3 weeks later. One week after that, the first blood sample (A) was taken. Eight weeks later, the same number of cells were injected intravenously. One week later, the second blood sample was taken to obtain serum B, which was used in the experiments in a dosage of 1.5 ml per kg body weight 3 times weekly. Two days before the transplantation, a double dose was given once, and the following single dose was given on the day of the operation.

3. RESULTS

3.1. Total pancreatectomy

All 77 dogs survived the first 48 post-operative hours. After this interval, 6 dogs died of hypo- or hyperglycemia, 2 died of peritonitis due to a duodenal perforation, and 3 died as the result of intussusception.

With respect to the first 6 dogs, the following points are relevant. As already mentioned, all of the animals received an amount of insulin adjusted to the serum glucose level. However, the appetite of the dogs after the operation varied widely, and a number died of hypo- or hyperglycemia with blood sugars varying from 16 to 720 mg %.

With respect to the 2 deaths due to peritonitis, the excision of the recipient's pancreas had left the duodenal circulation inadequate because the superior pancreaticoduodenal artery was ligated, leading in these cases to perforation of the duodenum. As far as the last 2 animals are concerned, it may be noted that after both total pancreatectomy and transplantation our dogs showed an elevated tendency to intussusception (see Table VI, page 64).

After the total pancreatectomy, most of the dogs did well on the following regimen (per 10 kg body weight): Morning: 8 U insulin novo lente (Novo Industrie A/S, Copenhagen, Denmark), 4 U insulin (Organon, Oss, The Netherlands). Evening: 4 U Insulin (Organon). In addition, food was given *ad lib* with 16 tablets Combizym and 8 tablets Cotazym. In a number of cases the solid food had to be replaced temporarily by the liquid diet (Wessanen) in which 5 g pancreatin and 50 mg lipase was dissolved. All of the dogs lost weight after the pancreatectomy, on average 23 per cent of the pre-operative weight in 3 weeks. After the transplantation the lost weight was recovered on the same enzyme regimen.

Two of the dogs became severely cachetic and had to be killed. In one the post-operative function determination showed that there was still some endocrine and exocrine function; this dog was not considered for transplantation.

Thus, 64 dogs remained available for transplantation. These dogs satisfied the already-mentioned criteria: a) K value of intravenous GTT = 0; b) serum insulin peak value < 3 μ U/ml after glucose loading; c) proteolytic enzymes in the feces < 1 U.

3.2. General transplantation results

3.2.1. Surgical mortality

Four dogs died during the operation or within the first 24 hours, three due to an electrocardiographically demonstrated ventricular fibrillation caused by hypokalemia and one from severe post-operative hypoglycemia despite the administration of more than the standard amount of glucose.

As a result of these complications, which occurred in the first 12 cases, the serum glucose, serum potassium, and serum insulin levels were determined in the next 15 transplantations at the following times: a) shortly before the preparatory anesthesia; b) shortly before revascularization of the transplant; c) immediately after revascularization of the transplant; d) at the termination of the anesthesia.

The results are shown in Fig. 13, where the composition of the infusions administered during the operation are also given. At the start of anesthesia the mean potassium content of the blood was 5.1 mmol/litre and the glucose con-

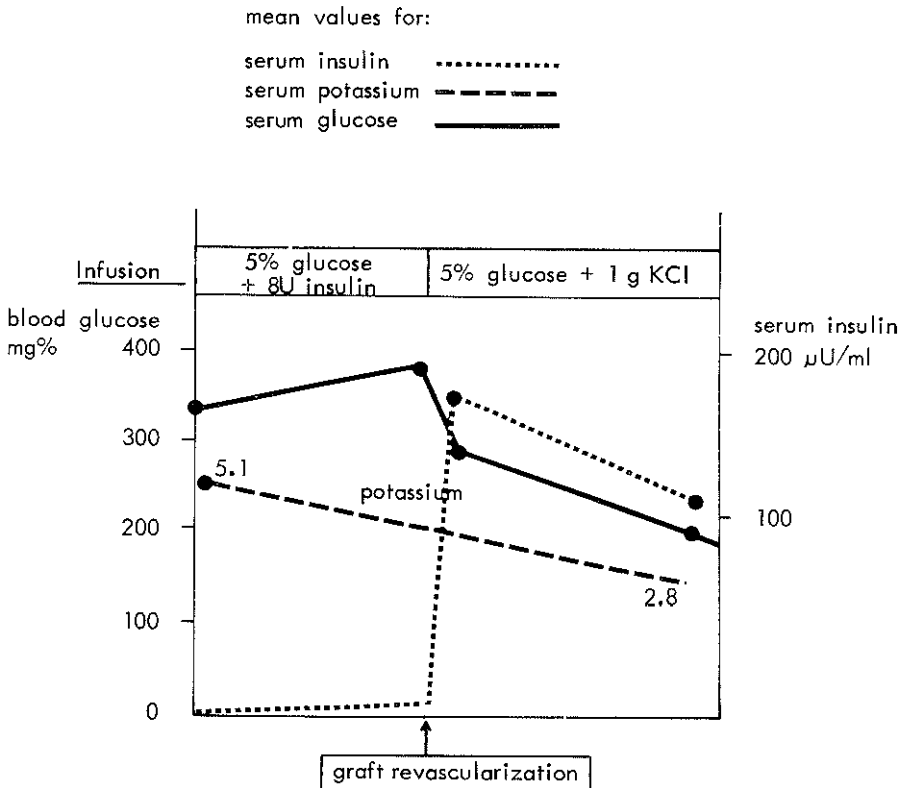


Fig. 13. Average course of the insulin, potassium, and glucose levels in the serum of 15 dogs given a pancreas allotransplant.

tent 330 mg %. There was then no endogenous insulin in the circulation. After revascularization the picture changed radically and a good insulin level with a mean value of 175 μ U/ml developed. This led to a sharp drop in the serum potassium and glucose levels despite the administration of both by infusion. In addition, most of the dogs proved to be slightly acidotic just before being anesthetized.

On the basis of these findings, pathological changes in the serum potassium and glucose levels and/or the pH value of the blood occurring during or immediately after the operation were corrected, after which the surgical mortality dropped to nil.

3.2.2. Post-operative course

a. Serum glucose level

The course of the mean serum glucose level is shown in Fig. 14. It is evident

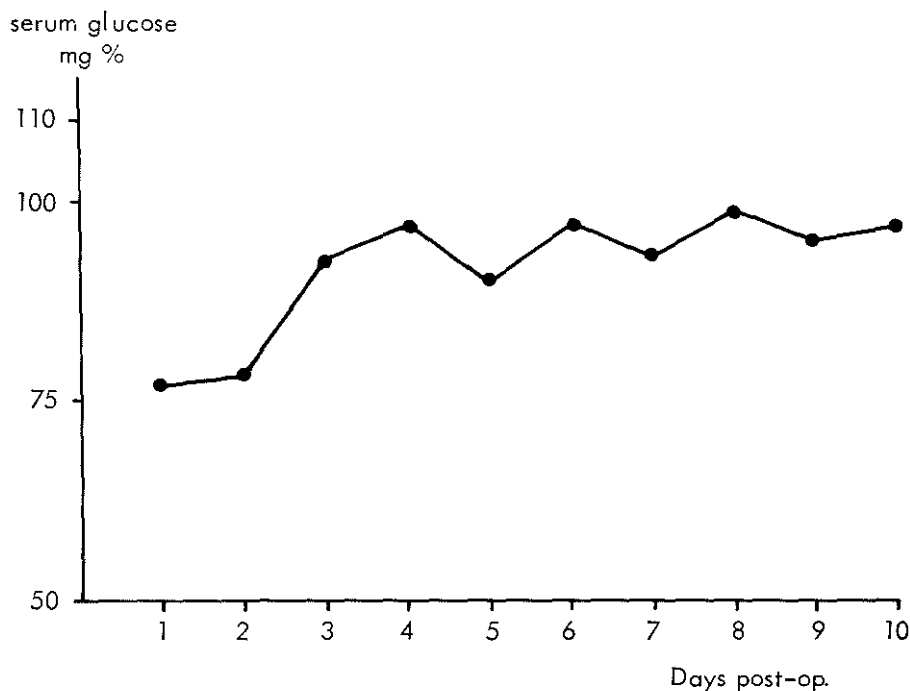


Fig. 14. Mean fasting serum glucose levels for 20 dogs after pancreas allotransplantation.

that despite the administration of glucose the values were lower on the first two post-operative days than on the following days. This pattern was the same for all of the groups. On the third or fourth post-operative day a GTT was done in each dog and the K value calculated; the results will be discussed separately under *Experimental groups*.

b. Hemoglobin level

The hemoglobin content of the blood varied only on the first two days after the operation. The pancreatitis led to elevated hematocrit values, which in turn caused sludging, but this was sometimes camouflaged by post-operative bleeding. This picture was the same for all groups except group 3e, in which a toxic side-effect of the administration of ALS led to further depression of the hemoglobin level. We shall return to this point below.

c. Serum amylase level

The serum amylase level varied widely in all of the groups. On the third post-operative day, for instance, the values ranged from 512 U to 32,768 U Wohlgemuth. In the individual dogs, however, there was a gradual rise, the highest

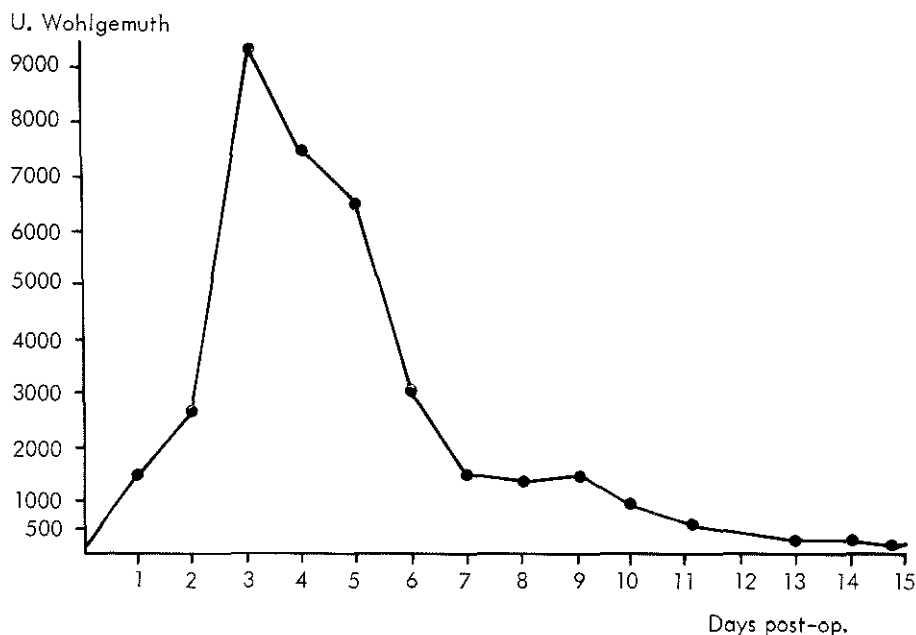


Fig. 15. Mean serum amylase levels for 20 dogs after pancreas allotransplantation.

values usually occurring on the third post-operative day. After the tenth day, elevated values were only seen twice. The course of the mean serum values of 20 randomly selected dogs representing all of the groups is shown in Fig. 15.

d. Serum insulin level

As can be seen from Fig. 14, the serum insulin level was high immediately after the operation. On the first post-operative day the serum insulin level determined arbitrarily without stimulation still had a mean value of 40 μ U/ml. The insulin peak value is, however, much more important for the evaluation of the endocrine pancreas function, and will be discussed on the basis of the individual dogs under *Experimental groups*.

e. Other determinations

The results of the other determinations mentioned in Chapter II were not divergent after transplantation with the exception of a number of hematological values in dogs treated with immunosuppressive drugs, which will be discussed under the relevant groups.

f. Deaths shortly after transplantation

Of the 60 dogs which could be followed after the transplantation, two had glucose values varying from 280 to 310 mg % immediately after the operation.

TABLE VI

Endocrine function and cause of death in cases of early mortality after pancreas transplantation.

Dog nr.	Nr. of haplo- types diff. between littermates	Nr. of days post- oper. survival	Endocrine function at time of death	Cause of death	Post-mortem findings
B 9.227	2	2	normal blood sugar values	unknown	<i>Cause of death:</i> splenic hemorrhage. <i>Pancreas:</i> some interstitial edema and mild superficial pancreatitis; no signs of rejection.
BD 9.178	2	7	K = 1.6 IPV = 240 μ U/ml	intussus- ception	<i>Cause of death:</i> intussusception. <i>Pancreas:</i> no pancreatitis; fibroid degeneration of acinar tissue; signs of onset of rejection.
B 0.195	2	2	normal blood sugar values	unknown	<i>Cause of death:</i> unknown. Venous anastomosis shows fresh thrombus. <i>Pancreas:</i> normal appearance except for some edema in interstitial tissue and very slight cellular infiltration.
BD 0.337	1	7	normal blood sugar values	intussus- ception	<i>Cause of death:</i> 75 cm gut necrotic. <i>Pancreas:</i> edema with loss of granulation; slight indications of pancreatitis; atrophic acini; no signs of rejection.
B 1.247	1	8	K = 3.5 IPV = 130 μ U/ml	intussus- ception	<i>Cause of death:</i> intussusception. Anastomoses patent. <i>Pancreas:</i> signs of pancreatitis; infarctions; almost no cellular infiltration; extensive arteritis with intimal swelling and fibroid necrosis.
BD 1.219	1	6	K = 3.5 IPV = 71 μ U/ml	unknown	<i>Cause of death:</i> hemorrhage arterial anastomosis. Venous anastomosis patent. <i>Pancreas:</i> mild edema; mild pancreatitis; no signs of rejection. Pancreas picture normal.

(Continued on p. 65)

TABLE VI. Continued

Dog nr.	Nr. of haplo-types between littermates	Nr. of days post-oper. survival	Endocrine function at time of death	Cause of death	Post-mortem findings
BD 0.301	0	1	normal blood sugar values	relaparotomy for volvulus followed by arterial thrombosis transplant	<i>Cause of death:</i> art. thrombosis after relaparotomy. Status after resection large intestine. <i>Pancreas:</i> extensive arterial thrombosis and pancreatitis. No signs of rejection.
BD 9.157	0	5	normal blood sugar values	hypoxemia	<i>Cause of death:</i> hypoxemia. <i>Pancreas:</i> pancreatitis and venous thrombosis. No signs of rejection.
B 0.177	0	7	K = 2.1 IPV = 190 μ U/ml	bleeding from venous anast.	<i>Cause of death:</i> hemorrhage. <i>Pancreas:</i> fibrotic replacement of acinar tissue; extensive mononuclear cell infiltration; intimal swelling.
Non-littermates					
B 0.260	incompatible	7	1 day normal blood sugar values, then high	killed because trans. failed to function	<i>Pancreas:</i> arterial thrombosis at sutures; complete necrosis.
BD 0.193	incompatible	5	K = 2.0 blood sugar values normal	unknown	<i>Cause of death:</i> hemorrhagic pancreatitis. Anastomoses patent. <i>Pancreas:</i> hemorrhagic necrosis with infarction; perivascular cellular infiltration.

These dogs were killed on the second post-operative day, and both were found to have thromboses in the venous vascular anastomosis. These were the only animals in which the transplant had not functioned at all. Of the other 58 dogs, 11 died in the first week after the first few post-operative days. Some data on these 11 dogs, including the endocrine pancreas function and cause of death, are shown in Table VI, from which it can be seen that thrombosis in an anastomosis was only found once after the first 24 hours and that intestinal strangulation was the cause of death in four cases. Thus, 47 dogs with a functioning transplant were available for further study.

