ORTHOTOPIC TRANSPLANTATION OF THE STORED LIVER

THESIS

FOR THE DEGREE OF MASTER OF SURGERY

Presented by

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GENERAL INTRODUCTION

The replacement of diseased tissue and organs with sound parts has stimulated the imagination of man for many centuries. Mention was made in Greek Mythology of transplants from animal to man, and early Christian legends and folk tales of the middle ages tell of successful transplants, the best known being the legend of Saints Cosmos and Damian and their successful use of a cadaver limb to replace one affected with malignancy. By the end of the 19th century, skin, cartilage, bone, nerve and fragments of glandular tissue had been transplanted, but whole organ transplants with its associated technical difficulties is a child of our present century.

Alexis Carrel (1902), described the first successful transplant of an autogeneic kidney to the cervical vessels of a dog using vascular sutures, yet it was more than 50 years later that the first description of transplantation of the whole canine liver was published (Welch, 1955). Further experimental work (Goodrich et al. 1966; Moore et al. 1959; Starzl et al. 1960) soon followed to ascertain the technical feasibility of liver transplantation, but the initial clinical application proved disastrous (Starzl et al. 1963; Demirleau et al. 1964; Moore et al. 1964).

More recent experience was summarised at the Cambridge Liver Transplantation Conference in April, 1969. At that time a total of 55 orthotopic liver homotransplants had been performed in man. Twenty-two of these patients survived more than one month, seven more than six months and three more than one year. These results reflect the status of renal transplantation of almost a decade ago. Several reasons were invoked for this obvious lag, the chief being the lack of effective supporting treatment (something comparable to dialysis in kidney disease) for liver failure. In orthotopic liver transplantation immediate post-operative survival of the patient depends entirely upon the excellent initial

function / . . .

function of the graft.

The present improved results (Starzl et al. 1969; Williams 1970), whilst also due to several factors is principally related to a greater awareness of the extreme metabolic fragility of the liver. To overcome this several methods, some simple (Schalm 1968; Spilg et al. 1971), and others complicated (Kestens et al. 1966; Mieny et al. 1968a; Hobbs et al. 1968; Brettschneider et al. 1968b; Slapak et al. 1969; Belzer et al. 1970), have been developed to preserve the liver in a functional state for several hours.

CHAPTER TWO

CHAPTER TWO

TECHNIQUES OF LIVER TRANSPLANTATION

The liver may be transplanted in the normal position (orthotopic transplantation). This requires preliminary hepatectomy of the recipient but has the great advantage that the liver is easily accommodated and the vascular connections lie in a normal anatomical situation. Alternatively, the liver may be transplanted in an abnormal situation (heterotopic transplantation). The advantage of this technique is that preliminary hepatectomy is not necessary, but the disadvantages are that it may be very difficult to accommodate a large organ satisfactorily in the abdomen and there may also be problems in providing satisfactory vascular inflow and outflow and bile drainage with the liver in an abnormal situation.

ORTHOTOPIC ALLOTRANSPLANTATION:

Orthotopic allotransplantation was first reported by Moore et al. (1959) and Starzl et al. (1960) in dogs with total hepatectomy of the recipient. Moore et al. (1959) used two external shunts from the inferior vena cava and the portal vein to the jugular veins. Starzl et al. (1960) performed a temporary portal-caval anastomosis and used a single external shunt between the femoral and jugular veins.

Bonillanaar and Alvarez-Vasquez (1963) and Fonkalsrud et al. (1967), described a technique of orthotopic transplantation without interruption of the recipient's vena cava. In the various techniques described, all the vascular anastomoses are re-established. The hepatic artery may be anastomosed with the right renal artery (Moore et al. 1959), splenic artery, hepatic artery or with the recipient's aorta (Starzl et al. 1960).

More recently orthotopic liver allotransplantation was described in pigs (Cordier et al. 1966; Calne et al. 1967; Mieny et al. 1967b;

Terblanche / . . .

Terblanche et al. 1968a; Starzl and Putnam 1969; Belzer et al. 1970; Huguet et al. 1970; Dent et al. 1971a). In this animal only a single external porto systemic shunt is necessary and a surprisingly prolonged survival is obtained without the use of immunosuppression.

ORTHOTOPIC AUTOTRANSPLANTATION:

The results of this procedure have been generally poor (Moore et al. 1960). Only Alican and Hardy (1967) have achieved real successes with the technical variant developed by them.

HETEROTOPIC ALLOTRANSPLANTATION:

This has been extensively studied in dogs (Welch 1955; Goodrich et al. 1956; Sicular et al. 1963; Bengoechea-Gonzalez et al. 1967; Mehrez et al. 1964; Marchioro et al. 1963, 1965; Hagihara and Absolon 1964; Absolon et al. 1965; Leger et al. 1965; Thomford et al. 1965; Slapak et al. 1970). The different techniques vary in the method of revascularisation of the liver, particularly with regard to the portal vein; it was ligated in the original technique by Welch (1955), anastomosed to an iliac vein or vena cava (Sicular et al. 1963; Mehrez et al. 1964), or connected with the splenic vein or mesenteric vein (Marchioro et al. 1963, 1965; Hagihara and Absolon 1964).

Heterotopic liver transplantation may be performed either as an auxiliary or non-auxiliary procedure. In the former the functional evaluation of the heterotopic liver with a normal liver in situ is extremely difficult.

BILIARY DRAINAGE:

Independent of whether the siting of the transplanted liver is orthotopic or heterotopic, special attention should be paid to reconstruction of the biliary apparatus. Simple ligature and diversion of the common bile duct may cause necrosis and leakage of the ligated

stump, as shown by observations of liver transplantation in man (Starzl et al. 1964). Cholecystoduodenostomy is a further source of complication; it may cause purulent cholangitis and sepsis in both the dog (Brettschneider 1968b) and in the pig (Calne et al. 1967b). This complication can be avoided by retaining the function of the sphincter of Oddi.

CHAPTER THREE

CHAPTER THREE

REVIEW OF THE LITERATURE ON LIVER STORAGE

INTRODUCTION:

Research in the preservation of tissues and organs, initiated by the now classic work of Carrel and Lindbergh (1938), proceeded at a leisurely pace until success in human kidney allotransplantation emphasised the clinical need for cadaveric organs. Extension of human transplantation to the lung, liver, heart and pancreas has further accentuated this need. As a result, a large share of developments in this field have only recently been reported, though there are a number of background studies upon which this present progress rests.

It must be emphasised at this juncture, that unicellular organisms and cell suspensions such as blood or bone marrow differ greatly from whole organs in their ability to withstand storage. When all cells of a tissue are similar, considerable numbers may succumb, but the preservation may still be regarded as successful. Therefore, methods suitable for cell preservation may not necessarily be successful in preserving whole organs.

CLASSIFICATION OF METHODS OF STORAGE:

Any method of storage is designed to accomplish either a reduction in the metabolic demands by the organ, or an increase in its metabolic supply. These methods may be classified accordingly.

1. REDUCTION / . .

1. REDUCTION OF METABOLIC DEMAND

(a) Simple hypothermia

(b) Deep hypothermia: (i) Freezing
(ii) Supercooling

(c) Pharmacological metabolic inhibition.

2. INCREASE OF METABOLIC REQUIREMENTS

(a) Perfusion

(b) ? Hyperbaric oxygen

SIMPLE HYPOTHERMIA - PHYSIOLOGICAL BASIS OF HYPOTHERMIA

The lowering of metabolism by hypothermia is the primary physiological basis for its use (McQuiston 1950; Bigelow et al. 1954; Blair 1960). The fall in oxygen uptake by tissue is said to be exponential with the fall in core temperature (Fuhrman and Crimson 1947; Adolph 1950; Spurr et al. 1954). Extrapolation of an exponential curve (from data obtained up to 20°C) shows that oxygen uptake ceases at 10°C (Spurr et al. 1954). This is not true, as actual studies at this level revealed a continuous uptake of between 4 and 11 percent (Civallero et al. 1962). The Van't Hoff Arrhenius rule (the rate of a chemical reaction is doubled for each 10°C rise in temperature) applies only within certain limits. The greatest fall occurs between 37°C and 30°C, below this level the decrease may well be exponential to 20°C; thereafter the decline levels out. The data derived from several sources of animal studies under controlled conditions (Adolph 1950; Bigelow et al. 1950; Civallero et al. 1962) show the oxygen requirements to be reduced as follows:

at / . . . . .

at 32°C	65 - 70%	
30°C	50 - 55%	
28°C	40%	Oxygen consumption % of normal
25°C	20 - 25%	
10°C	10%	
5°C	5%	

BIOCHEMICAL CHANGES IN THE LIVER DUE TO HYPOTHERMIA

(i) OXYGEN CONSUMPTION -

The measure of oxygen consumption as an index of the total metabolic state of the liver is not entirely accurate, since it does not account for anaerobic metabolic pathways. Fisher et al. 1956, demonstrated that after a six-hour period of hypothermia some oxidative mechanism was interfered with and that there was no direct relationship between the liver oxygen uptake and the glycogen level in the liver.

(ii) CARBOHYDRATE METABOLISM -

The glucose tolerance curve is flattened below 28°C (Wynn 1964), suggesting almost complete block of glucose utilisation. Glycogen stores in the liver become depleted after 5 hours of hypothermia and are not restored to normal on rewarming even when the blood glucose level is maintained by constant infusion of glucose.

(iii) B.S.P. UPTAKE -

Disodium phenoltetrabromphthalein sulphonate (B.S.P.) uptake was shown to be 50% of normal at 23°C (Brauer et al. 1959), however there was increased extrahepatic removal of B.S.P. as the hours of hypothermia progressed (Fisher et al. 1956). This, therefore, invalidates the use of B.S.P. to determine the estimated hepatic blood flow (E.H.B.F.) under conditions of hypothermia. Of greater importance than the

quantitative / . .

quantitative validity of B.S.P. during hypothermia is the fact that rewarming following prolonged cooling results in prompt return of normal blood flow to the liver.

(iv) BILE FLOW -

Bile production was reduced with temperature decrease, though continued to respond to choleretic stimuli (Cornelius et al. 1955). Rewarming resulted in a return of bile flow to normal after a delay of 12 to 24 hours.

(v) CONJUGATION AND DETOXIFYING ACTIVITIES -

These functions were significantly affected by hypothermia (Brauer et al. 1959; Rusk et al. 1956). The half life of morphine was increased from a normal of three to seven minutes to ninety-seven minutes at 24°C. Similar prolongation of barbiturate levels also occurred.

(vi) ENZYMES -

The Q 10 of enzyme reactions is 2 to 3, therefore it would appear that the extent of modification by cold should be easily predicted. However, reports indicate the customary degree of conflicting results and conclusions much of which are due to the differences in species and techniques used in individual studies. In rats, no alteration was found in succinic dehydrogenase, cytochrome oxidase or alkaline phosphatase levels (Kaufman et al. 1958), whilst in dogs there was an increase in alkaline phosphatase and decrease in cytochrome oxidase and succinic dehydrogenase.

In dogs the serum glutamic oxalo acetic transaminase content was elevated after 6 hours of hypothermia at 25°C (Wendel 1961). The mechanism of this is obscure in the absence of cellular necrosis. It has been postulated that the enzyme in the cell escapes into the

blood / . . .

blood stream because of altered cellular permeability.

HISTOLOGICAL CHANGES IN THE LIVER DUE TO HYPOTHERMIA

As with the biochemical investigations so also the morphological studies appear contradictory.

No obvious histological changes were reported by Delmore 1952; Hallet 1954; and Fisher et al. 1957, in the canine liver under hypothermic conditions. However, Knocker (1955) observed an increase in lipid deposition in the same species which she attributed to anoxia, whilst Sarajas et al. (1954), and Sarajas (1956) described degenerative changes in the central portions of the hepatic lobule on the basis of micro-embolisation.

LIVER SURVIVAL WITHOUT A BLOOD SUPPLY - NORMOTHERMIA

The extent of ischaemic damage in cadaver transplantation of any organ is extremely important and has probably been responsible for more early failures in clinical liver transplantation than has immunological reactions.

The first report on temporary occlusion of the hepatic artery and portal vein is attributed to Duchinova (1925). He occluded the hepatoduodenal ligament to obtain a bloodless field during surgery on the liver. Although he achieved his experimental aim, he found that fatalities occurred when the period of occlusion exceeded 30 minutes. Raffucci and Wangensteen (1950) found that occlusion of the afferent circulation to the liver beyond 20 minutes was associated with hepatic necrosis and prolongation to one hour under normothermia resulted in 100% mortality. Comparison of these results with those obtained during total occlusion of the renal pedicle under the same conditions (Bogardus and Schlosser 1956; Mitchell and Woodruff 1957; Stueber et al. 1958; Calne et al. 1963) suggests that the liver is more sensitive than the kidney to normothermic ischaemia.

Van Wyk et al. (1965a) showed that if the pig liver was left for graded periods of ischaemia within the cadaver, cooling was very slow (1°C per minute). Such livers tested on a pump disc oxygenator perfusion system were found to be functionally active for half an hour after death. Residence in the cadaver beyond this period was associated with functional dissolution and electron microscopic studies (Slagel and Eiseman 1968) showed all the hallmarks of hepatocyte ischaemic necrosis. When the liver was excised from the cadaver immediately after death and left at room temperature (van Wyk et al. 1965b), cooling was much more rapid (2.5°C per minute) and functional integrity was retained for three hours, but after six hours a severe metabolic derangement occurred. It is striking how this slight temperature difference affords such a marked protective effect on the liver.

Swenson et al. 1967, studied the immediate and long-term effects of acute hepatic ischaemia. They established a porto systemic shunt in the dog prior to occluding the afferent ciruclation to the liver. After a two-hour period of ischaemia, the biochemical changes were only moderately affected and did not appear to influence the animal's subsequent health. The pertinent observations of this study, however, were the progressive vascular changes found later in the liver. These authors conclude that this period of ischaemia in an otherwise unprotected liver would preclude satisfactory long-term survival.

Goodrich et al. (1956) failed to obtain a single survivor in their liver transplantation experiments when the total anoxic period at room temperature exceeded 33 minutes.

LIVER SURVIVAL WITHOUT A BLOOD SUPPLY - HYPOTHERMIA

Bernhard et al. (1955; 1957) found that whole body hypothermia (24°C-27°C) during afferent circulation occlusion for one hour protected the liver against ischaemic damage with no biochemical or

histological / . .

histological evidence of hepatic cell necrosis. Using differential hypothermia to below 26°C, Mito et al. (1965) were able to occlude the afferent circulation for 90 minutes.

OPTIMUM TEMPERATURE AND MAXIMUM DURATION OF PROTECTION OF THE LIVER WITH HYPOTHERMIA

Sicular and Moore (1961) emphasised the temperature to time relationship for tissue preservation. At 4°C they found that liver slices were still capable of adequate glucose utilisation after 24 hours. Van Wyk and Eiseman (1966) in an extension of their previous work excised pig livers, rinsed them free of blood with a cold Ringer lactate solution and maintained the organs at 4°C in a refrigerator. These livers retained their functional integrity for 12 hours.

OPTIMUM METHOD OF COOLING

Sicular and Moore (1961) compared the functional activity of liver slices following various methods of cooling, viz.

(a) rapid and immediate postmortem cooling via the portal vein;

(b) immediate freeze locker cooling;

(c) surface cooling by peritoneal lavage;

(d) isolated liver perfusion with a pump oxygenator.

Optimum function was obtained when the liver was cooled via the portal vein with a physiological salt solution (4°C) for a period of 6-8 minutes

PERFUSION PRESSURE QUANTITY OF PERFUSATE AND COMPOSITION OF PERFUSATE

The optimum perfusion pressure is not well documented and in most instances no measurement is recorded. The quantity used is also not

considered / . . .

considered to be of great importance provided it is sufficient to remove all the blood from the organ. The composition of the perfusate, however, has become increasingly more important and may be one of the prime factors in contributing to the success of a storage procedure. To understand the rationale for the variation in composition of different perfusates, an understanding of the pathophysiology of ischaemic injury is essential.

CONSEQUENCES OF ISCHAEMIC INJURY:

When the liver or any other organ is removed from the body, it is subjected to many different stresses:

(i) Mechanical Damage:

Damage is inevitable in the handling and removal of the organ. If excessive, this leads to disruption in tissue continuity and subsequent compromise of its viability.

(ii) Physical:

Organs in storage are subjected to extrémes of rapid fluctuation of temperature not normally occurring in vivo. One of the injurious consequences of cold is the damage induced in the walls of small blood vessels (Moritz and Davis 1966) which may result in extensive fluid transudation into the tissues. Mild hypothermia may also cause coagulation of protein, inactivation of enzyme systems and induce a hypermetabolic state which results in a relative tissue hypoxia Robbins 1967).

(iii) / . . .

(iii) Chemical Injury:

In isolated organs, the intracellular enzymes are the most vulnerable targets. Chemicals may enter by direct contamination or by leakage in an isolated perfusion system. The absence of essential factors however, such as hormones and specific metabolites are a more important consideration in producing chemical injury.

(iv) Infection:

Bacterial and viral infections are two potential causes of tissue damage. Bacteria may elaborate toxins, or destroy cells by direct invasion, whilst viruses may cause cell lysis. Infection in the isolated organ must be dealt with in the absence of antibody producing or phagocytosing cells and other normal host defences.

(v) Hypoxia:

One of the commonest causes of cell death in vitro is hypoxia. The pathophysiology of hypoxic death appears to centre on decreased energy production. The metabolism of glucose in the presence of oxygen provides most of this energy. Approximately 45% of the energy is successfully captured in high energy bonds available for later use in the cell (White et al. 1964). In the absence of oxygen, energy is derived from anaerobic glycolysis which is inefficient in energy production and also results in a fall of cellular pH due to excessive lactic acid production. Increased acidity in itself has been shown to inhibit the oxygen consumption of isolated tissue (Enerson and Berman 1966). The addition of oxygen alone does little to preserve

oxidative / . . .

oxidative phosphorylation if a substrate such as glucose is not present as well (Gallagher et al. 1956).

The maintenance of cellular volume is a continuously active energy consuming process which is dependent on the active extrusion of sodium at the cell membrane (Leaf 1959). The lack of energy resulting from hypoxia soon causes the sodium pump to fail and as a consequence, sodium and water move into the cell and the cell begins to swell. This correlates with the microscopic swelling which occurs in ischaemic isolated tissue and which is reversible in its early stages (Baker 1956) and also with the increase in the weight found in hypoxic tissues in vitro (Enerson 1966). Thus, two distinct primary events consequent to hypoxia

(a) energy depletion, and
(b) blockage of active transport

can both lead to failure of the sodium pump, following which the same pathological phenomenon of cellular swelling occurs. The cellular swelling following hypoxia has been implicated as a possible cause of breaks sometimes seen in the cell membrane (Ashford and Burdette 1965). This explains the cytoplasmic leakage of potassium (Flink et al. 1950), protein (King et al. 1959) and enzymes (Rees 1962).

The fate of an ischaemic organ is thus determined by the variety and severity of the noxious stimuli to which it is subjected. This is a quantitative phenomenon as the cellular metabolic processes have a degree of resiliency which allows some alteration and adaptation to

new / . . . .

new steady states. The steps towards death taken by the injured cell may arbitrarily be divided into three phases:

(i) THE PERIOD OF REVERSIBLE DETERIORATION in which injury is done to the cell, but damage is not so extensive that recovery is impossible.

(ii) THE PERIOD OF DYING in which the cell sustains sufficient injury to pass the point of no return and cannot be retrieved.

(iii) THE PERIOD OF DEATH in which cellular organisation is so disrupted that integrated cellular function immediately ceases.

In the stored liver, one encounters all three phases. Reversible deterioration is diagnosed in an animal which shows the initial biochemical changes of tissue injury and recovers spontaneously within a few days. With irreversible injury, the animal may be resuscitated by massive transfusions of blood or other fluids and a variety of clot promoting agents only to die within the first 10 days in a cachectic state with histological evidence of severe parenchymal injury rather than rejection. Established cell death during storage manifests almost immediately after complete revascularisation of the liver as an uncontrollable bleeding diathesis.

In attempting to provide the ideal preserving solution for use in in vitro storage, consideration must be given to these changes.

(i) It should remove all the red blood cells, free haemoglobin and other cellular debris and prevent the formation of thrombi in the microvasculature.

(ii) It should buffer adequately in the absence of a haemoglobin-oxygen system.

(iii) / . . .

(iii) It should be isotonic with respect to critical anions and cations.

(iv) It should be isosmotic with the intracellular fluid.

(v) It should contain the substrates and nutrients necessary during the period of in vitro storage.

(vi) Antibiotics should be added to prevent infection.

Manax et al. (1965c) compared 10 different solutions for preserving kidneys and obtained the best results with a buffered balanced salt solution containing 5% low molecular weight dextran and heparin.

Keeler et al. (1966) found that prolonged continuous perfusion of kidneys with a physiological solution removes essential substances, particularly potassium and magnesium from the cytoplasm, which can be prevented by using a solution containing cations in quantities approaching those normally present in the cell. On the basis of this work, Collins et al. (1969) prepared a solution rich in potassium and magnesium with a low sodium content and obtained satisfactory kidney storage by simple cooling for 30 hours. The efficacy of this solution was confirmed by Collste et al. (1970) with successful kidney preservation for 48 hours.

Liver storage with a cold ($4^{o}C$) Ringer Lactate solution (Starzl et al. 1960) failed to provide a satisfactorily functioning organ beyond a two-hour period of cold ischaemia. Schalm (1968) initially cooled the liver by perfusion with a non physiological solution of dextran, glucose and bicarbonate. He then infused a "preserving" solution of plasma, bicarbonate, dextrose, procaine, hydrocortisone and penicillin via the portal vein. By this method he was able to preserve a canine liver for a maximum period of 3.5 hours. There

have / . . . .

have been no studies reported on the use of a Collins, Bravo-Shugarman, Terasaki type of solution in liver storage.

SUMMARY:

(i) The liver is extremely sensitive to ischaemic injury at 37°C.

(ii) Simple hypothermia protects the liver against ischaemic injury.

(iii) Simple hypothermia per se does not produce any metabolic or morphological injury to the liver with the exception of depleting it of glycogen.

(iv) The optimum temperature for preserving the liver is 0 - 5°C but adequate protection is obtained in the range of 5 - 15°C.

(v) Simple hypothermia rapidly induced by core cooling and maintained at 4°C protects the liver for a maximum period of 12 hours when assessed biochemically.

(vi) A canine liver protected only by simple hypothermia does not retain its functional integrity beyond 3.5 hours when tested by transplantation into an anhepatic recipient.

DEEP/. . .

DEEP HYPOTHERMIA

INTRODUCTION:

A logical extension of simple hypothermia is storage at temperatures below zero. John Hunter in 1776 was probably the first to experiment and apply this method, to provide a "stepping stone" to the prolongation of life. He froze 2 carp in river water in an attempt to keep the fish "alive" in the frozen state. Currently, many tissues including skin (Billingham and Medawar 1952), glands (Playfair and Davies 1964) and cell suspensions of semen and blood (Polge 1957; Sherman 1957; Sherman and Lin 1958; Brown and Hardin 1953; Jones et al. 1957; O'Brien and Watkins 1960; Weiss and Ballinger 1958) are stored by freezing. However, with few exceptions no-one has successfully frozen and resuscitated a large organ.

FREEZING:

Mechanism of Freezing:

There are three methods by which water may enter the solid state (Karow and Webb 1964).

(i) Heterogenous nucleation: occurs when cooling is slow, with ice forming around large molecular inclusions of non aqueous substances. The freezing ordinarily found in nature results from heterogenous nucleation, because the cooling rates rarely exceed $1^{\circ}C$ per minute.

(ii) Homogenous nucleation: This results from faster rates of cooling (50 - $100^{\circ}C$ per minute) when innumerable minute crystals form around the natural molecular clumps of water.

(iii) / . . .

(iii) Vitrification: entails the transformation of water into an amorphous state, rather than a crystal; this can only be achieved through ultra rapid cooling (Meryman 1956, 1957, 1960, 1962). Theoretically, vitrified water enters the solid phase without deadly effects on the tissues. To say the least, it is extremely difficult to achieve as water must be cooled to below -130°C in one second and maintained well below that temperature. Only two well documented reports of this achievement are to be found and it is possible that vitrification was not complete even in these cases (Burton and Oliver 1935; Pryde and Jones 1952).

THERMAL INJURY

Profound cooling per se appears to adversely affect the living organism. The irreversible and lethal effects of extreme change in temperature termed "thermal shock", appears to be the product of rapid cooling rather than of thawing (Lovelock 1953, 1959) and are unrelated to the differential slowing of biochemical reactions (Walton 1957).

Motility, respiratory activity and glycolysis are reduced in spermatozoa cooled faster than 0.25°C per minute (Blackshaw and Salisbury 1957). Red blood cells may undergo haemolysis (Lovelock 1955) and even such relatively stable molecules as nucleoprotein may be affected (Lovelock 1957). Lochte et al (1958) found that sudden chilling of human bone marrow mixed with glycerol slightly inhibits deoxyribonucleic acid synthesis, but repeated chilling depresses deoxyribonucleic acid synthesis "appreciably".

Many theories have been propounded on both a chemical and physical basis to explain the mechanism of thermal injury. However, none is wholly acceptable.

CRYOPHYLACTIC SOLUTE MODERATORS

The discovery that certain chemical solutes abrogate the fatal effects of freezing has made possible the bulk of practical and theoretical advances in cryobiology.

Polge et al. (1949) reported on the successful freezing of spermatozoa and erythrocytes with glycerol and were the first to recognise the specific cryophylactic action of this substance. A natural consequence was the screening of a large number of related substances, but only a few were found to be of comparable efficacy. The most promising being dimethyl sulfoxide (DMSO) reported by Lovelock and Bishop (1959). Some of the mechanisms by which DMSO protects against freezing damage almost certainly are similar to those of glycerol, but in addition this substance exhibits a bewildering array of other properties which are at present under intensive clinical investigation.

The most critical property of cryophylactic substances is their ability to retard and limit the extraction of water by ice crystal formation, this represents the closest approach to vitrification possible. However, no single solute moderator can be used with equal success for all tissues, since tissues vary in their sensitivity to a particular agent and it must be matched to the organ being preserved. This relationship still requires intensive investigation.

SUPERCOOLING

Is an attempt to extrapolate hypothermic slowing of metabolism to temperatures below the normal freezing point while avoiding the penalties of freezing. This is done simply by depressing the freezing point and storing the specimen just above the new freezing temperature.

Depression of the freezing point can be accomplished by the addition of solutes. Their effect on the freezing point is a colligative property. The ideal solute for this purpose should be non

toxic / . . .

toxic, readily diffusible and very soluble, to allow maximal depression of the freezing point with the least harm to the specimen. Desphande and Jacobs (1963) and Robertson et al. (1964) have pursued super cooling with dog hearts and kidneys perfused with 15% DMSO and dimethyl sulphone. These organs could be stored at -6°C to -8°C with no freezing. Hearts were resuscitated after storage in these circumstances for as long as 30 hours. Kidneys survived storage for up to 8 hours and sustained life when auto-transplantation and subsequent contralateral nephrectomy was performed. Storage at -7°C for 72 hours allowed good perfusion and immediate production of glomerular filtrate with reimplantation, but these organs would not support life. Another method of depressing the freezing point is by dehydration, which effectively increased solute concentration. Barsamian et al. (1959, 1960) found that puppy hearts will survive deliberate dehydration up to 55% of vaporizable water. These organs can then be immersed in 70% glycerol and stored at -8°C for up to 20 hours with resuscitation of contractions.

EFFECTS OF THAWING

It is impossible to judge the viability of a frozen specimen unless it is first thawed. The thawing of frozen biologic material would seem to be just the reverse of freezing it; but in several important respects this is not so. Indeed, many investigators consider that a disproportionate fraction of the nett damage induced by freezing preservation is incurred during the period of thawing. Opinion is nearly unanimous that thawing should be as rapid as possible and therein lies the problem. For reasons both physical and biological, rapid thawing is more difficult to achieve than rapid freezing. Using hampsters, four methods of warming were studied (Lovelock and Smith 1956). Some animals were allowed to rewarm spontaneously at room temperature; others were warmed either by immersion in a warm

water / . . .

water bath at 37°C or by heating with a 120 Watt diathermy unit. Diathermy was by far the most successful method.

The reasons for apparent failure in freezing whole organs is not likely to be elucidated by experimentation exclusively with cell systems. This failure is probably related to functional organisation at levels higher than the single cell. Factors which may be crucial in this respect include the functional integrity of the micro circulation and the recovery of normal membrane potentials. These aspects will require more experimental attention.

LIVER PRESERVATION WITH DEEP HYPOTHERMIA

Moss et al. (1966) stored canine livers with glycerol for periods varying between 24 hours and 2 weeks. Two of the livers showed reduced though significant B.S.P. uptake. Within an hour following transplantation of these livers, the animals became acidotic and shocked, and died within 6 hours from a bleeding diathesis. Brown et al. (1966, 1967) stored canine livers for as long as 45 days at -6°C immersed in either 2M glycerol or 2M DMSO solution without freezing. Activity of the four urea cycle enzymes studied remained normal during storage. In seven experiments livers preserved for as long as 5 days were tested by auxiliary heterotopic transplantation, these survived one to six additional days as judged by gross appearance, patency of blood vessels and survival of recipients.

This latter method or some refinement of it appears to have the potential for prolonging liver preservation in the future.

PHARMACOLOGICAL METABOLIC INHIBITION

A reversible drug induced inhibition of metabolism should, theoretically, prolong tolerance to ischaemic injury in the same way as hypothermia but with the advantage that these drugs can be administered to a prospective donor before death with the hope of protecting the organ not only after death, but principally during agonal deterioration and before other organ preserving measures can be instituted.

De Duve et al. (1955) first recognised the existence of intracellular particles in rat livers which were termed lysosomes. Their early work favoured the view that damage to the lysosomal membrane led to the liberation of degradative enzymes with subsequent cell destruction. They also drew attention to the possibility that segregation of such enzymes might be a mechanism to control and restrict autolysis. Janoff (1964) summarises the problem as follows: "A key event in cell autolysis accompanying alteration in tissues is an increase in the rate of leakage of lysosomal enzymes through the lipoprotein membranes of the particles. An intriguing speculation that was raised by the discovery of lysosomes concerned the possibility that lysosomal hydrolytic enzymes are not only released at cell death, but may also be released prior to cell death as a result of cellular anoxia or some other form of injury. Such changes if they occur might constitute a critical event in the progression of cell injury towards an irreversible outcome. Furthermore, the action of the released enzymes, both extracellularly and intracellularly, could contribute to the propagation of injury from cell to cell." Weissmann (1964) reviewed the agents which appear to inhibit the release of enzymes from lysosomes in vitro and concluded that such agents may play a similar role in the living organism. Today numerous pharmacological substances are known which act in this manner, these include the phenothiazines, magnesium, steroids and phenoxybenzamine.

(i) / . . . .

(i) CHLORPROMAZINE

Chlorpromazine, along with many other of the phenothiazines, has a general inhibitive effect on cellular metabolism (Dawkins et al. 1959; Eyal and Eyal-Giladi 1963), mitochondrial swelling (Judah 1960) and the leakage of lysosomal enzymes (Guth et al. 1953) which tend to destroy other intracellular structures. Phenothiazines have also been demonstrated to aid in the maintenance of normal membrane permeability (Guttman and Freedman 1963) and in the prevention of electrolyte shifts and cellular swelling. Perhaps their most striking action was demonstrated by Rees et al. (1961) in preventing cellular necrosis in livers treated concomitantly with carbon tetrachloride and promethazine. Hershey et al. (1955) demonstrated the beneficial action of pretreatment with chlorpromazine on survival following graded haemorrhage in rats and Eyal et al. (1965a, 1965b) reported that the preservation quality of preserved hearts and small intestine with or without hyperbaric oxygen is improved by the addition of chlorpromazine. This was confirmed by Turner et al. (1970) who successfully stored kidneys for 24 hours with hypothermia and chlorpromazine. This could not be achieved by hypothermia alone. Under normothermic conditions, Fonkalsrud et al (1969) demonstrated the value of pretreatment with chlorpromazine in dogs subjected to hepatic ischaemia.