3.3. Experimental groups

The 47 surviving dogs were distributed over the groups as follows:

Group 3a: unrelated, not identical	9
Group 3b: related, not identical (differing by 2 haplotypes)	9
Group 3c: related, identical (differing by 0 haplotypes)	13
Group 3d: related, not identical (differing by 1 haplotype, treated with azathioprine and prednisone)	9
Group 3e: related, not identical (differing by 1 haplotype, treated with ALS)	7

The results will be discussed for each group separately and the data shown in 2 Tables for each group. The first of these Tables gives the highest serum glucose values for the first 2 post-operative days, when these values were relatively too low. The hemoglobin values of these 2 dogs are also shown, because this is the period in which the level fluctuated. The endocrine function is reflected in the K values and the IPV; the highest values registered in the first month are given but not those of the first post-operative week for the dogs with a longer survival time. The IPV invariably corresponded with the K value (for the course of the endocrine function in these five groups, see § 4). The clinical cause of death is also indicated. The second Table gives the duration of transplant function, the criteria for zero or almost zero function, the histological data on the transplant, and the final cause of functional failure. To permit comparison of the histological data of the various groups, the following grading was applied:

Pancreatitis:

Very mild (only interstitial edema)	+
Moderate (edema and local parenchymal necrosis due to auto-digestion)	++
Severe (edema and local parenchymal necrosis with hemorrhages)	+++

Rejection:

Distinction of an interstitial component with infiltration of mononuclear cells (lymphocytes, lymphoblasts, plasma cells) in tissue and perivascularly, and a vascular component with swelling of the intima, fibrinoid necrosis of the media, and thrombus formation in the smaller blood vessels.

a. *Interstitial rejection phenomena*

Mild (local cellular infiltration limited to the superficial area of the organ)	+
Moderate (general cellular infiltration but mainly on and near the organ surface)	++

Severe (cellular infiltration throughout, particularly between and around the blood vessels)	+++
b. <i>Vascular rejection phenomena</i>	
Mild (only swelling of the intima)	+
Moderate (intimal swelling with fibrinoid necrosis in the media)	++
Severe (initial swelling, fibrinoid necrosis, thrombus formation)	+++

3.3.1. Group 3a: unrelated, non-identical beagles

The data for this group are shown in Tables VII and VIII. In general, the K values lie under and the IPV above the normal level. Dogs BD 1.222 and B 171

TABLE VII
Post-operative values and endocrine function in group 3a.

Dog nr.	Blood sugar values (mg %)		Hemoglobin content (g %)		Highest K value %/min.	Insulin peak values (μU/ml)	Cause of death
	1st day	2nd day	1st day	2nd day			
BD 1.195	56	68	13.4	10.9	3.4	163	Killed
BD 1.271	74	83	13.6	13.6	2.2	135	Killed
B 0.335	77	67	10.2	12.8	2.4	240	Unknown
B 1.183	79	59	15.7	13.1	2.4	240	Killed
BD 0.314	67	61	14.6	13.6	3.3	133	Killed
BD 578	65	56	13.9	14.4	2.0	210	Killed
BD 1.222	48	68	13.1	13.0	3.5	57	Killed
B 171	56	81	18.2	15.5	3.0	170	Killed
B 184	25	25	11.7	11.6	4.1	—	Killed

had fasting blood sugar values of 90 mg % and 85 mg %, respectively, at a time when the K value had reached zero and the IPV was very low. In this group rejection was the cause of the loss of the endocrine pancreas function. As a rule, this was accompanied by a mild pancreatitis. The mean transplant survival time was 9.6 days.

3.3.2. Group 3b: related, non-identical beagles differing by 2 haplotypes

The data on these dogs are shown in Tables IX and X. In dog nr. BD 0.284 the values on the sixth post-operative day were $K = 0$ and $IPV = 0$, indicating that the transplant had been rejected. Insulin could not be determined in nr. BD 382 because antibodies against the administered insulin were demonstrated in the period without the pancreas (41 days). The K value of B 0.217 could not

TABLE VIII

Transplant survival and post-mortem findings in group 3a.

Dog nr.	Nr. days transplant function	Clinical indications for abolition of function	Post-mortem findings (with grades)**	Final diagnosis
BD 1.195	11	K = 0 Ins.: not demonstrable	Pancreatitis ++ Interstitial +++ Vascular — Anastomoses intact	Rejection with pancreatitis
BD 1.271	8	High blood sugar values*	Pancreatitis + Interstitial ++ Vascular ++ Anastomoses intact	Rejection with pancreatitis
B 0.335	10	Normal blood sugar values	Pancreatitis ++ Interstitial ++ Vascular + Anastomoses intact	Rejection with pancreatitis
B 1.183	15	K = 0 Ins.: 2 μ U/ml	Pancreas: cysts Pancreatitis + Interstitial +++ Vascular + Anastomoses intact	Rejection with pancreatitis
BD 578	8	K = 0 Ins.: 4 μ U/ml	Early fibrosis of acinar tissue Pancreatitis — Interstitial ++ Vascular + Anastomoses intact	Rejection
BD 0.314	11	High blood sugar values*	Anastomoses intact No histological data (technical failure)	Rejection
BD 1.222	8	K = 0 Ins.: 2 μ U/ml	Early fibrosis of acinar tissue Pancreatitis + Interstitial +++ Vascular + Anastomoses intact	Rejection
B 171	8	K = 0 Ins.: 10 μ U/ml	Pancreatitis — Interstitial + Vascular ++ Anastomoses intact	Rejection
B 184	8	K = 0 Ins.: 6 μ U/ml	Pancreatitis + Interstitial ++ Vascular ++ Anastomoses intact	Rejection with pancreatitis

* Blood samples too small to permit calculation of K value.

** Rejection phenomena shown as interstitial and vascular components.

Grading: — = none
+ = mild
++ = moderate
+++ = severe

TABLE IX

Post-operative values and endocrine function in group 3b.

Dog nr.	Blood sugar values (mg %)		Hemoglobin content (g %)		Highest K value (% min)	Insulin peak value (μ U/ml)	Cause of death
	1st day	2nd day	1st day	2nd day			
BD 0.283	113	101	11.3	13.4	2.7	anti- bodies	killed
BD 0.190	76	81	12.9	12.9	2.6	49	hyperglycemia
BD 0.284	54	20	21.0	19.4	—	—	hyperglycemia
B 0.248	85	79	11.6	14.2	2.2	92	killed
B 0.284	61	87	17.7	13.3	1.8	57	killed
BD 0.213	79	88	16.1	12.6	3.9	92	killed
BD 0.237	74	97	11.8	12.8	2.3	—	killed
BD 0.206	59	56	16.9	15.1	3.0	—	hyperglycemia
B 0.217	65	65	10.6	12.8	—	38	hyperglycemia

be calculated because the GTT showed unexplained fluctuations, but the IPV could be determined.

In this group, too, the main causes of the loss of the endocrine pancreas function were rejection and pancreatitis. The mean transplant survival time was 10.5 days.

3.3.3. Group 3c: related, identical beagles differing by 0 haplotypes

For this group the data are shown in Tables XI and XII. Here, the IPV could not be determined in 2 dogs (B 0.340 and BD 9.141) after the transplantation, because 27 and 47 days, respectively, after the administration of insulin was started antibodies were found to be present. At the end of the study, dogs BD 622 and BD 617 were still alive, so no autopsy data are available.

Of the 13 dogs in this group, 7 were killed because the organ was rejected. The two which survived the first year have a good endocrine function. If, however, the survival time of the transplant in these two cases is taken equal to that of BD 9.161, which lived 69 days, the mean survival time of the transplants in this group amounts to 42.9 days.

3.3.4. Group 3d: related, non-identical beagles differing by 1 haplotype and treated with azathioprine and prednisone

Tables XIII and XIV show the data concerning these dogs. The immunosuppressive drugs were given for 42 days. This duration of treatment was chosen as being virtually equal to that of the mean transplant survival time of group 3c.

TABLE X

Transplant survival and post-mortem findings in group 3b.

Dog nr.	Nr. days transplant function	Clinical indications for abolition of function	Post-mortem findings (with grades)**	Final diagnosis
BD 0.283	29	Polydipsia High blood sugar values K = 0	Pancreatitis + Interstitial + Vascular ++ Anastomoses intact	Rejection with pancreatitis
BD 0.190	10	K = 0 Ins. = 0	Pancreatitis + Interstitial +++ Vascular ++ No acinar tissue Extensive fibrosis with autolytic cysts Anastomoses intact	Acute rejection, enzymic auto- digestion
BD 0.284	5	K = 0 High blood sugar values	Pancreatitis ++ Interstitial ++ Vascular + Anastomoses intact	Rejection with pancreatitis
B 0.248	12	High blood sugar values K = 0 Ins. = 0	Pancreatitis + Interstitial +++ Vascular + Anastomoses intact	Rejection with pancreatitis, local throm- bosis
B 0.284	9	High blood sugar values K = 0 Ins. = 4 μ U/ml	Pancreatitis ++ Interstitial + Vascular + Anastomoses intact	Rejection with pancreatitis
BD 0.213	6	High blood sugar values K = 0 Ins. = 0	Pancreatitis ++ Interstitial ++ Vascular — Thrombotic anast.	Rejection, pancreatitis, thrombosis
BD 0.237	7	High blood sugar values K = 0	Pancreatitis + Interstitial ++ Vascular — Severe pneumonia with abscess formation Anastomoses intact	Rejection with pancreatitis
BD 0.206	8	High blood sugar values*	Pancreatitis + Interstitial ++ Vascular + Anastomoses intact	Rejection with pancreatitis
B 0.217	9	High blood sugar values K = 0 Ins. = 0	Pancreatitis — Interstitial + Vascular ++	Rejection with pancreatitis

* Blood samples too small to permit calculation of K values.

** Rejection phenomena shown as interstitial and vascular components.

Grading:

— = none

+ = mild

++ = moderate

+++ = severe

TABLE XI

Post-operative values and endocrine function in group 3c

Dog nr.	Blood sugar values (mg %)		Hemoglobin content (g %)		Highest K value (% min)	Insulin peak value (μ U/ml)	Cause of death
	1st day	2nd day	1st day	2nd day			
B 619	67	92	15.4	13.6	2.0	62	killed
BD 0.294	59	94	18.2	15.8	3.8	—	killed
BD 9.161	90	92	14.3	13.1	3.2	123	killed
BD 0.164	74	72	16.8	15.2	4.6	240	killed
B 0.262	65	74	18.5	17.1	2.6	160	killed
B 578	65	101	14.9	11.8	3.4	82	unknown
B 556	65	103	18.2	10.1	1.8	—	hemorrhage
BD 622	86	70	12.6	8.8	4.6	224	still alive
B 558	97	90	10.9	12.5	3.5	24	killed
B 0.340	86	65	12.6	13.7	3.8	antibodies	killed
BD 617	88	94	14.2	13.8	4.0	240	still alive
BD 9.276	65	65	18.4	16.8	1.8	80	unknown
BD 9.141	34	52	15.6	17.6	2.8	antibodies	pneumonia

Nr. B 649 is a good example of a dog in such poor condition that sufficient blood for the GTT and IPV determination could not be taken at the scheduled times. No hematological anomalies were found.

Of the 9 dogs in this group, 8 were killed because the endocrine function disappeared due to rejection of the transplant. The mean survival time of the transplants was 34.2 days.

3.3.5. Group 3e: related, non-identical beagles differing by 1 haplotype and treated with ALS

The data for this group are shown in Tables XV and XVI. The very high IPV are again striking. Dog nr. BD 0.201 is the only one in this group to die with a functioning pancreas; the cause of death proved to be a defective arterial anastomosis. Of these 7 dogs, 5 were killed because of rejection of the transplant. Of the two remaining dogs, one bled to death and one is still alive even though ALS was withdrawn on the 42nd post-operative day. We have equated the survival time of this dog with that of the dog with the longest transplant survival time ending in rejection at 90 days. On this basis, the mean transplant survival time for this group is 57.0 days.

The unfavorable effect of ALS on the hemoglobin level and the morphological blood picture was unmistakable. Fig. 16 shows the course of the mean of the hemoglobin level in this group up to the 7th post-operative week, the first value indicated being the pre-operative level and the second the result of the

TABLE XII

Transplant survival and post-mortem findings in group 3c.

Dog nr.	Nr. days transpl. function	Clinical indications for abolition of function	Post-mortem findings (with grades)*	Final diagnosis
B 619	22	K = 0 IPV = 0	Pancreatitis — Interstitial + Vascular + Extensive fibrotic areas with intact islets Anastomoses intact	Rejection
BD 0.294	12	K = 0 High blood sugar values Polydipsia	Pancreatitis — Interstitial + Vascular ++ Moderate fibrosis, islets intact Anastomoses intact	Rejection
BD 9.161	69	K = 0 IPV = 0	Pancreatitis — Interstitial ++ Vascular + Total fibrotic degen. of acinar tissue with recognizable islet cells Anastomoses intact (Plates 12 & 15)	Rejection
BD 0.164	59	(Persisting function)	Pancreatitis — Interstitial + Vascular + Total fibrotic degen. of acinar tissue with intact islet cells Anastomoses intact (Plates 13 & 14)	Killed because of multiple abscesses after relaparotomy for severe ileus (resection of entire small intestine)
B 0.262	39	K = 0 IPV = 24	Pancreatitis — Interstitial ++ Vascular + Total fibrotic degen. of acinar tissue, islets intact Anastomoses intact	Rejection
B 578	12	(Persisting function)	Pancreatitis — Interstitial +++ Vascular ++ Fibrosis, intact islets in surviving acinar tissue Anastomoses intact	Rejection

(Continued on p. 73)

TABLE XII. Continued.

Dog nr.	Nr. days transpl. function	Clinical indications for abolition of function	Post-mortem findings (with grades)*	Final diagnosis
B 556	8	(Persisting function)	Pancreatitis — Interstitial + Vascular — Early fibrosis Arterial anas. ruptured	Bleeding from arterial anastomosis
BD 622	365	(Persisting function)	—	Still alive
B 558	36	K = 0 IPV = 0	Pancreatitis — Interstitial ++ Vascular — Total fibrotic degen. of acinar tissue, islets intact Anastomoses intact	Rejection
B 0.340	42	K = 0 antibodies	Pancreatitis — Interstitial ++ Vascular ++ Total fibrotic degen. of acinar tissue, islets intact Anastomoses intact	Rejection
BD 617	365	(Persisting function)	—	Still alive
BD 9.276	11	(Persisting function)	Pancreatitis — Interstitial + Vascular + Extensive fibrosis of acinar tissue showing islets Signs of pulm. embolism and pneum. foci	Pulmonary embolis and pneumonia
BD 9.141	35	(Persisting function)	Pancreatitis — Interstitial — Vascular — Total fibrotic degen. of acinar tissue, islets recognizable Pulmonary congestion, pneum. foci	Pneumonia

* Rejection phenomena shown as interstitial and vascular components.

Grading: — = none
+ = mild
++ = moderate
+++ = severe

TABLE XIII

Post-operative values and endocrine function in group 3d

Dog nr.	Blood sugar values (mg %)		Hemoglobin content (g %)		Highest K value (% min)	Insulin peak value (μ U/ml)	Cause of death
	1st day	2nd day	1st day	2nd day			
BD 653	40	79	16.8	13.4	2.6	135	killed
B 2.152	40	79	13.4	15.5	3.5	120	killed
B 2.206	70	97	15.8	13.9	4.1	179	killed
BD 2.219	70	84	12.6	12.3	4.1	107	killed
B 2.228	56	72	17.4	14.4	2.6	48	killed
BD 2.204	65	65	13.1	12.3	2.8	90	killed
B 2.213	90	100	11.0	11.5	4.3	76	killed
B 649	54	67	17.4	14.9	—	—	killed
B 1.180	97	86	13.3	13.0	2.6	87	killed

TABLE XIV

Transplant survival and histological findings in group 3d

Dog nr.	Nr. days transpl. function	Indications for abolition of function	Post-mortem findings (with grades)**	Final diagnosis
BD 653	8	K = 0 Ins. not demon- strable	Pancreatitis + Interstitial — Vascular + + Extensive fibrosis Thromb. art. anas.	Arterial anastomoses thrombotic
B 2.152	62	K = 0 Ins.: 2 μ U/ml	Pancreatitis — Interstitial — Vascular + + + Extensive necrosis due to vas- culitis and thrombus formation Anastomoses intact	Rejection
B 2.206	73	High blood sugar values*	Pancreatitis — Interstitial + Vascular + + + Total fibr. degen. of acinar tissue Anastomoses intact	Rejection
BD 2.219	26	K = 0 Ins.: 4 μ U/ml	Pancreatitis — Interstitial — Vascular + + + Total fibr. degen. of acinar tissue; islet tissue here and there Anastomoses intact	Rejection
B 2.228	7	K = 0 Ins.: 10 μ U/ml	Pancreatitis — Interstitial + Vascular + + Total fibr. degen. of acinar tissue, no islets Anastomoses intact	Rejection

(Continued on p. 75)

TABLE XIV. Continued

Dog nr.	Nr. days transpl. function	Indications for abolition of function	Post-mortem findings (with grades)**	Final diagnosis
BD 2.204	27	K = 0 Ins.: 16 μ U/ml	Pancreatitis — Interstitial + Vascular +++ Moderate fibrosis, some recog- nizable acinar tissue and islets Anastomoses intact	Rejection
B 2.213	31	K = 0 Ins.: 12 μ U/ml	Pancreatitis — Interstitial + Vascular +++ Total fibr. acinar tissue, some recogn. islets Anastomoses intact	Rejection
B 649	21	High blood sugar values*	Pancreatitis + Interstitial + Vascular +++ Total fibr. acinar tissue, some islets present Anastomoses intact	Rejection
B 1.180	27	K = 0 Ins.: 2 μ U/ml	Pancreatitis — Interstitial + Vascular +++ Total fibr. acinar tissue, islets easily recognizable Anastomoses intact (Plates 18-21)	Rejection

* Blood samples too small to permit calculation of K value.

** Rejection phenomena shown as interstitial and vascular components.

Grading: — = none
+ = mild
++ = moderate
+++ = severe

TABLE XV

Post-operative values and endocrine functions in group 3e

Dog nr.	Blood sugar values (mg %)		Hemoglobin content (g %)		Highest K value (% min)	Insulin peak value (μ U/ml)	Cause of death
	1st day	2nd day	1st day	2nd day			
B 626	92	81	9.1	11.8	3.8	240	killed
BD 619	74	70	14.2	14.6	3.9	16	killed
B 0.211	54	76	13.6	13.3	3.0	240	hyperglycemia
B 614	67	56	13.9	11.8	2.6	46	killed
B 591	67	83	9.1	9.3	2.3	205	still alive
B 616	29	95	11.8	11.7	3.8	240	killed
BD 0.201	61	61	12.6	16.0	3.7	100	bleeding from anastomosis

TABLE XVI

Transplant survival and post-mortem findings in group 3e

Dog nr.	Nr. days transpl. function	Indications for abolition of function	Post-mortem findings (with grades)*	Final diagnosis
B 626	68	K = 0 IPV = 12 μ U/ml	Pancreatitis — Interstitial — Vascular ++ Total fibr. degen. of acinar tissue, easily recognized islets Anastomoses intact	Rejection
BD 619	33	K = 0 IPV = 0	Pancreatitis — Interstitial + Vascular ++ Acinar tissue almost entirely replaced by connective tissue, islet tissue in most areas Anastomoses intact (Plates 16 & 17)	Rejection
B 0.211	90	K = 0 IPV = 0	Pancreatitis — Interstitial + Vascular ++ Total fibr. degen. of acinar tissue, sparse islets Anastomoses intact	Rejection
B 614	35	K = 0 IPV = 0	Pancreatitis — Interstitial — Vascular ++ Total fibr. degen. of pancreas Anastomoses intact	Rejection
B 591	365	(Persisting function)	Still alive	Good pancreatic function
B 616	28	K = 0 Ins.: 6 μ U/ml	Pancreatitis — Interstitial + Vascular ++ Total fibr. degen. of acinar tissue, islets virtually indistinguishable Anastomoses intact	Rejection
BD 0.201	14	(Persisting function)	Pancreatitis — Interstitial + Vascular + Total fibr. degen. of acinar tissue, islets clearly recognizable Arterial anastomosis partially ruptured	Fatal hemorrhage

* Rejection phenomena shown as interstitial and vascular components.

Grading:

— = none

+ = mild

++ = moderate

+++ = severe

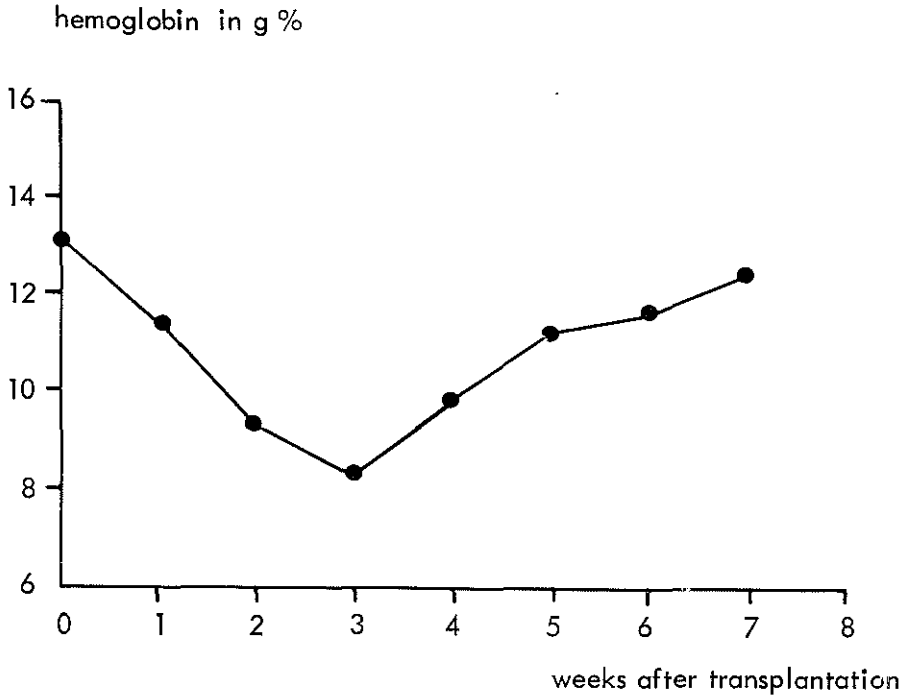


Fig. 16. Average course of the hemoglobin level in group 3e.

determination at the end of the first post-operative week. The most pronounced drop in the hemoglobin level occurred in the third post-operative week, despite blood transfusion. The amount of blood given after the second post-operative day is shown in Table XVII. Figs. 17, 18, and 19 give the results of the leukocyte, lymphocyte, and thrombocyte counts made at the same time as the hemoglobin determinations.

4. THE ENDOCRINE FUNCTION

In the dogs with a brief duration of transplant function the GTT and IPV determination could only be performed once or twice and there was little point in analysing the endocrine pattern. For the dogs with longer transplant function, the same picture was found in all groups. The first post-operative K value was usually slightly under the normal and the IPV often above the normal level. The K value returned to normal during the first 4 weeks; and the IPV dropped to a slightly lower level. Both values dropped one or two weeks before rejection.

TABLE XVII

Amount of blood administered to dogs of group 3e after the 2nd post-operative day

Dog nr.	Amount of blood (in ml)
B 626	550
BD 619	1000
B 0.211	3200
B 614	2900
B 591	700
B 616	700

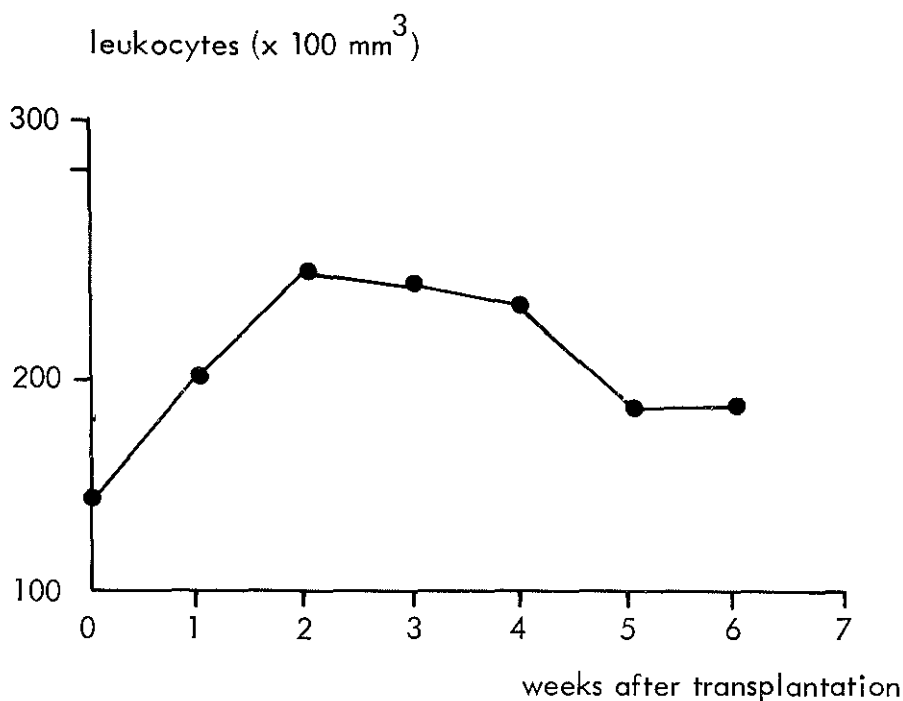
*Fig. 17. Average course of the leukocyte counts in group 3e.*

Fig. 20 shows the course of these values for group 3c, but these means should only be considered to give a rough impression because they concern different numbers of animals.

As can be seen from Fig. 20, the mean IPV rose again just before the dogs were killed. This elevation was due to a sudden rise of the IPV at a low K value in one dog belonging to this group. The course of the IPV in this dog (BD 0.164)

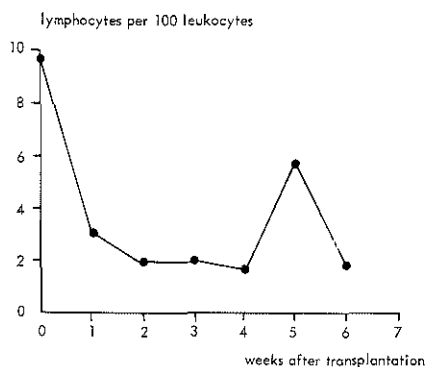


Fig. 18. Average course of the lymphocyte counts in group 3e.

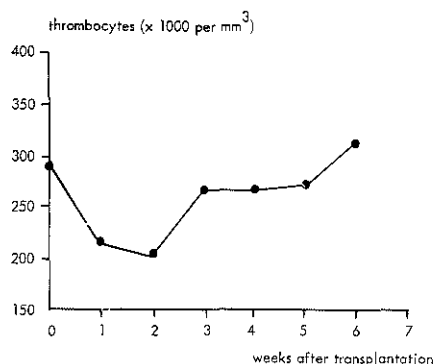


Fig. 19. Average course of the thrombocyte counts in group 3e.

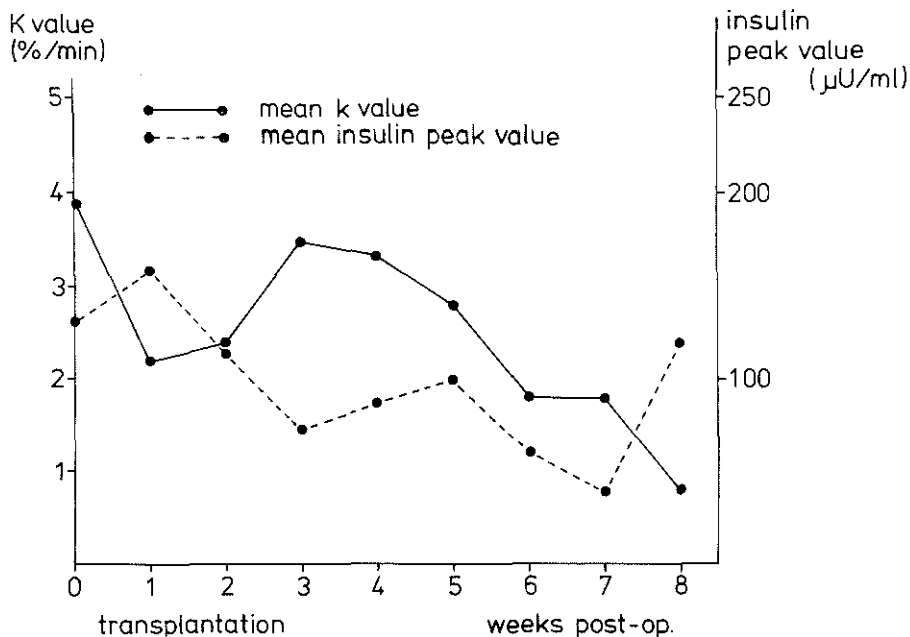


Fig. 20. Variation in the mean K value and insulin peak values after pancreas allotransplantation in group 3c.

is shown in Fig. 21. Seen retrospectively, the relevant blood samples might have been collected just before rejection occurred; since the IPV was only determined once a week, it was pure chance that the determination was made at this time.

The occurrence of high insulin levels just prior to rejection was first discussed

by GRENIER *et al.* (51), who explained it as due to the measurement not of insulin but of proinsulin liberated by cell damage associated with rejection. This is supported by the absence of a drop in the serum glucose level in the presence of high IPV.

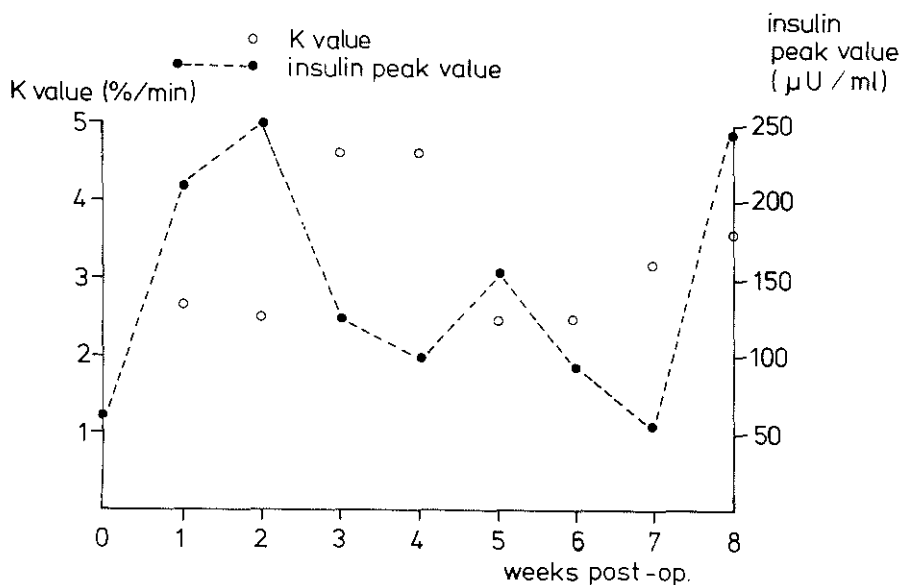


Fig. 21. Variation in the K value and insulin peak value in dog. nr. BD 0164 after pancreas allotransplantation.

Because the endocrine function showed the same pattern in all of the cases of long transplant survival, we shall limit ourselves to a detailed discussion of the group with the longest survival time, as exemplary, and of the dogs with a transplant that functioned more than a year.

4.1. Endocrine function in group 3e

Fig. 22 shows the course of the K values in this group. The two high values in the 8th post-operative week concern the two dogs with the longest survival time. In Fig. 23 the course of the IPV and the corresponding K values are given for 5 dogs separately. The IPV for the first week is missing for BD 619 and B 626 because sampling could not be performed at the right time in the usual way. The sixth dog in this group (B 591) is not included because, for the same reason, there were too few IPV available; this animal is discussed in § 4.2.

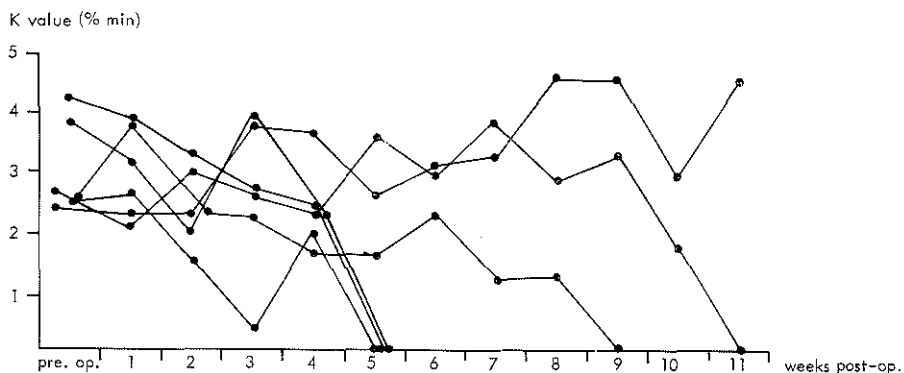


Fig. 22. K values of 6 beagles of group 3e before and after pancreas allotransplantation.

Dog. nr.	Pre-op. value	Weeks post-op.											
		1	2	3	4	5	6	7	8	9	10	11	
B 591	2.4	2.3	2.0	3.8	3.7	2.6	3.1	3.3	4.6	4.6	2.9	4.6	
BD 619	4.2	3.9	3.3	2.7	2.4	0							
B 626	2.6	3.8	2.4	2.2	1.7	1.6	2.3	1.2	1.3	0			
B 616	3.8	3.2	2.0	3.8	2.3	0							
B 0211	2.6	2.3	3.0	2.6	2.3	3.6	3.0	3.8	2.9	3.3	1.8	0	
B 614	2.5	2.6	1.5	0.3	2.0	0							

4.2. The endocrine function one year after transplantation

Although no special attempt was made to achieve very long survival times – all immunosuppressive drugs were withheld starting in the sixth week – there were three dogs in which the transplant was not rejected, i.e. nrs. B 591, BD 617, and BD 622.