(ii) MAGNESIUM

Holland (1964) demonstrated that magnesium is capable of reducing membrane permeability to potassium which would tend to maintain a more normal membrane potential. Magnesium is known to be essential to nearly all vital metabolic systems (Aikawa 1963) acting as a catalyst in many of the enzyme systems: in phosphate metabolism, in the pentose-monophosphate shunt and in certain stages of the Kreb's cycle. Webb et al. (1966) found that the heart could be more readily revived after normothermic ischaemia if preceded by perfusion with magnesium sulphate. This effect, however, must be regarded as being specific to the heart and possibly to the kidney but not to the liver where magnesium has no metabolic

inhibitory / . . .

inhibitory action (Potter 1951).

(iii) STEROIDS

De Duve (1959) observed that steroids stabilised lysosomes against incubation at acid pH and Weissmann (1964) showed that cortisol in high concentration retards the release of lysosomal enzymes, acid phosphatase and Beta glucuronidase from the lysosome granules. However, on taking into account the high concentration of steroid required in vitro to achieve this inhibition and extrapolating it to in vivo, it is unlikely that lysosomes throughout the body can be coated by such amounts of protective steroids.

(iv) PHENOXYBENZAMINE

Investigations by Manax and Liliehei (1969) suggest that phenoxybenzamine is also a metabolic inhibitor in a manner similar to chlorpromazine. Fonkalsrud et al. (1969) showed that pretreatment of livers with phenoxybenzamine prior to inducing ischaemia gave results comparable to that obtained with chlorpromazine.

It is interesting that chlorpromazine, steroids and phenoxybenzamine are also effective in the treatment of shock where ischaemic anoxic damage to cells is a consistent pahtological feature. The mode of action of these so-called inhibitors may therefore be related principally to their adrenergic blocking effect enhancing the blood flow to the organ during the agonal premortem period. Although each of these agents have been shown to be effective in some ways, there remains conflicting evidence which probably generates from experimental design as well as the organ in question. It is clear, however, that while no drug yet described is dramatically useful by itself, such agents may enhance the quality of the stored organ when used in combination with other methods of storage.

PERFUSION / . . .

PERFUSION STORAGE

INTRODUCTION:

The concept of maintaining alive a portion of the body in order to study its function is not a new one. In 1812 Le Gallois, the French physiologist, expressed his belief in the feasibility of artificially maintaining an organ indefinitely by substituting the heart with a perfusion system. Initially, another animal was introduced as the pump oxygenator, but Hooker (1910) carried the concept of perfusion to its ultimate by introducing a system which could both aerate the blood and pump it at a variable pulse pressure through an organ with a vascular pedicle.

Using a variety of animals, isolated liver perfusion was first used as a standard means of studying hepatic physiology and biochemistry (O'Donnell and Schiff 1965; Shoemaker 1964; Gerber 1964).

In 1958 Otto et al., studied the clearance of ammonia in an isolated canine perfused liver, perfused with blood of a recipient dog with an elevated blood ammonia. The liver was able to remove 85% of the ammonia from the blood. Eiseman et al. (1961) used the isolated pig liver and showed total clearance of acute loads of 2 Gm. of ammonia citrate in 20 to 30 minutes. These studies stimulated numerous programmes of isolated liver perfusion with varying degrees of success for the treatment of otherwise terminal hepatic coma (Eiseman et al. 1965; Eiseman et al. 1966; Norman et al. 1966b; Sen et al. 1966; Van Wyk 1966; Watts et al. 1967; Mieny et al. 1968b; Abouna et al. 1969b; Hickman et al. 1970).

With the current surge of interest in organ transplantation, and the success achieved in perfusion storage of kidneys both experimentally and clinically (Humphries et al. 1964; Humphries et al. 1968a; 1968b; Belzer et al. 1967; Belzer and Kountz 1970), perfusion storage of the liver as a method of preservation prior to transplantation is currently being extensively investigated (Kestens et al. 1966; Brettschneider

1968b; Hobbs et al. 1968; Meiny and Eiseman 1967; Belzer et al. 1970; Abouna et al. 1969a).

Continuous perfusion should, theoretically, fulfil the same purpose as the circulation of blood, namely the continuous and adequate supply of oxygen and nutrients and the simultaneous removal of carbon dioxide and other end products of metabolism. Carrel and Lindberg (1938) set down the factors which determine successful prolonged perfusion of an organ. . "The fluid must be free of floating particles that may act as emboli. If blood is used, there should be no agglutinated corpuscles. The temperature, the osmotic pressure, the pH of the fluid, the pulse rate, the maximum pressure, the minimum pressure, have to be exactly adjusted. The chemical composition of the nutrient medium and its oxygenation are of capital importance. Moreover, it is imperative that the organ be completely protected against bacteria. Even if all conditions except one are satisfactory, the results of the experiment is utter failure." This adequately describes the technical and mechanical problems involved in an artificially produced system.

CADAVER PERFUSION

In trying to extend the safe ischaemic period of approximately 2 hours Marchiomo et al. (1963) described a method of whole body extracorporeal perfusion to obtain postmortem liver homografts. The grafts were transplanted orthotopically into 10 animals after perfusions ranging from 71 to 416 minutes. Five of the animals survived the operation, but none beyond 5 days. An uncontrollable bleeding diathesis was the main cause of death being particularly prominent in those animals perfused in excess of two hours. Histologically all the livers showed evidence of hepatic cell necrosis. In a comparative study of renal transplants from cadaver donors by the same method, ten of eleven animals produced urine immediately post operatively and maintained good urinary

volumes until death.

Veith et al. (1969) used an Anstadt Mechanical Ventricular Assisstor and perfused animals for 5 to 6 hours prior to transplantation of the liver. They failed to obtain a survivor beyond 3 days.

Whole body cadaver perfusion may offer an increase in the number of suitable donor organs by limiting the degree of ischaemic damage before harvesting, but appears to have a limited application in liver storage.

ISOLATED LIVER PERFUSION STORAGE

A method of in situ isolated perfusion originally described by Kestens et al. (1961) was used (Kestens et al. 1966) to store livers for varying periods between 90 and 325 minutes. Biochemical assays during the perfusion showed a normal potassium concentration in the plasma and the levels of SGOT, SGPT and LDH remained below the upper limits of normal. Nine of 12 dogs survived orthotopic transplantation for periods longer than 5 days.

Mieny and Eiseman (1967) perfused isolated pig livers for periods of 18 to 24 hours and described adequate function when assessed by oxygen consumption, volume of bile production, bromsulphalein clearance, ammonia clearance and adenosine triphosphate regeneration. However, when the perfusion stored livers were tested by orthotopic transplantation, all 7 animals receiving livers perfused for 18 to 24 hours died within 5 hours of the operation with a generalised bleeding diathesis. Animal survival only occurred when storage was limited to 6 to 8 hours (Mieny et al. 1968a).

Slapak et al. (1969) preserved canine livers by hypothermic asanguinous perfusion for 6 hours; of 20 animals, only 3 survived orthotopic transplantation longer than 5 days. They were unable to find any correlation between oxygen consumption, bile production, lactic pyruvate and potassium levels, during the period of perfusion

storage / . . .

storage and successful preservation.

Hobbs et al. (1968) satisfactorily perfused isolated porcine livers for 6 hours using hypothermia (8°C) and low flow perfusion with autogenous blood diluted with Rheomacrodex. They assessed function biochemically and histologically. Using livers stored by this method for periods of 24 to 410 minutes for orthotopic transplantation (Peacock et al. 1969) had 2 of 9 living donors and one of 6 cadaver donors surviving more than 24 hours.

Brettschneider et al. (1968a), in 3 experiments using perfusion storage for periods of 20½ to 25½ hours had no survivors.

Belzer et al. (1970) used an isolated prefusion storage method to preserve pig livers. They failed to obtain consistently successful survival beyond a 9 to 10 hour period of storage and subsequent orthotopic transplantation. Most of the animals transplanted with organs stored for 24 hours died of a bleeding diathesis. The limiting factor which prevented consistent preservation beyond 10 hours appeared to be related to/inability to maintain endothelial integrity of the sinusoids. Damage to the endothelium began within 8 hours after preservation and consisted of swelling and disruption of the Kupffer cells. Endothelial lining damage was not encountered in their kidney isolation perfusion studies even when perfusion was maintained for 72 hours. Grana et al. (1968) described similar changes which were attributed to anoxia - however, the venous effluents in Belzer's experiments were found to have consistently high pO_2 and they concluded that hypothermia per se or in combination with denervation produced undesirable shunting of blood leading to inadequate regional hepatic perfusion and localised tissue anoxia to account for this damage.

METHODS /. . . .

METHODS OF ASSESSING LIVER VIABILITY DURING PERFUSION STORAGE

(i) MACROSCOPIC EXAMINATION

An experienced person is able to draw conclusions about the degree of damage from the initial hypothermic perfusion flush out. Speedy perfusion with uniform discoloration of the whole organ indicates very little or no damage; slow perfusion with spotty discoloration due to irregular washing out of the organ suggests severe ischaemic damage. During continuous perfusion the hallmarks of organ distress are the triad of rising perfusion pressure, falling venous output and increasing oedema.

(ii) BIOCHEMICAL INVESTIGATIONS OF LIVER FUNCTION DURING PERFUSION STORAGE

Despite the now wide usage of liver perfusion, there has been little agreement concerning adequate methods for measurement of hepatic viability in the isolated state. It is generally agreed that the rates of oxygen consumption and bile production are two simple but reliable indices. However, because of the multiplicity and complexity of liver cell function, a more extensive assessment is usually desirable and a large variety of tests have been used for this purpose. These include, B.S.P. clearance (Chapman et al. 1960), the clearance of ammonia (Eiseman et al. 1961) and the clearance of lactate and pyruvate (Schimassek 1965). The rate of synthesis of glycogen from glucose (Craig 1966), the synthesis of urea from ammonia (Hems et al. 1966), the rate of elimination of galactose (Tygstrup 1966), the rate of glucose oxidation (Sicular and Moore 1961), and the rate of uptake of potassium (Flink et al. 1950). Other methods are designed to measure the degree of liver damage as reflected by the release of specific hepatocyte enzymes (McLean et al. 1965; Gallagher et al. 1956; Brown et al.

1966). Whilst in a trend to even more fundamental measures of cellular function, several investigators have turned their attention to the energy producing processes of the hepatocyte (Vang and Drapnas 1966; Vang et al. 1966) and have used both the content and regeneration of high energy phosphate compounds in the liver as measures of hepatocyte integrity. Unfortunately, conflicting views are held with regard to the value of some of these tests.

(a) Oxygen Consumption - A great deal of available information indicates that oxygen uptake by the liver falls coincidentally with irreversible ischaemic damage - but though an index of cellular damage, it is not a reliable index of cellular function (Hambrick and Myers 1953; Murphy and Muntz 1957). Furthermore, oxygen consumption estimation may give misleading information when there are metabolic blocks present such as uncoupling of phosphorylative oxidation (Lambotte 1970).

(b) Bile Flow - Without continual stimulation, bile output falls because of the interruption of the enterohepatic circulation by hepatectomy. Thus a fall in bile output does not necessarily reflect liver damage (Wheeler and Ramos 1960; Presig et al. 1961).

(c) B.S.P. Clearance - The clearance of a single dose of B.S.P. by the liver depends on three important factors. The rate of blood flow, the conjugating capacity of the liver and the B.S.P. transport activity across the bile canaliculi, the latter being the rate limiting step, since it depends upon

A.T.P. availability. Bromberg et al. (1968), failed to correlate B.S.P. extraction with any other parameter of liver function. Segal (1961), has suggested that since saturation of the hepatocyte with bromsulphalein occurs readily, a period of at least 24 hours should elapse between consecutive B.S.P. determinations. Abouna et al. (1969a), suggests that the measurement of concentration of B.S.P. in the bile is a more reliable index than the measurement of B.S.P. retention alone. Drapnas et al. (1966), holds the view that B.S.P. clearance can be carried out by a dying liver.

(d) Ammonia Clearance - Ammonia extraction by the liver has the limitation that even the dead or the irreversibly damaged liver will actively take up ammonia, even though it will not synthesise urea from it. This is explained on the basis that the dying liver cell rapidly establishes a large pH gradient with the surrounding medium (Barnett et al. 1958) in which the intracellular pH will be substantially lower than the medium pH - ammonia follows this concentration gradient and becomes concentrated in the more acid intracellular milieu.

(e) Bile Clearance - The metabolsim of bilirubin appears to follow a similar pattern to that of B.S.P. Drapnas et al. (1966), have shown that both the B.S.P. and bilirubin clearance can be carried out by a dying liver. Abouna (1969a) however, does not

agree / . . .

agree with Drapnas on this latter point. Van Wyk and Eiseman (1966) used both the clearance of bilirubin and of ammonia as criteria of liver viability.

(f) Potassium Uptake - In an attempt to apply the known ionic shift which occurs with cell injury, the loss of hepatocyte potassium and consequent gain of potassium in the perfusate is used by some as an index of liver function (Stewart et al. 1953; Thiers and Valliee 1957; McLean 1960). This finding is disputed by Buckberg et al. (1968).

(g) Enzyme Studies - Hypoxia, ischaemia and the administration of toxic agents which interfere with cell metabolism have all been demonstrated to result in marked increases of the levels of serum enzymes normally active only within the intracellular environment (McLean et al. 1965). Such changes in the serum enzyme content are sensitive indicators of the degree of damage to the functioning parenchyma. However, these parameters do not give any indication of the functional ability of the organ. Abouna et al. (1969a) found enzyme studies generally unhelpful and furthermore found their interpretation difficult in the presence of an appreciable degree of haemolysis occurring during the perfusion. These observations are in agreement with previous transplantation experiments which have shown that the rise in plasma enzymes becomes evident only when the damaged liver is rewarmed and not during the period of hypothermic perfusion (Mikaeloff et al. 1965).

(iii) / . .

(iii) BIOPHYSICAL INVESTIGATION

Couch and Middleton (1968) described the potentiometric measurement of surface hydrogen ion concentration as pH or redox potential. They maintained that with increasing anoxic injury there is increasing surface hydrogen ion concentration which indicated an ability to undergo anaerobic metabolism and that the capacity for anaerobic metabolism means metabolic viability. However, no work has been reported using this technique in correlation with the transplanted organ or in preservation experiments other than with hypothermia.

In a recent publication Lambotte (1970) described the measurement of membrane potential in isolated hypothermic canine livers using a micro electrode. He correlates vital cellular activity with the maintenance of a stable potential. This investigation has also only been confined to the isolated perfused liver.

(iv) HISTOLOGICAL ASSESSMENT DURING ISOLATED LIVER PERFUSION

(a) Light Microscopy - It is now generally accepted that light microscopy is not a reliable index of cell damage. In many cases normal liver architecture is seen in spite of significant alterations in biochemical parameters.

(b) Electron Microscopy - Its use in the cytochemical localisation of metabolic processes within the liver may enable one to recognise subtle changes in the biochemical processes of subcellular components, thereby constituting a "biochemical dissection" of the hepatocyte. This may be of retrospective value in the experimental laboratory, but is not feasible for routine assessment in the clinical situation.

(v) / . . .

(v) TRANSPLANTATION

Transplantation of the perfusion stored liver into an anhepatic recipient has recently been emphasised as being the only test to determine the functional viability of the organ (Abbott and Sell, 1968; Mieny et al. 1968a; Belzer et al. 1970). All other methods described in the preceding paragraphs are only of importance if they coincide with the survival of the animal.

SUMMARY OF PERFUSION STORAGE

1. Extracorporeal whole cadaver perfusion has a place in the harvesting of organs, but is of limited value in preserving the liver beyond a few hours.

2. The isolated liver adequately perfused under hypothermic conditions can retain functional integrity for periods of 8 to 10 hours.

3. No test other than transplantation into an anhepatic recipient can at present be used with any degree of accuracy to determine functional viability of the perfusion stored liver.

HYPERBARIC OXYGEN

PRESSURE TERMINOLOGY

As a rule, the degree of pressurisation is referred to in terms of "atmospheres". A vacuum - as in space - is spoken of as having a pressure of zero atmospheres absolute. At sea level, the weight of air pressing on each square inch of the earth's surface in 14.7 lbs. (760 mm. Hg.) which is referred to as one atmosphere absolute. However, confusion is possible because at one atmosphere absolute the gauges used to measure pressure show a zero reading; hence, one atmosphere absolute is equal to zero atmosphere gauge pressure.

Most basic research concerned with man's ability to survive under increased atmospheric pressures has been related to underwater diving (a dive of 33 feet is equivalent to a gauge pressure of one atmosphere or two atmospheres absolute).

THE PHYSIOLOGICAL BASIS OF HYPERBARIC OXYGENATION

Oxygen is transported in the circulation, both chemically bound to haemoglobin and also physically dissolved in the blood; but normally, only the former is important for oxygen transfer. However, the amount of physically dissolved oxygen is directly proportional to the percentage of oxygen inspired and to the atmospheric pressure.

Breathing air at normal atmospheric pressure, the partial pressure of oxygen (pO_2) in arterial blood is 100 mm. Hg. When breathing 100% oxygen at the same pressure, the pO_2 becomes 675 mm. Hg. (760 mm. Hg. minus the partial pressures of carbon dioxide (40 mm. Hg.) and water vapour (45 mm. Hg.)). When breathing 100% oxygen at an ambient pressure of 3 atmospheres absolute, the arterial pO_2 becomes 2,195 mm. Hg. (3 X 760 minus 40 mm. Hg. CO_2 minus 45 mm. Hg. H_2O), which is approximately

twentyfold / . . .

twentyfold that breathing air at normal pressure.

At normal body temperatures, the absorption coefficient for physical solution in blood is 0.022. When 100% oxygen is administered in a hyperbaric chamber pressurised to 3 atmospheres absolute (3 ATA), the amount of physically dissolved oxygen will thus be raised to approximately 6 volumes %. Since the arteriovenous difference in oxygen content is approximately 6 volumes % for most organs, theoretically it is possible to dispense with haemoglobin as a means of oxygen transport. This was demonstrated by Boerema et al. (1960) in his paper "Life without Blood", describing experiments where the blood of pigs was replaced by plasma or dextran solutions while the animals breathed oxygen at 3 ATA.

EFFECTS OF HYPERBARIC OXYGEN ON TISSUE METABOLISM

Lavoisier demonstrated that the active biological component of air was oxygen and that the breathing of pure oxygen did not alter any of the vital processes. Bert (1878), however, observed changes in the consumption of oxygen by tissues exposed to a range of oxygen pressures less than one atmosphere absolute. He concluded that there was an optimum concentration for biologic oxidation of about 45% of one atmosphere absolute and that depression of oxygen consumption occurred if tissues were exposed to higher or lower oxygen concentrations. Bert's observations have subsequently been confirmed by numerous workers (Bean 1929; Stadie et al. 1945a; Cruickshanks and Trotter 1956; Jacobson et al. 1963) and there is now universal agreement that the metabolism of tissue exposed to pressures of oxygen in excess of one atmosphere is inhibited.

The order of sensitivity of tissues to the toxic effects of hyperbaric oxygen was demonstrated by Dickens (1946a) to be brain more than spinal cord more than liver. Norman et al. (1966) confirmed the sensitivity of the liver to hyperbaric oxygen to an extent of a 34% reduction in oxygen consumption in rat liver homogenates exposed to two atmospheres of oxygen.

The / . . . .

The ultimate basis for the toxic action of oxygen within the cell may not be due to a single mechanism. Dickens (1946a, 1946b) suggested that the metabolic depression was due to inactivation of certain enzymes, notably pyruvate oxidase. Stadie et al. (1945a, 1945b, 1945c) and Haugaard (1946) showed that all the enzymes containing thiol groups are inactivated by high pressures of oxygen, whilst Gershman (1964) considers the toxic action to be related to an excess production of free radicals in the affected tissue. Although it is possible that the clinical manifestations (Paul Bert effect and the Lorrain-Smith effect) of oxygen poisoning are due to these mechanisms, the tensions of oxygen and duration of exposure necessary to cause impaired tissue respiration and enzyme damage in vitro are far in excess of those that cause convulsions in vivo.

EFFECTS OF HYPERBARIC OXYGENATION ON TEMPERATURE

In 1965 Norman suggested that at reduced temperatures an increase in oxygen tension causes an increase in oxygen consumption until a critical oxygen tension is reached (500 mm. Hg. at 28°C and 100 mm. Hg. at 15°C); thereafter, the expected fall occurs. This theory supports the work of Grossman and Penrod (1949), who observed that hypothermia prolongs the period for which rats can be exposed to high pressures of oxygen before signs of oxygen poisoning occur. Stimulation of oxygen consumption at hypothermic levels, in response to moderate increases in pressure, suggests that the reduction in oxygen consumption brought about by hypothermia is not only due to the decreased oxygen requirements by the tissue, but also to a progressive inability of the tissues to utilise the oxygen supplied at low temperatures. In this way a high tension of oxygen may be necessary to ensure adequate oxygenation at low tissue pressure.

HYPERBARIC OXYGEN AND HYPOTHERMIA AS A MEANS OF ACHIEVING CIRCULATORY ARREST

Meinje et al. (1962) described their experimental work in dogs using hypothermia (19 to 23°C) in association with hyperbaric oxygen. Their main conclusion was that hyperbaric oxygen was able to compensate for reduced perfusion due to inflow stasis. Thomas et al. (1966) subjected dogs to circulatory arrest for periods of 2.5 hours at 3°C and 3 ATA. Of 25 dogs, 17 were partially revived, 5 could be taken off the bypass and 3 were well enough to leave the hyperbaric chamber. One of these dogs remained well for 10 months following this procedure.

HYPERBARIC OXYGEN AND HYPOTHERMIA IN ORGAN PRESERVATION

Because frozen storage of whole organs with glycerol and similar hygroscopic additives as a method of preservation was temporarily frustrated by an inability to solve the problems inherent in thermal damage, Woodruff suggested that storage with hypothermia and hyperbaric oxygen was the next logical method to try.

In 1964 Manax et al., published their first report on the techniques and results of the use of the combination of hypothermia and hyperbaric oxygen in the preservation of whole organs and clearly demonstrated the advantage of this combination over either of the two modalities used by itself. Lillehei et al. (1964) theorised on the possible mechanisms through which hyperbaric oxygen exercised its effects and concluded that it was probably due to the toxic effects of the oxygen on the tissue enzymes which interrupted the metabolism of the organ and in this way potentiated the preserving effect of hypothermia. In further papers by Bloch et al. (1964); Manax et al.(1965a, 1965b); Eyal et al. (1965a, b) and Lyons et al. (1966) successful storage of the heart and segments of small intestine under hypothermia and hyperbaric oxygen were reported and these various authors recorded excellent functional and structural preservation. At the time they did not consider the addition of perfusion necessary during the storage period, and the simplicity of this

mode / . . .

mode of preservation represented a great advance on previous efforts. Bloch et al. (1964), in discussing the role oxygen plays in the preservation of organs, favoured the concept of hyperbaric oxygenation supporting oxidative metabolism. In the absence of perfusion and active transport, it must be presumed that the oxygen under pressure is capable of diffusion through the tissues. These authors, in their experiments on the dog's heart, demonstrated that at pressures of 7 ATA oxygen can diffuse to a depth of 1 cm. Matloff et al. (1966), however, showed that oxygen under a pressure of up to 4 ATA failed to penetrate the renal capsule. Lyons et al. (1966), supported the suggestion of diffusion and demonstrated that when oxidative phosphorylation was blocked by using iodo acetate and dinitrophenol, they obtained poor results. However, these same authors also noted that hyperbaric nitrogen and helium were as effective as oxygen for cardiac preservation; hearts preserved for 24 hours with hypothermia and either hyperbaric oxygen, nitrogen or helium all resumed a forceful contraction upon removal from the hyperbaric chamber. Subsequently, Groenewald et al. (1970) have also shown that inert gases under hyperbaria can effectively preserve kidneys. The hypothesis, therefore, that pressure itself exerts a favourable effect is probably the more reasonable in that the whole parenchyma, independent of its thickness, is uniformly exposed to the increased pressure. Cellular swelling is a final common pathway in oxygen deprived tissue (Wickman et al. 1967) and it has been suggested that this may be slowed in a high pressure environment.

There appears, however, to be a definite optimum above which hyperbaric oxygen becomes detrimental. For lungs, the optimum pressure is in the region of 3 atmospheres (Largiadèr et al. 1965; Lyons et al. 1965). This has subsequently been confirmed for other organs - e.g. small intestine (Momose and Salerno, 1968). For thicker organs such as the heart, excess pressures of over 10 atmospheres exerts an unfavourable effect, it is conceivable that under these conditions oxygen toxicity due to enzyme block occurs.

Many / . . . .

Many other workers have confirmed the beneficial effect of hyperbaric oxygen in the preservation of heart, kidney and lung (Lempert et al. 1965; Blumenstock et al. 1965; Ackermann and Barnard 1966a; Almond et al. 1966; Ladaga 1966; Khastagir 1968); However, very few reports are available on its efficacy in liver preservation.

Slapak et al. (1967), used pulsatile hypothermic perfusion with a balanced salt solution at 3 ATA to store canine livers for 24 hours. Decompression was over a period of 20 minutes and the livers were transplanted into the neck of the recipient and assessed for 48 hours, after which the animals were sacrificed. Successful preservation was presumed on the ability of the liver to secrete bile during preservation and post transplantation. Eleven of the 19 experiments were unsuccessful. Brettschneider et al. (1968b), preserved 3 livers unsuccessfully for 24 hours using hypothermia and hyperbaric oxygen.

COMBINED PRESERVATION TECHNIQUES

Ackermann and Barnard (1966b) first described the use of the combined technique of hypothermia, hyperbaric oxygen and low flow perfusion using diluted blood and successfully stored kidneys for 24 hours. It was the application of this same method which produced the best results in liver storage (Brettschneider et al. 1968b). Consistently successful preservation was obtained using canine livers stored for 8 to 9½ hours. These livers showed an average weight loss of 4.2% during residence in the chamber. In 5 experiments, preservation was extended to 24 hours; 2 of the animals died within 24 hours but the remaining 3 survived between 8 days and 4 months. In this latter group, there was an average weight gain of 4.3%.

The authors emphasised that slow decompression over a period of 3 hours was an important factor in achieving success, though Ackermann and Barnard decompressed kidneys over a period of 15 minutes without complications of air embolisation.

The / . . . .

The loss of weight occurring during successful storage confirms the hypothesis that hyperbaric oxygen in in vitro storage limits cellular swelling.

SUMMARY OF RESULTS OF SUCCESSFUL LIVER STORAGE

In the foregoing sections, the various techniques used for in vitro liver preservation have been discussed; and the results achieved by various workers incidentally mentioned. It is generally accepted that the only reliable method to assess successful liver storage is to transplant the previously stored liver into an anhepatic recipient and observe its ability to maintain life. The results of such experiments prior to the commencement of this study (January, 1970) are presented (Table I), giving only the broad principles and the results.

TABLE 1

SUCCESSFUL LIVER STORAGE PRIOR TO THIS STUDY

NAME AND YEAR	STORAGE TIME HRS.	HYPOTHERMIA	PERFUSION	HYPERBARIC OXYGEN	SURVIVORS (4 DAYS AND MORE)
Starzl et al. (1960)	>>2	10-20°C	-	-	No survivors
Schalm (1968)	3.5	4°C	-	-	4 out of 5
Kestens et al. (1966)	1.5 - 5.5	10-18°C	Fresh Blood	-	9 out of 12
	6				4 out of 7
Mieny et al. (1968)	8	10°C	Diluted Blood	-	2 out of 3
	24				None out of 24
Slapak et al. (1969)	6	11°C	Asanguinous	-	3 out of 20
Peacock et al. (1969)	5 - 7	6°C	Plasma	-	2 out of 9
	8 - 10				5 out of 5
Belzer et al. (1970)	24	8-10°C	Plasma	-	Occasional survivor
Brettschneider et al (1968)	8 - 9½	4°C	Diluted Blood	3-4 ATA	5 out of 5
	24				3 out of 5

CHAPTER FOUR

CHAPTER FOUR

EXPERIMENTAL AIM; MATERIAL AND METHODS

EXPERIMENTAL AIM:

The aim of this study was to store the liver by a simple and clinically applicable method for a minimum period of 6 hours.

To achieve this, two simple methods of organ storage were assessed: simple hypothermia and simple hypothermia combined with hyperbaric oxygen. In addition, a control group of experiments of immediate transplantation and a group which received perfusion stored livers were included for comparison. Every effort was made to ensure that the only variation in these experiments constituted the method of storage.

ISCHAEMIC TIME

In these experiments, "ischaemic time" denotes the period between donor death and revascularisation of the portal vein. It does not include the periods of relative warm ischaemia during exsanguination (average 7 minutes) nor the interval between portal vein revascularisation and complete revascularisation (25 minutes).

EXPERIMENTAL / . .

EXPERIMENTAL GROUPS

	Temperature °C	Ischaemic Time	No. of Exprs.
GROUP 1) IMMEDIATE TRANSPLANT	12-15	40-45 min.	4
GROUP 2) IMMERSION STORAGE		HRS	
(a) Ringer Lactate Solution	8-12	6-8	3
(b) Solution II (room temp.)	18-20	6-8	3
(c) Solution II	8-12	6-8	10
(d) Solution II	8-12	10	4
(e) Solution II	8-12	12	3
(f) Solution II	2-5	12	3
(g) Pretreatment with cell membrane stabilising drugs and Solution III	8-12	10	3
(h) Solution IV	8-12	10	3

GROUP 3) HYPOTHERMIC IMMERSION WITH HYPERBARIC OXYGEN	Temperature °C	Pressure ATA	Ischaemic HRS	No. of Exprs.
(a) Solution II	2-5	3	12-12.5	5
(b) Solution II	2-5	3	12-24	2
GROUP 4) PERFUSION STORAGE				
(a) Normothermic	37	-	6-7	3
(b) Hypothermic	8-12	-	6-7	10

MATERIAL / . . .

MATERIAL

Pigs obtained from local farmers weighing between 15 Kg. and 40 Kg., of Large White, Landrace or Crossed Landrace/Large White breed were used. A weight difference of 5 Kg. between donor and recipient was preferred. The animals were of either sex; some transplants were between litter mates, and others between animals not related, either of the same or of different breed.

SURGICAL TECHNIQUE

ANAESTHESIA

The pigs were fasted for 24 hours before operation. No premedication was given. The animals were induced with 3% halothane in a nitrous oxide and oxygen mixture (5 and 2 litres/minute respectively) administered through a nose can. As soon as muscle tone was abolished, the animals were placed on the operating table, and maintained in the supine position by securing the legs to the operating table. The trachea was intubated with a cuffed endotracheal tube and anaesthesia maintained with 0.5 to 2% halothane in the same nitrous oxide and oxygen mixture, administered through a non-return circuit using a Blease pulmoflator.

DONOR OPERATION

All operative procedures were carried out with full sterile precautions. The neck, chest, anterior abdominal wall and groin were shaved and prepared with a 2% iodine in alcohol solution. A catheter was inserted into the left femoral artery for blood sampling and constant arterial pressure monitoring and a Portex[1] (FG 9)feeding tube into the right subclavian vein for the administration of fluids and drugs. A Ringer lactate 10% Invert Sugar solution was administered at the rate of 5 to 10 drops per minute during the donor operation.

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[1]Portex Ltd. Hythe Kent. England.

The abdomen was opened with a midline incision extending from the xiphisternum to the pubis, in males the incision was diverted to the right to avoid injury to the penis. The edges of the incision were widely retracted and by displacing the intestine to the left, the vena cava was identified and the peritoneal reflection along the right border of the liver overlying the vena cava divided up to the diaphragm above and down to the adrenal gland below. With slight traction applied to the stomach, the gastrohepatic ligament was next dissected: the common bile duct was identified and traced to the superior surface of the duodenum, divided as low as possible and allowed to drain freely into the peritoneal cavity. Only the distal end attached to the duodenum was ligated. The peritoneum lying in front of the portal vein was divided longitudinally from the porta hepatis to the pancreas and all its tributaries ligated and divided. Several large lymphatics were usually found loosely connected to the portal vein and hepatic artery which were easily stripped out by blunt dissection. The hepatic artery was then dissected free from surrounding structures: branches to the lesser curve of the stomach were divided, the gastroduodenal artery was identified, ligated and divided, the artery was then freed from its adventitial connection to the portal vein and after division of the right crus, the coeliac artery was reached. The stomach, spleen and intestine were then retracted sharply to the right and the coeliac axis dissected free from its origin to its trifurcation where the splenic and left gastric arteries were ligated and divided. Division of the left coronary ligament, left triangular ligament and falciform ligament completed the skeletonization of the liver. The donor was then heparinized (1 mgm/2 Kg.) the intestines displaced cranially and the lower abdominal aorta proximal to its bifurcation dissected free. Via an arteriotomy, the blunt end of a standard intravenous infusion set was inserted into the abdominal aorta and a ligature tied around it to hold it in position and to prevent haemorrhage. The animal was exsanguinated and the blood (1 to 1.5 litres) collected in a sterile bottle. This blood, without grouping or crossmatching, was used for transfusion

in / . . . . .

in the recipient operation. When the animal was bled out, the ventilator was disconnected. The liver was then rapidly cooled by intraportal infusion through a transverse portal vein venotomy with 3 litres of Solution I (3 litres Tis-U-Sol[2], 90 ml. Rheomacrodex[3], 10% W/V in dextrose) at 4°C with a hydrostatic pressure of 60 cms. using gravity drainage from a drip stand and run in through polyvinylchloride tubing (internal diameter 8 mm.).

The final preparation of the donor liver was completed during cooling. The abdominal contents were retracted to the right, the left diaphragm was divided radially from abdominal aorta to costal margin, the lung was retracted with the left lateral lobe of the liver and the aorta mobilised by division and ligation of its intercostal branches from the arch to below the coeliac axis.

The portal vein was then divided below the perfusing tubing, the infrahepatic vena cava at the level of the right adrenal gland and the suprahepatic vena cava in the abdomen flush with the diaphragm. The liver was transferred to a sterile basin and the perfusion continued until completed. The liver was then transferred to a sterile plastic bag containing 1 litre cold (4°C) normal saline, and the liver perfused via both the portal vein and hepatic artery with 500 ml. "preserving solution", run in over a period of 20 minutes. A temperature probe was tied into the infrahepatic vena cava and the bag closed with an umbilical tape tie. The bag, with its contents, was then placed in a metal basin surrounded by ice to maintain the liver temperature between 8 and 12°C (Fig. 1)

Fig. 1 / . . . .

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[2]Baxter Laboratories Ltd., Morton Grove, Illinois, U.S.A.
[3]Pharmacia, Uppsala, Sweden.

Fig. 1

HYPOTHERMIC IMMERSION STORAGE

COMPOSITION OF THE "PRESERVING" SOLUTIONS

1) RINGER LACTATE SOLUTION

400 ml. Ringer Lactate[4]
80 ml. 5% Dextrose Water[5]
20 ml. 4.2% Sodium Bicarbonate[6]
10 mgm. Heparin
1 Mega U Penicillin G
1 G. Streptomycin

2) SOLUTION II

400 ml. A.C.D. freshfrozen pig plasma
80 ml. 5% Dextrose Water
20 ml. 4.2% Sodium Bicarbonate
10 mgm. Heparin
1 Mega U Penicillin G
1 G. Streptomycin

PREPARATION OF PLASMA

A fully grown pig was anaesthetised and, under sterile conditions, a catheter was inserted into the femoral artery. The animal was then heparinised (1 mgm/lb.) and the blood collected into sterile A.C.D. bottles. These were centrifuged at 1,800 r.p.m. to pack the red cells, the supernatant plasma was drawn off aseptically and stored in a deep freeze at -20°C to -40°C. Prior to use, the plasma was thawed in a water bath at 37°C and then refrigerated at 4°C.

3) / . . . . . .

[4,5,6] Saphar Laboratories, Johannesburg, Tvl., S.A.

3) SOLUTION III

400 ml. Fresh Frozen A.C.D. Pig Plasma
80 ml. 5% Dextrose Water
20 ml. 4.2% Sodium Bicarbonate
50 mgm. Chlorpromazine
50 mgm. Phenoxybenzamine
1 G. Magnesium Sulphate (50% W/V)
10 mgm. Heparin
100 mgm. Hydrocortisone
1 Mega U Penicillin G
1 G. Streptomycin

4) SOLUTION IV

400 ml. Kidney Preserving Solution[7]
20 cc. 50% Dextrose
3 G. Magnesium Sulphate (50% W/V)
10 mgm Phenoxybenzamine
1 Mega U Penicillin G
1 G. Streptomycin

Each 100 ml. of Kidney Preserving Solution contains:

Potassium Acid Phosphate	205 mgm.
Potassium Phosphate Dibasic .$3H_2O$	970 mgm.
Potassium Chloride	112 mgm.
Sodium Bicarbonate	84 mgm.
Procaine HCl.	10 mgm.
Heparin	500 U.

VARIATIONS / . . .

[7]Saphar Laboratories Ltd., Johannesburg, Tvl., S.A.

VARIATIONS IN PREPARATION OF THE DONOR LIVER AND STORAGE TECHNIQUE

1) IMMEDIATE TRANSPLANTATION.

After perfusion with Solution I, the liver was immediately transplanted into a previously prepared recipient.

2) IMMERSION STORAGE AT ROOM TEMPERATURE.

All solutions used were maintained at room temperature and no ice was used to surround the basin during preservation.

3) PRETREATMENT WITH CELL MEMBRANE STABILISING DRUGS AND SOLUTION III

Chlorpromazine 3 mgm/Kg. and Phenoxybenzamine 2.5 mgm/Kg. diluted in 500 ml. 5% Dextrose Water was administered intravenously to the donor one hour prior to hepatectomy. Solution III was used as the preserving solution.

4) IMMERSION STORAGE WITH SOLUTION IV

This solution was used instead of Solution I for the initial perfusion of the liver prior to hepatectomy and also as the preserving solution.

5) THE HYPERBARIC CHAMBER (Fig. 2)

The hyperbaric oxygen chamber used in this study was designed originally for kidney storage and used by Dr. J.R.W. Ackermann (1966a) to store kidneys by the combined technique of hypothermia, hyperbaria and continuous perfusion.

The chamber is cylindrical, made of stainless steel (grade 316), 12 inches high with an internal diameter of 9 inches.

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The dished lid can be secured with four swing bolts. Within the chamber, there is a perforated removable bottom below which a thermometer projects into the chamber. The fixtures on the chamber comprise:- Pressure guage, reading up to 60 lbs/sq. inch; thermometer (40^{o}F to 110^{o}); two perfusion nipples, 2 mm. internal diamter which provide access to the chamber. These were closed off for this study. Quick coupling oxygen connector for compression; exhaust valve; safety valve operating at 52 lbs/sq. inch. A standard oxygen cylinder of 2,000 lbs/sq. inch was used for compression by way of a reducing valve.

The chamber and its components were sterilised with the lid off and then precooled before use. The chamber was used in two ways during these experiments:

(a) NON PRESSURISED
(b) PRESSURISED

NON PRESSURISED

The plastic bag containing the liver was inserted into the chamber. The lid was screwed down and the chamber placed in a cold room at 2^{o}C to 5^{o}C.

PRESSURISED

The plastic bag containing the liver was left untied. The chamber was compressed to 3 ATA and then placed in a cold room at 2^{o}C to 5^{o}C. At the end of storage, decompression of the chamber was effected via the exhaust valve over a period of 10 to 20 minutes.

Fig.2 / . . . . .

Fig. 2

HYPERBARIC OXYGEN CHAMBER

6) / . . .

6) PERFUSION STORAGE

These livers were perfused by Dr. Hickman using an isolated perfusion system described by Hickman et al. (1970). The main features of the perfusion were:-

(a) PERFUSATE — Abattoir pig blood with added Tis-U-Sol and sodium bicarbonate to obtain a P.C.V. of 30 to 35% and a pH 7.4

(b) FLOW RATE — 0.3 ml/G/min. with 70 to 80% of the flow directed through the portal vein.

(c) PRESSURE — Hepatic artery: 20 to 30 mm.Hg.
Portal Vein: 2 to 6 cm. H_2O.

RECIPIENT OPERATION

The initial preparation was as for the donor operation; in addition, electrocardiogram leads were attached and an oesophageal thermometer inserted. The left femoral artery was again used for constant arterial pressure monitoring and the right subclavian vein for fluid and drug administration. In the neck dissection, the right jugular vein was also adequately exposed for the splenojugular bypass. The abdomen was opened through a midline incision from the xiphisternum to the pubis and the spleen mobilised by division and ligation of the gastrosplenic ligament. The bowel was retracted to the left, and the peritoneal reflection along the right border of the liver overlying the vena cava divided up to the diaphragm above and down to the adrenal gland below. With slight traction applied to the stomach, the gastrohepatic ligament was next dissected: the hepatic artery was divided between clamps distal to its gastroduodenal branch, the common bile duct was divided just below its origin, leaving the distal end untied and the portal vein was dissected

free / . . . .

free of adventitia and glands from the porta hepatis to the pancreas without dividing any of its tributaries. Division of the left coronary, left triangular and falciform ligaments completed the skeletonisation of the liver, leaving the liver attached by the suprahepatic vena cava above, and infrahepatic vena cava and portal vein below. The animal was then heparinised (1 mgm/2 Kg.) and a splenojugular shunt was inserted between the splenic vein and the right jugular vein. The shunt consisted of two pieces of silastic tubing (internal diameter 4mm) connected by a plastic T piece. The side arm of the connector was attached to a bottle of heparinised normal saline to prime the shunt. The liver was then excised between vascular clamps, the splenojugular shunt was opened after applying clamps to the portal vein.

The donor liver was placed in the orthotopic position. The vascular anastomoses were sutured with 4-0 atraumatic black silk using a continuous over-and-over technique. After completing the suprahepatic vena caval and portal vein anastomoses, the portal vein clamp was removed, the splenojugular shunt clamped off and approximately 100 ml. of blood was allowed to flush through the liver and discharge through the infrahepatic vena cava. The remaining vascular anastomoses (end-to-end infrahepatic vena cava and end-to-side donor aorta to recipient aorta) were completed whilst the liver was perfused with donor blood and returned to the heart.

After complete revascularisation of the liver, a splenectomy was performed. Biliary drainage was established by an end-to-end choledochodochodochostomy using interrupted 4-0 black silk sutures and finally a gastroduodenostomy, with a 5 cm. stoma between the posterior portion of the antrum of the stomach and the mobile third part of the duodenum.

The abdomen was closed with a dermalon (1) running suture, through peritoneum, muscle and anterior rectus sheath. The shunt was removed from the right jugular vein which was then ligated, and the catheter in the right subclavian vein brought out behind the ear through a subcutaneous tunnel for postoperative fluid administration. The arterial pressure catheter was

removed / . . .

removed and the left femoral artery ligated. All skin incisions were closed with a continuous dermalon (2-0) running suture. Skin allografts were simultaneously performed in most cases.

INTRA OPERATIVE PARENTERAL ADMINISTRATION AND MONITORING

An intravenous infusion of Ringer Lactate 10% Invert Sugar was commenced as soon as the catheter was inserted into the subclavian vein. The pH pCO_2 and base deficit were determined at the commencement of the recipient operation using an arterial blood sample. These were repeated after revascularisation of the liver with portal blood and finally before removing the arterial pressure catheter. Any base deficit was corrected with a 4.2% sodium bicarbonate solution. Providing the splenojugular shunt functioned adequately, no more than 100 mEqs. of sodium bicarbonate were required to maintain a normal astrup.

Donor blood was commenced as soon as the animal's blood chemistry was stabilised and approximately 300 ml. administered prior to recipient hepatectomy. A total of 1 litre of blood was given during the whole operation.

Following portal vein revascularisation, 20 ml. 50% dextrose and 10 mgm. vitamin K_1 and after complete revascularisation, 1 mgm./Kg. of protamine sulphate were given.

A unit (200 ml.) of fresh frozen pig plasma was commenced at the end of the operation; and thereafter, a vacolitre of Ringer Lactate 10% Invert Sugar containing 1 G. chloromycetin, 1 mega U penicillin G and 0.5 G. Streptomycin was given daily for 10 days.

POST OPERATIVE CARE

The pig was returned to a warmed recovery cage and the endotracheal tube removed when the animal recovered completely from the anaesthetic. Oral fluids were commenced on the second day and a standard pig diet on the third. The neck vein catheter was removed on the tenth day; thereafter, all blood samples were obtained by jugular venous puncture under general anaesthetic. All sutures were removed on the seventeenth day.

Animals / . . . .

Animals failing to thrive post operatively were sacrificed and a complete postmortem was performed in all cases.

CRITERIA OF SUCCESSFUL STORAGE

A pig cannot survive without a liver beyond 72 hours. Any technical error incompatible with life will manifest within the same period. However, rejection or pulmonary embolisation may cause the death of the animal by the fifth post operative day. Therefore, in this study survival beyond 4 days in conjunction with adequate post storage liver function were the main criteria of successful preservation.

CHAPTER FIVE

RESULTS

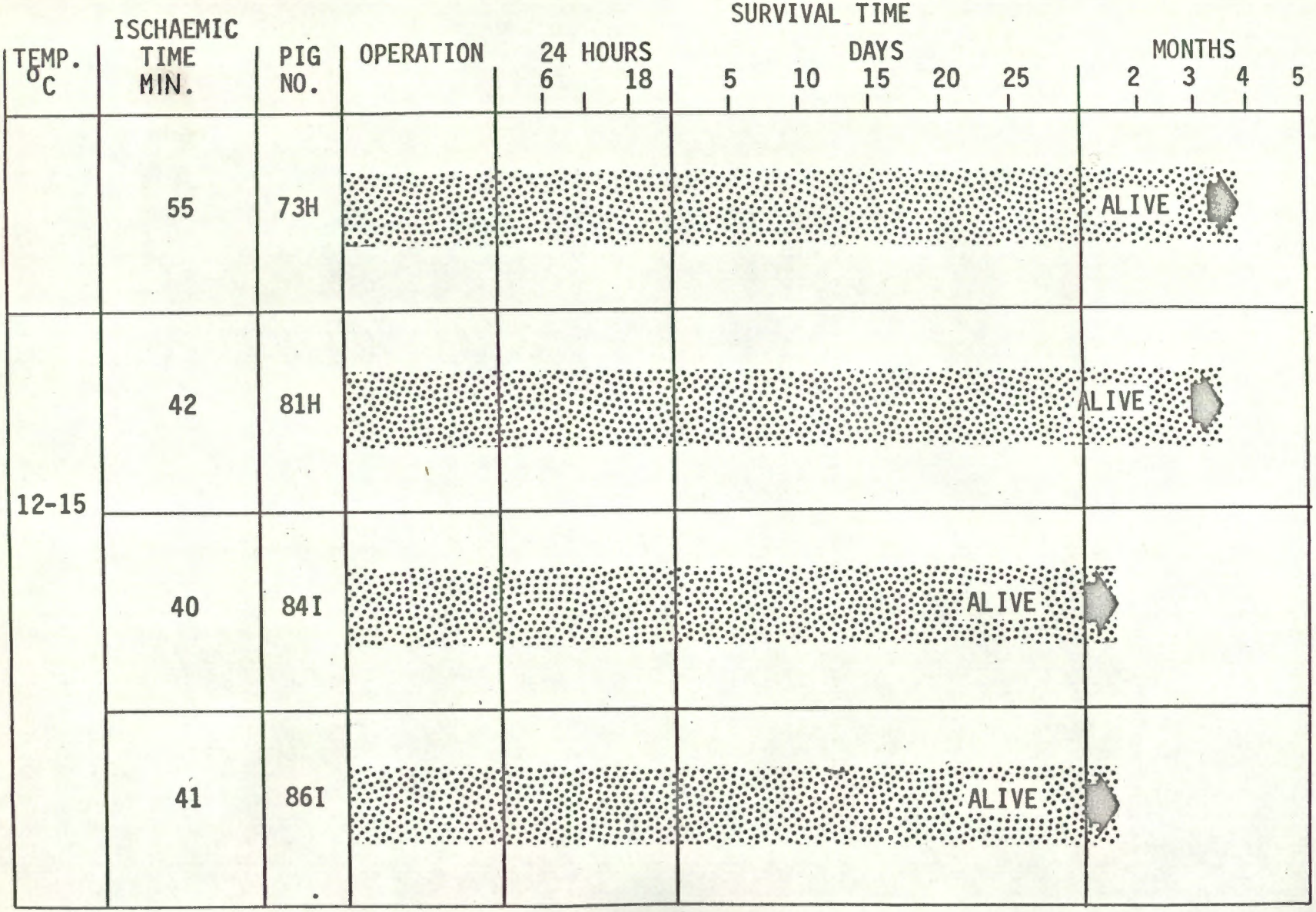
GROUP I (TABLE 2)

IMMEDIATE LIVER TRANSPLANTATION

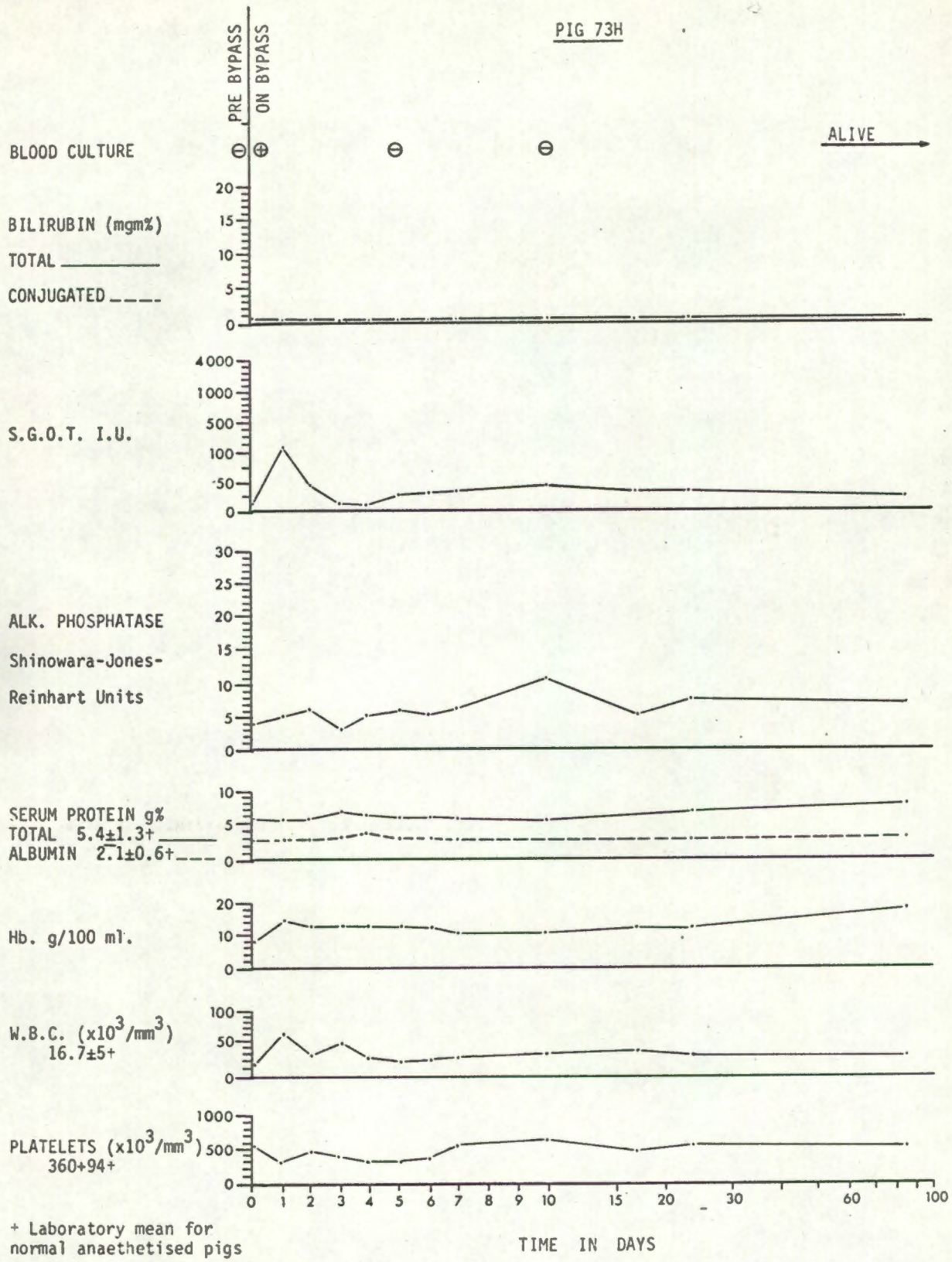
All four animals are alive and thriving 6 weeks to 4 months post transplantation.

TABLE 2

IMMEDIATE TRANSPLANT



	DONOR	RECIPIENT
	Landrace	Landrace
	15.9 Kg.	18.1 Kg.
	Female	Female
ISCHAEMIC TIME		55 minutes
DURATION OF SURVIVAL		Alive and thriving 4 months post transplantation
POSTOPERATIVE COURSE		Uncomplicated.
BIOCHEMISTRY		Except for the initial elevation of the S.G.O.T. and alkaline phosphatase, there were no significant biochemical changes.



+ Laboratory mean for normal anaethetised pigs

LIVER BIOPSY 2 MONTHS POST TRANSPLANTATION

(Fig. 3)

Shows minimal evidence of rejection.

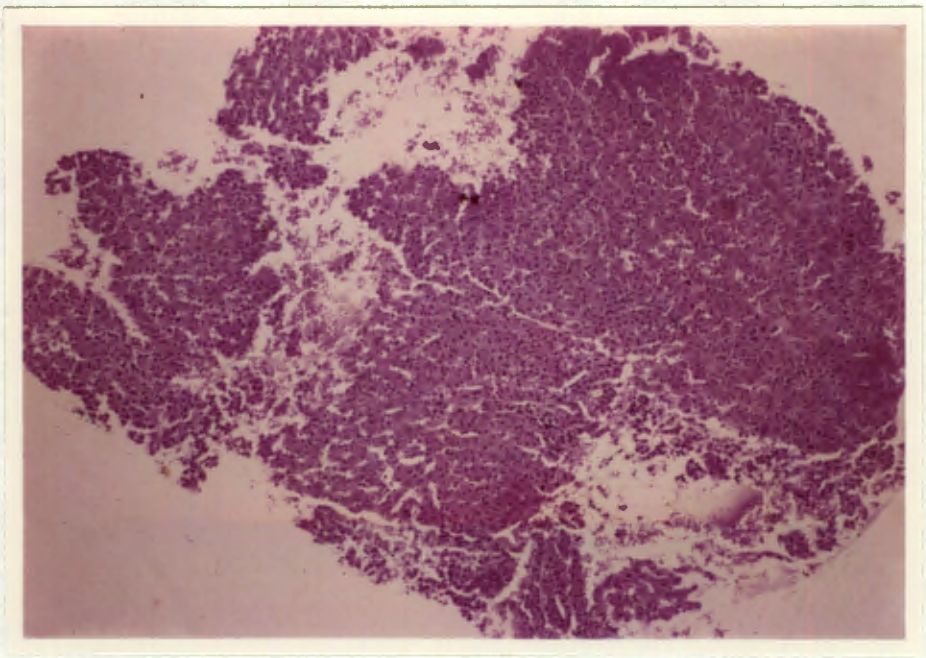
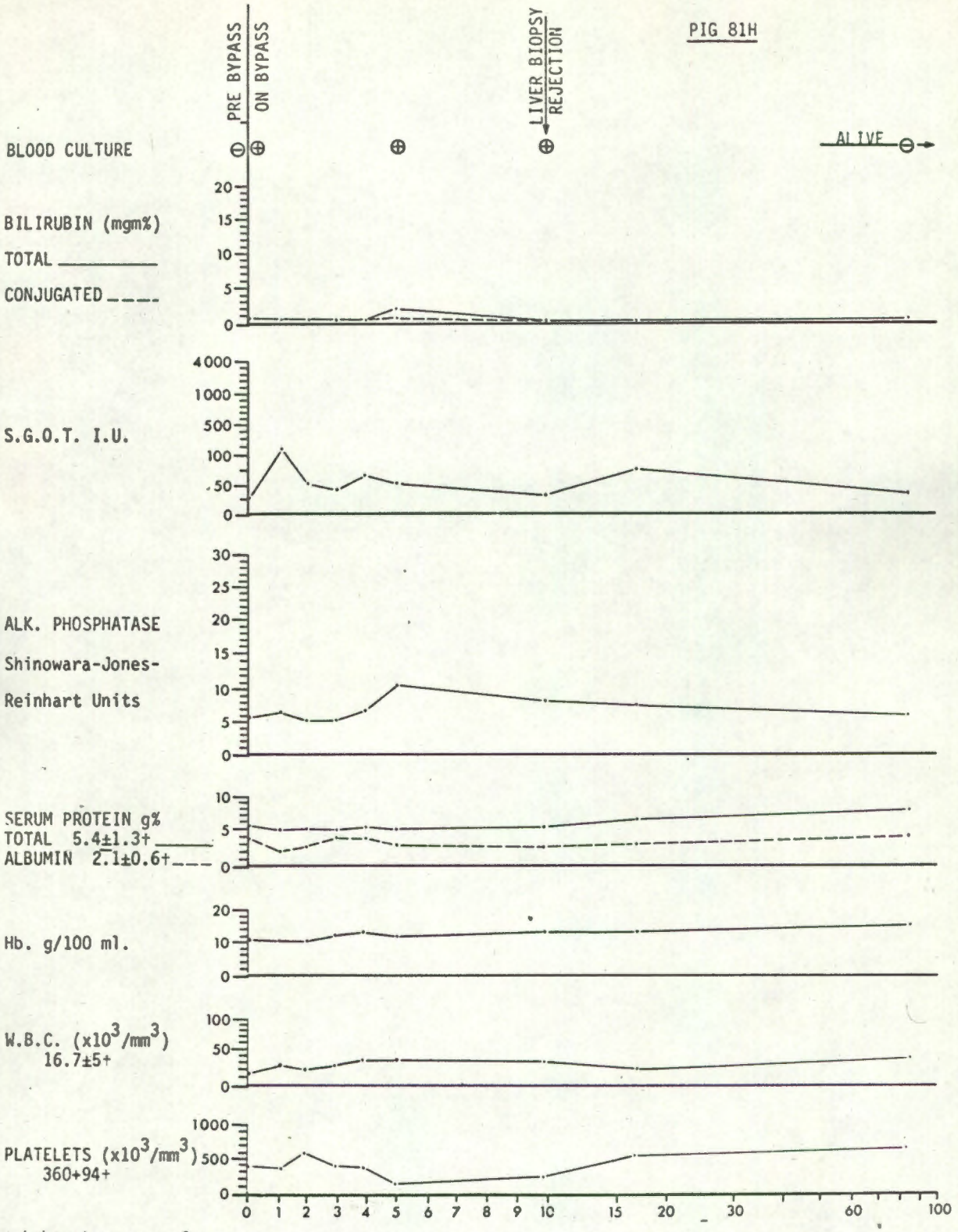


Fig. (3) Mag. X 25

	DONOR	RECIPIENT
	Landrace	Landrace
	22.7 Kg.	24.9 Kg.
	Female	Female
ISCHAEMIC TIME		42 Minutes
DURATION OF SURVIVAL		Alive and thriving 4 months post transplantation.
POST OPERATIVE COURSE		A transient episode of rejection occurred during the first and second week which remitted spontaneously.
BIOCHEMISTRY		There was minimal elevation of the S.G.O.T. and alkaline phosphatase following transplantation which returned to normal on the second day. This was followed by elevation of serum bilirubin, alkaline phosphatase and S.G.O.T. between the 4th and 17th day.
HAEMATOLOGY		Thrombocytopenia and leucocytosis coincided with the episode of rejection.

PIG 81H



+ Laboratory mean for normal anaethetised pigs

LIVER BIOPSY - 10TH DAY (Fig. 4)

There was round cell infiltration in the portal tracts which extended into the sinusoids. Areas of focal necrosis were also present. These changes were in keeping with the clinical and biochemical findings of rejection.

LIVER BIOPSY - 2.5 MONTHS POST TRANSPLANTATION (Fig. 5)

Showed a more prominent round cell infiltration in the portal tracts and incipient vascular changes of rejection with prominent vascular endothelium and endarteritis. The Kupffer cells were also prominent. These changes were not associated with any biochemical evidence of disturbed liver function.