Figs. 24, 25, and 26 show the course of the K values and IPV in these animals during the first year, and Fig. 27 gives the GTT and insulin secretion patterns found a year after the transplantation. At that time the fecal proteolytic enzymes were again determined; the values are shown in Table XVIII.

5. DISCUSSION

5.1. Weight loss

In the 3 weeks after the pancreatectomy the mean weight loss amounted to 23 per cent. After the transplantation there was no further loss of weight in the dogs with a longer survival time and the body weight returned to the level

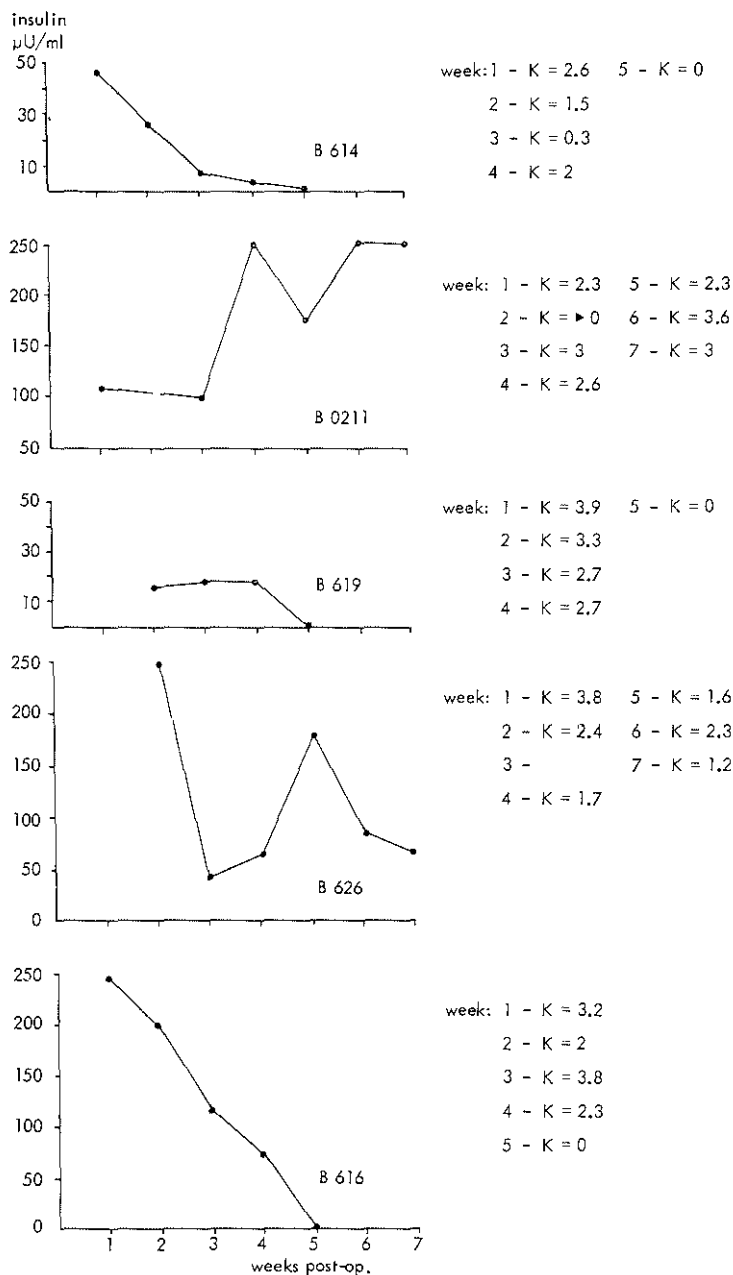
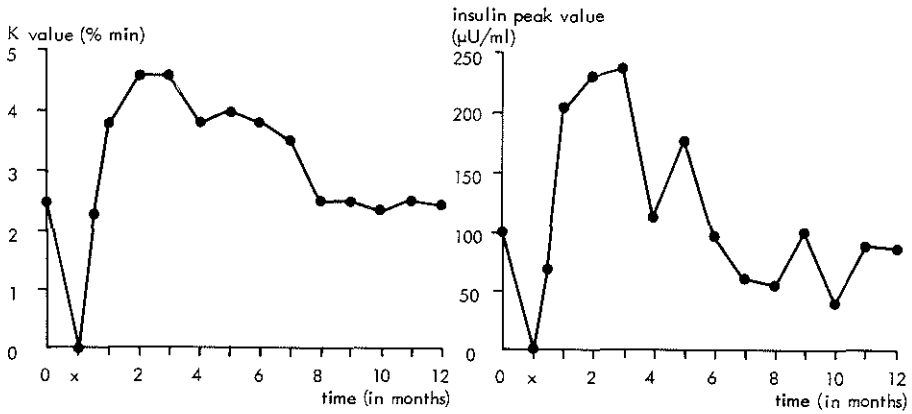
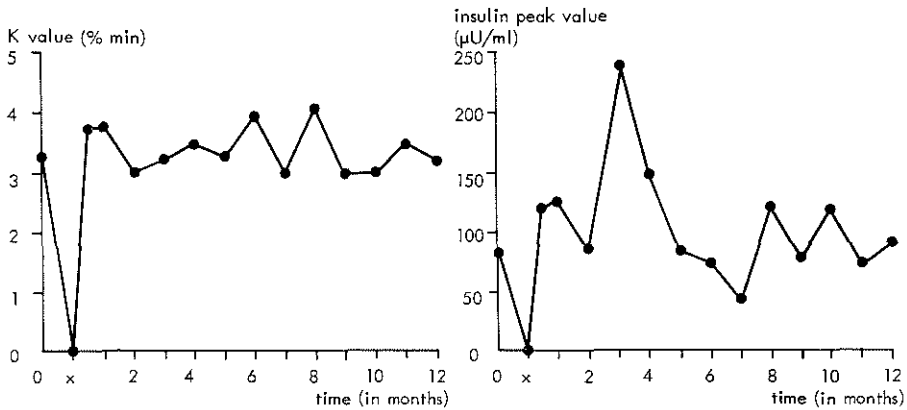


Fig. 23. Insulin peak values of 5 dogs in group 3e after pancreas allotransplantation.

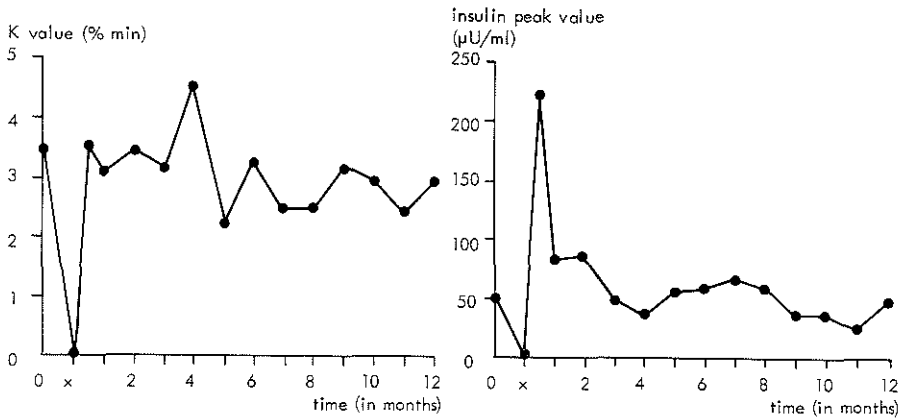
BD 591



BD 617



BD 622



Figs. 24, 25, and 26. Endocrine function of pancreas allotransplant over a period of a year in 3 dogs.

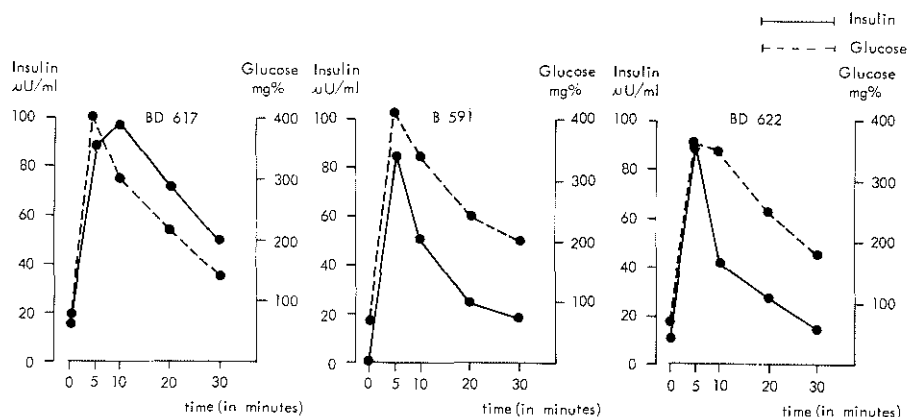


Fig. 27. The intravenous GTT values and the insulin secretion pattern a year after pancreas allotransplantation in 3 dogs.

TABLE XVIII

Fecal proteolytic enzyme levels one year after transplantation (in IU)

Dog nr.	α -chymotrypsin	trypsin
B 591	1.3	0.0
BD 617	0.0	0.0
BD 622	0.4	0.0

prevailing before the pancreatectomy. Since there was no loss of weight in the group of dogs in which only the pancreatic ducts were ligated, we are of the opinion that loss of weight is caused solely by the diabetes mellitus and not by the absence of the exocrine function of the pancreas.

5.2. Endocrine function

We found that immediately after transplantation a high level of insulin secretion occurred which could lead to hypoglycemia in the first two days after transplantation. CERRA *et al.* (21) showed that there is a relationship between the duration of the ischemic time and the level of the insulin secretion, and concluded that the elevated serum insulin level is the direct result of cell damage caused by ischemia. On the other hand, GRENIER *et al.* (51) observed high insulin levels just prior to rejection but without any change in the serum glucose level. On one occasion we by chance measured a very high IPV probably just before rejection; this could possibly be explained by the measurement of proinsulin rather than insulin. With the immunological method we applied, however, proinsulin cannot be distinguished from insulin.

In the dogs with a longer transplant survival before rejection there was a gradual decrease in the K values and the IPV a few weeks before rejection, as seen in groups 3c and 3e. For the dogs whose transplant functioned longer than a year, the K values were the same a year after transplantation as pre-operatively but the IPV lay higher than the pre-operative values, although within normal limits.

5.3. Histocompatibility

The mean transplant survival time of group 3a (unrelated, non-identical beagles) was 9.6 days, that of group 3b (related, non-identical beagles differing by 2 haplotypes) 10.5 days, and that of group 3c (related, identical beagles with 0 haplotype difference) 42.9 days. Fig. 28 shows these results graphically. The difference between groups 3a and 3b is not significant ($P = 0.33$), as can be

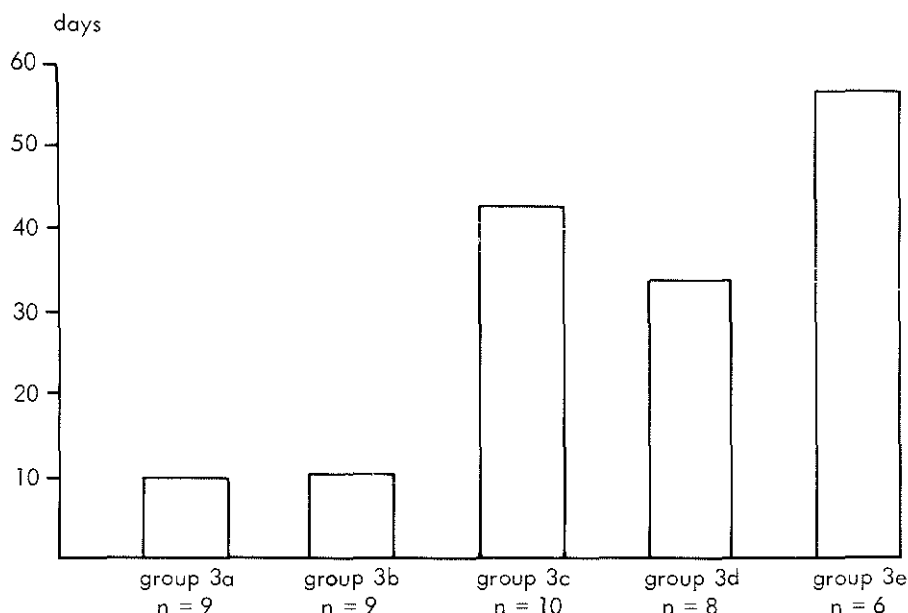


Fig. 28. Mean survival time of duct-ligated pancreas allografts in beagles.

Group 3a: unrelated, non-identical beagles. MST = 9.66 (SD 2.39)

Group 3b: related, non-identical beagles (2 hapl. diff.). MST = 10.55 (SD 7.22)

Group 3c: related, identical beagles (0 hapl. diff.). MST = 42.90 (SD 22.9)

Group 3d: related, identical beagles (1 hapl. diff.) given azathioprine and prednisone.
MST = 34.20 (SD 22.0)

Group 3e: related, non-identical beagles (1 hapl. diff.) given ALS. MST = 57.30
(SD 29.0)

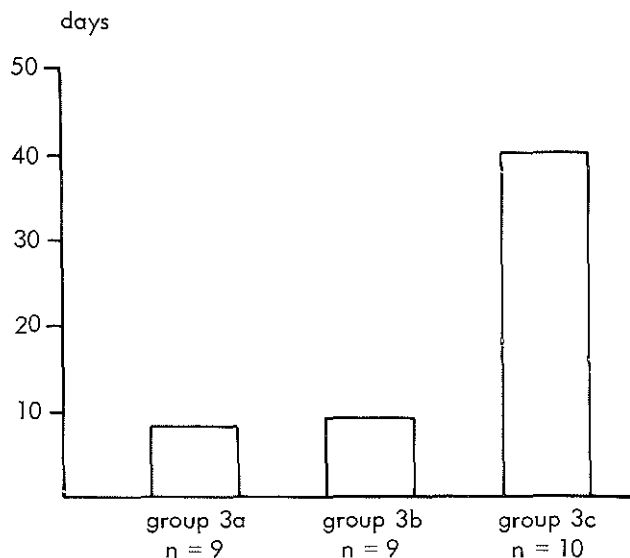


Fig. 29. Median survival time of duct-ligated pancreas allografts in the absence of immunosuppression. (Wilcoxon's 2-sample test, one-sided.)

Group 3a - Group 3b $P = 0.33$ (not significant)

Group 3c - Group 3a $P = 0.0005$ (significant)

Group 3c - Group 3b $P = 0.0005$ (significant)

seen from Fig. 29. The significance of the differences in survival time between groups 3a, 3b, and 3c was calculated with Wilcoxon's two-sample test. The difference between groups 3c and 3a and between groups 3c and 3b is significant ($P = 0.0005$). These results indicate the influence of compatibility for the histocompatibility system on the transplant survival time, and are analogous to the findings for heart (15), kidney (147), skin (142), and intestine (146) allotransplantations. There is no reason to assume that in this respect the pancreas allotransplant reacts differently from otherwise vascularized allotransplants.

5.4. Immunosuppression

For group 3d (related, non-identical beagles differing by 1 haplotype, treated with azathioprine and prednisone) the mean transplant survival time was 34.2 days, for group 3e (related, non-identical beagles differing by 1 haplotype, treated for 6 weeks with ALS) 57.3 days. The median survival times are shown in Fig. 30. In the dosages applied, ALS was more effective than the combination of azathioprine and prednisone. ALS also gave better results, even with the short period of administration in the non-identical group, than in the identical

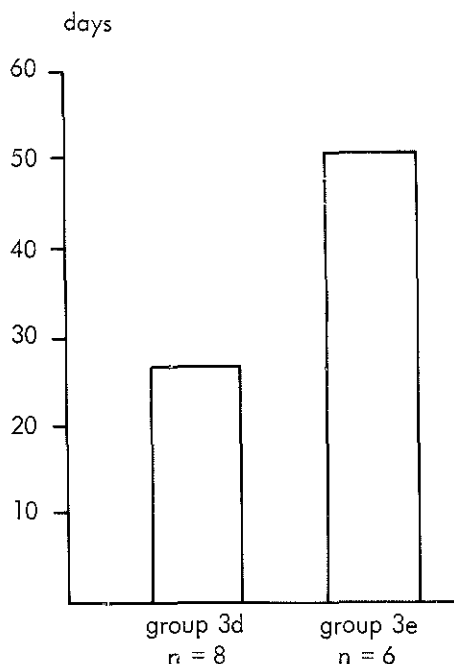


Fig. 30. Median survival time of duct-ligated pancreas allografts under immunosuppression. (Wilcoxon's 2-sample test, one-sided.)