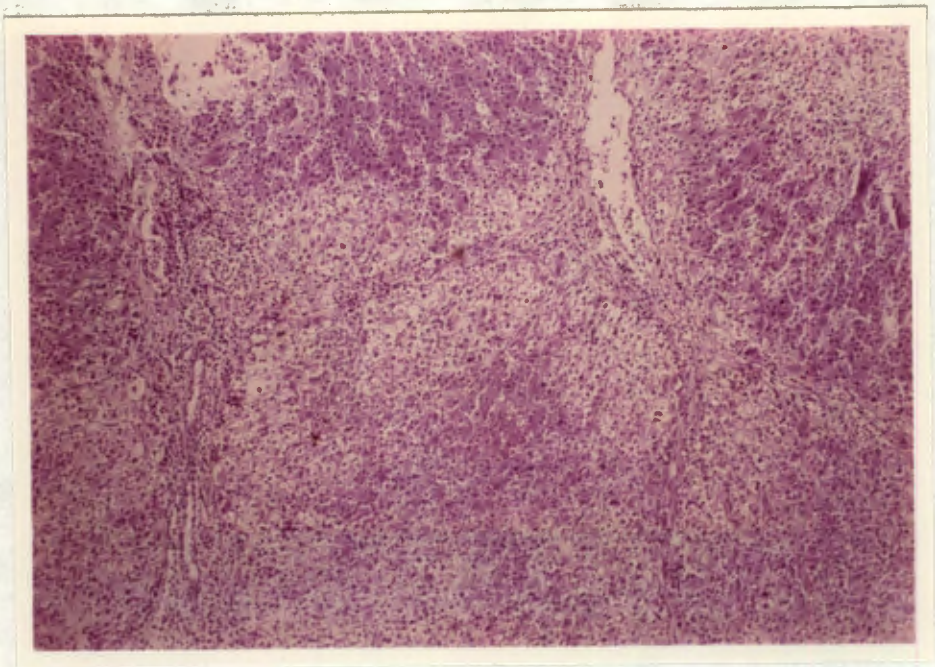


Fig. 4 Mag. X 25

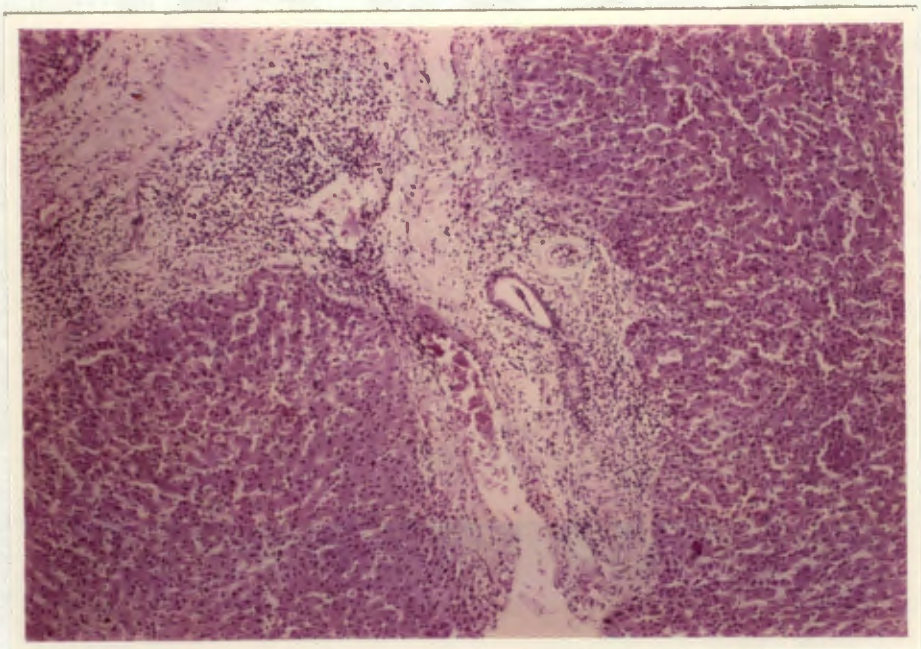
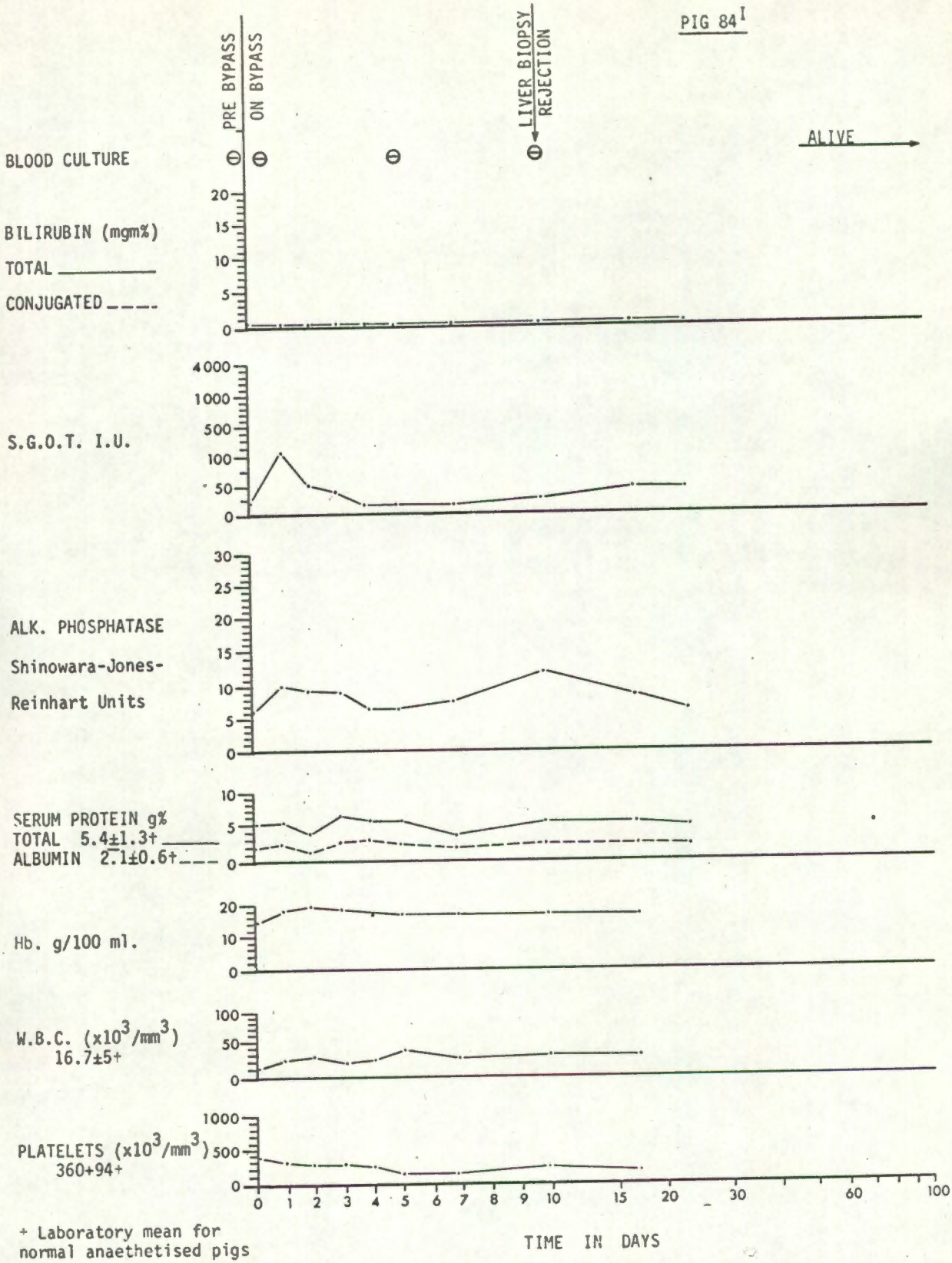


Fig. 5 Mag. X 25

	DONOR	RECIPIENT
	Landrace	Landrace
	15.9 Kg.	20.4 Kg.
	Male	Female.
ISCHAEMIC TIME		40 minutes
DURATION OF SURVIVAL		Alive and thriving 6 weeks post transplantation.
POST OPERATIVE COURSE		Was uncomplicated and this animal is alive and well 6 weeks following transplantation.
BIOCHEMISTRY		The initial post operative elevation of S.G.O.T. and alkaline phosphatase returned to normal levels by the 4th day. During the 2nd week a slight elevation of S.G.O.T. and a more marked elevation of the alkaline phosphatase occurred.
HAEMATOLOGY		The platelet count remained depressed during the 2nd week.

PIG 84[I]



+ Laboratory mean for normal anaethetised pigs

LIVER BIOPSY - 10TH DAY (Fig. 6)

There was a prominent round cell infiltration in the portal tracts. The sinusoids were widened and contained round cells and red blood cells but there was minimal evidence of hepatocyte necrosis. The histology suggests moderate rejection.

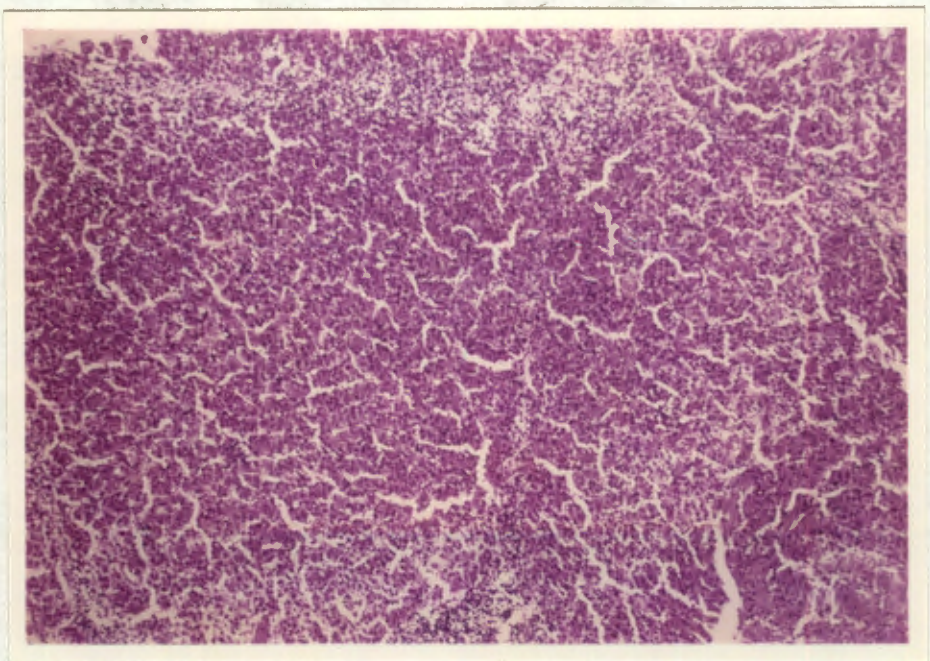
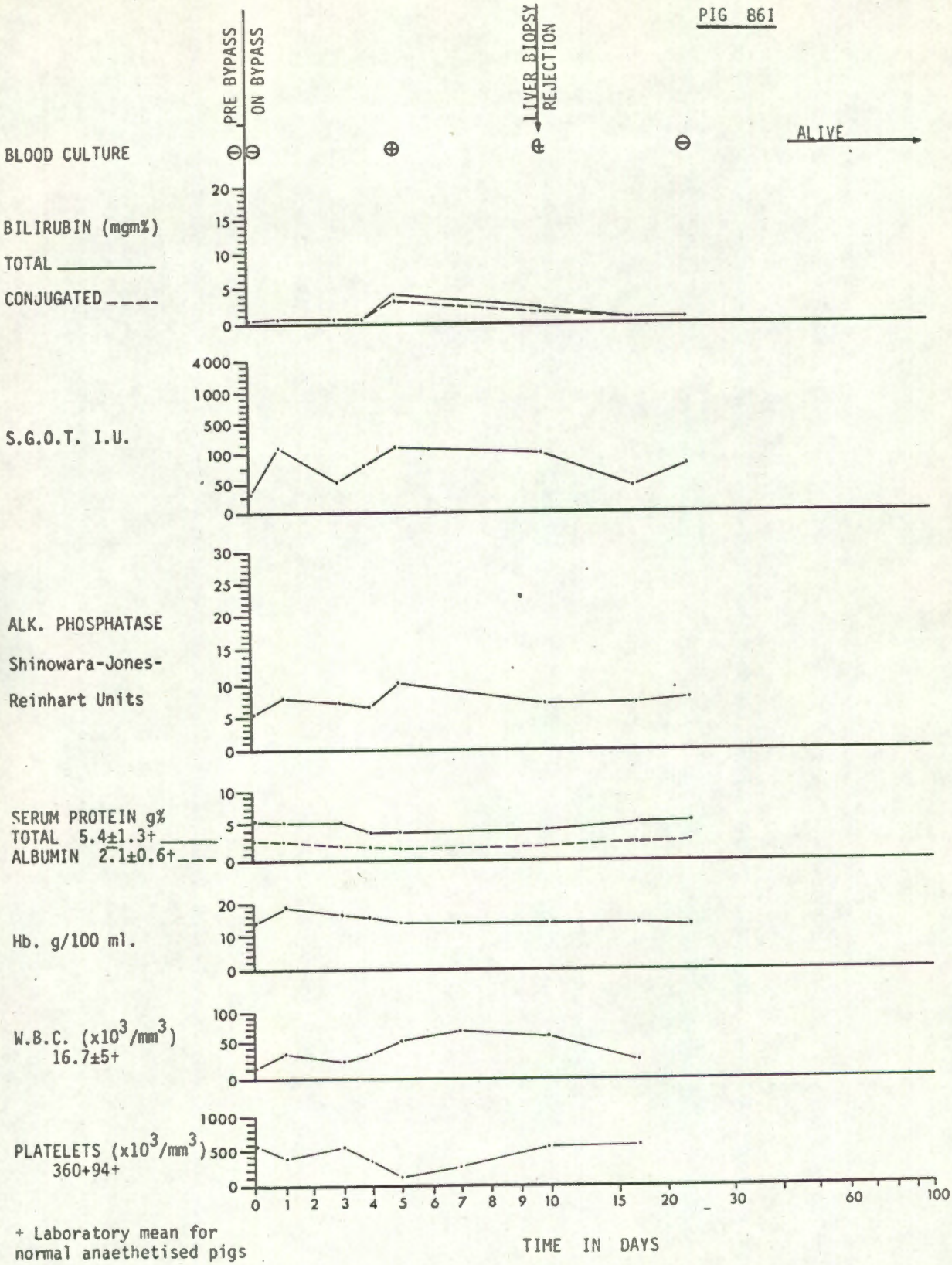


Fig. 6 Mag. X 25

	DONOR	RECIPIENT
	Landrace	Landrace
	15.9 Kg.	22.7 Kg.
	Male	Female
ISCHAEMIC TIME		40 Minutes
DURATION OF SURVIVAL		Alive and thriving 6 weeks post transplantation.
POST OPERATIVE COURSE		Uncomplicated until the 5th day then anorexic and jaundiced. This remitted spontaneously by the end of the 2nd week.
BIOCHEMISTRY		The S.G.O.T. and alkaline phosphatase were initially elevated; these returned to normal levels by the 3rd day. On the 5th day, a significant rise in serum bilirubin, S.G.O.T. and alkaline phosphatase occurred which gradually returned to normal levels by the middle of the 3rd week.
HAEMATOLOGY		Thrombocytopenia and leucocytosis coincided with the clinical and biochemical evidence of rejection.

PIG 86I



+ Laboratory mean for normal anaethetised pigs

LIVER BIOPSY -10TH DAY (Figs. 7 & 8)

Showed a profuse round cell infiltration of the portal tracts which extended into the sinusoids with focal areas of hepatocyte necrosis. The most striking features of the section however, were the numerous giant cells and mitotic figures indicating simultaneous regeneration. The histology was in keeping with the clinical and biochemical evidence of rejection.

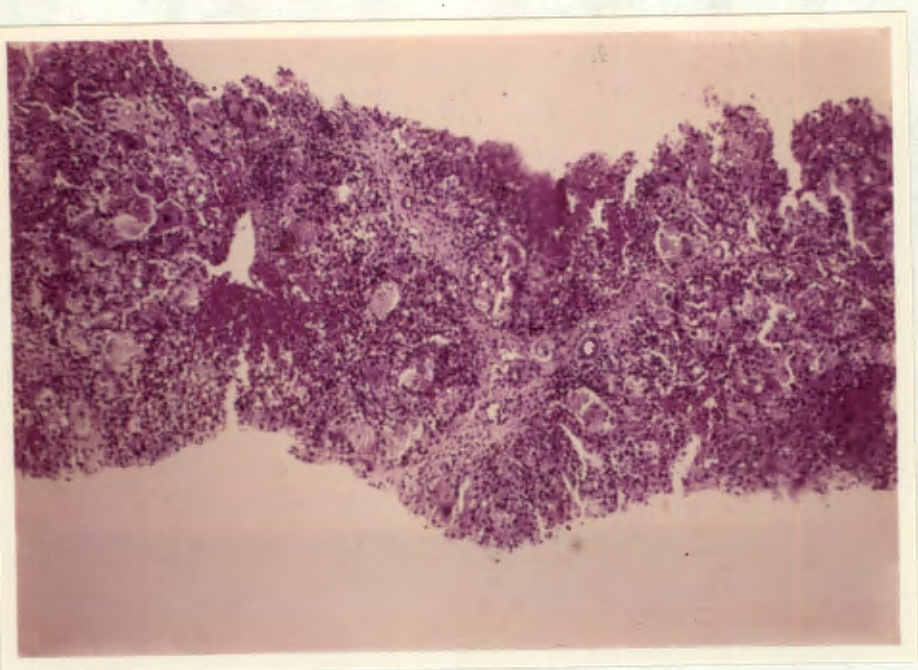


Fig. 7 Mag. X 25

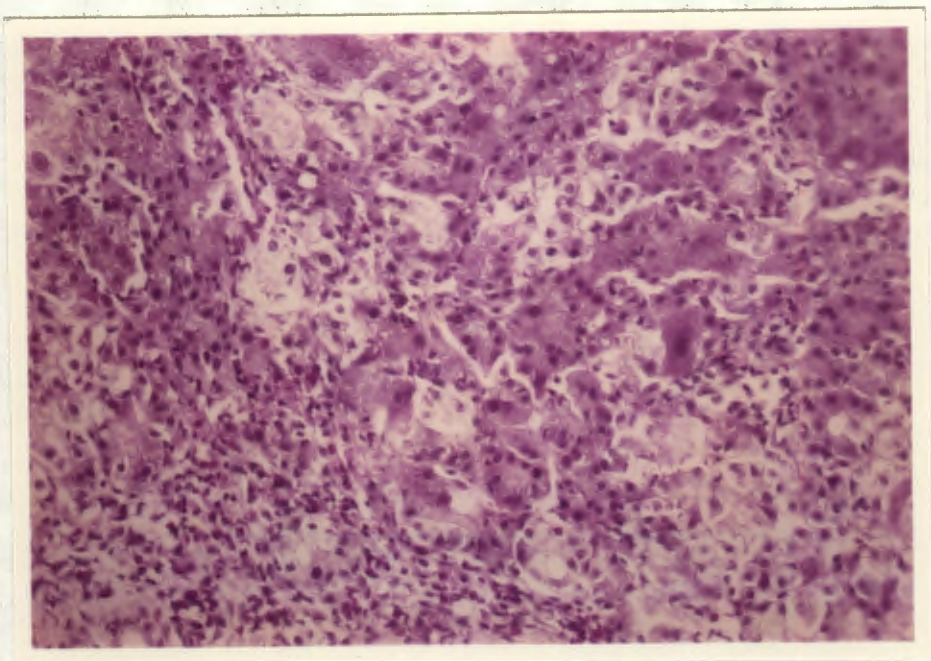


Fig. 8 Mag. X 64

DISCUSSION

The clinical course followed by these four animals was similar to that found in other studies (Calne et al. 1968a; Terblanche et al. 1968b; Cordier et al. 1966; Starzl and Putnam 1969; Huguet et al. 1970; Dent et al. 1971) and confirmed that the method used for orthotopic liver transplantation under ideal laboratory conditions was compatible with a protracted and healthful survival.

GROUP II

IMMERSION STORAGE

GROUP II - IMMERSION STORAGE (TABLE 3)

1. RINGER LACTATE SOLUTION 6 - 8 hours at $8^{o}C$ - $12^{o}C$

All the animals survived the operation ; but none lived beyond 48 hours. All died from a generalised bleeding diathesis.

2. SOLUTION II 6 - 8 hours at $18^{o}C$ - $20^{o}C$

Two of the animals failed to survive the operation and the third died shortly after its completion. All three had a generalised bleeding diathesis.

3. SOLUTION II 10 hours at $8^{o}C$ - $12^{o}C$

Only one of four animals survived and is alive and well 7 months post transplantation. One death was due to a technical error (tear in the liver). Of the remaining animals, one developed a massive ascites and died on the 3rd day and the other a generalised bleeding diathesis.

4. SOLUTION II 12 hours at $8^{o}C$ - $12^{o}C$

Two animals died from a bleeding diathesis and massive ascites was found in the third.

5. SOLUTION II 12 hours at $2^{o}C$ - $5^{o}C$

Two animals did not survive the operation. All three died from a generalised bleeding diathesis.

6. PRETREATMENT WITH CELL MEMBRANE STABILISING DRUGS AND PRESERVATION WITH SOLUTION III 10 hours at 8 - $12^{o}C$

All three animals died from a generalised bleeding diathesis shortly after completing the operation.

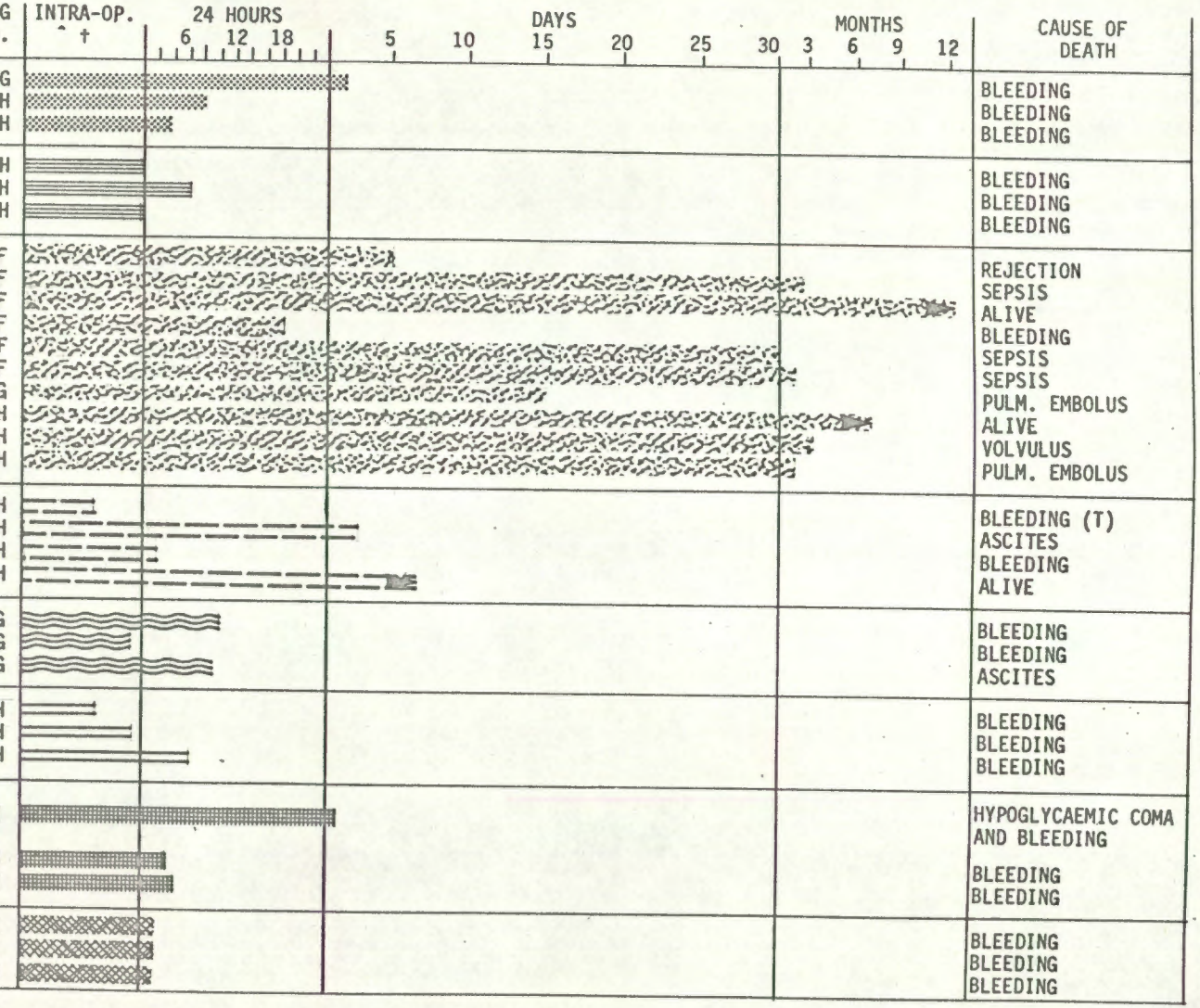
7. SOLUTION IV 10 hours at $8^{o}C$ - $12^{o}C$

All three animals survived the operation but died shortly afterwards from a generalised bleeding diathesis.

TABLE 3

IMMERSION STORAGE

STORAGE SOLUTION	TEMP. °C	ISCHAEMIC TIME HRS.	PIG NO.	SURVIVAL TIME	CAUSE OF DEATH
RINGER LACTATE	8-12	6 - 8	79G	INTRA-OP.	BLEEDING
			3H	24 HOURS	BLEEDING
			14H		BLEEDING
SOLUTION II	18-20	6 - 8	34H		BLEEDING
			38H		BLEEDING
			50H		BLEEDING
SOLUTION II	8-12	6 - 8	43F		REJECTION
			21F		SEPSIS
			56F		ALIVE
			66F		BLEEDING
			86F		SEPSIS
			63F		SEPSIS
			76G		PULM. EMBOLUS
			12H		ALIVE
			24H		VOLVULUS
			28H		PULM. EMBOLUS
SOLUTION II	8-12	10	5H		BLEEDING (T)
			10H		ASCITES
			20H		BLEEDING
			17H		ALIVE
SOLUTION II	8-12	12	65G		BLEEDING
			68G		BLEEDING
			70G		ASCITES
SOLUTION II	2-5	12	31H		BLEEDING
			47H		BLEEDING
			62H		BLEEDING
CELL MEMBRANE STABILISERS + SOLUTION III	8-12	10	38I		HYPOGLYCAEMIC COMA AND BLEEDING
			42I		BLEEDING
			46I		BLEEDING
SOLUTION IV	8-12	10	26I		BLEEDING
			31I		BLEEDING
			35I		BLEEDING



^ PORTAL VEIN REVASCULARISATION
† HEPATIC ARTERY REVASCULARISATION
(T) TECHNICAL

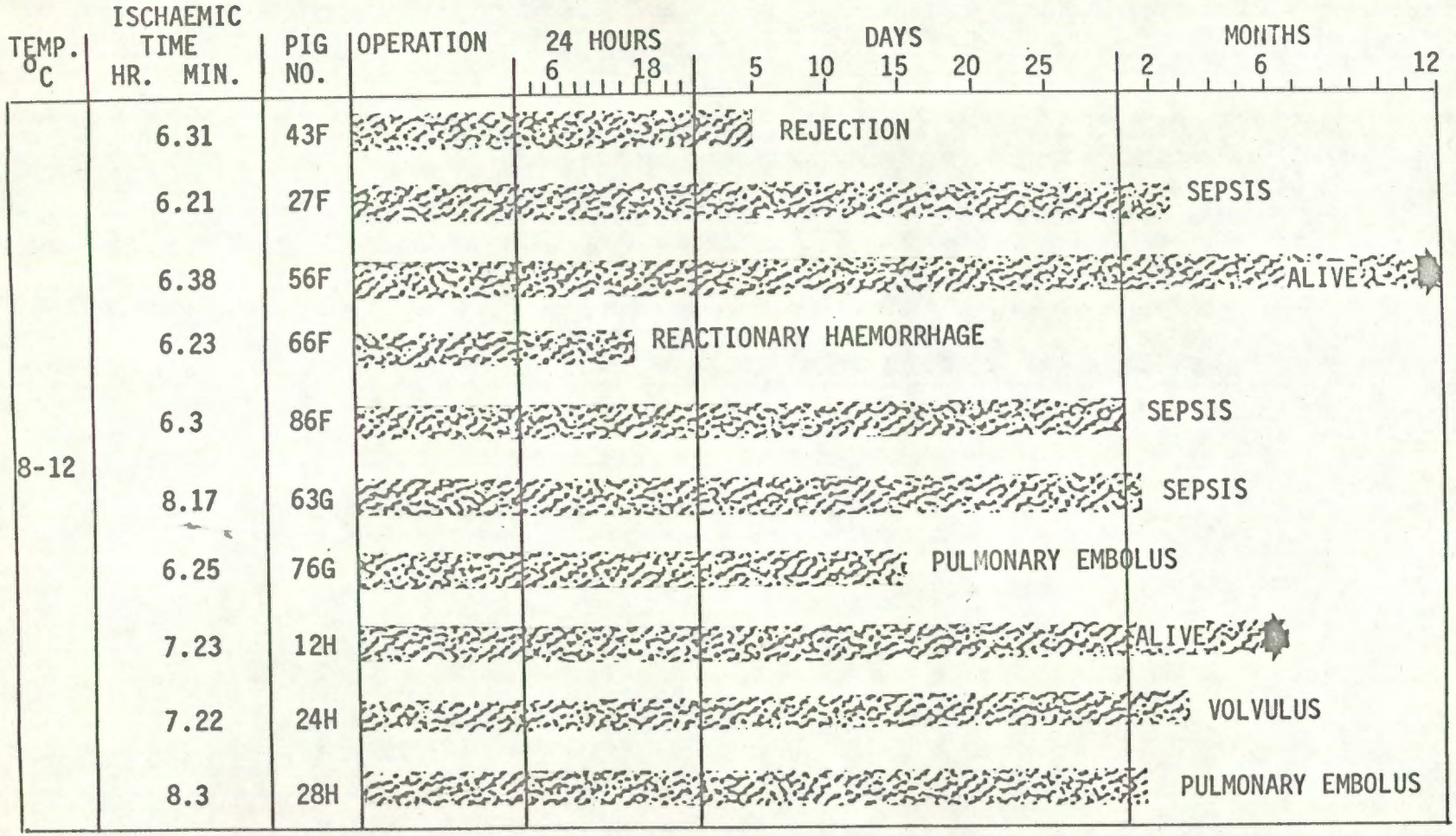
GROUP II - Immersion Storage SOLUTION II 6 - 8 Hours at 8 - 12°C

(TABLE 4)

In this group of experiments, nine of ten animals survived 5 or more days. Two of the animals are still alive one year and seven months respectively after transplantation.

TABLE 4

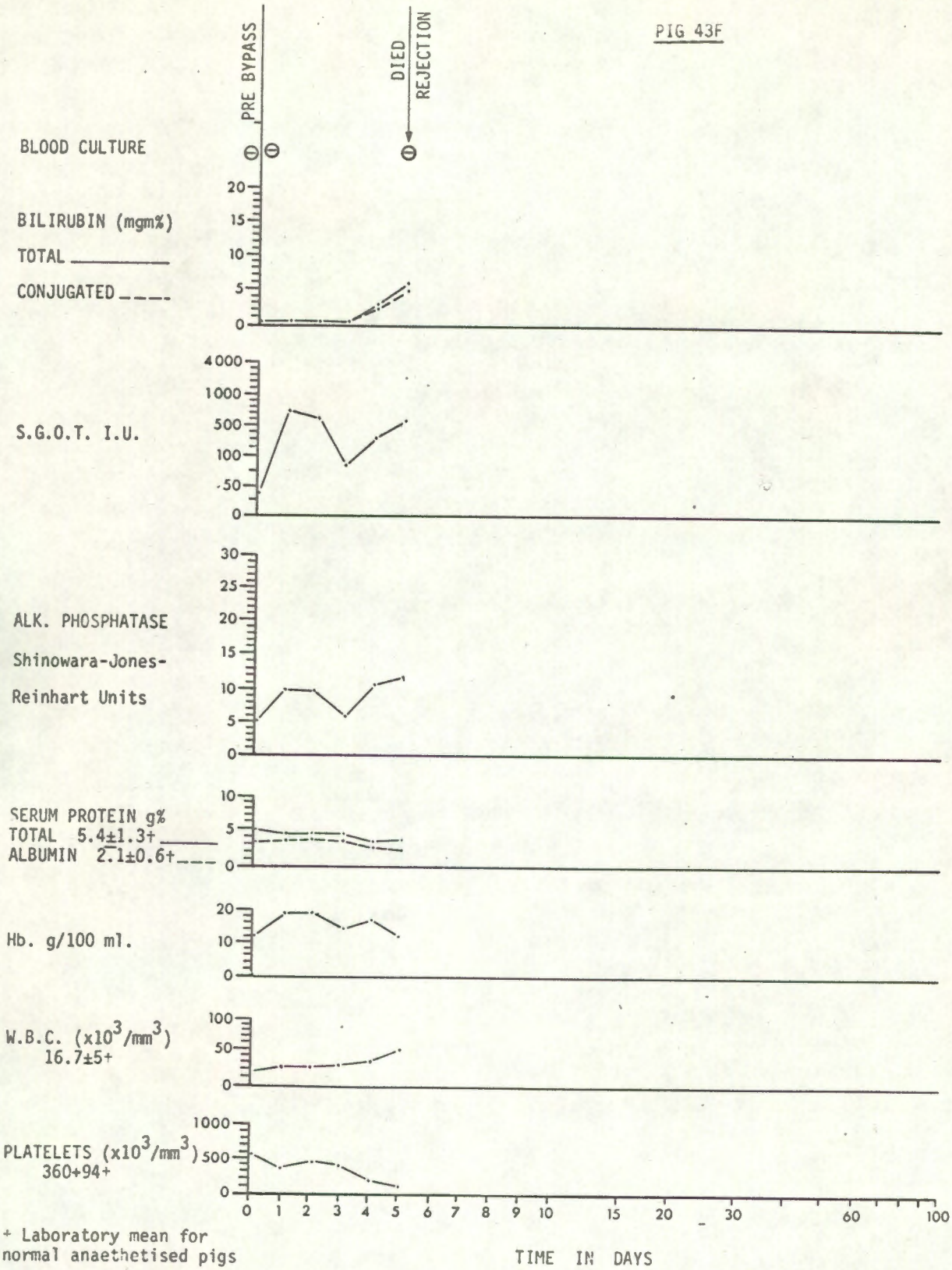
HYPOTHERMIC IMMERSION STORAGE (SOLUTION II*)



* 400 ml RECONSTITUTED A.C.D. FRESH FROZEN PIG PLASMA
80 ml 5% DEXTROSE WATER
20 ml 4.2% SOD. BIC.
10 mgm HEPARIN
1 Mega u PENICILIN: 1 g STREPTOMYCIN

	DONOR	RECIPIENT
	Landrace/Large White	Landrace
	20.4 Kg.	24.9 Kg.
	Female	Female
ISCHAEMIC TIME		6 Hours 31 Minutes
DURATION OF SURVIVAL		5 Days
POST OPERATIVE COURSE		Uncomplicated until the 4th day. The animal then became anorexic and jaundiced and by the following day the jaundice had deepened and was associated with hypoglycaemia (dextrostix less than 40 mgm %). Later in the day the animal lapsed into a coma, became markedly acidotic and died.
BIOCHEMISTRY		The immediate post operative levels of the S.G.O.T. and alkaline phosphatase were higher than in the controls. These returned to normal by the third day. Terminally there was hyperbilirubinaemia with elevated S.G.O.T. and alkaline phosphatase.
HAEMATOLOGY		Thrombocytopenia and leucocytosis coincided with the clinical and biochemical changes.
POSTMORTEM		The peritoneal cavity contained 200 ml. of ascitic fluid with a protein content of 0.5 Gm/100 ml. There was a small straw coloured effusion in the right pleural cavity. The liver macroscopically was normal.

PIG 43F



+ Laboratory mean for normal anaethetised pigs

POSTMORTEM HISTOLOGY OF LIVER (Fig. 9)

There was extensive cellular necrosis of hepatocytes with haemorrhage, mononuclear cell infiltration into the lobule and prominence of the Kupffer cells. The portal tracts were infiltrated with mononuclear cells and the wall of some vessels were oedematous with round cells lying between the endothelial lining and the vessel wall. These changes were in keeping with severe rejection.

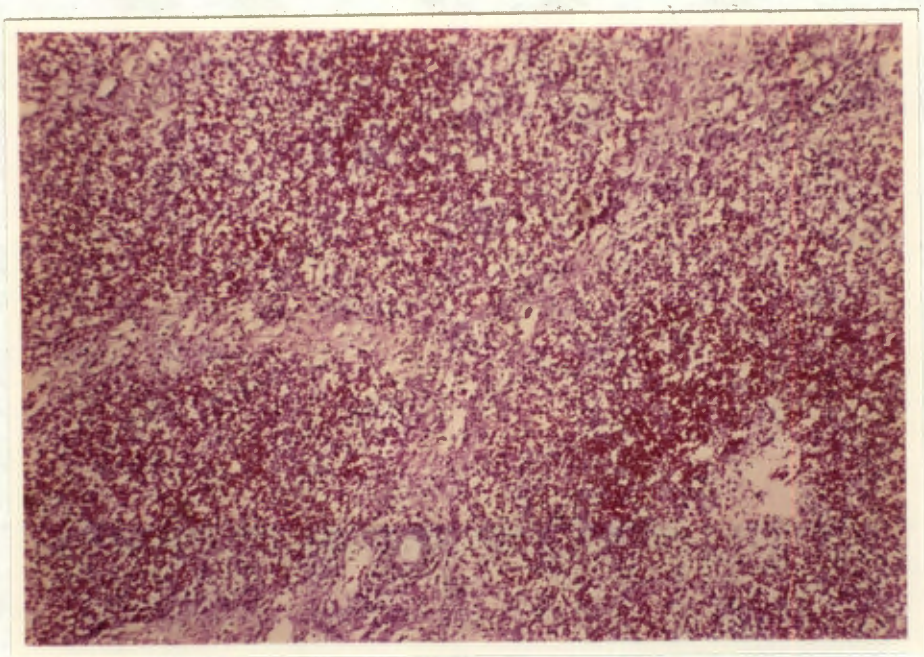
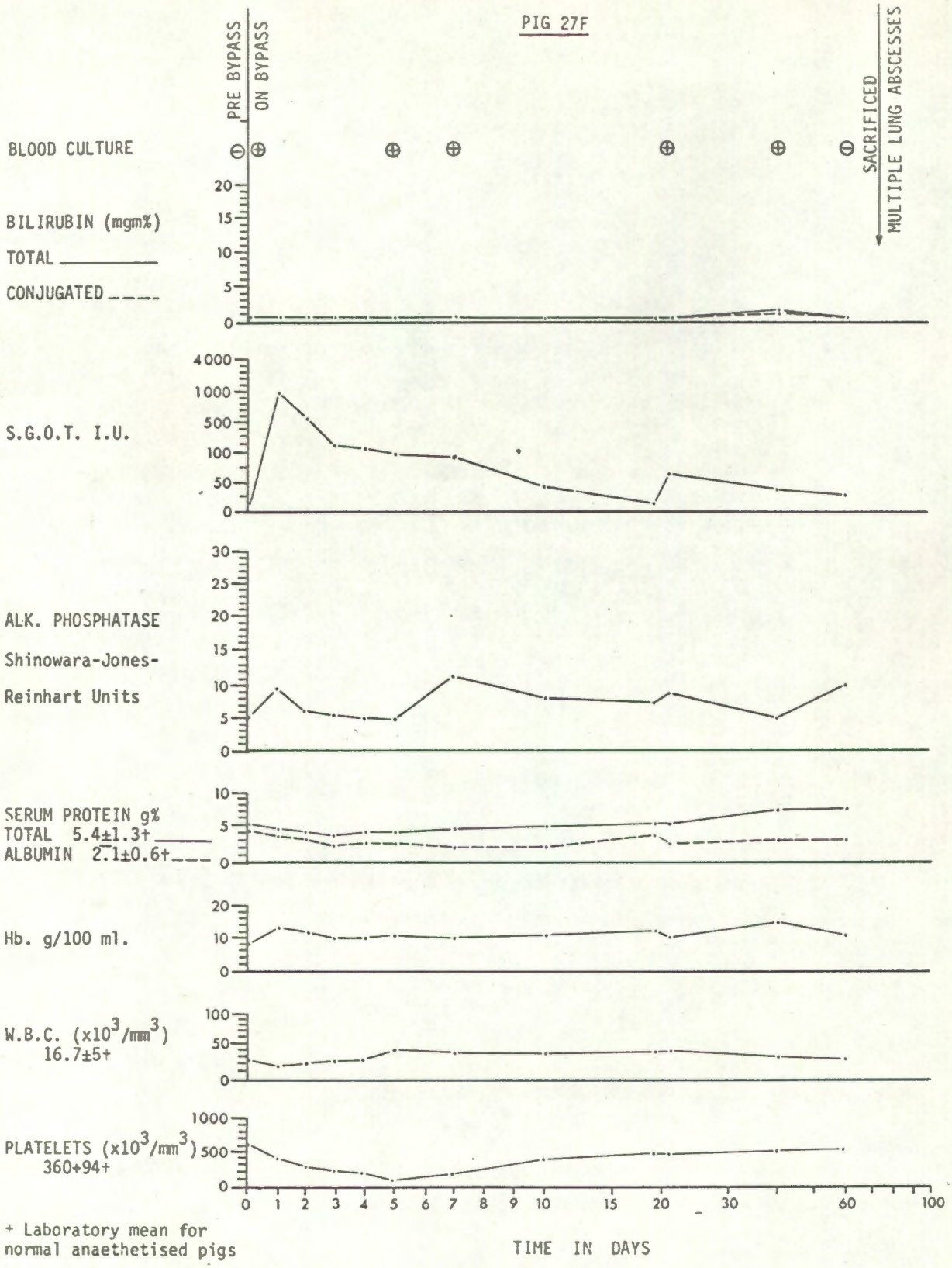


Fig. 9 Mag. x 25

	DONOR	RECIPIENT
	Landrace	Landrace/Large White
	19 Kg.	22.7 Kg.
	Male	Male
ISCHAEMIC TIME		6 Hours 21 Minutes
DURATION OF SURVIVAL		74 Days (sacrificed)
INTRA OPERATIVE COURSE		A spontaneous right sided pneumothorax occurred intra operatively and was decompressed with an underwater chest drain which was removed at the end of the operation.
POST OPERATIVE COURSE		Uncomplicated until the 3rd post operative week. The animal then became pneumonic and responded to a course of antibiotics but relapsed a few weeks later. Thereafter its general condition deteriorated and it was therefore sacrificed.
BIOCHEMISTRY		The initial levels of the S.G.O.T. and alkaline phosphatase were again higher than in the controls. The changes during the 5th to 10th day were suggestive of rejection but thereafter were rather non specific.
HAEMATOLOGY		Thrombocytopenia and leucocytosis were present on the 5th day.
BLOOD CULTURES		Were positive throughout the post operative course.
POSTMORTEM		Both lungs contained multiple lung abscesses. The liver macroscopically was normal.



+ Laboratory mean for normal anaethetised pigs

POSTMORTEM LIVER HISTOLOGY (Fig. 10)

The hepatocytes were normal in appearance though there was some atrophy of the lobule. The portal tracts were not thickened and there was minimal round cell infiltration.

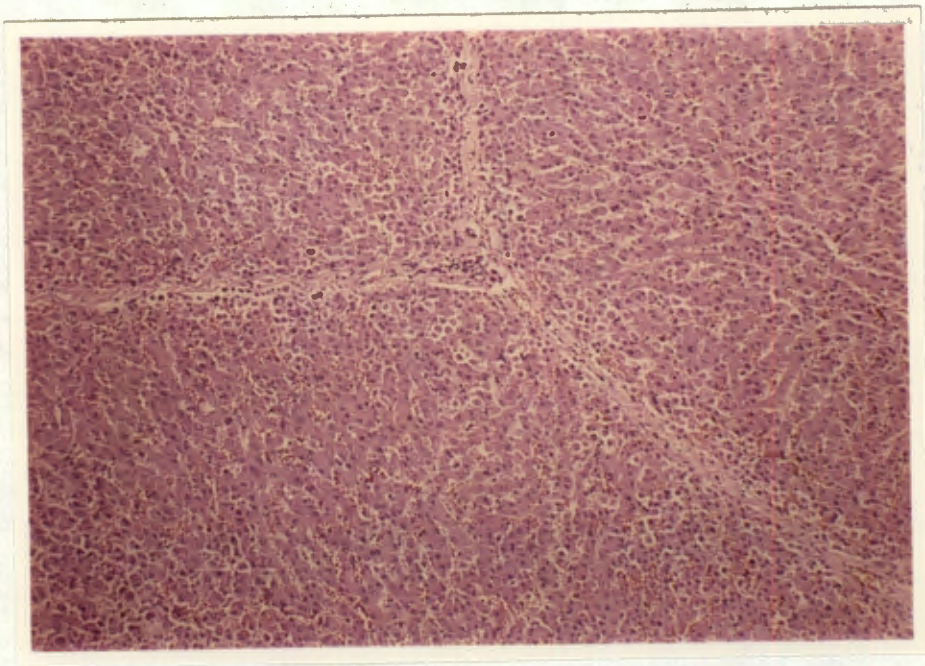
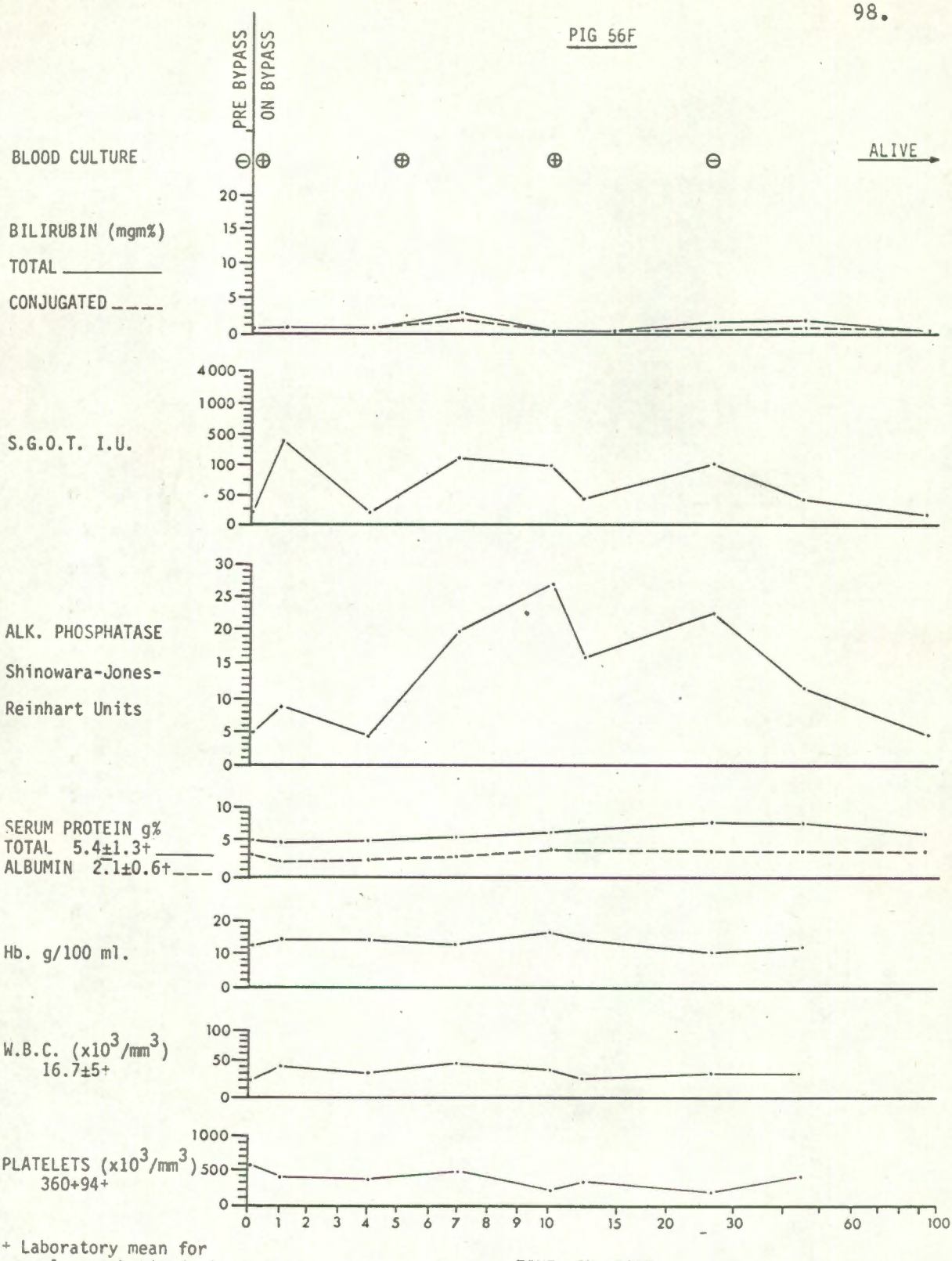


Fig. 10 Mag. x 25

	DONOR	RECIPIENT
	Landrace/Large White	Landrace
	25.8 Kg.	33 Kg.
	Female	Male
ISCHAEMIC TIME		6 Hours 38 Minutes
DURATION OF SURVIVAL		Alive and thriving 12 months post transplantation.
POST OPERATIVE COURSE		Two transient episodes of jaundice occurred during the 2nd and 3rd weeks. Thereafter, the animal has remained well. The present weight is estimated to be $\pm$ 150 kg.
BIOCHEMISTRY		The initial elevated levels of the S.G.O.T. and alkaline phosphatase returned to normal by the 4th day. During the 2nd and 3rd weeks, elevation of the serum bilirubin, S.G.O.T. and alkaline phosphatase occurred but remitted spontaneously. Liver biopsies were not done to confirm whether these changes were due to rejection.
HAEMATOLOGY		Thrombocytopenia coincided with the changes in S.G.O.T. and alkaline phosphatase.

PIG 56F



+ Laboratory mean for normal anaethetised pigs

LIVER BIOPSY 4 MONTHS POST TRANSPLANTATION (Fig. 11)

Showed normal hepatocytes with minimal round cell infiltration into the portal tracts or liver sinusoids.

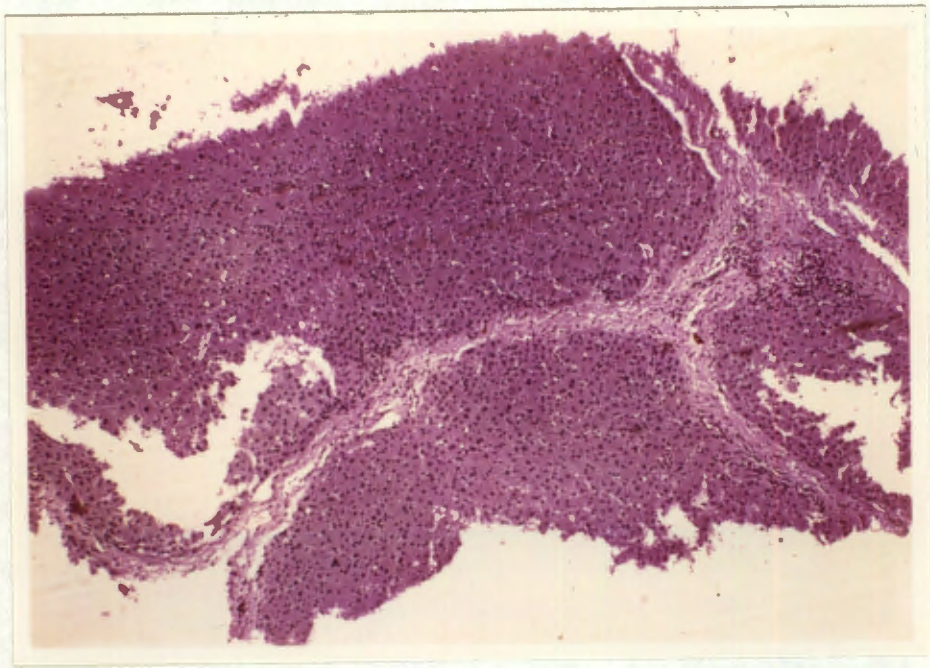


Fig. 11 Mag. x 25

	DONOR	RECIPIENT
	Landrace	Landrace
	21.3 Kg.	27.2 Kg.
	Male	Male
ISCHAEMIC TIME		6 Hours 23 Minutes
DURATION OF SURVIVAL		15 Hours
POST OPERATIVE COURSE		This animal was fully awake 10 minutes after completing the operation. The biochemistry 3 hours later showed serum bilirubin 0.7 mgm %, serum alkaline phosphatase 13.8, S.G.O.T. 456 I.U. and blood sugar 90 mgm %. The clotting time was 3 minutes. Fifteen hours post transplantation the animal was perfectly well and moving freely in its cage. It died suddenly 10 minutes later.
POSTMORTEM		The peritoneal cavity contained 700 ml. of fresh blood (Hb 8.6 Gm %); no specific bleeding point was found. The liver appeared macroscopically normal.
HISTOLOGY (Figs. 12 & 13)		There was evidence of some cellular damage with haemorrhage into the lobule but the striking feature was the degree of cellular regeneration occurring so soon after the injury.

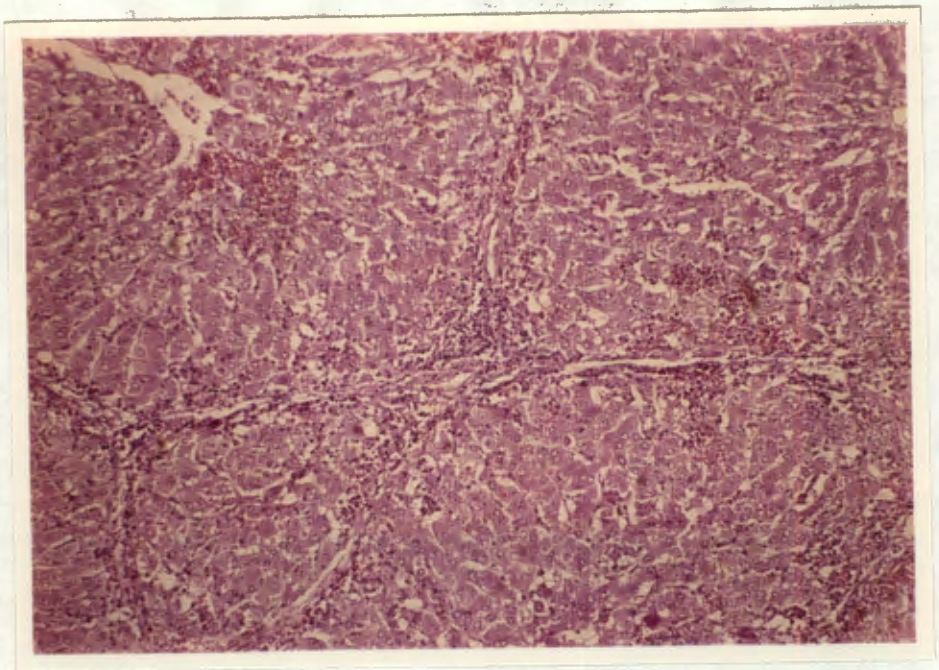


Fig. 12 Mag. x 25.

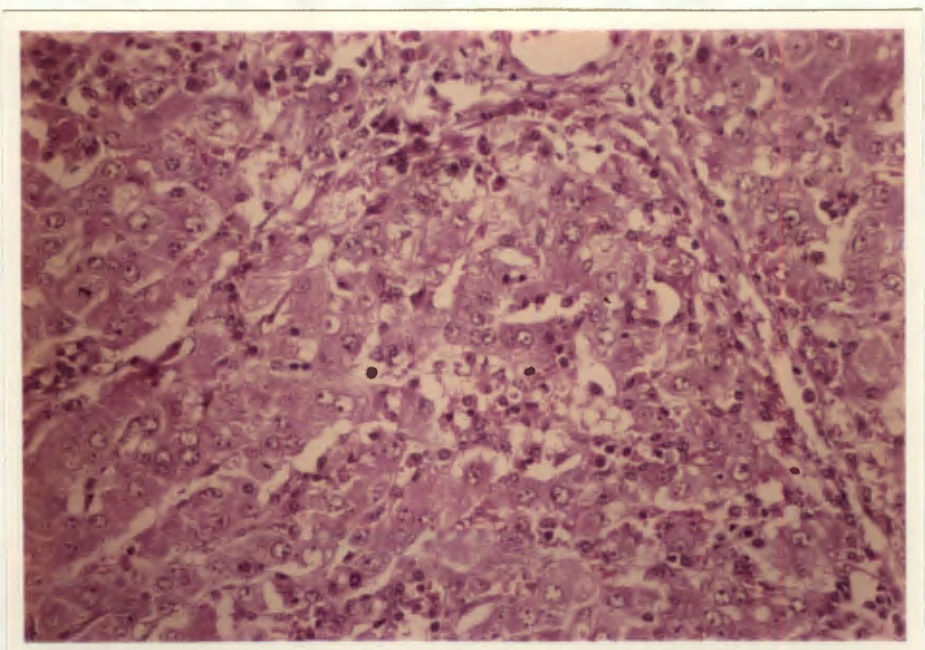
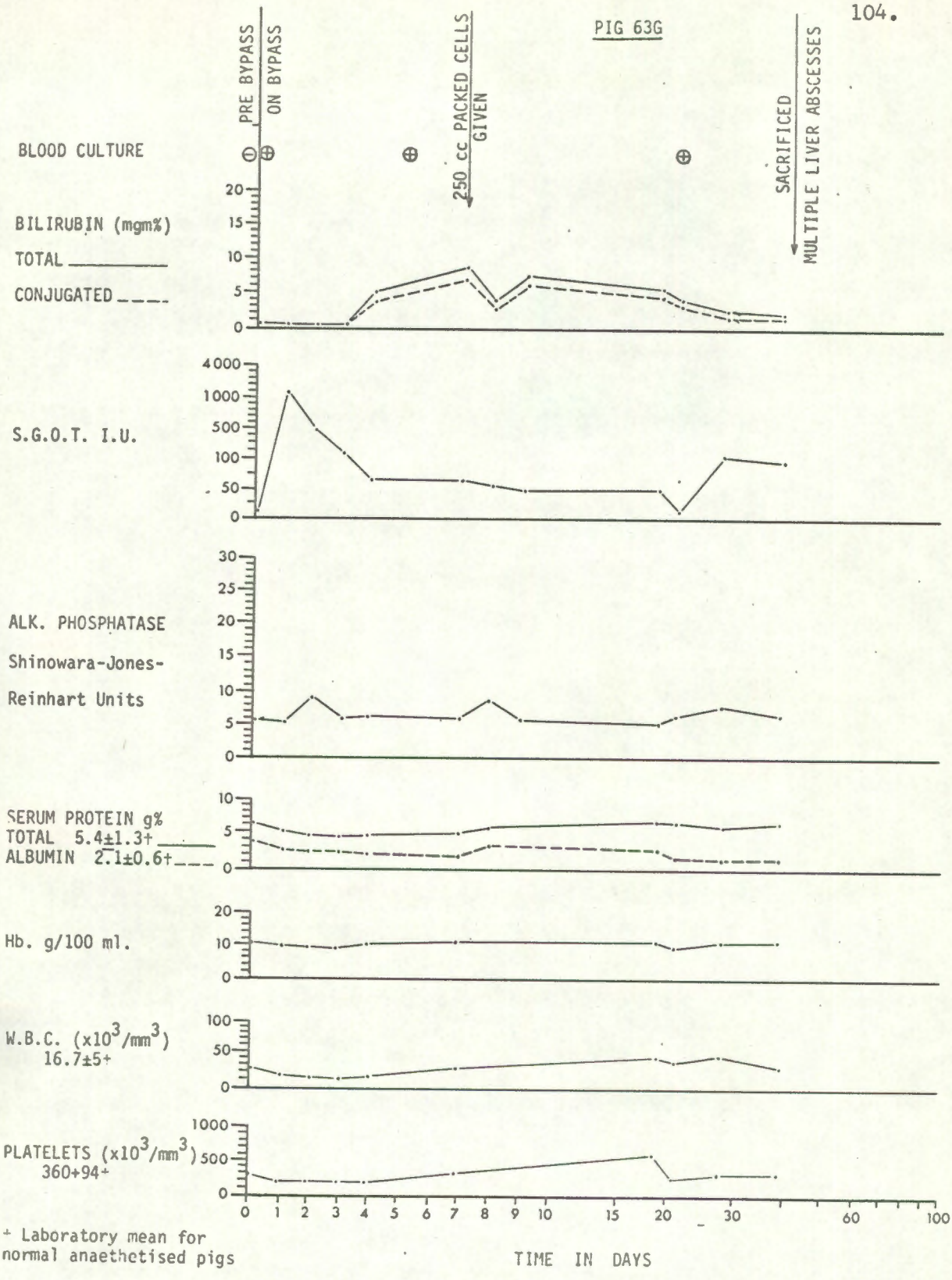


Fig. 13 Mag. x 64.

	DONOR	RECIPIENT
	Landrace/Large White	Landrace
	25.8 Kg.	27.2 Kg.
	Female	Female
ISCHAEMIC TIME		8 Hours 17 Minutes
DURATION OF SURVIVAL		38 Days (sacrificed)
POST OPERATIVE COURSE		Uncomplicated until the 5th day; therafter, anorexic and jaundiced associated with failure to thrive which persisted until sacrifice.
BIOCHEMISTRY		Following the return to normal levels of the S.G.O.T. and alkaline phosphatase by the 4th day, the serum bilirubin became elevated (predominantly conjugated) but the S.G.O.T. and alkaline phosphatase changes thereafter were non specific.
BACTERIOLOGY		Blood cultures were positive throughout the post operative course.
POSTMORTEM		The liver contained multiple abscesses in all the lobes from which a mixture of organisms (Beta strep., enterococci, proteus and coliforms) were cultured.



+ Laboratory mean for normal anaethetised pigs

LIVER BIOPSY - 10TH DAY (Fig. 14)

There was a moderate infiltrate of round cells into the portal tract and fatty vacuolation of the hepatocytes. Bile plugs were scattered throughout the lobule both intracellularly and in the sinusoids.

POSTMORTEM LIVER BIOPSY (Fig. 15)

Shows one of the liver abscesses.