Group 3e - Group 3d $P = 0.021$ (significant)

group without immunosuppression (see Fig. 28). The use of ALS had the disadvantage, however, of the toxic effect on the blood cells of the recipient. Furthermore, it is not known to what extent subsequent blood transfusions, which these animals received, have a positive or negative effect on the survival time of the transplant. Further discussion of this problem would, however, take us beyond the scope of this investigation.

5.5. Microscopical appearance

The findings made in the microscopical investigation fall into two main groups: 1) changes caused by pancreatitis resulting from ligation of the pancreatic ducts, and 2) changes associated with rejection of the transplant.

5.5.1. Histological changes due to pancreatitis and duct ligation

One of the first signs of pancreatitis was the occurrence of interstitial edema, followed some time later by infiltration of polymorphonuclear cells and dilatation of the capillaries. This was in turn followed in some cases by necrosis

of the acinar cells in which it was often uncertain whether the cause was auto-digestion or acute ischemia. This parenchymal necrosis was usually limited to a few areas but in a few cases more extensive necrosis had affected the capillaries as well, resulting in local hemorrhages. This process progressed until all of the acinar tissue was replaced by connective tissue. The islet tissue, however, remained intact. In our material hemorrhagic pancreatitis was only observed once (BD 0.193). At autopsy, extensive loss of acinar cells with early proliferative fibrosis was observed in this dog a few days after transplantation.

In general, pancreatitis with mild interstitial edema was observed up to the 15th day, occurring later only once, on the 29th day (BD 0.283). Six cases showed not only interstitial edema but also focal necrosis of the parenchyma. A small amount of acinar tissue was found in one dog (BD 2.204) 27 days after duct ligation. All pancreases examined later than that showed complete fibrosis of the acinar tissue, with in one case cyst formation (B 1.183).

5.5.2. Histological changes due to rejection

In general, these changes were characterized by the infiltration of mononuclear cells (lymphocytes, lymphoblasts, and plasma cells) in the interstitium, initially at the periphery and later penetrating through the acinar tissue. After that, the islets were infiltrated by these cells. The first evidence of damage to the capillaries was swelling of the endothelium and blood stasis. In the small arteries intimal swelling was followed by fibrinoid necrosis of the media and, in a later stage, thrombus formation.

In these untreated cases with short transplant survival (e.g. groups 3a, 3b) the interstitial cellular rejection picture predominated. One dog in group 3b (BD 0.283), however, survived 29 days, and here the vascular component dominated. In the dogs of group 3c (untreated dogs with longer transplant function), the interstitial component was in general slightly less pronounced and such vascular lesions as intimal swelling and fibrinoid necrosis were more evident. This was particularly marked in group 3d (non-identical, treated with azathioprine and prednisone), where little mononuclear cell infiltration was seen, but the vasculitis was so strong that it led to thrombosis and such extensive necrosis that the islets could no longer be recognized microscopically.

A different picture was seen in group 3e (non-identical, treated with ALS). These transplants showed more mononuclear cell infiltration and the vascular component was weaker. Thrombosis and necrosis did not occur, but intimal swelling and fibrinoid necrosis of the media developed. As a result, the structures within the fibrotic areas could be distinguished and intact islets were seen frequently.

6. CONCLUSIONS

In the first 3 weeks after total pancreatectomy the mean weight loss amounted to 23 per cent. After transplantation of a pancreas with only the endocrine function persisting, the weight increased. Thus, the primary cause of the loss of weight must be diabetes mellitus and not enzyme deficiency. Furthermore, it also indicates that the transplant can regulate the diabetes better than parentally administered insulin does.

Three weeks after the administration of insulin was started in pancreatectomized dogs, antibodies to porcine insulin could already be demonstrated in 3 of the 47 dogs. In allotransplantations of a pancreas with ligated ducts large amounts of insulin reach the circulation immediately after revascularization, which can lead to hypoglycemia and hypokalcemia. Equilibrium between insulin production and the serum glucose level is only reached 48 hours after an allotransplantation, and only then can the parenteral administration of glucose be stopped.

There is no significant difference with respect to the mean transplant survival under non-identical conditions between related and unrelated, DL-A non-identical pairs of beagles. Related, DL-A identical beagle donor-recipient pairs had a significantly longer transplant survival. Low dosages of azathioprine and prednisone also have a favorable effect on the mean transplant survival time, but not to the same degree as ALS. Since a mild pancreatitis developed during the first 14 days after transplantation and only one case was seen after that, it is possible that in the cases of shorter survival pancreatitis may be a factor which, together with rejection, leads to the elimination of the endocrine function of the transplant.

The rejection of the pancreas was characterized by mononuclear cell infiltration, first in the interstitium and then also in the acinar tissue and perivascularly. The islet tissue is not infiltrated until a later stage is reached. The vascular anomalies predominate more strongly the longer the survival of the transplant. These phenomena were extremely pronounced in the dogs given azathioprine and prednisone.

In the animals treated with ALS the histological results showed intimal swelling and fibrinoid necrosis without thrombosis and parenchymal necrosis, in contrast to the animals treated with azathioprine and prednisone.

If rejection does not occur, the acinar tissue is replaced by connective tissue in which islets are clearly distinguishable. This fibrosis resulting from the ligation of the pancreatic ducts has no influence on the endocrine function of the pancreas when an adequate diet is provided.

A year after heterotopic allotransplantation of a canine pancreas with ligated ducts, the endocrine function is normal.

General discussion

The experiments reported in the preceding chapters were performed to determine whether duct ligation influences the function of the pancreas and whether the combination of duct ligation and allotransplantation of the pancreas has a harmful effect on the endocrine function of the organ. From the results described in three chapters certain relationships can be drawn which provide answers to the questions posed in the introduction.

1. THE EFFECT OF LIGATION OF THE EXOCRINE DUCT ON THE ENDOCRINE FUNCTION OF THE PANCREAS

After the ligation of the exocrine duct of the canine pancreas *in situ* we observed a distinct reduction of endocrine function of the organ. After some time, however, this function improved and reached about 75 per cent of the pre-operative values. At no time, however, was diabetes mellitus observed.

There is no consensus of opinion in the literature with respect to the effect of ligation of the exocrine ducts on the endocrine function of the pancreas. A number of investigators (2, 35, 74) saw an unequivocal reduction of the endocrine function and even diabetes mellitus, but others found no diminishment (4, 22, 66, 121, 127). One objection which can be raised to the studies of the former group of authors is that after elimination of the exocrine function no pancreatic enzymes were supplied or only inadequate amounts, which leads to reduced conversion of carbohydrates into glucose and this in turn can result in endocrine hypofunction. With respect to the studies of the latter group of investigators, objections can be raised to the parameters applied for the evaluation of the endocrine function. In some cases only the possible presence of

diabetic blood sugar values was investigated (121), but usually non-diabetic GTT curves were compared without calculation of the K values. Our results indicate that the endocrine function indeed diminishes in connection with the pancreatitis, but a substantial part of this function is recovered when an adequate diet is provided. This fact is not mentioned by any of the authors referred to above. The mild pancreatitis that develops after ligation of the pancreatic ducts also does not have an unfavorable effect on the functioning of the beta cells. From the literature (8,109) and our results (Chapter IV) it seems that in all probability the more severe the pancreatitis the greater the drop in endocrine function after duct ligation. Therefore, the way in which the pancreatitis resulting from the ligation is treated can constitute one of the important factors determining the ultimate endocrine function of the pancreas.

In sum, it may thus be said that ligation of the pancreatic duct leads directly to pancreatitis which causes a reduction of the endocrine function of the organ, but a large part of this function can be restored by adequate diet and treatment.

2. THE ENDOCRINE FUNCTION OF THE PANCREAS AFTER HETEROTOPIC TRANSPLANTATION OF THE ORGAN WITH LIGATED EXOCRINE DUCTS

The authors who ligated the pancreatic ducts some time before the transplantation, e.g. BEAVEN *et al.* (8) in autotransplantations and SEDDON & HOWARD (127) in allotransplantations, obtained very good results. This can be attributed, in our opinion, to the distinction made between two kinds of factors which induce pancreatitis, on the one hand ischemia and surgical trauma and on the other duct ligation.

In Chapter IV mention was made of the reasons why the surgical trauma to the organ is less severe in allotransplantation than in autotransplantation. In addition, with the technique we applied the blood supply to the allotransplant is better than that to an autotransplant and, furthermore, the venous drainage via the portal vein is such that it does not promote pancreatitis (136). In our opinion, these factors account for the higher percentage of success in allotransplantations as compared with autotransplantations. After allotransplantations there is also hardly any loss of endocrine function. This can be completely explained by the less severe pancreatitis as compared with the form seen after autotransplantations.

If, however, we compare the endocrine function in the group of dogs in which only the pancreatic ducts were ligated with that of the allotransplantation group, the pancreatitis no longer offers a valid explanation of the differences.

In view of the methods applied there is no reason to expect a more severe form of pancreatitis in one of these 2 groups. The reason for the better post-operative endocrine function after allotransplantation can be sought much more logically in the heterotopic localization of the organ, by which the insulin reaches the peripheral circulation directly, avoiding the liver which would take at least 50 per cent of the supply (80). This would also explain the high insulin values found in the course of time, as described by BERGAN & TEIXEIRA (12). Their results are, however, neither confirmed nor contradicted by the findings in our material.

In the phase immediately after the operation, thus, the loss of endocrine function due to the pancreatitis caused by the duct ligation would be compensated for by an elevated insulin level in the peripheral blood, as has also been described by HEARN & PATON (61) and LEVEEN *et al.* (91). The fact that we did not find high insulin peak values shortly after autotransplantation can be satisfactorily explained by the higher number of beta cells rendered defective by the pancreatitis.

3. INFLUENCE OF TISSUE TYPING ON THE SURVIVAL TIME OF A HETEROTOPIC PANCREAS ALLOTRANSPLANT

By the use of tissue typing for SD determinants of the DL-A complex we hoped to obtain prolonged transplant survival while avoiding the influence of immunosuppressive drugs on the results of the function tests. Our experiments indeed showed a significant difference in transplant survival between related DL-A identical beagles on the one hand and related and unrelated DL-A non-identical beagles on the other. This is in agreement with the results obtained in dogs with kidney transplantations (144), heterotopic gut-segment transplantations (145), and orthotopic heart transplantations (15).

The use of beagles typed for SD determinants of the DL-A complex made it possible to follow a number of animals for a very long time after pancreas allotransplantation. Three of the dogs could even be followed for more than a year and at the time of writing were still alive with good endocrine function.

4. INFLUENCE OF IMMUNOSUPPRESSIVE DRUGS ON THE HETEROPTOPIC PANCREAS ALLOTRANSPLANT

The results obtained in the group of dogs which were not given immunosuppressive drugs and had a long transplant survival and those obtained in the

group with a long transplant survival after treatment with such drugs, were analysed in an attempt to distinguish a possible relationship between histocompatibility and an influence of the immunosuppressive drugs on the endocrine function of the pancreas. No information is available about the influence of ALS and azathioprine on this endocrine function, but prednisone is known to have a distinct diabetogenic effect (88).

Because we had shown that with respect to tissue typing the pancreas allotransplant does not differ from other organs, it could be assumed that the mean transplant survival time for a difference of one haplotype would lie between that of the identical dogs and those differing by two haplotypes (142). Treatment of recipients differing by one haplotype from the donor with a low dose of immunosuppressive drugs was expected to give a mean survival time approaching that of the identical group, thus permitting comparison. In comparison with the untreated group, there proved to be no difference in the course of the endocrine function in the ALS-treated group or the group treated with a combination of azathioprine and prednisone, but there was divergence in the histological findings. It must be kept in mind here, however, that we used a lower dosage of prednisone than had been applied by other investigators (51, 62, 76, 94, 152). This also means that little importance should be attached to the fact that longer transplant survival times were obtained with ALS than with azathioprine and prednisone in combination, since a higher dosage of the latter would probably have led to longer survival. The histological differences are, however, important. They showed that the vascular rejection phenomena were most prominent in the group treated with azathioprine and prednisone.

5. IMPLICATIONS OF THE RESULTS

It seems justified to conclude from the results of the present study that transplantation of the entire pancreas with ligated pancreatic ducts is perfectly feasible, at least in the dog, and that the pancreas transplant behaves in the same way as other vascularized transplants. This means that it is unnecessary to perform the complicated operations described in the literature for the preservation of enzyme secretion. In addition, the pancreatitis which develops proved to have little importance. It is not known whether this would also be the case for the human pancreas, but the acute pancreatitis seen in animals is reported to be anatomically, histologically, biochemically, and physiologically indistinguishable from that in man (9).

On this basis we are of the opinion that for vascularized pancreas transplantation in patients, preference should be given to a transplant consisting of the entire organ with ligated ducts.

Experiments reported by a number of investigators (6,23,90) in which islets or even beta cells in suspension were transplanted allogeneically by applying them intraperitoneally or injecting them into the portal circulation, were only successful in isologous rat strains. This raises questions concerning, for instance, the regenerative capacity of beta cells in rats and whether degeneration of these cells would occur eventually because they reach an isolated location or because the total volume is too small (88,97). Furthermore, in view of the technical difficulties involved in obtaining a sufficient number of beta cells from a single pancreas to provide adequate endocrine function after transplantation, it seems possible that transplantation of a vascularized pancreas will ultimately prove to be a more feasible method, the more so because preservation and perfusion of the pancreas for 24 hours is possible. This subject lies outside the present scope, but has been discussed by the author elsewhere (55,56).

Experimentally, it is now possible to perform pancreas allotransplantation in dogs with spontaneous juvenile diabetes. We are continuing this experimental work too, in the hope of finding answers to a number of questions concerning the pathophysiology of diabetes mellitus.

Summary

The relevance of pancreas transplantation is discussed in the Introduction to this thesis. Chapter I gives the historical background, the specific problems in relation to previous research, the surgical techniques applied, and a review of the clinical pancreas transplantation performed so far. The conflicting results published by various investigators concern the long-term quality of the endocrine function of the organ when the exocrine function is abolished by ligation of the pancreatic ducts. As a result, in extensive series of experimental pancreas transplantations and a number performed in patients the endocrine function was maintained intact, which can only be achieved by including the duodenum, applying the Roux technique for anastomosis with the pancreas, or establishing an anastomosis between the pancreatic duct and the ureter. The disadvantages of these methods are discussed and the advantages of transplanting the pancreas with ligated ducts are indicated. The latter method requires only a vascular anastomosis and is in fact even less complicated than a kidney transplantation. On the basis of these considerations we thought it worthwhile to investigate the effects of transplantation of the pancreas with ligated ducts. This study was performed in dogs, and the effect of tissue typing for SD determinants of the DL-A complex on allograft survival was included in the investigation.

The experimental design and the techniques applied are discussed in Chapter II. Three groups of dogs were formed: one group to be given only duct ligation, one for autotransplantation with ligated ducts, and the third for heterotopic allotransplantation with ligated ducts. This last group was subdivided according to compatibility or non-compatibility for the major histocompatibility complex (DL-A) and the use or omission of immunosuppressive drugs. The method of anesthesia, the histological methods, and the laboratory determinations (with special attention to the K values of the glucose tolerance test and the insulin peak values) are described, as well as a method for the

determination of the proteolytic enzymes in the feces as an indication of the exocrine function. The importance of these enzyme determinations is also discussed.

The experiments performed to study the effect of pancreatic duct ligation on long-term endocrine function are discussed in Chapter III. Sixteen mongrel dogs underwent ligation of the pancreatic ducts and were followed for 2 years. It was found that the operation according to Markowitz did not completely eliminate the exocrine function in a number of cases. After duct ligation the endocrine function of the pancreas dropped sharply and then began to improve, reaching about 75 per cent of the original value within a year and remaining there throughout the second year. The results led us to conclude that in the dog ligation of the pancreatic ducts does not cause diabetes mellitus in the long run.

Pancreas autotransplantations performed in 17 mongrel dogs are described in Chapter IV. The operation was successful in 5 cases, but only one dog could be followed for 5 years. At the end of the fifth year this dog was killed and functioning pancreatic tissue was found at the site of the autotransplant. The vascular relationships which contributed to the low percentage of successful canine pancreas autotransplantations are discussed.