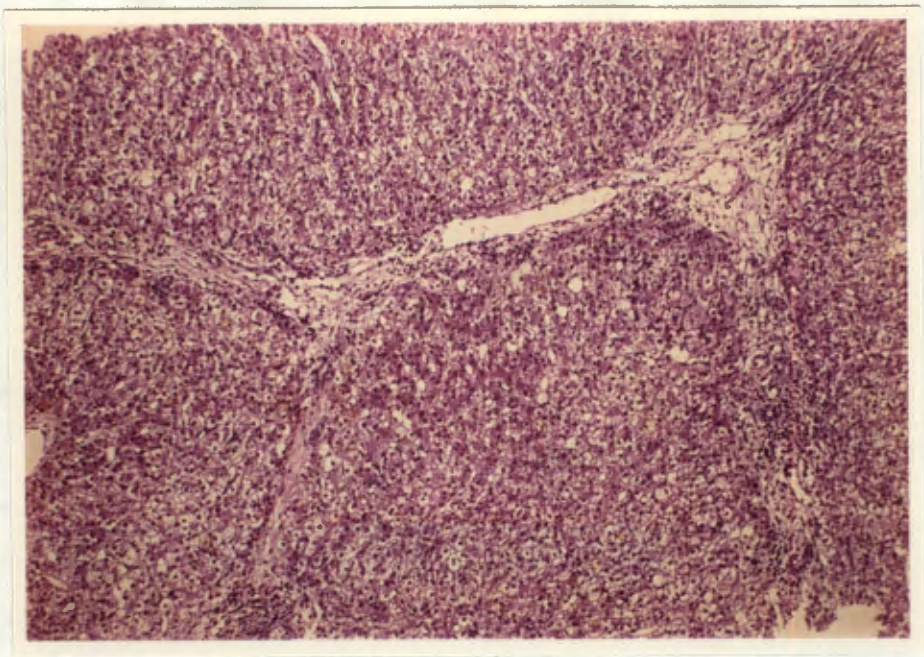


Fig. 14 Mag. x 25

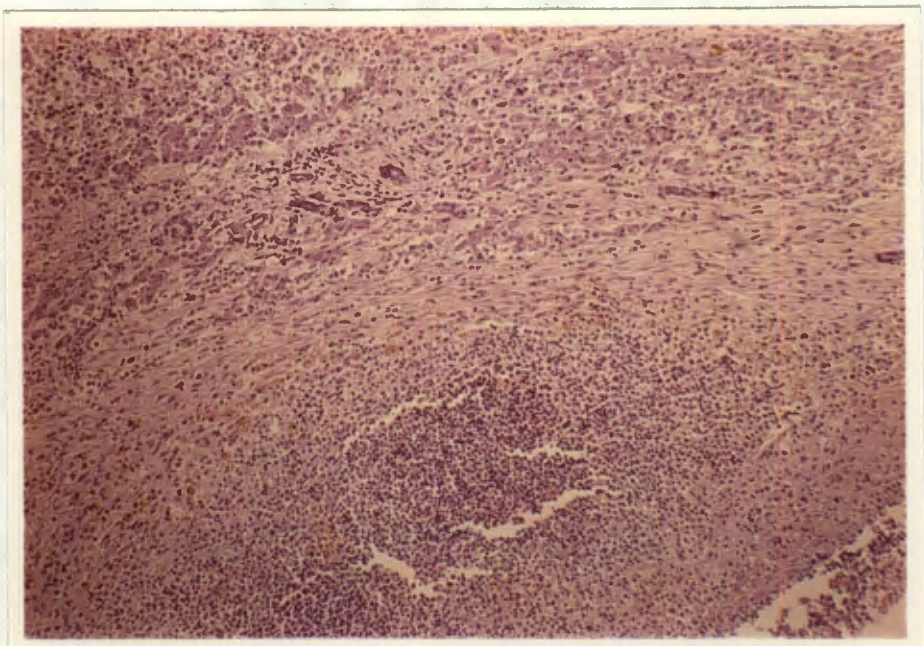
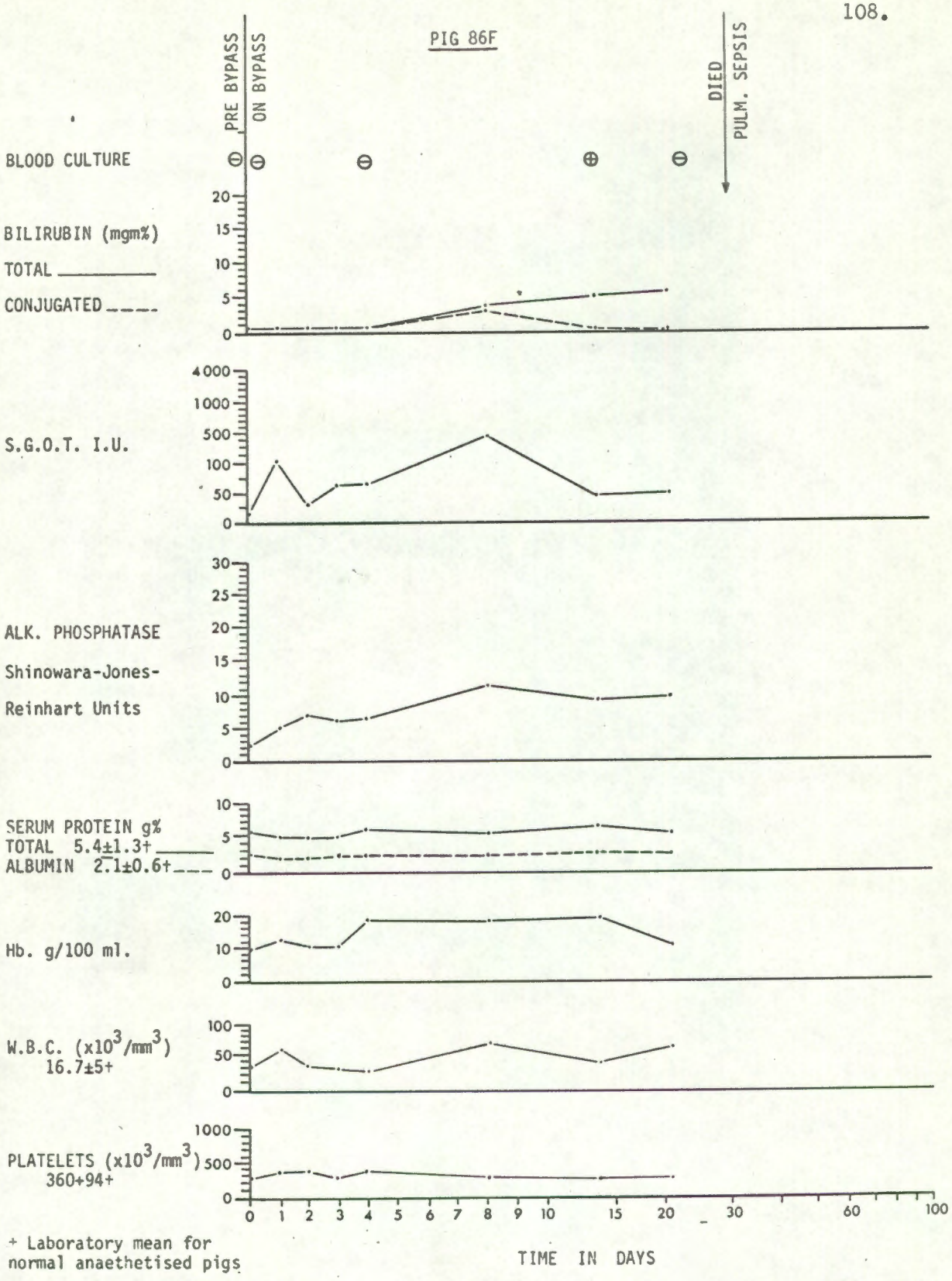


Fig. 15 Mag. x 25

	DONOR	RECIPIENT
	Landrace	Landrace
	19 Kg.	21.3 Kg.
	Male	Male
ISCHAEMIC TIME		6 Hours 3 Minutes
DURATION OF SURVIVAL		30 Days
POST OPERATIVE COURSE		Uncomplicated until the 8th day. Thereafter, anorexic, jaundiced and generally failed to thrive. Died on the 30th day post transplantation.
BIOCHEMISTRY		The S.G.O.T. and alkaline phosphatase were initially less elevated than previously encountered in this group. Between the 5th and 10th day, the raised serum bilirubin, S.G.O.T. and alkaline phosphatase were suggestive of rejection; but thereafter were more in keeping with a septicaemia.
BACTERIOLOGY		The blood culture became positive and remained so after the 2nd week.
POSTMORTEM		Both lungs contained multiple abscesses from which a species of klebsiella was cultured. A large septic thrombus (enterococci) was found in the superior vena cava. The liver was macroscopically normal.



+ Laboratory mean for normal anaethetised pigs

POSTMORTEM LIVER HISTOLOGY (Fig. 16)

The lobules contained numerous bile lakes, with infiltration by polymorphonuclear leucocytes and occasional foreign body giant cells. The sinusoids were congested with red blood cells and there was minimal infiltration of the portal tracts with round cells. The histology suggests an extrahepatic biliary obstruction, but this was not demonstrated at postmortem.

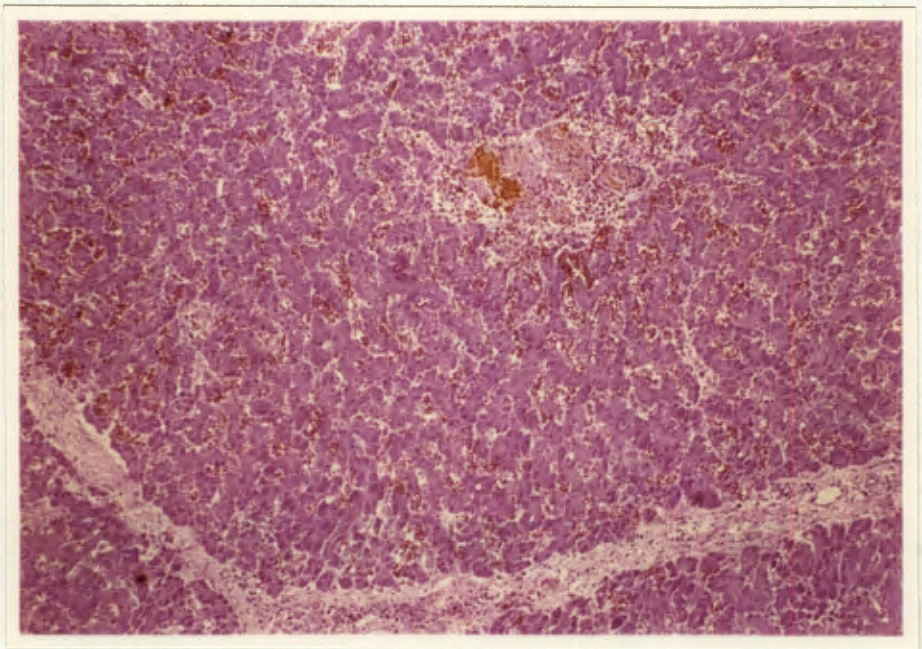
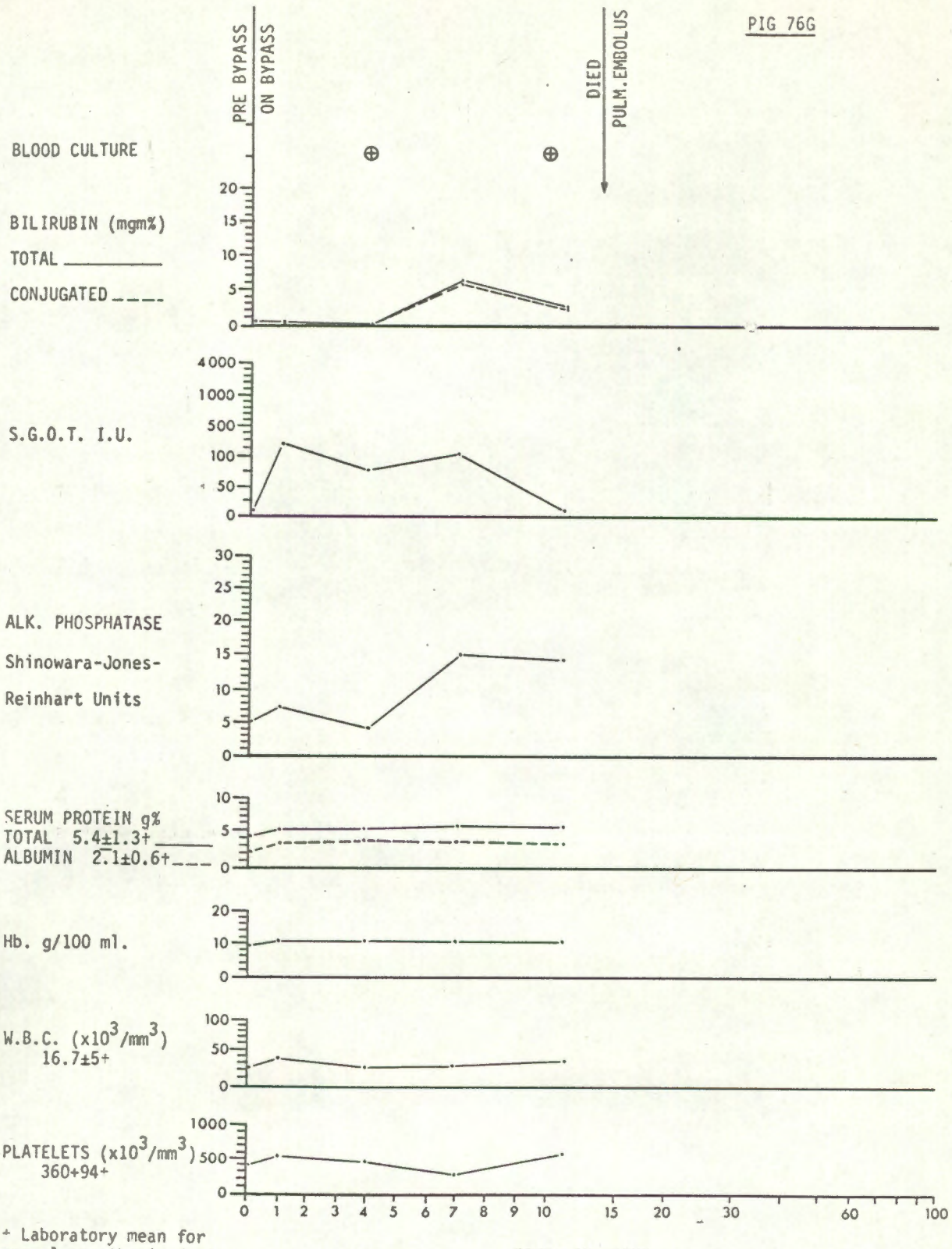


Fig. 16 Mag. x 25

	DONOR	RECIPIENT
	Landrace/Large White	Landrace/Large White
	27.2 Kg.	30.9 Kg.
	Female	Female
ISCHAEMIC TIME		6 Hours 25 Minutes
DURATION OF SURVIVAL		14 Days
POST OPERATIVE COURSE		The early post operative course was uneventful. The animal then became jaundiced on the 7th day. This was resolving spontaneously when it suddenly died.
BIOCHEMISTRY		The initial changes in the S.G.O.T. and alkaline phosphatase were similar to that seen previously in this group. The changes thereafter were suggestive of rejection.
HAEMATOLOGY		Thrombocytopenia without leucocytosis was prominent on the 7th day.
POSTMORTEM		A large pulmonary embolus was found occluding the pulmonary artery. No source for the embolus was found. The liver was macroscopically normal.

PIG 76G



+ Laboratory mean for normal anaethetised pigs

POSTMORTEM LIVER HISTOLOGY (Fig. 17)

There were areas of focal necrosis of the lobule with sinusoidal congestion. A notable round cell infiltration was present in the portal tracts and there was some evidence of biliary stasis as indicated by the presence of bile plugs. These features were in keeping with the clinical and biochemical evidence of moderate rejection.

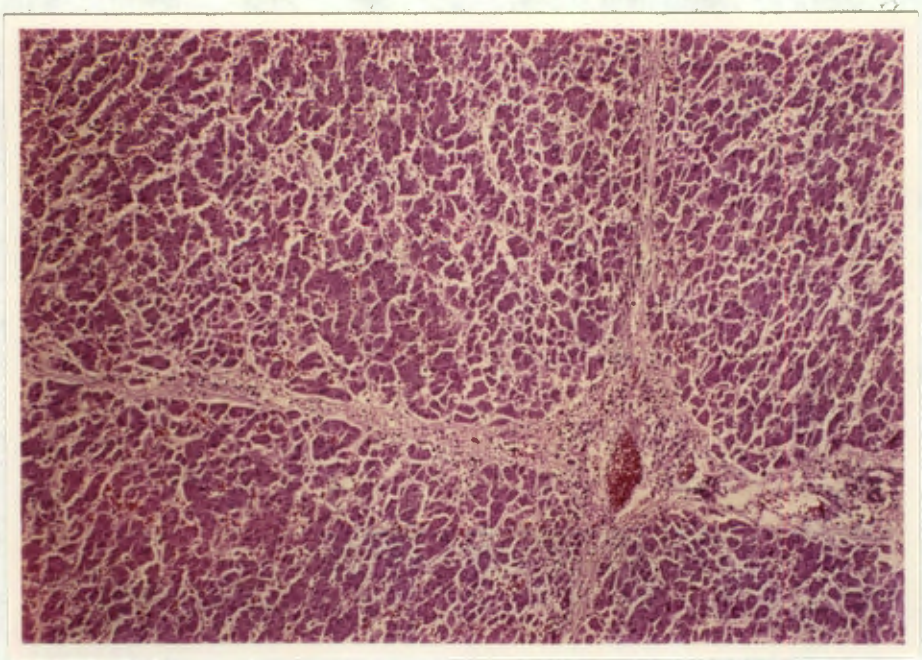
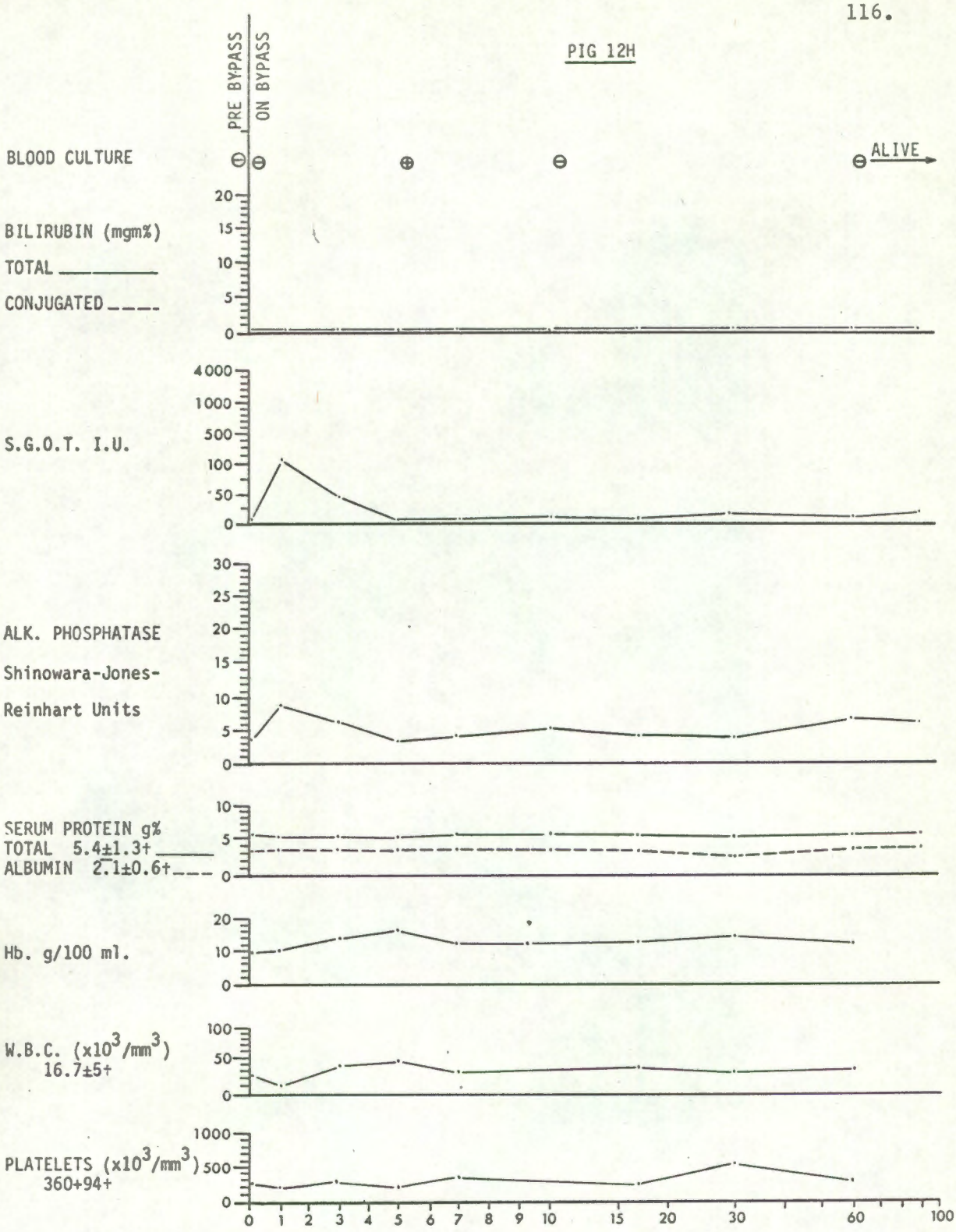


Fig. 17 Mag. x 25

	DONOR	RECIPIENT
	Landrace	Landrace
	22.7 Kg.	24.5 Kg.
	Female	Male
ISCHAEMIC TIME		7 Hours 23 Minutes
DURATION OF SURVIVAL		Alive and thriving 7 Months post transplantation.
POST OPERATIVE COURSE		Has been uncomplicated throughout.
BIOCHEMISTRY		Following the initial elevation of S.G.O.T. and alkaline phosphatase which returned to normal by the 5th day, there have been no further specific biochemical changes.

PIG 12H



+ Laboratory mean for normal anaethetised pigs

LIVER BIOPSY - 3 MONTHS POST TRANSPLANTATION (Fig. 18)

Shows normal hepatocytes and lobules with minimal round cell infiltration.

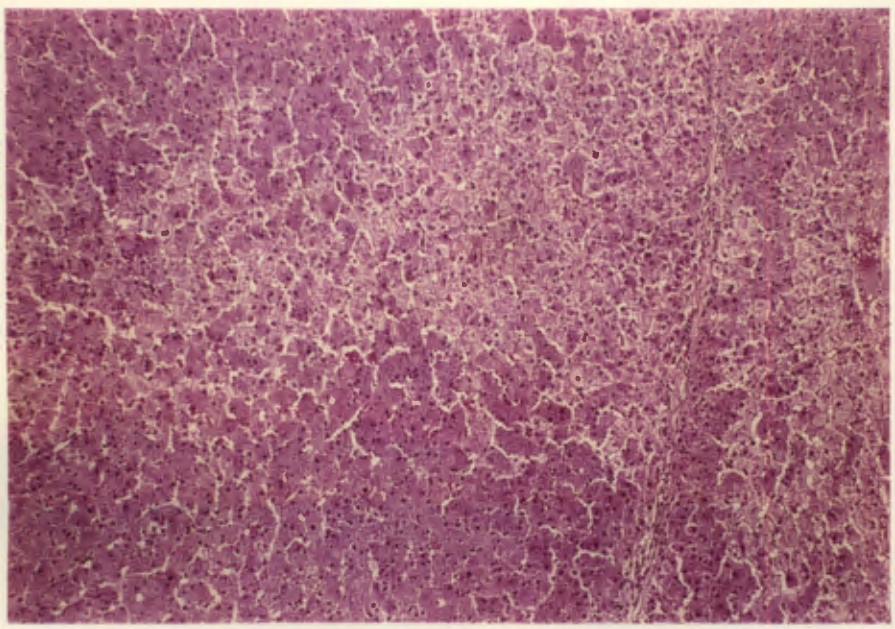
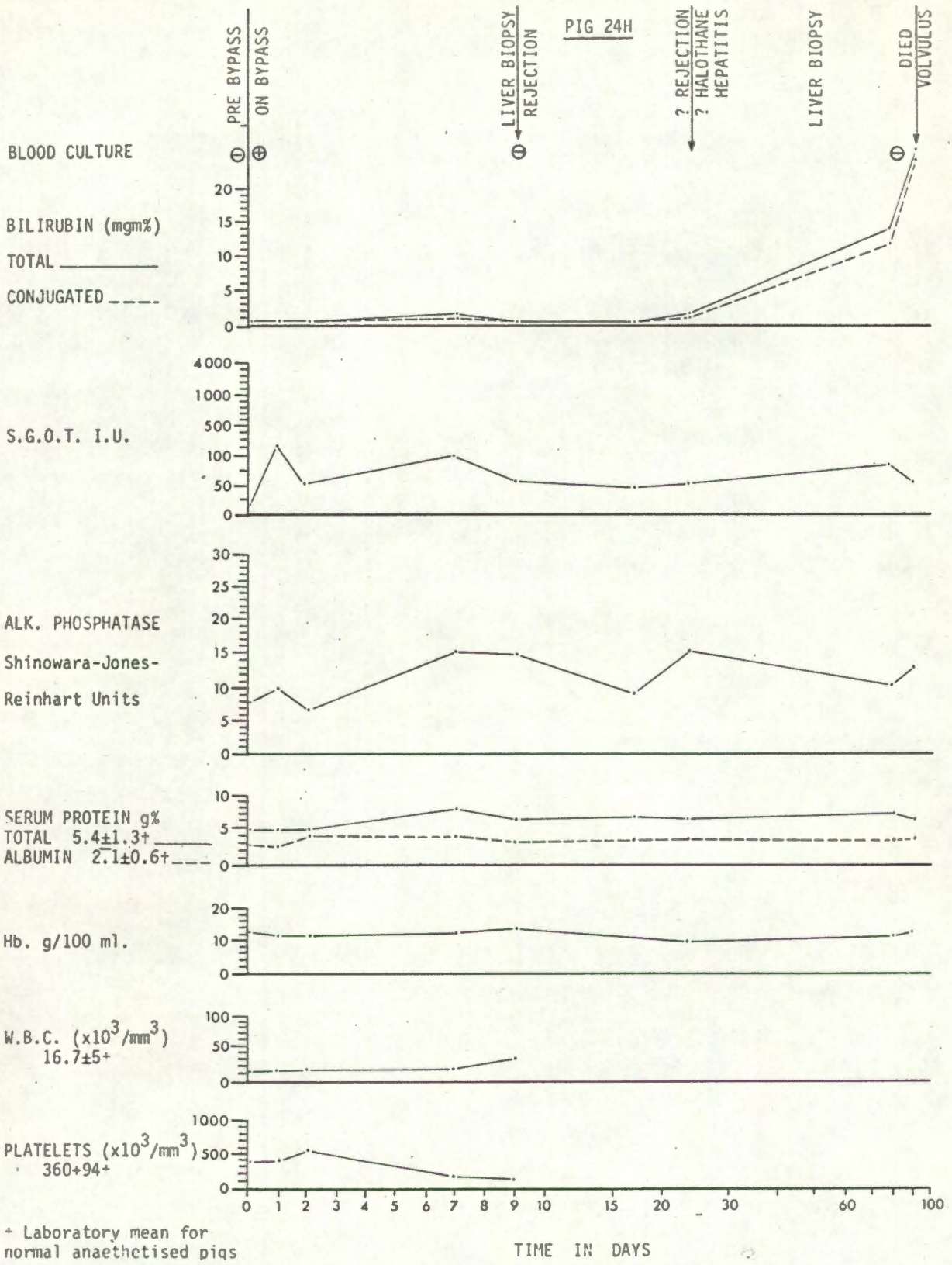


Fig. 18 Mag. x 25.

	DONOR	RECIPIENT
	Landrace	Landrace
	18.1 Kg.	22.7 Kg.
	Male	Female
ISCHAEMIC TIME		7 Hours 22 Minutes
DURATION OF SURVIVAL		88 Days
POST OPERATIVE COURSE		Uncomplicated during the first week, then transiently jaundiced; this resolved spontaneously within a few days. Following a 4th halothane anaesthetic the animal manifested signs and symptoms of malignant hyperpyrexia with a C.P.K. greater than 2,000; three days later it became jaundiced. Terminally the jaundice deepened and was associated with abdominal distension.
BIOCHEMISTRY		During the first week and early part of the third week the biochemical changes were suggestive of rejection, though one could not exclude a halothane hepatitis after the 3rd week. Terminally, the sudden rise in the serum bilirubin and alkaline phosphatase without biochemical evidence of hepatocellular disease suggested an acute extrahepatic biliary obstruction.
HAEMATOLOGY		Thrombocytopenia and leucocytosis occurred with the first episode of jaundice; thereafter these parameters could not be determined as the specimens of blood became auto agglutinated.
POSTMORTEM		A volvulus of small bowel was found obstructing the afferent and efferent limbs of the duodenal loop forming the gastroduodenostomy.



+ Laboratory mean for normal anaethetised pigs

LIVER BIOPSY - 10TH DAY (Fig. 19)

This showed focal necrosis of the hepatocyte with areas of regeneration and prominence of the Kupffer cells. The portal tracts were infiltrated with round cells which extended into the sinusoids. The features are in keeping with clinical and biochemical evidence of rejection.

LIVER BIOPSY - 3RD WEEK (Fig. 20)

In this biopsy, there was no evidence of cell necrosis and only a few round cells were present in the portal tracts.

POSTMORTEM LIVER HISTOLOGY (Fig. 21)

The acute liver cell necrosis was in keeping with an acute anoxic death. There was no evidence of rejection.

This series of biopsies demonstrates that the cellular and morphological changes of rejection can also remit spontaneously.

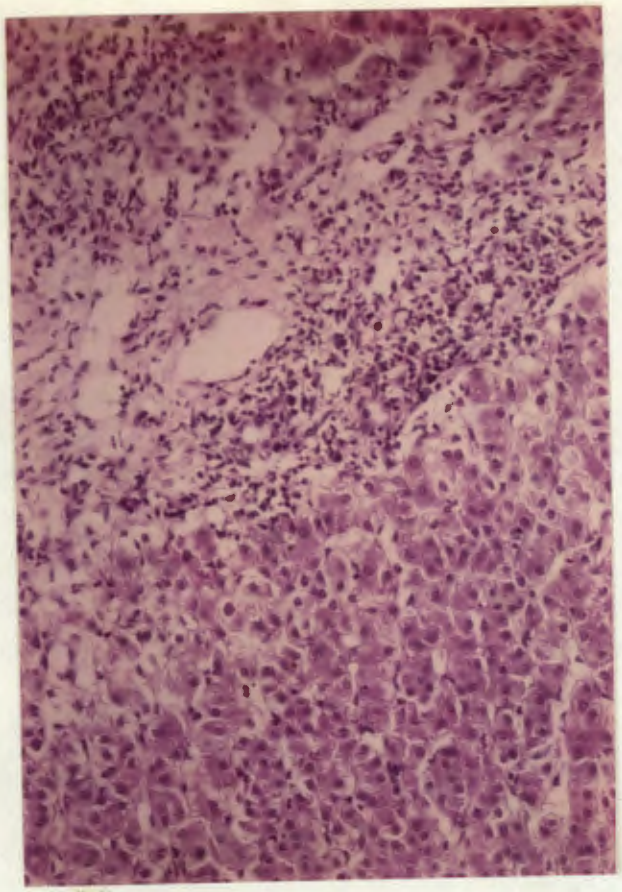


Fig. 19 (mag.x 25)

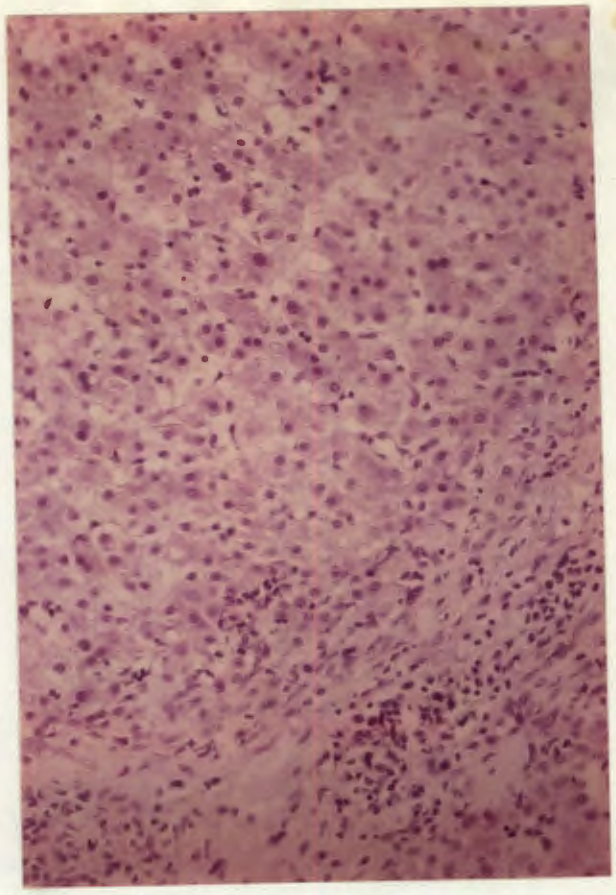


Fig.20 (mag.x 25)

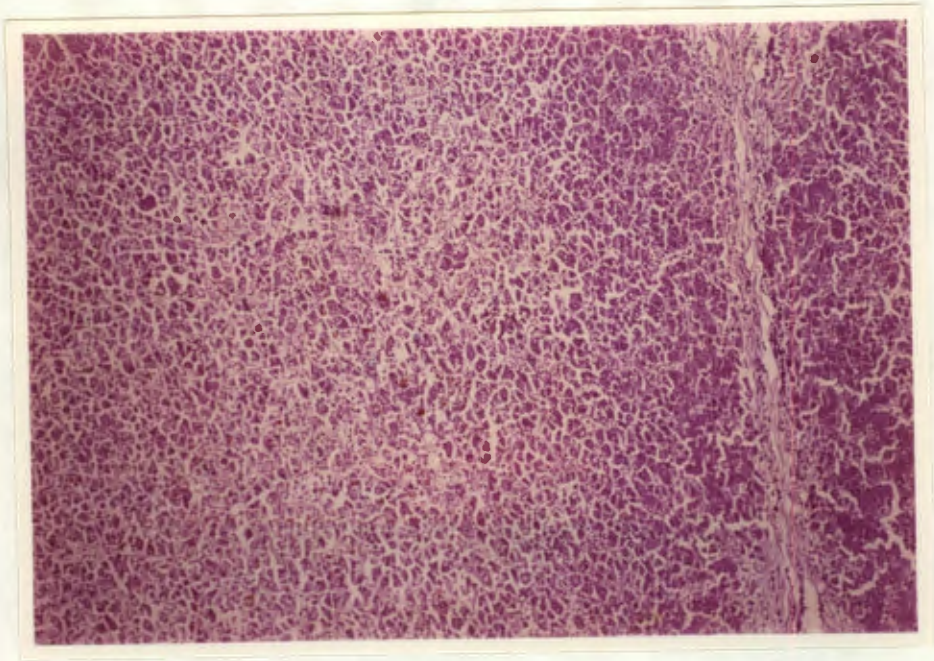
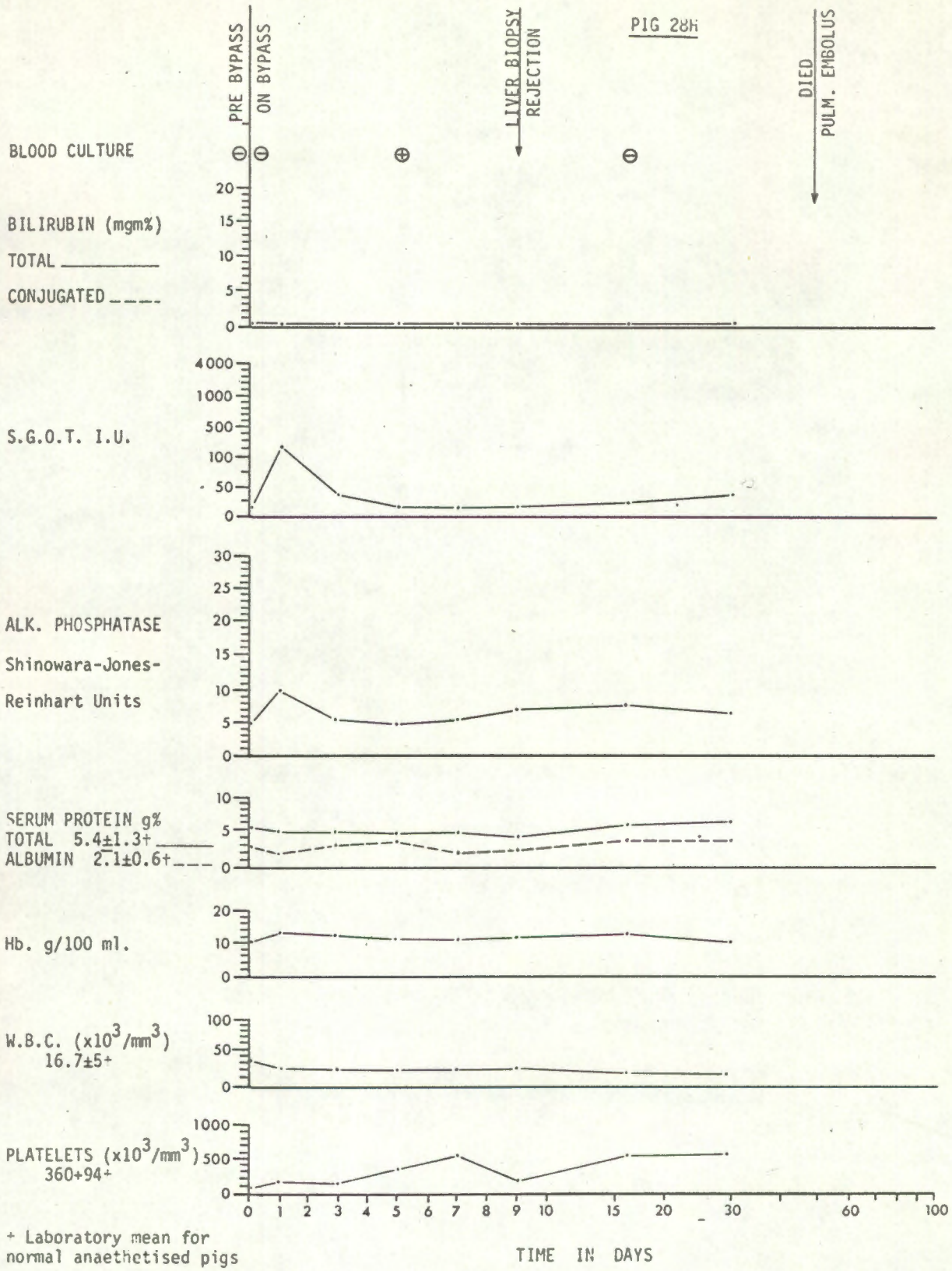


Fig. 21 (mag.x 25)

	DONOR	RECIPIENT
	Landrace	Landrace
	20.1 Kg.	24.9 Kg.
	Female	Male
ISCHAEMIC TIME		8 Hours 3 Minutes
DURATION OF SURVIVAL		48 Days
INTRA OPERATIVE COURSE		The donor liver had no infrahepatic vena cava; therefore the recipient infrahepatic vena cava was ligated. This precluded flushing of the liver with blood prior to removing the supra hepatic vena caval clamp. A temporary pulsus alternans occurred following portal vein revascularisation; but this was not associated with any change in the serum chemistry.
POST OPERATIVE COURSE		Was completely uncomplicated. The animal died suddenly and unexpectedly 48 days post transplantation.
BIOCHEMISTRY		After the initial biochemical changes following the transplant had returned to normal, only a slight rise in alkaline phosphatase occurred between the 9th and 15th day.
HAEMATOLOGY		A noticeable thrombocytopenia occurred on the 9th day.
POSTMORTEM		A large pulmonary embolus was found in the right ventricle occluding the pulmonary outflow tract. A bland thrombus was also present in the superior vena cava.



+ Laboratory mean for normal anaethetised pigs

LIVER BIOPSY - 10TH DAY (Fig. 22)

There were areas of focal necrosis of hepatocytes and moderate infiltration of the portal tracts with round cells. The features were in keeping with moderate rejection.

LIVER BIOPSY - 10TH DAY BEST CARMINE STAIN (Fig. 23)

This demonstrates the presence of glycogen granules in the liver cells post transplantation. The animal was starved for 12 hours prior to taking the biopsy.

POSTMORTEM LIVER HISTOLOGY (Fig. 24)

There was evidence of centrilobular necrosis with a polymorphonuclear cell infiltration. The portal tracts were widened but there was less round cell infiltration present. The histological features were in keeping with sudden anoxic death rather than rejection.

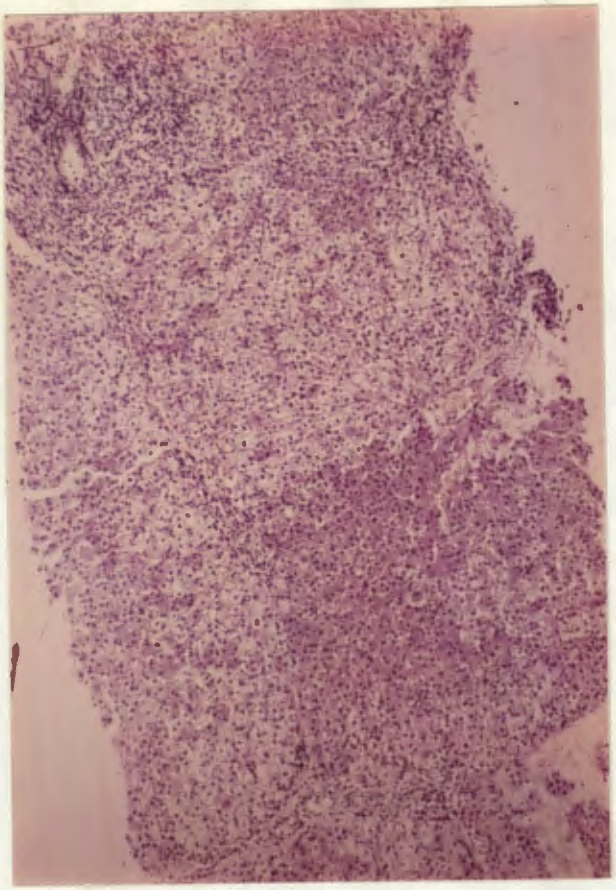


Fig. 22 (mag.x 25)

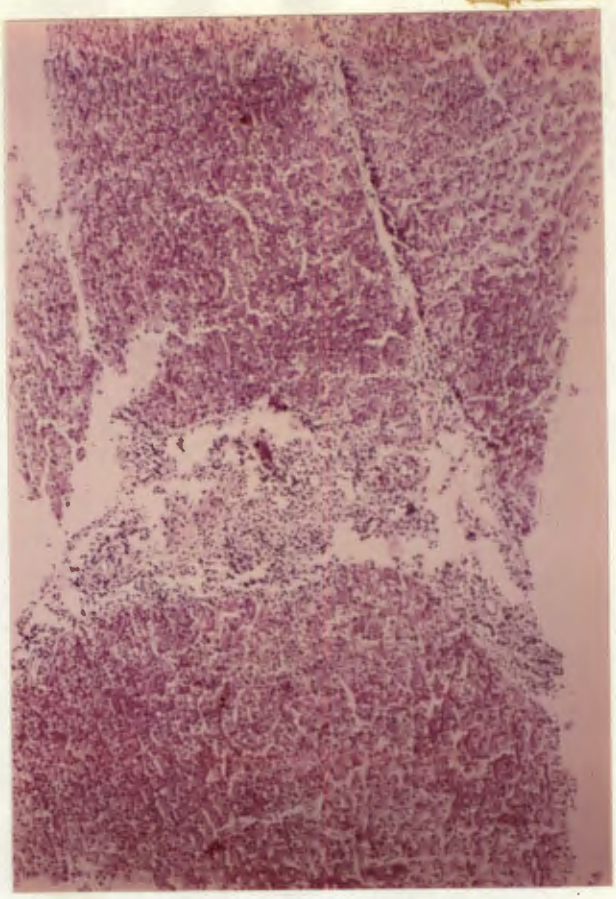


Fig. 23 (mag.x 25)

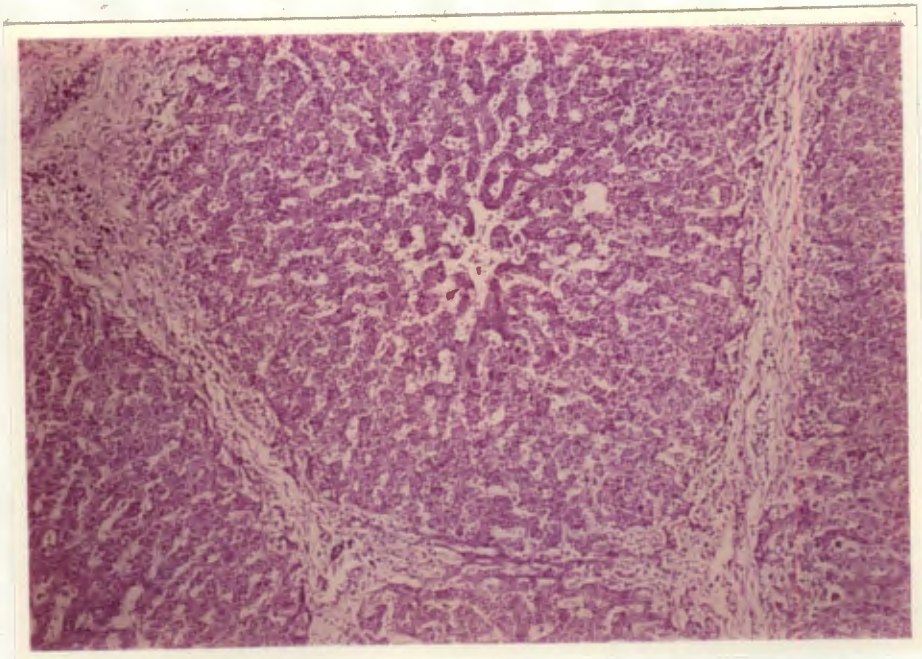


Fig. 24 (mag.x 25)

DISCUSSION

In this group of experiments, four basic preserving solutions were assessed at varying temperatures: a Ringer Lactate solution, plasma (Solution II), plasma with cell membrane stabilising drugs (Solution III) and a solution similar in cation composition to intracellular fluid (Solution IV).

Ringer Lactate solution at 8°C - 12°C and Solution II at room temperature (18°C - 20°C) were uniformly unsuccessful.

Consistently successful results, however, were obtained with Solution II following a 6 - 8 hour period of storage at 8°C - 12°C. In the only unsuccessful experiment in this subgroup, the clinical course, the postmortem findings and even the histology of the liver suggested that death was due to a reactionary haemorrhage rather than a primary bleeding diathesis.

In trying to extend the period of preservation to 10 hours, only one animal in four experiments survived and is still alive 7 months post transplantation. When cell membrane stabilising drugs were used in combination with a plasma solution for 10 hours storage, the animals did not wake up promptly from the anaesthetic and assisted respiration was required for at least one hour post operatively. Hypoglycaemia was a notable feature in these animals, the pig surviving 28 hours died in hypoglycaemic coma.

Solution IV was no better in achieving extended preservation but did not produce the gross metabolic disturbance of the previous solution.

At 8°C - 12°C for 12 hours with Solution II preservation was uniformly unsuccessful and even reduction of the temperature to an "optimum" level (2°C - 5°C) failed to influence the results.

The major limiting factor to successful storage was a bleeding diathesis which unfortunately, due to lack of facilities, could not be adequately investigated in the course of this study. Generalised

bleeding / . . .

bleeding became evident shortly after complete revascularisation of the liver. Initially, it manifested as a slow ooze from all vascular anastomoses and from the skin incisions. Later petechial haemorrhages appeared on the parietal peritoneum and after a variable period appreciable amounts of blood and lymph collected in the paracolic gutters. Following the administration of protamine, no clotting occurred and in many instances this drug appeared to aggravate the situation rather than improve it. The average quantity of blood found in the peritoneal cavity was 700 ml. with a haemoglobin content ranging between 1.9 G% and 3.4 G%. At postmortem blood stained effusions were frequently present in both the pleural and pericardial cavities in addition to the haemoperitoneum.

A pure ascites was found in only two animals (1.5 L and 3 L). In both the clinical picture was that of acute hypovolaemic shock. The protein content of the ascitic fluid was 2G/100 ml.

Macroscopically it was not possible to assess whether the liver was adequately preserved or not. Following revascularisation with portal blood, all livers appeared uniformly perfused; but after complete revascularisation obvious differences became evident. The well preserved organ became hyperaemic and maintained a uniform turgor, whereas the poorly preserved liver remained dusky and appeared "collapsed".

The histological features of the various subgroups are shown (Fig.25 to 31). The extensive morphological changes which occurred following the use of cell membrane stabilising drugs were noteworthy and were in keeping with the metabolic disturbance encountered.

The postoperative course of animals surviving 6-8 hours storage with Solution II at 8-12^{o}C was very similar to the controls. The immediate postoperative levels of the alkaline phosphatase and S.G.O.T. were slightly higher but these returned to normal values within 4 days. Thereafter in uncomplicated cases the clinical behaviour and the biochemical and histological findings were comparable.

CONCLUSION / . . .

CONCLUSION

Successful storage of the pig liver for 6 - 8 hours was achieved using simple hypothermia at $8^{o}C$ - $12^{o}C$ and a modified plasma preserving solution.

RINGER LACTATE SOLUTION 6-8 HOURS STORAGE AT $8^{\circ}C$-$12^{\circ}C$ (Fig. 25)

Shows extensive centrizonal necrosis.

SOLUTION II 6-8 HOURS STORAGE AT $18^{\circ}C$-$20^{\circ}C$ (Fig. 26)

There was centrilobular necrosis and haemorrhagic congestion of the sinusoids.

SOLUTION II 10 HOURS STORAGE AT $8^{\circ}C$-$12^{\circ}C$ (Fig. 27)

There was considerable oedema of the lobule and the sinusoids contained both red cells and polymorphonuclear leucocytes. Peripherally some cells showed early changes of necrosis.

SOLUTION II 12 HOURS STORAGE AT $8^{\circ}C$ - $12^{\circ}C$ (Fig. 28)

There was oedema with early disorganisation of the lobule.

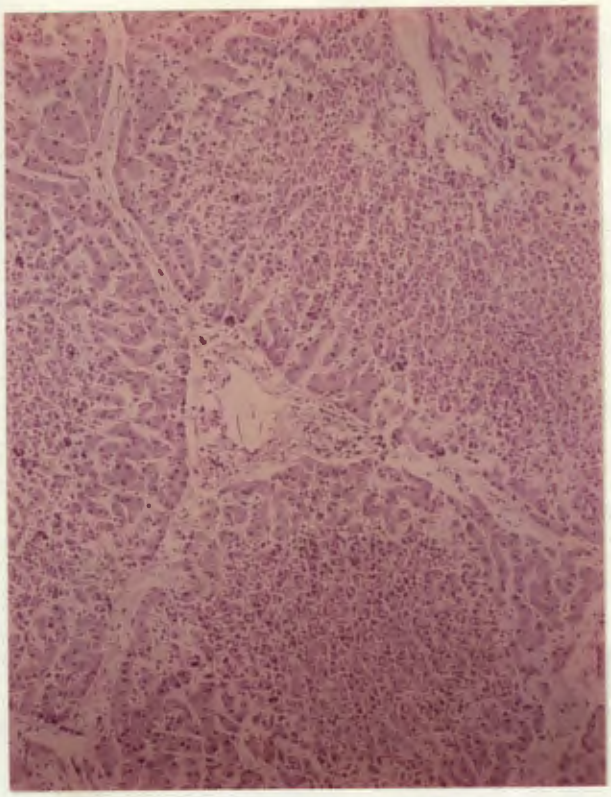


Fig. 25 (mag.x 25)

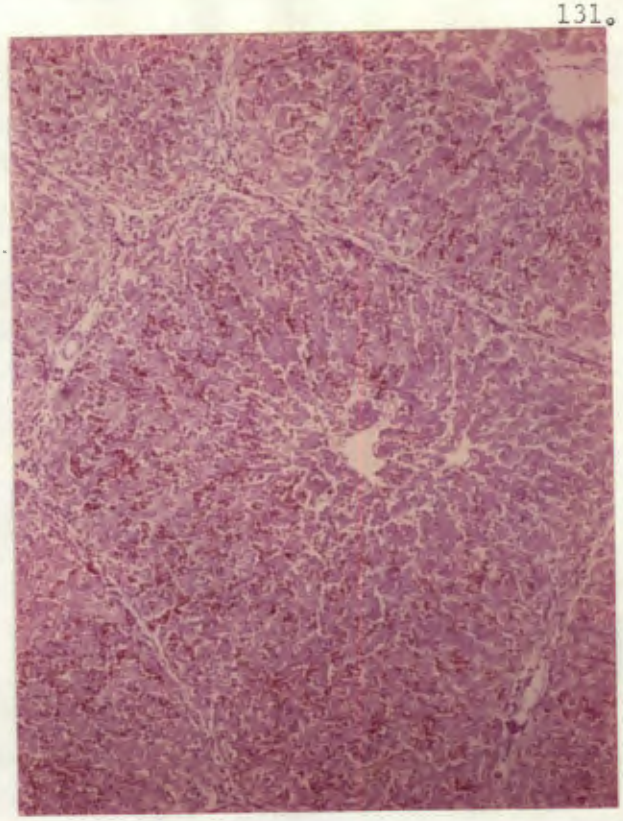


Fig. 26 (mag.x 25)

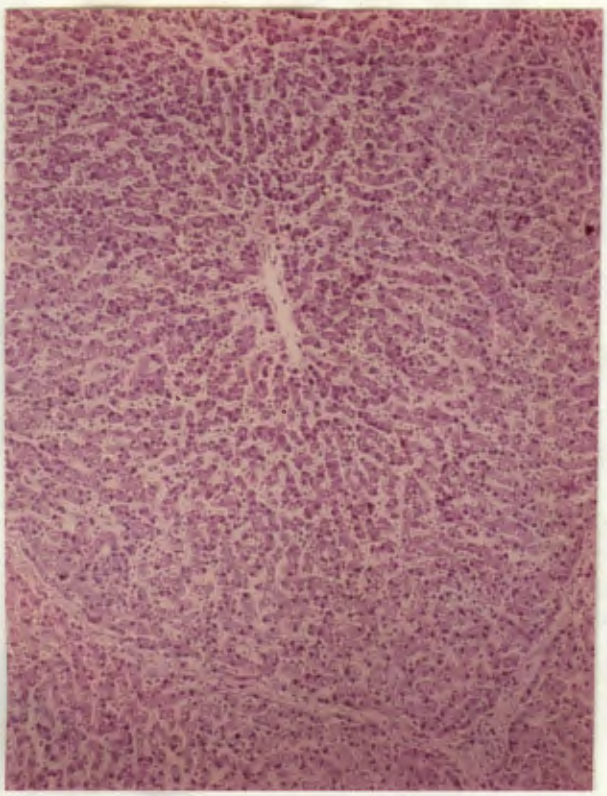


Fig. 27 (mag.x 25)

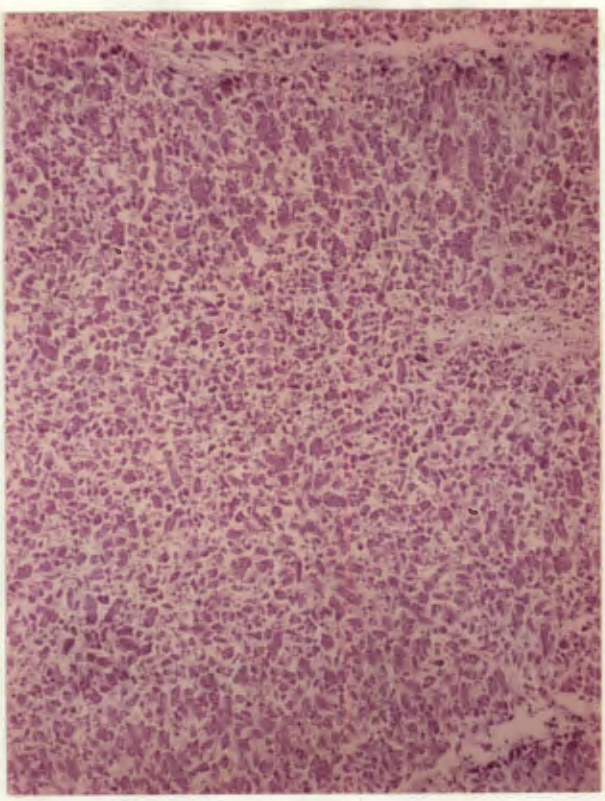


Fig. 28 (mag.x 25)

SOLUTION II 12 HOUR STORAGE AT 2°C - 5°C (Fig. 29)

There was extensive centrizonal necrosis with oedema.

PRETREATMENT WITH CELL MEMBRANE STABILISING DRUGS AND PRESERVATION WITH SOLUTION III 10 HOURS AT 8°C - 12°C (Fig. 30)

Extensive centrizonal necrosis with polymorphonuclear leucocyte infiltration and congestion of the sinusoids with red blood cells.

SOLUTION IV 10 HOUR STORAGE AT 8°C - 12°C (Fig. 31)

Shows extensive centrizonal necrosis and oedema of the lobule with early disorganisation.

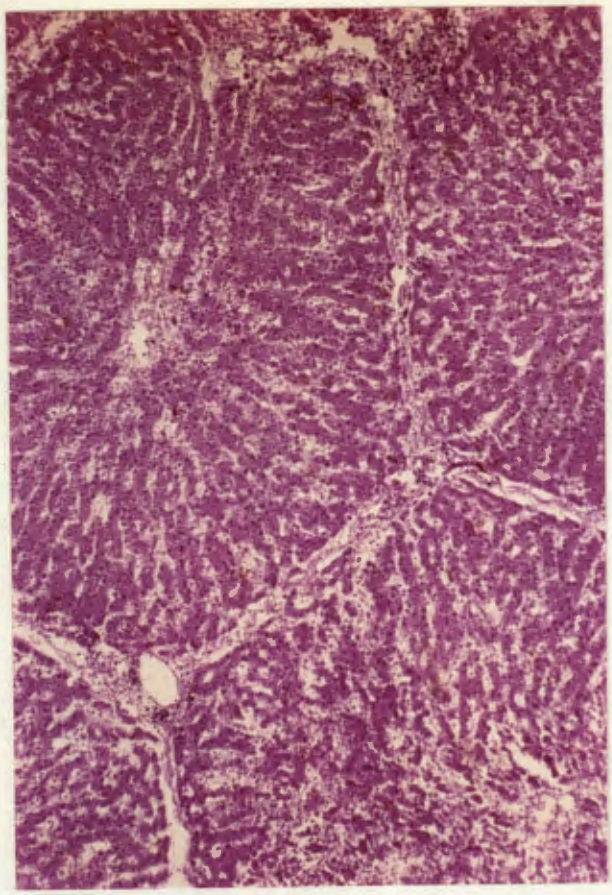


Fig. 29 (mag.x 25)

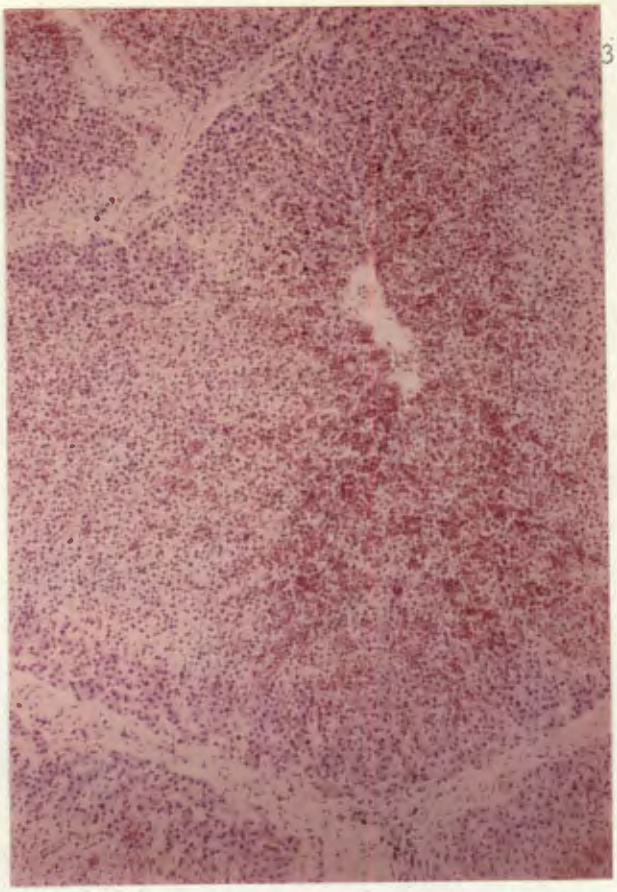


Fig. 30 (mag.x 25)

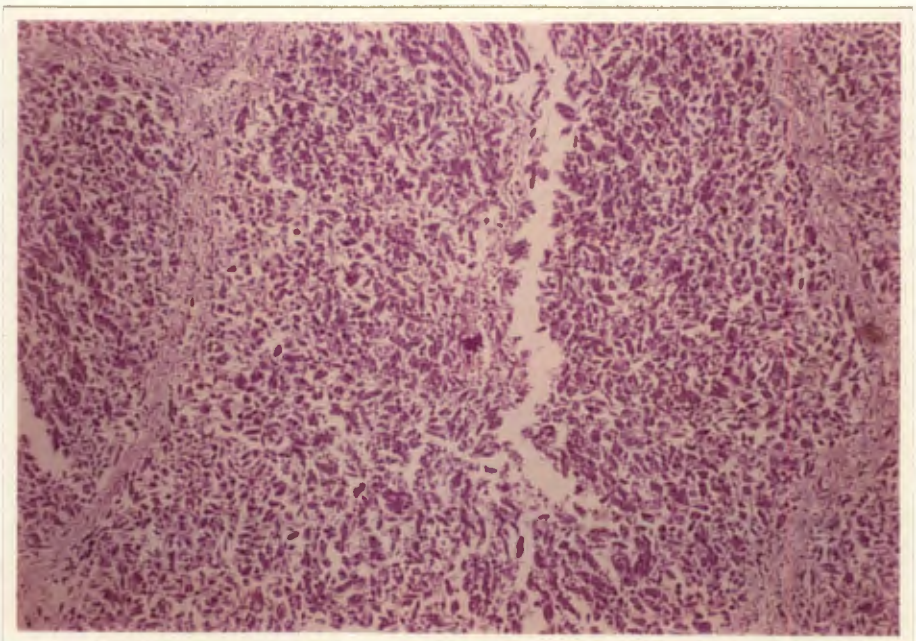


Fig. 31 (mag.x 25)

GROUP III

HYPOTHERMIC IMMERSION STORAGE WITH HYPERBARIC OXYGEN

HYPOTHERMIC IMMERSION STORAGE WITH HYPERBARIC OXYGEN (HBO) - TABLE 5

1. HYPOTHERMIC IMMERSION STORAGE 12 - 12.5 HOURS AT 2°C-5°C AND HYPERBARIC OXYGEN AT 3 ATA.

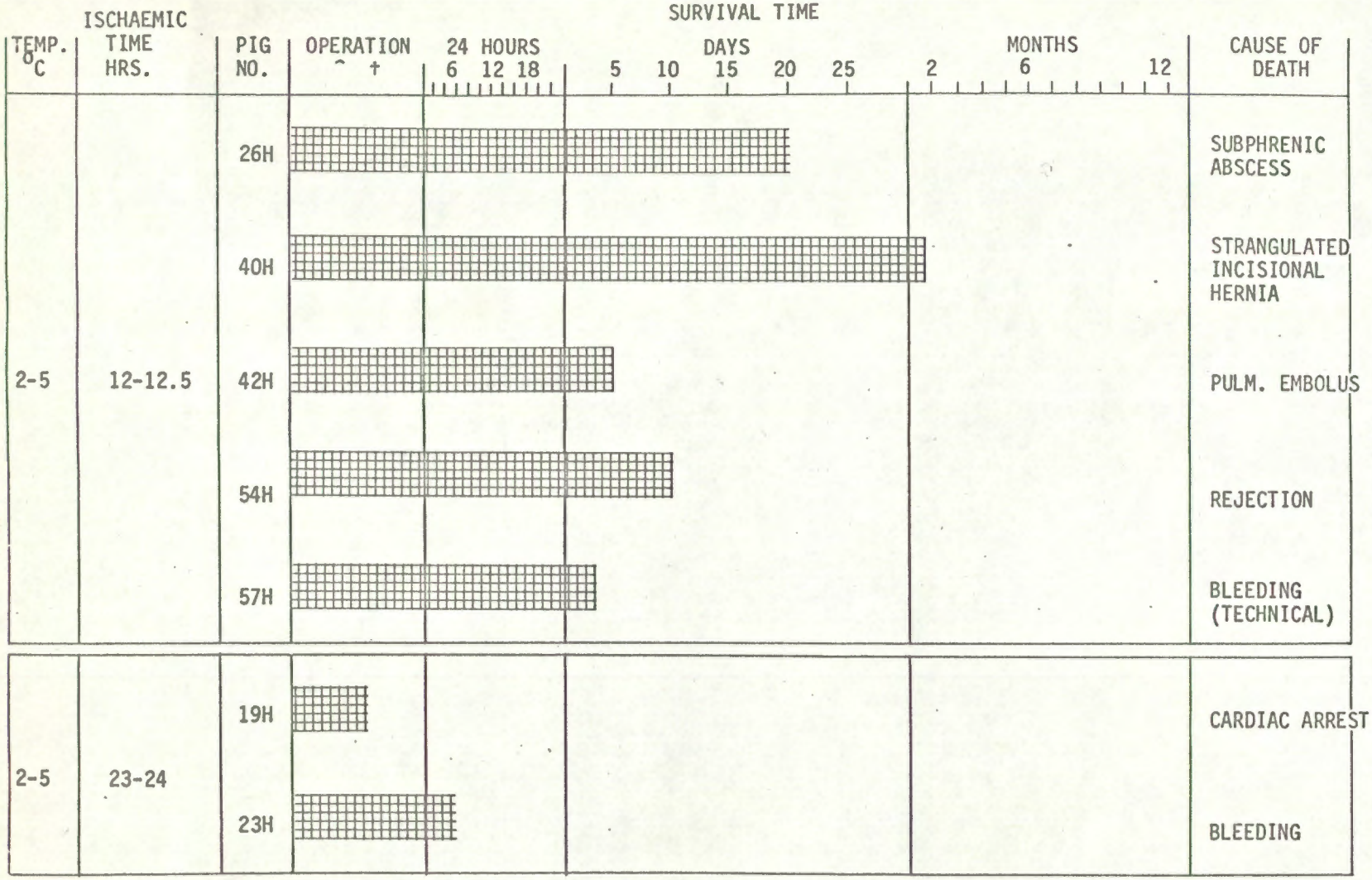
In four of five experiments successful storage was achieved.