The allotransplantations are dealt with in Chapter V, starting with the measures taken to maintain the animals in good physical condition after total pancreatectomy. The criteria that the pancreatectomized dogs had to satisfy to serve as recipient are discussed. The differences in histocompatibility, the immunosuppressive drugs used, the surgical technique, and the post-operative treatment are also discussed. The results obtained in 77 dogs subjected to total pancreatectomy are reported and the cause of death during or shortly after the transplantation is indicated. The 5 subgroups are then discussed separately. Tissue typing proved to have a significant influence on transplant survival. At the end of this chapter special attention is given to the endocrine function of 3 dogs with a survival time of more than a year; this function was found to be virtually the same as the level observed before the pancreatectomy.

Lastly, the theoretical background and the implications of the results are discussed in relation to divergent findings made by other investigators.

Samenvatting

Nadat in de inleiding is uiteengezet waarom pancreastransplantatie wenselijk is, wordt in het *eerste hoofdstuk*, na een kort historisch overzicht, een opsomming gegeven van de *specifieke problemen bij pancreastransplantaties* met een globaal overzicht van het onderzoek dat daarvoor al is verricht. De operatie technieken worden genoemd en er wordt een overzicht gegeven van de tot nu toe verrichte klinische pancreastransplantaties. Hieruit blijkt, dat er tegenstrijdige uitkomsten bestaan bij verschillende onderzoekers als het gaat om de kwaliteit van de endocriene functie van het pancreas op de lange duur, wanneer de exocriene functie is uitgeschakeld door middel van onderbinding van de ductus pancreaticus. Dit heeft ertoe geleid, dat bij grote series experimentele pancreastransplantaties en een aantal humane pancreastransplantaties deze exocriene functie intact is gehouden. Dit kan in de praktijk alleen door of het duodenum mee te transplanteren, of een technische procedure volgens Roux toe te passen, of door de ductus pancreaticus met de ureter te anastomoseran. Bij de probleemstelling in hoofdstuk I wordt op de nadelen van bovenvermelde technieken gewezen en de voordelen van een te transplanteren pancreas met onderbonden ductus pancreaticus vermeld. Voor een dergelijk transplantaat dient alleen een vaatanastomose te worden gemaakt en dit zou in feite eenvoudiger zijn dan een niertransplantatie. Op grond van deze overwegingen leek het ons de moeite waard een onderzoek in te stellen naar de mogelijkheid pancreastransplantaties met onderbonden afvoergang te verrichten. Dit onderzoek werd uitgevoerd met voor SD determinanten van het DL-A complex getypeerde honden.

In het *tweede hoofdstuk* worden de *proefopstellingen en gebruikte technieken* besproken. Drie groepen honden werden gevormd. Een groep waarbij alleen ductus onderbinding zou plaats vinden, een groep waarbij een auto-transplan-

tatie met ductus onderbinding zou worden verricht en tenslotte een groep honden waarbij een heterotopie pancreas allo-transplantatie met onderbonden ductus pancreaticus zou worden toegepast. De laatste groep werd uitgesplitst in subgroepen naar gelang de typering en eventueel gebruik van immuno-suppressiva. In dit hoofdstuk werden nog apart besproken de narcose, de histologische technieken en de laboratorium bepalingen, waarvan vooral van belang zijn de K-waarden van de GTT en de insuline piekwaarden. Verder wordt een methode beschreven om de proteolytische enzymen in de faeces te bepalen als controle op de exocriene functie en wat de betekenis hiervan is.

In *hoofdstuk III* worden de experimenten besproken, die bij 16 bastaardhonden zijn verricht. De *ductus pancreaticus* werd bij deze proefdieren onderbonden en vervolgens werd deze groep twee jaar lang bestudeerd. Het bleek, dat na loskoppelen van het pancreas volgens de methode van MARKOWITZ er toch in een aantal gevallen geen volledige uitval van de exocriene functie was. De endocriene pancreasfunctie daalde aanzienlijk na het onderbinden van de pancreasafvoergang, doch herstelde zich binnen het jaar tot ± 75 procent van de oorspronkelijke waarden. Deze functie handhaafde zich gedurende de tweejarige follow-up periode. Geconcludeerd werd, dat na onderbinding van de pancreasafvoergang bij de hond op den duur geen diabetes mellitus ontstond.

In *hoofdstuk IV* worden *pancreas auto-transplantaties* beschreven, die verricht zijn bij 17 bastaardhonden. De auto-transplantatie was succesvol by 5 honden, doch slechts één hond kon gedurende 5 jaar vervolgd worden. Na 5 jaar werd deze hond opgeofferd, waarbij functionerend pancreasweefsel werd aangetoond op de plaats waar het auto-transplantaat was ingebracht. Er wordt verder in dit hoofdstuk nog ingegaan op de vasculaire verhoudingen, die mede verantwoordelijk zijn voor het lage succes-percentages van de pancreas auto-transplantatie bij de hond.

In *hoofdstuk V* worden de *allo-transplantaties* besproken. Allereerst wordt beschreven hoe de proefdieren na totale pancreatectomie in goede conditie worden gehouden en welke criteria werden gehanteerd voor een pancreasloos proefdier om als orgaan-ontvanger te kunnen fungeren. Beschreven wordt verder de histocompatibiliteits verschillen, de gebruikte immuno-suppressieve middelen, de post-operatieve therapie en de operatie techniek. Eerst worden de resultaten genoemd bij 77 honden die een totale pancreatectomie ondergingen. Ingegaan wordt op de doodsoorzaak tijdens of direct na de transplantatie. De 5 getransplanteerde sub-groepen worden daarna afzonderlijk besproken. Het blijkt, dat weefseltypering een significante invloed heeft op de transplantatoeverleving. Aan het einde van dit hoofdstuk wordt nog ingegaan op de endocriene functie

van drie honden, die langer dan een jaar overleefden. Het blijkt, dat deze endocriene functie praktisch gelijk is aan de functie vóór de pancreatectomie.

In *hoofdstuk VI* tenslotte wordt aan de hand van resultaten van andere onderzoekers, voor zover die afwijken van de onze, op de theoretische achtergronden ingegaan en worden de consequenties van de resultaten besproken.

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Curriculum vitae

De auteur van dit proefschrift werd geboren in 1934 te Amsterdam.

Hij behaalde het eindexamen H.B.S.-B aan de eerste vijfjarige H.B.S. te Amsterdam in 1955.

Na het vervullen van de militaire dienstplicht begon hij met de studie in de Geneeskunde. Dit resulteerde in het behalen van het artsexamen in 1965, waarna hij arts-assistent werd op de afdeling 1-10 van het Binnengasthuis te Amsterdam (hoofd V. H. JUSTESEN). In september van datzelfde jaar ving de opleiding in de chirurgie aan onder leiding van C. VAN STAVEREN in het Gemeente Ziekenhuis Dijkzigt te Rotterdam. In 1968 werd deze opleiding overgenomen door Prof. Dr. H. MULLER in het Academisch Ziekenhuis Dijkzigt. Na inschrijving in het specialisten register in 1971, is de auteur als wetenschappelijk hoofdmedewerker verbonden aan de Erasmus Universiteit te Rotterdam.

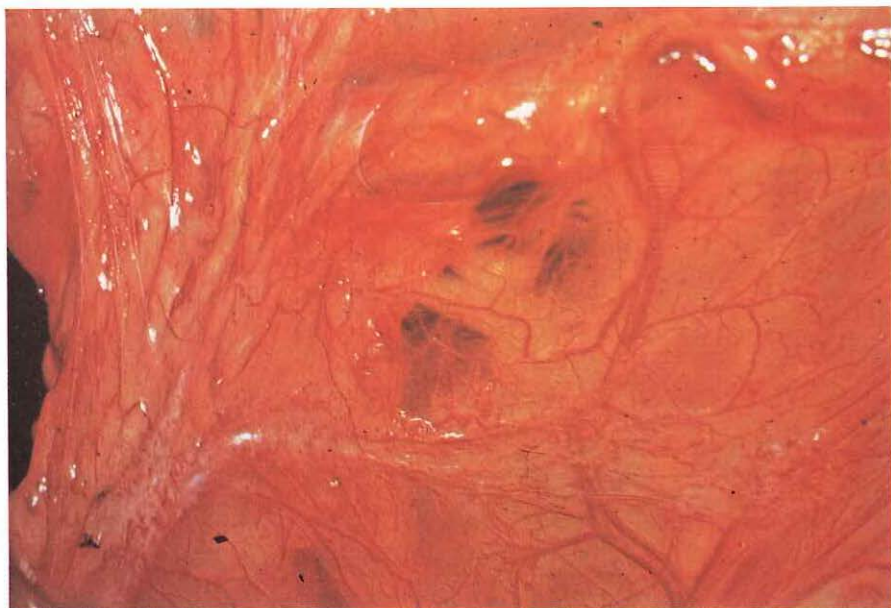


Plate 1. Pancreas 6 months after duct ligation (dog nr. 477).

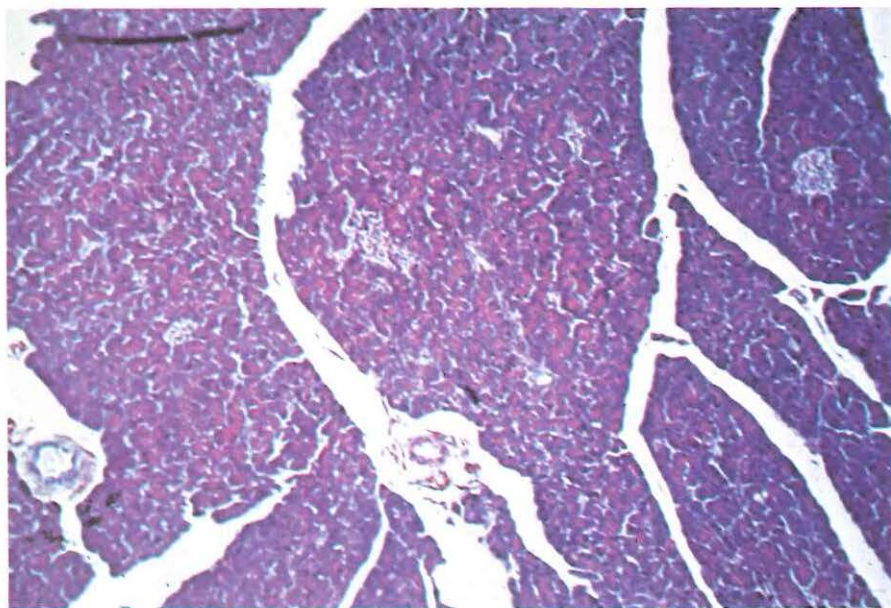


Plate 2. Normal canine pancreas. $\times 100$.

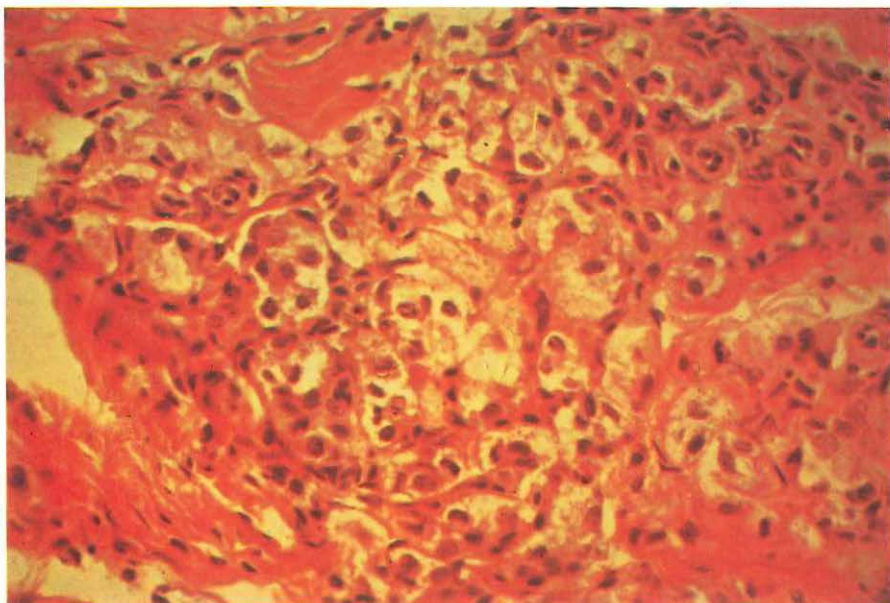


Plate 3. Islet tissue showing fibrosis of acinar tissue 6 months after duct ligation (dog nr. 477) $\times 250$.

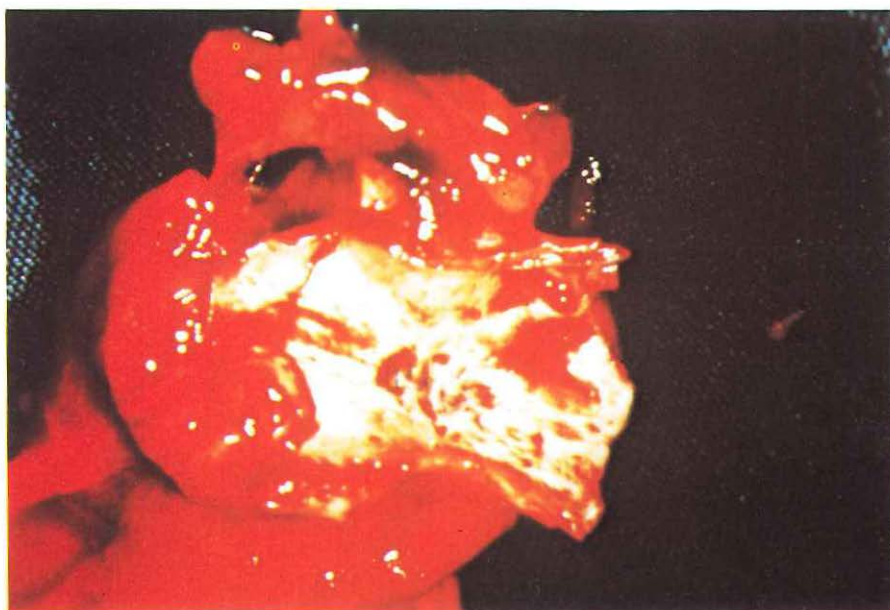


Plate 4. Vascular anastomosis 5 years after autotransplantation of pancreas with ligated ducts (dog nr. 16).

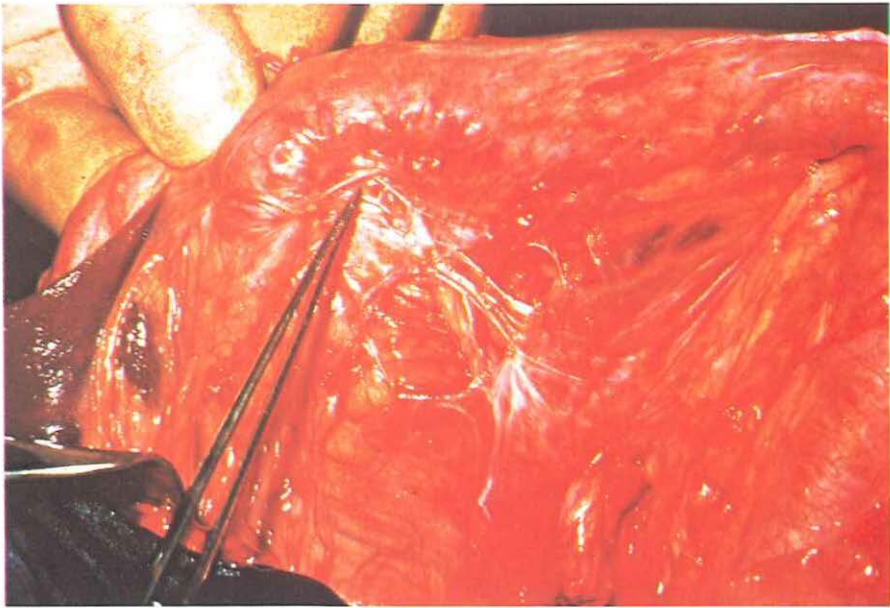


Plate 4a. Duodenum of dog nr. 16, 5 years after pancreatectomy for autotransplantation.

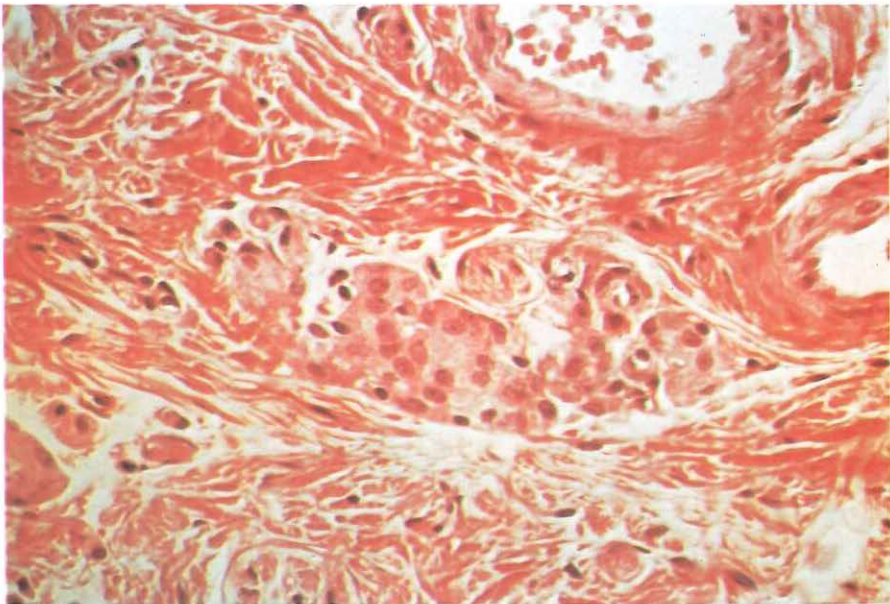


Plate 5. Islet tissue of a pancreatic autotransplant after 5 years, showing complete replacement of the acinar tissue by fibrotic connective tissue (dog nr. 16) $\times 250$.

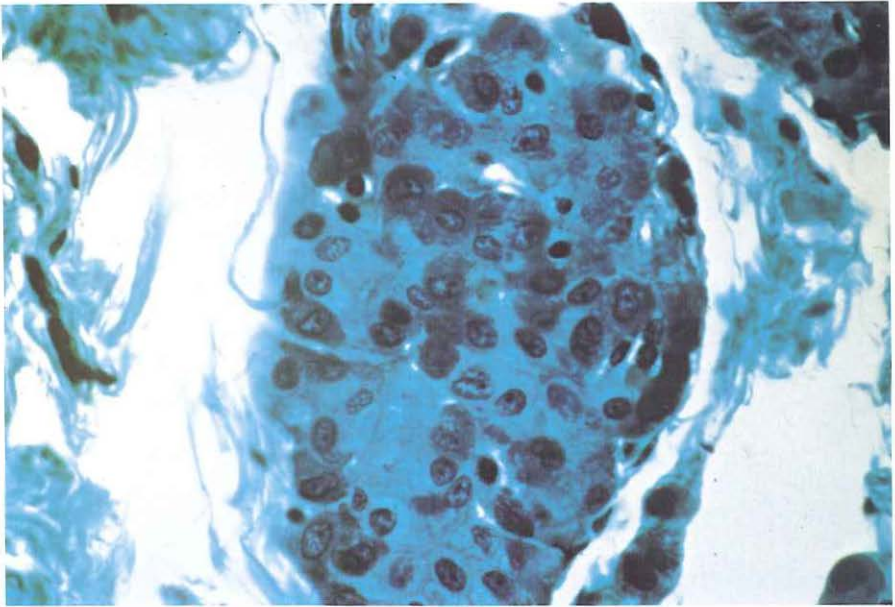


Plate 6. Islet tissue 5 years after autotransplantation (dog nr. 16; aldehyde fuchsin staining). Note alpha and beta cells.

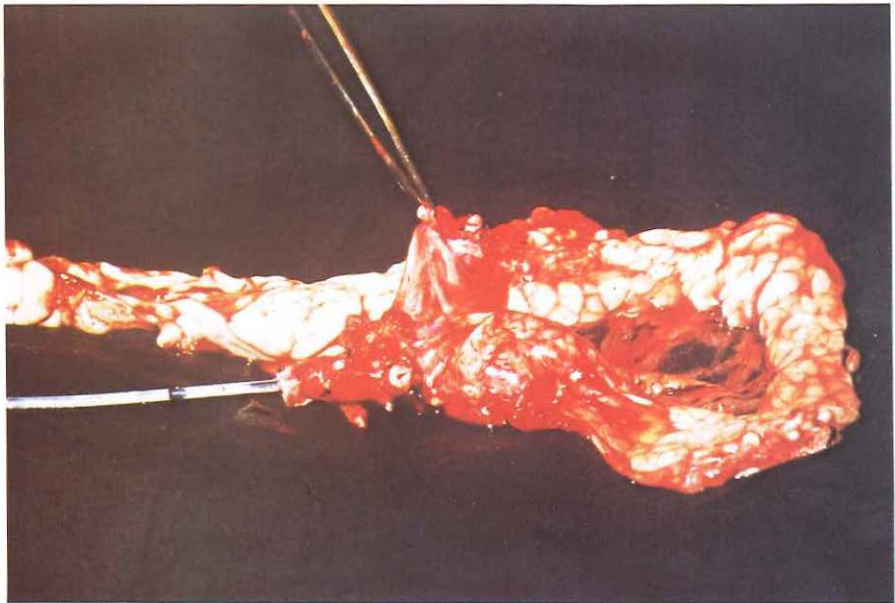


Plate 7. Pancreas with ligated ducts during flushing with cold saline.

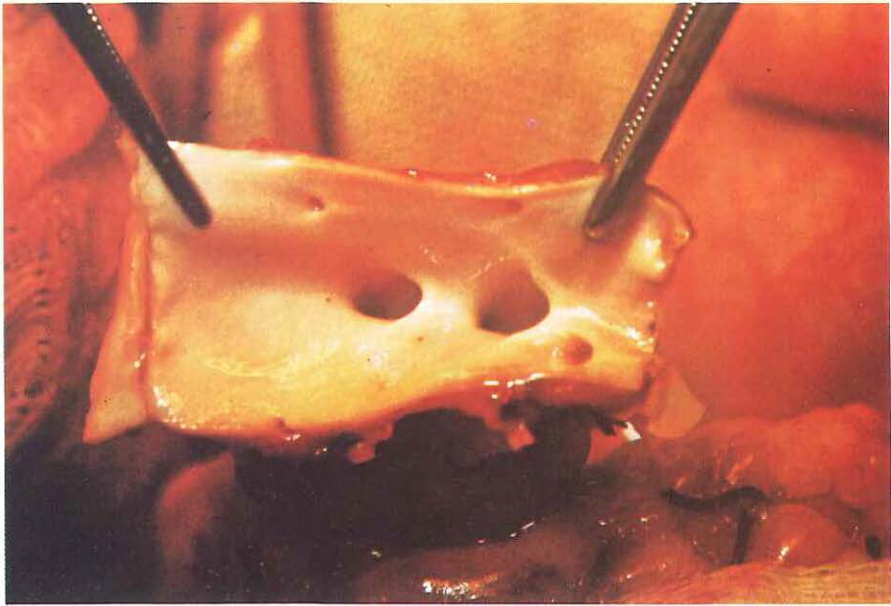


Plate 8. The aortic patch with the celiac and superior mesenteric arteries, before anastomosis.

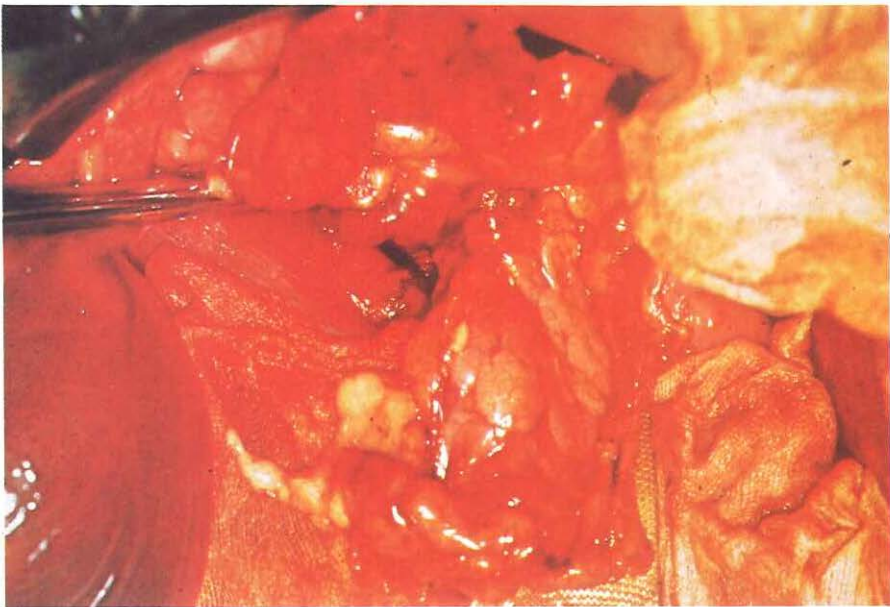


Plate 9. The same pancreas as in Plate 8, after revascularization.

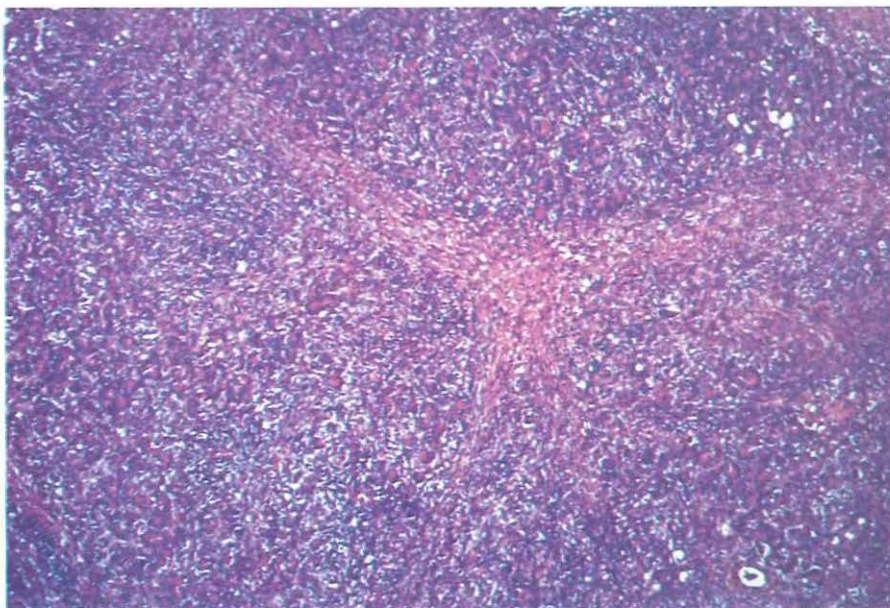


Plate 10. Representative histological picture of pancreas rejection after 5 days. $\times 40$.

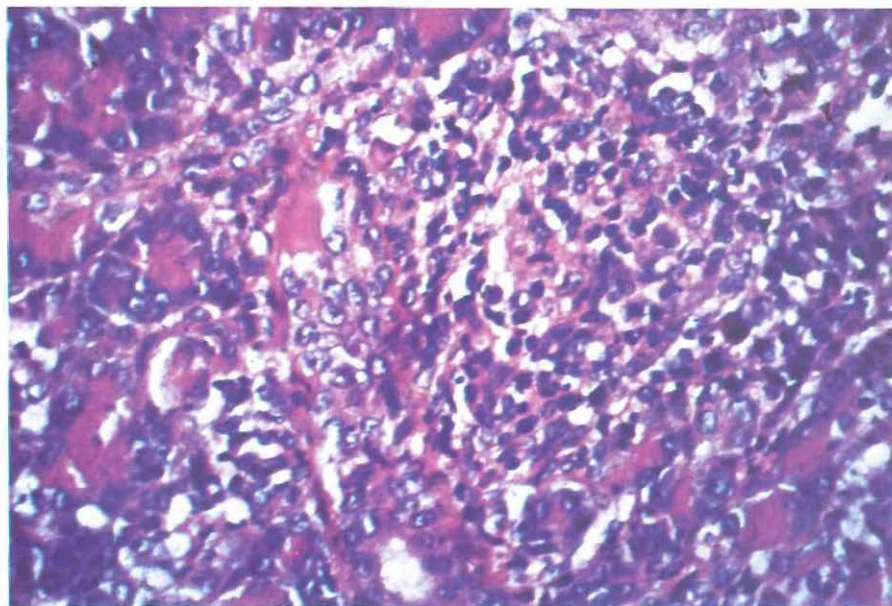


Plate 11. Detail of Plate 10. $\times 250$.

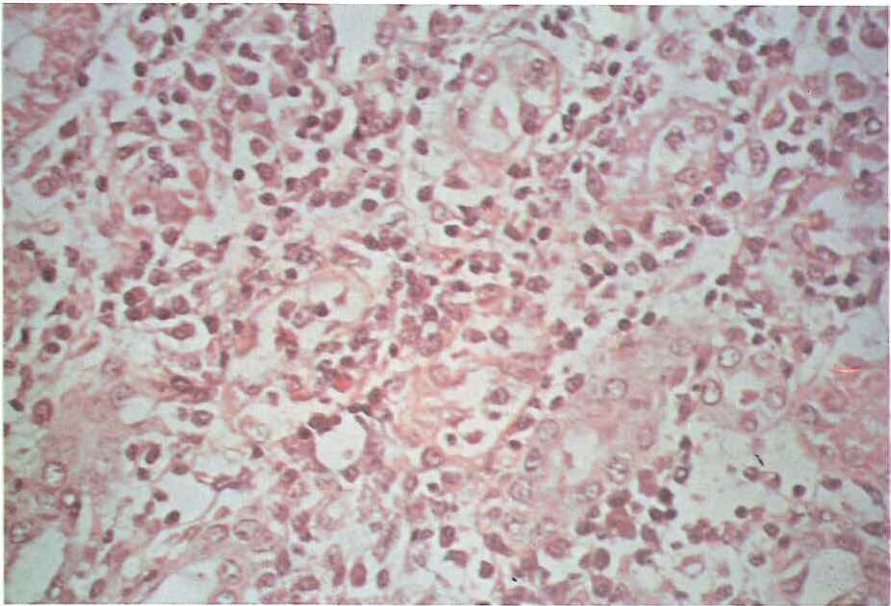


Plate 12. Allotransplant on 69th day, showing cellular infiltration of islet tissue, loss of acinar tissue, and survival of duct tissue. No immunosuppressive drugs given. (Nr. BD 9.161) $\times 250$.

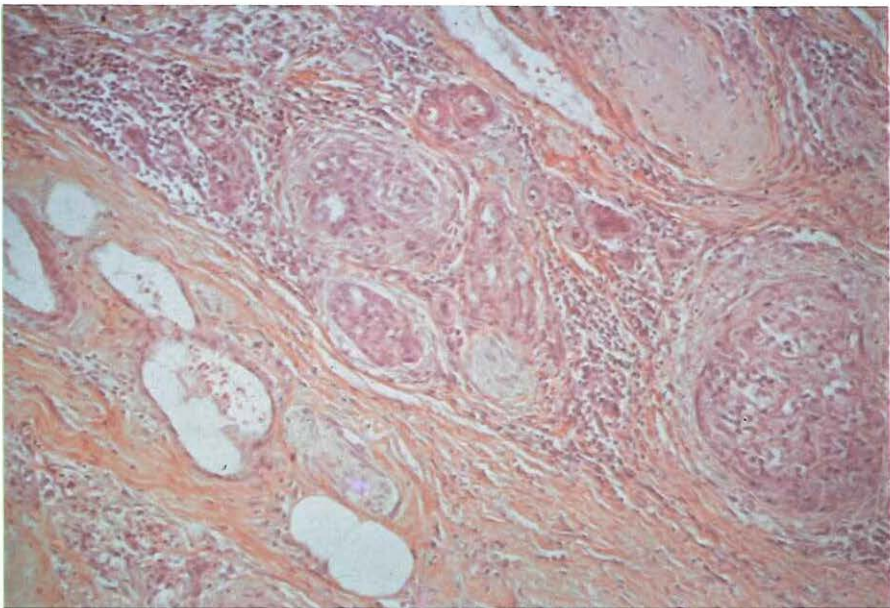


Plate 13. Allotransplant on 59th day, showing islet tissue and damaged acinar tissue with extensive fibrosis and moderate cellular infiltration. No immunosuppressive drugs given. (Nr. BD 0.164) $\times 100$.

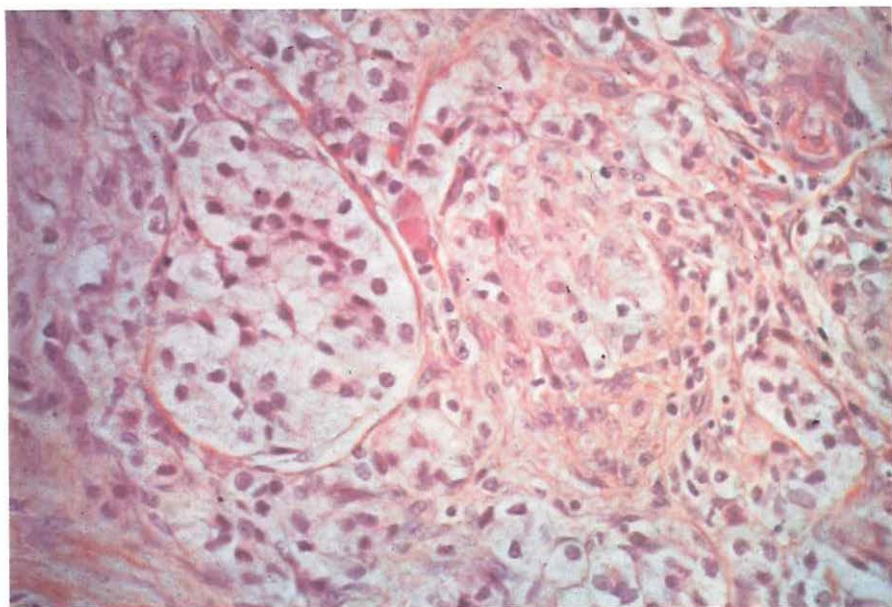


Plate 14. Detail of Plate 13, showing islet tissue and ductal proliferations (nr. BD 0.164) $\times 250$.

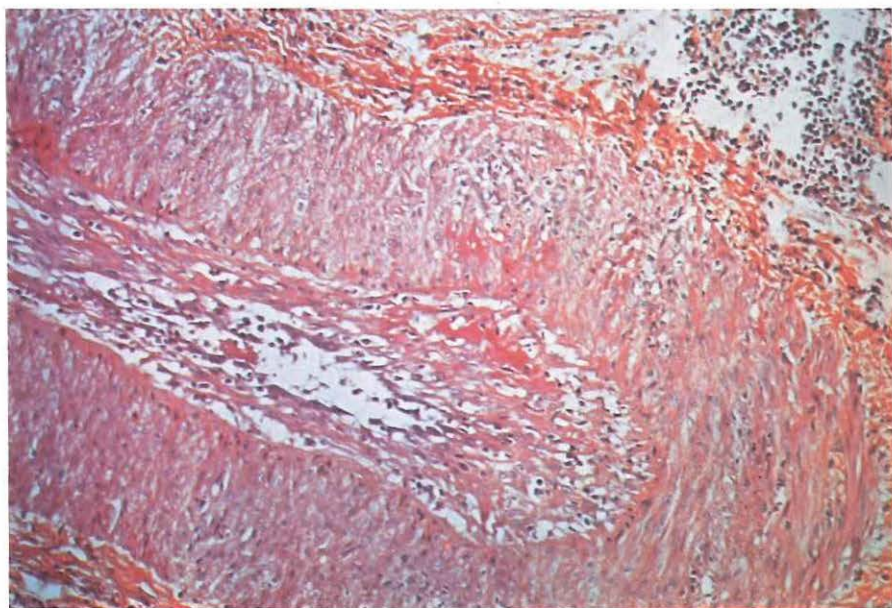


Plate 15. Case exemplifying severe arteritis and thrombosis with rejection phenomena, 69 days after transplantation (nr. BD 9.161) $\times 100$.

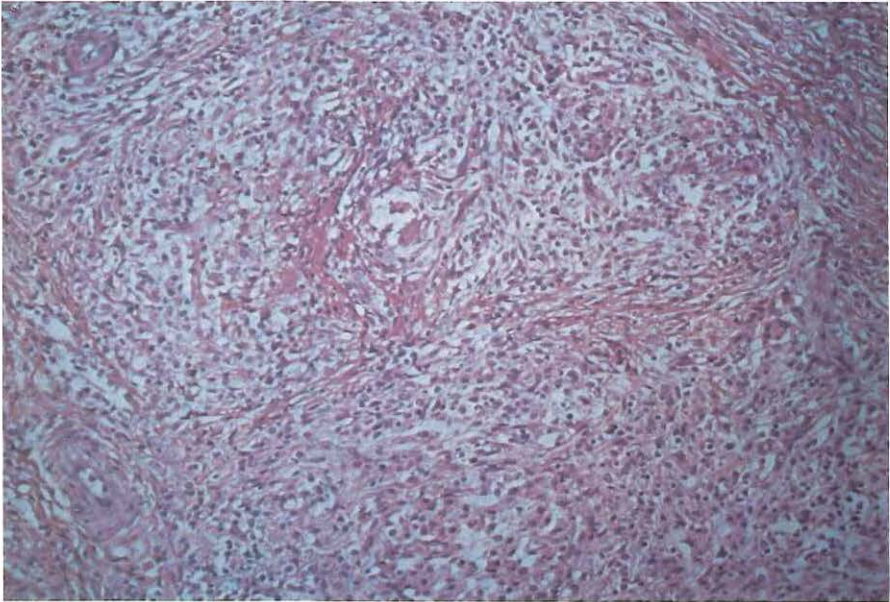


Plate 16. Representative histological picture of pancreatic allograft rejection under ALS treatment, after 33 days. Slight cellular infiltration and only a little swelling of the intima (nr. BD 619) $\times 100$.

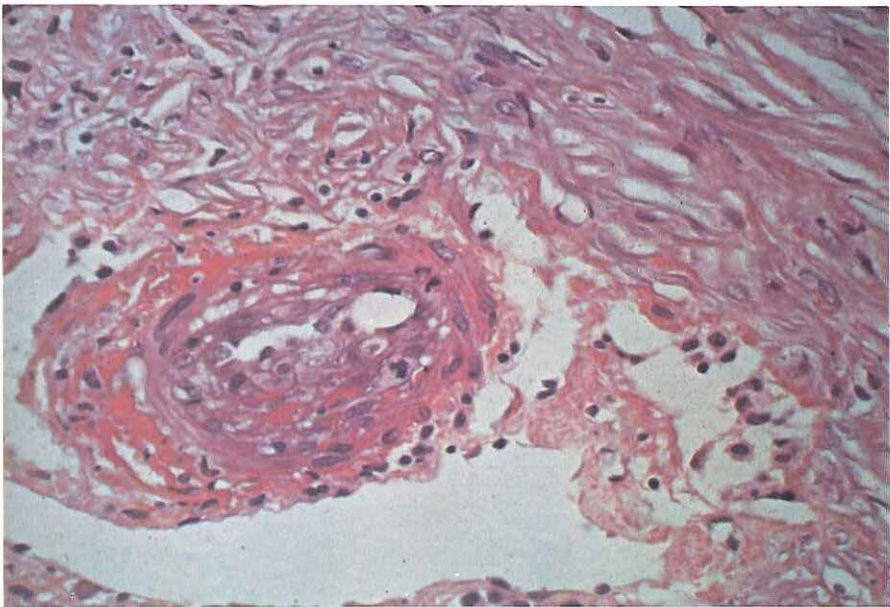


Plate 17. Arteritis with proliferation of intimal cells (nr. BD 619) $\times 250$.

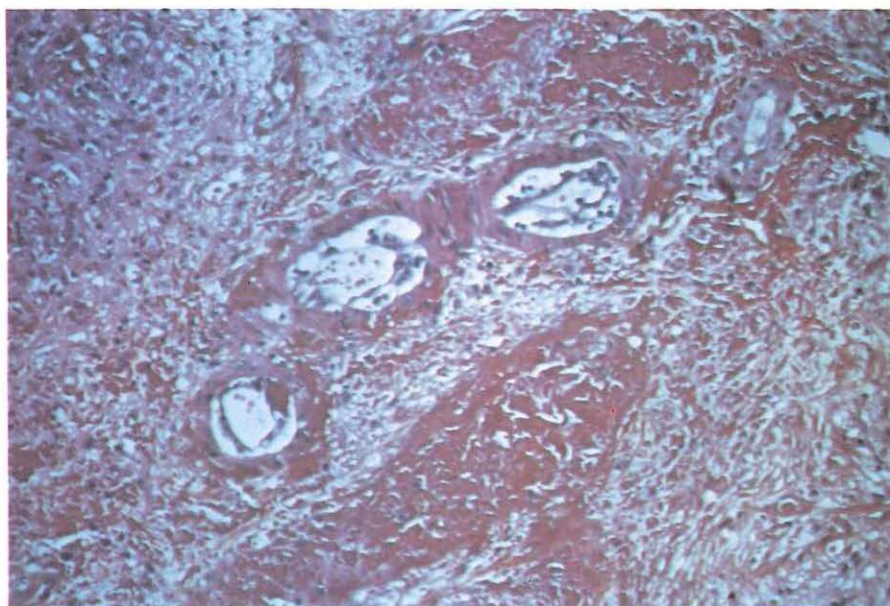


Plate 18. Vasculitis and fibrinoid necrosis after treatment with azathioprine and prednisone for 30 days (nr. B 1.180) $\times 100$.

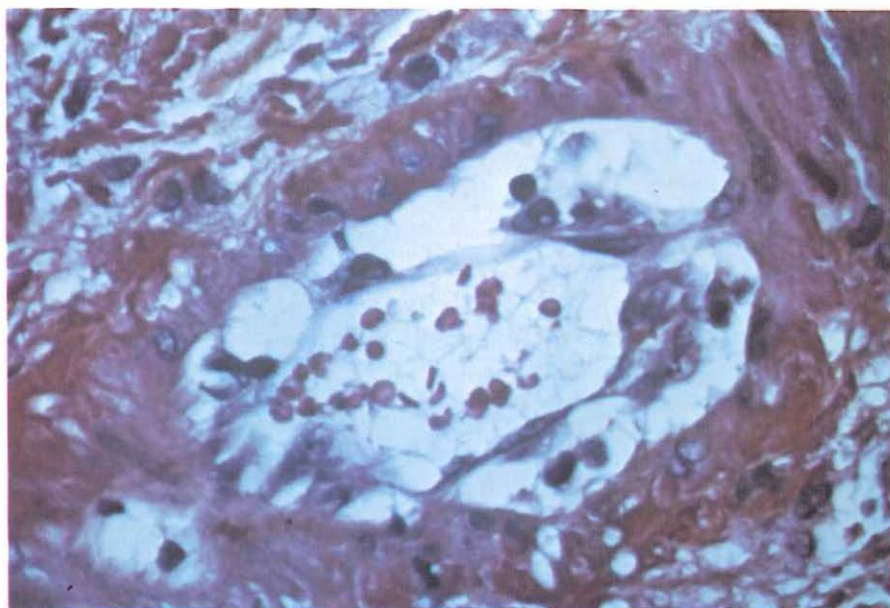


Plate 19. Detail of Plate 18. $\times 300$.

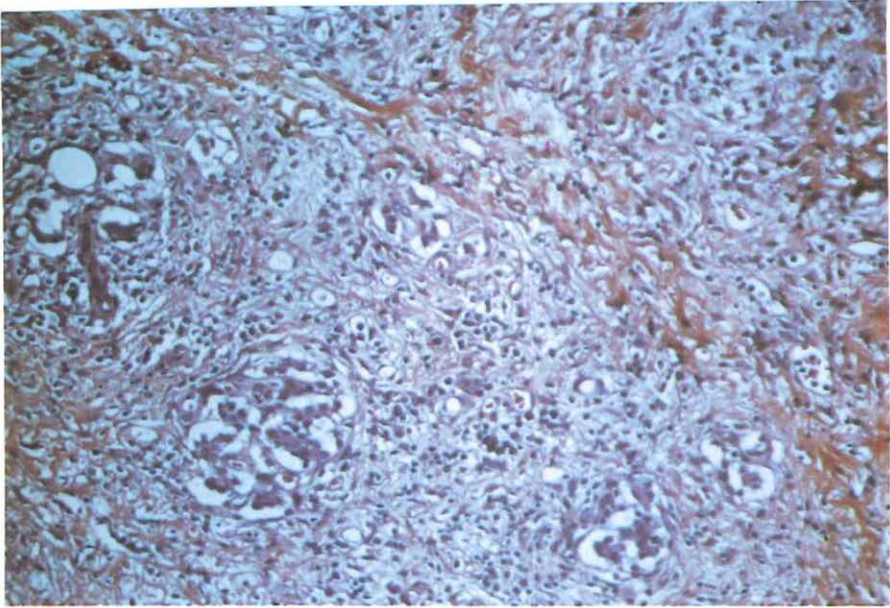


Plate 20. Pancreatic fibrosis in section showing intact islets (nr. B 1.180) \times 300.

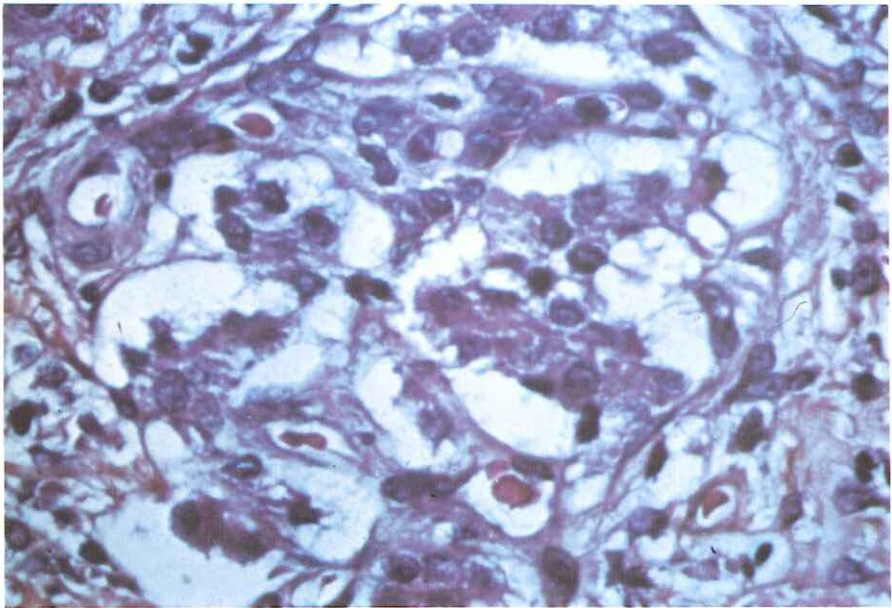


Plate 21. Islet in area of fibroid degeneration (nr. B 1.180) \times 300.

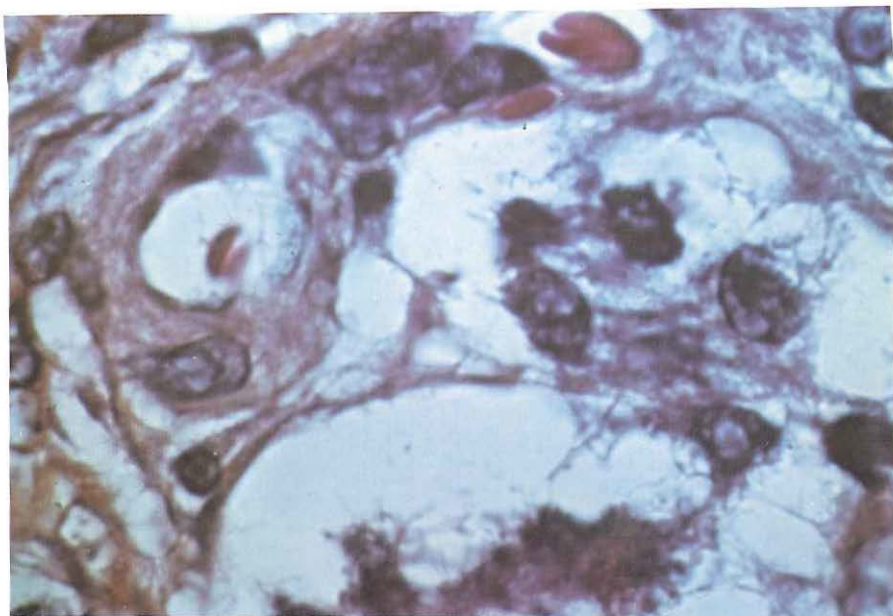


Plate 22. Detail of Plate 20, showing afferent arteriole. $\times 500$.



Plate 23. Contrast radiogram showing the arterial supply of the canine pancreas.