2. HYPOTHERMIC IMMERSION STORAGE 23 - 24 HOURS AT 2°C-5°C AND HYPERBARIC OXYGEN AT 3 ATA

Only two experiments were performed in this subgroup. The first animal had a cardiac arrest shortly after the liver was revascularised with portal blood. The second animal survived the operation but died from a generalised bleeding diathesis.

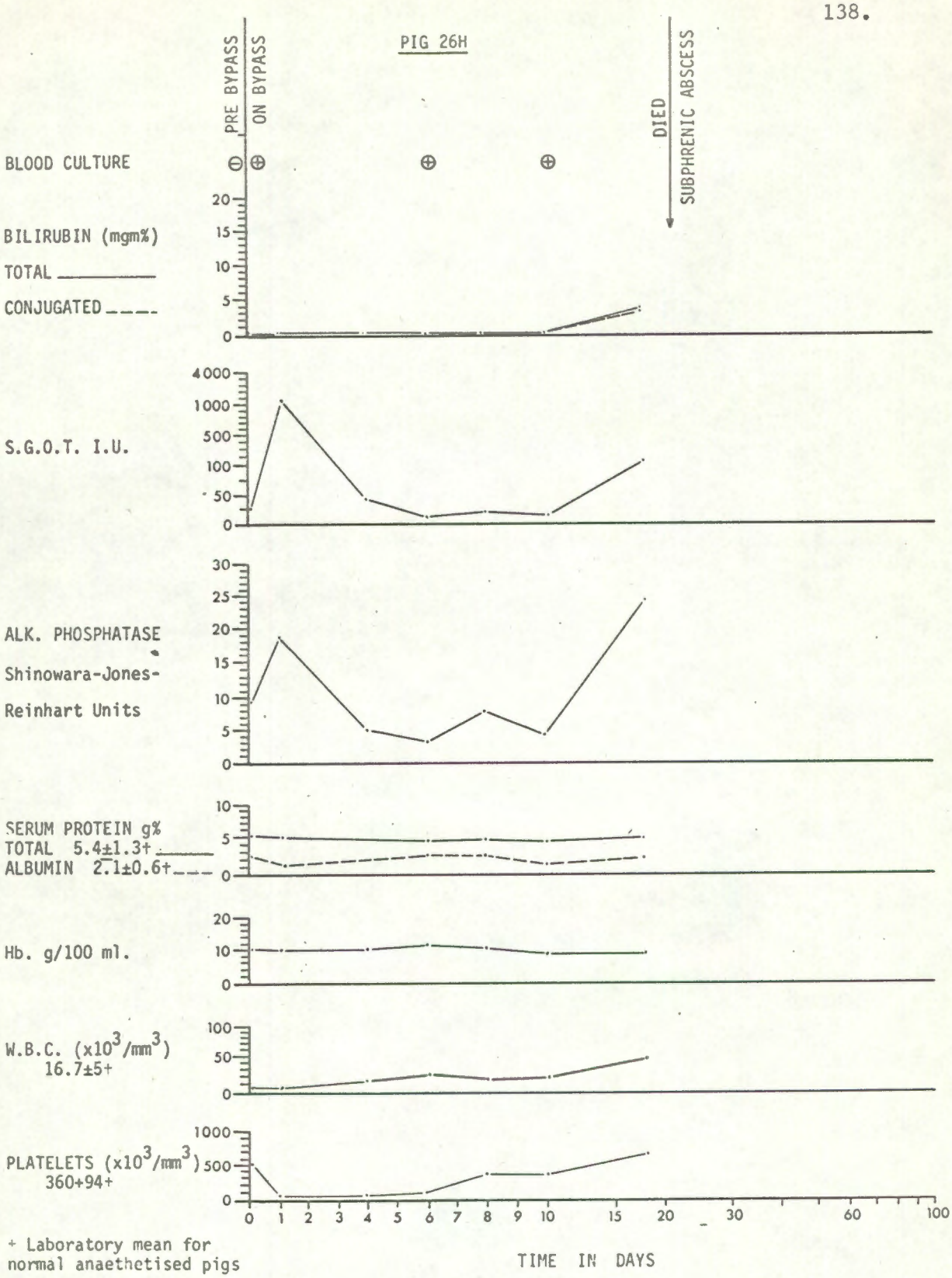
TABLE 5

HYPOTHERMIC IMMERSION STORAGE WITH H.B.O. (3 A.T.A.)



^ PORTAL VEIN REVASCULARISATION
† HEPATIC ARTERY REVASCULARISATION

DONOR	RECIPIENT
Landrace	Landrace
22.7 Kg.	24.5 Kg.
Male	Male
ISCHAEMIC TIME	12 Hours 31 Minutes
DURATION OF SURVIVAL	20 Days
HYPERBARIC PRESSURE	3 ATA
PRE STORAGE p.O2	240 mm.Hg.
POST STORAGE p.O2	1200 mm.Hg.
DECOMPRESSION TIME	10 Minutes
INTRA OPERATIVE COURSE	A spontaneous right sided pneumothorax occurred. This was decompressed after closure of the abdomen with a chest drain connected to an underwater seal. The drain was removed before the animal recovered from the anaesthetic.
POST OPERATIVE COURSE	Uncomplicated until the 10th day. Abdominal distension was then noted particularly on the right side which progressively increased. Terminally the animal became jaundiced and died 20 days post transplantation.
BIOCHEMISTRY	The initial post operative levels of the S.G.O.T. and alkaline phosphatase were higher than in the previous two groups, but returned to normal values by the 4th day. Terminally the bilirubin, S.G.O.T. and alkaline phosphatase all became elevated.
HAEMATOLOGY	The platelets remained depressed during the first 6 days.
BACTERIOLOGY	All blood cultures were positive throughout the post operative period.
POSTMORTEM	A large subphrenic abscess was present in Morrison's pouch which contained 3 litres of pus (coliforms).



+ Laboratory mean for normal anaethetised pigs

POSTMORTEM LIVER HISTOLOGY (Fig. 32)

Micro abscesses were present in the section and were probably pyaemic in origin from the subphrenic abscess. Purulent exudate was also present in the bile ducts. There was minimal histological evidence of rejection.

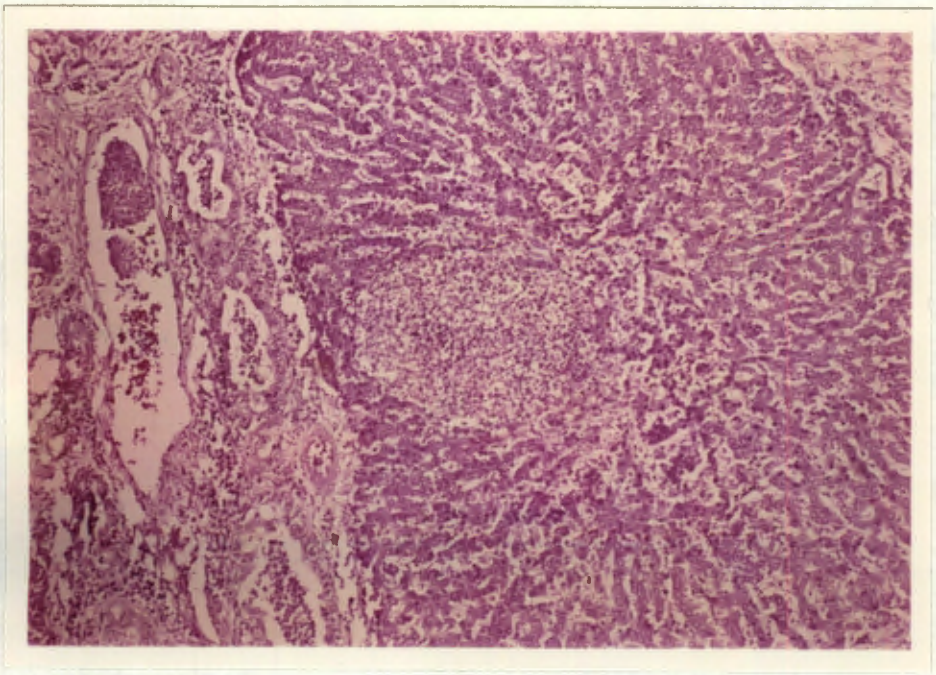
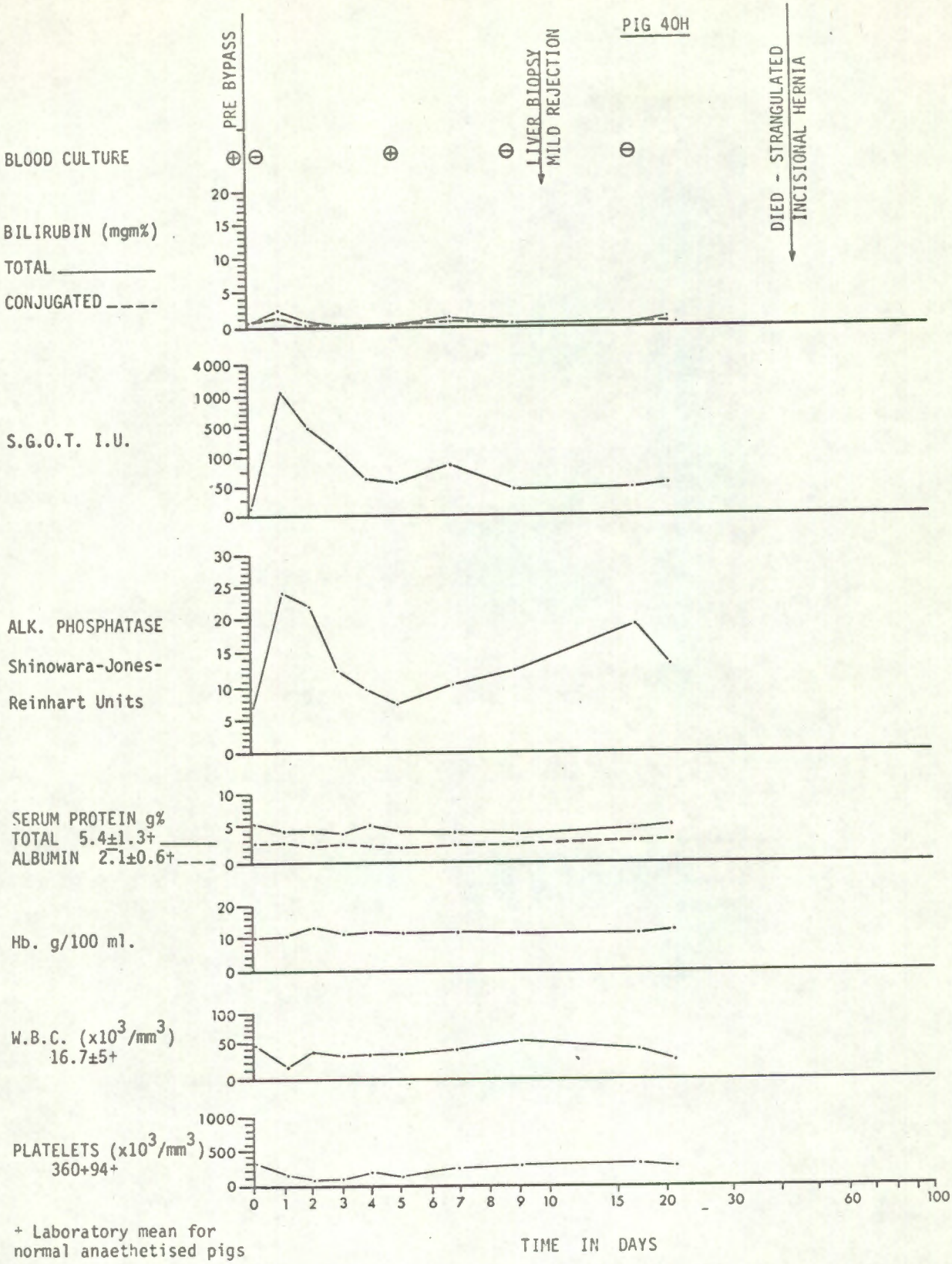


Fig. 32 Mag. x 25

DONOR	RECIPIENT
Landrace	Landrace
13.6 Kg.	18.1 Kg.
Male	Male
ISCHAEMIC TIME	12 Hours 13 Minutes
DURATION OF SURVIVAL	44 Days
HYPERBARIC PRESSURE	3 ATA
PRE STORAGE p.O2	180 mm.Hg.
POST STORAGE p.O2	950 mm.Hg.
DECOMPRESSION TIME	15 Minutes.
POST OPERATIVE COURSE	Was uncomplicated until the 25th day when an incisional hernia was noted. The animal died suddenly on the 44th day.
BIOCHEMISTRY	The higher initial levels of the S.G.O.T. and alkaline phosphatase combined with hyperbilirubinaemia in this case was again noted. These parameters returned to normal values by the 5th day. The changes during the 2nd week were suggestive of a low grade rejection process.
HAEMATOLOGY	The platelets remained depressed during the whole of the first week.
POSTMORTEM	A strangulated incisional hernia was found.



+ Laboratory mean for normal anaethetised pigs

LIVER BIOPSY 10TH DAY (Fig. 33)

There were areas of focal necrosis with active regeneration. Kupffer cells were prominent and a round cell infiltration was present. The features suggest moderate rejection.

POSTMORTEM LIVER HISTOLOGY (Fig. 34)

There was necrosis with haemorrhage and polymorphonuclear leucocyte infiltration. The features were in keeping with an anoxic death rather than rejection.

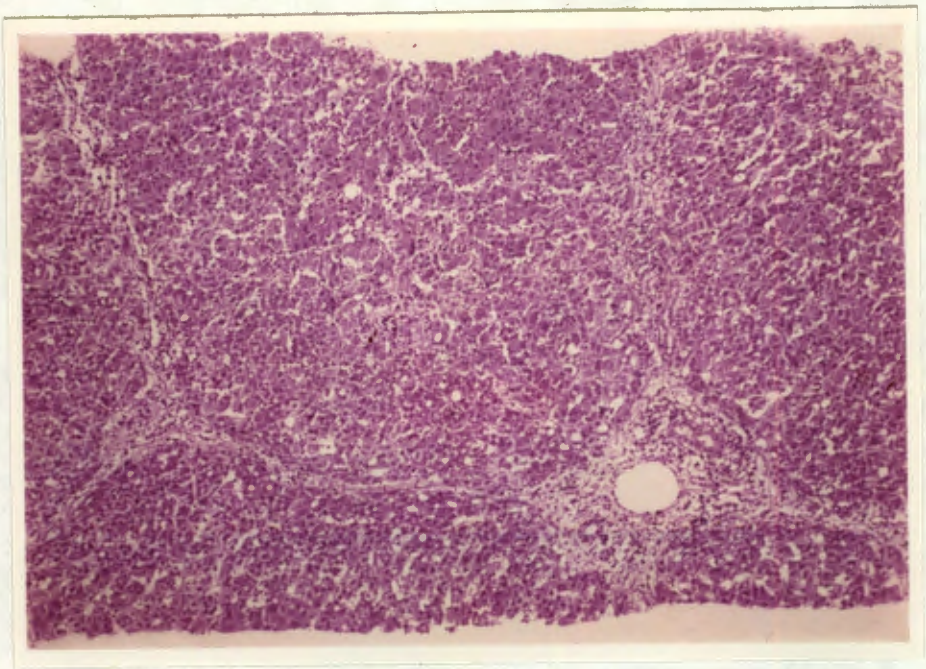


Fig. 33 Mag. x 25.

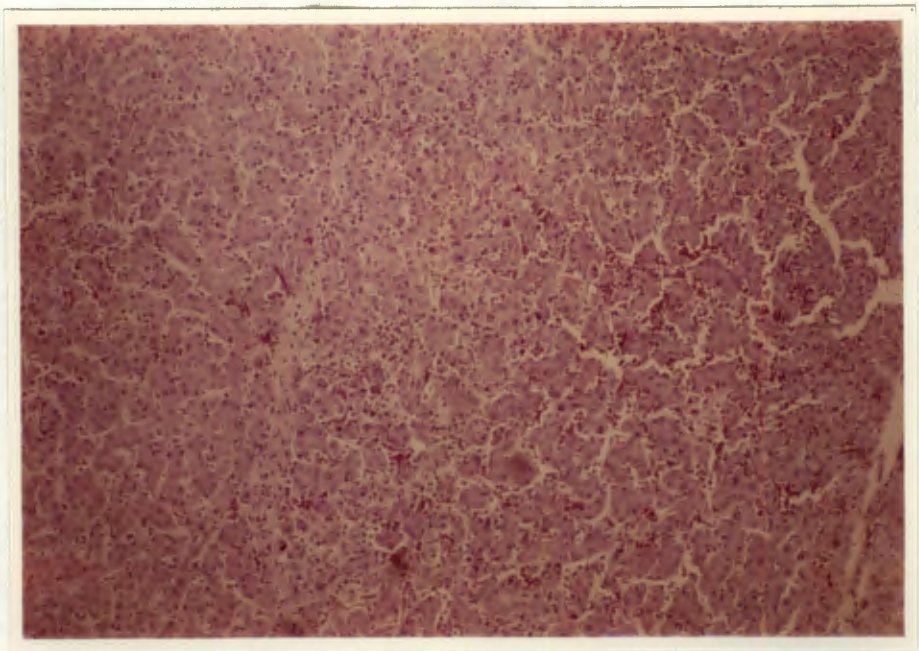
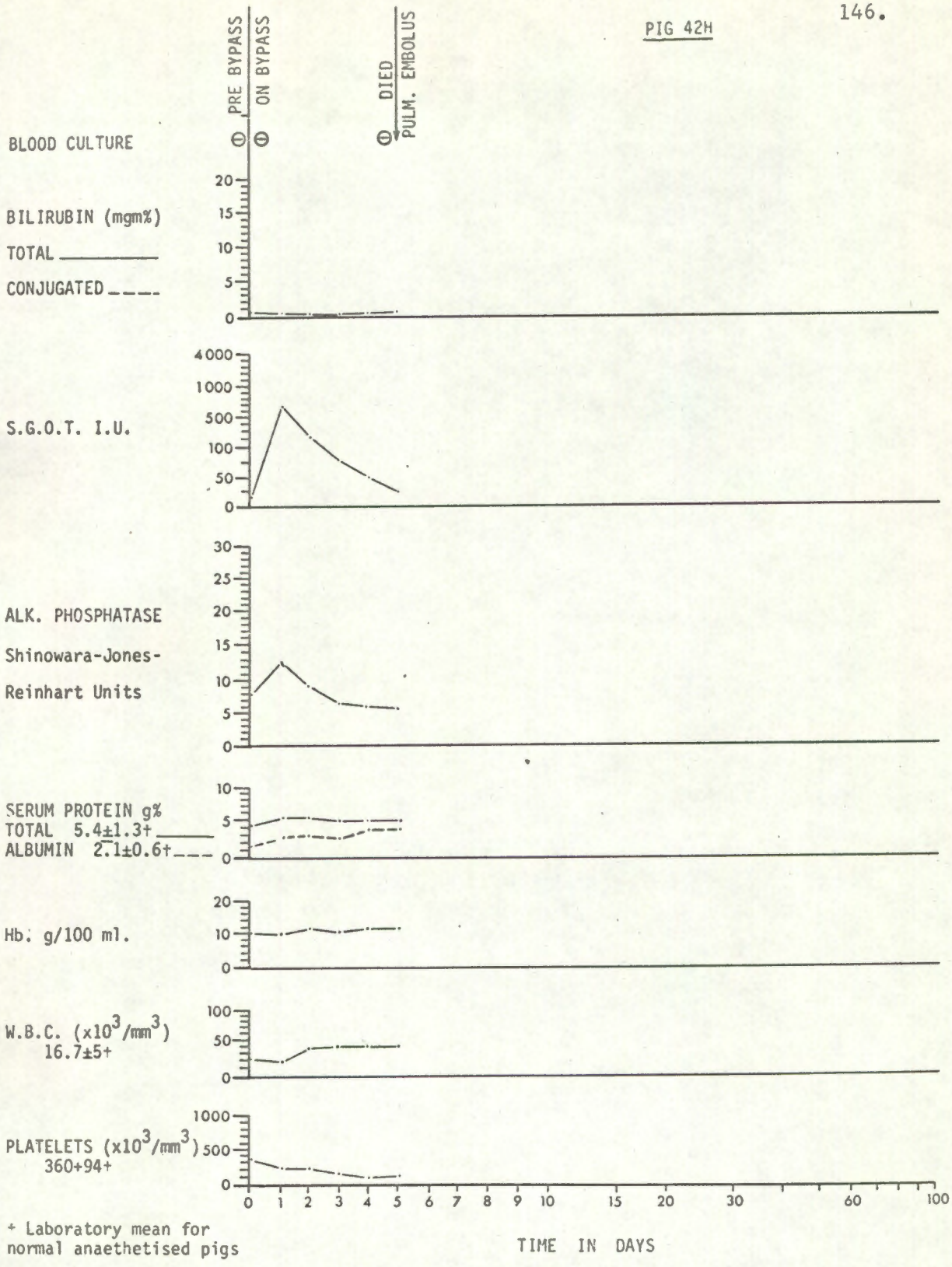


Fig. 34 Mag. x 25

	DONOR	RECIPIENT
	Landrace	Landrace
	15.9 Kg.	16.8 Kg.
	Male	Male
ISCHAEMIC TIME		12 Hours 20 minutes
DURATION OF SURVIVAL		5 Days
HYPERBARIC PRESSURE		3 ATA
PRE STORAGE p.O2		190 mm.Hg.
POST STORAGE p.O2		> 1200 mm.Hg.
DECOMPRESSION TIME		20 Minutes
POST OPERATIVE COURSE		Uncomplicated until the 5th day, then sudden onset of dyspnoea and cyanosis with no response to digitalisation and diuretics.
HAEMATOLOGY		Platelet count was depressed throughout the post operative course.
POSTMORTEM		A large thrombus was present in the right ventricle extending into the pulmonary outflow tract. No source for the embolus was found.

PIG 42H



+ Laboratory mean for normal anaethetised pigs

POSTMORTEM LIVER HISTOLOGY (Fig. 35)

The liver showed morphological changes in keeping with an anoxic death. There was minimal evidence of rejection.

Fig. 36

Demonstrates the thrombus occluding the pulmonary outflow tract found at postmortem.

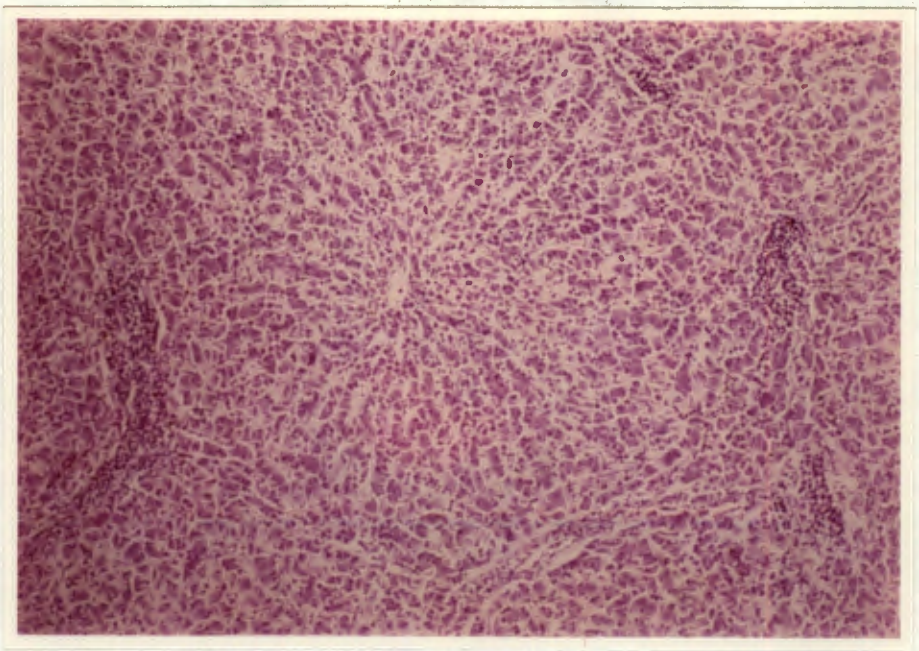


Fig. 35 Mag. x 25

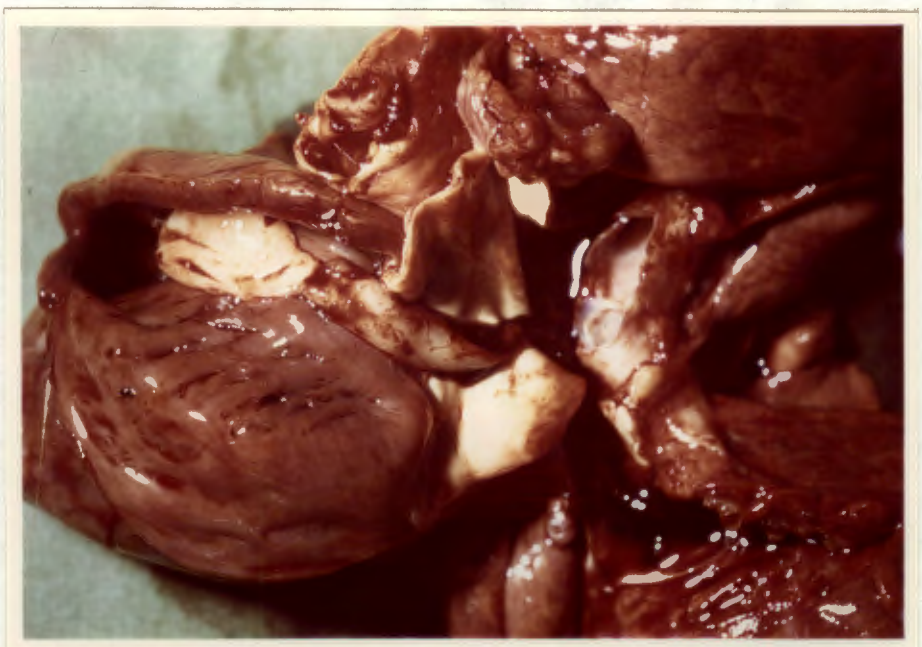
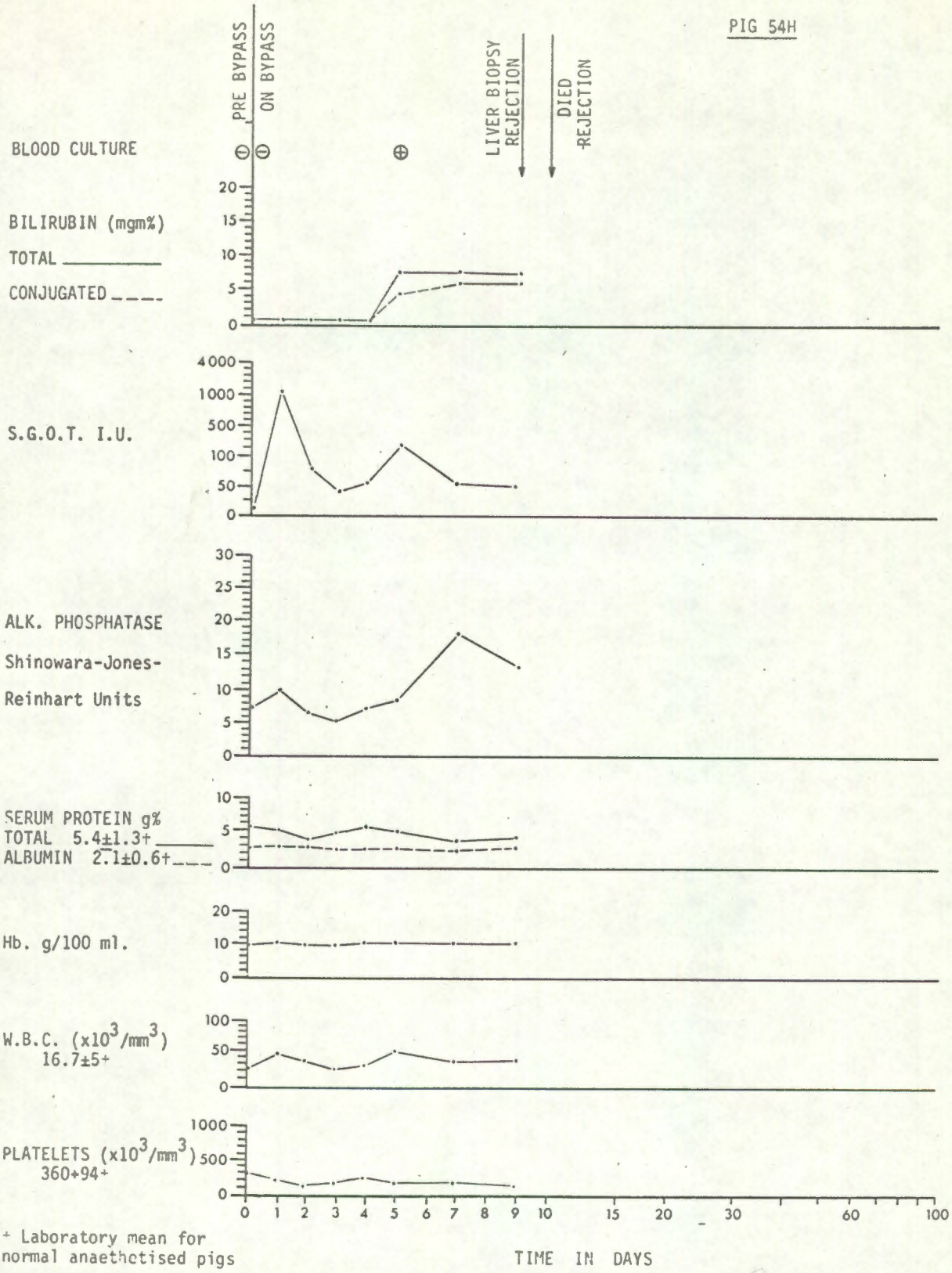


Fig. 36

	DONOR	RECIPIENT
	Landrace	Landrace
	18.1 Kg.	21.8 Kg.
	Female	Male
ISCHAEMIC TIME		12 Hours 22 Minutes
DURATION OF SURVIVAL		10 Days
HYPERBARIC PRESSURE		3 ATA
PRE STORAGE p.O2		200 mm.Hg.
POST STORAGE p.O2		900 mm. Hg.
DECOMPRESSION TIME		15 Minutes
POST OPERATIVE COURSE		Uncomplicated until the 5th day; thereafter progressive anorexia and jaundice. A liver biopsy was taken under G.A. on the 9th day. The animal died the following day.
BIOCHEMISTRY		The biochemical changes after the 5th day were strongly suggestive of rejection.
HAEMATOLOGY		The platelet count remained depressed throughout the post operative period.
POSTMORTEM		Nil abnormal was found. The liver was macroscopically normal in appearance.

PIG 54H



+ Laboratory mean for normal anaethetised pigs

LIVER BIOPSY 9TH DAY (Fig. 37)

In many areas there was extensive loss of liver cells and haemorrhage into the lobule; a few of the surviving cells were actively regenerating. The portal tracts were heavily infiltrated with round cells which extendedinto the liver sinusoids. There was active bile duct proliferation and some bile plugging. The features are in keeping with severe rejection.

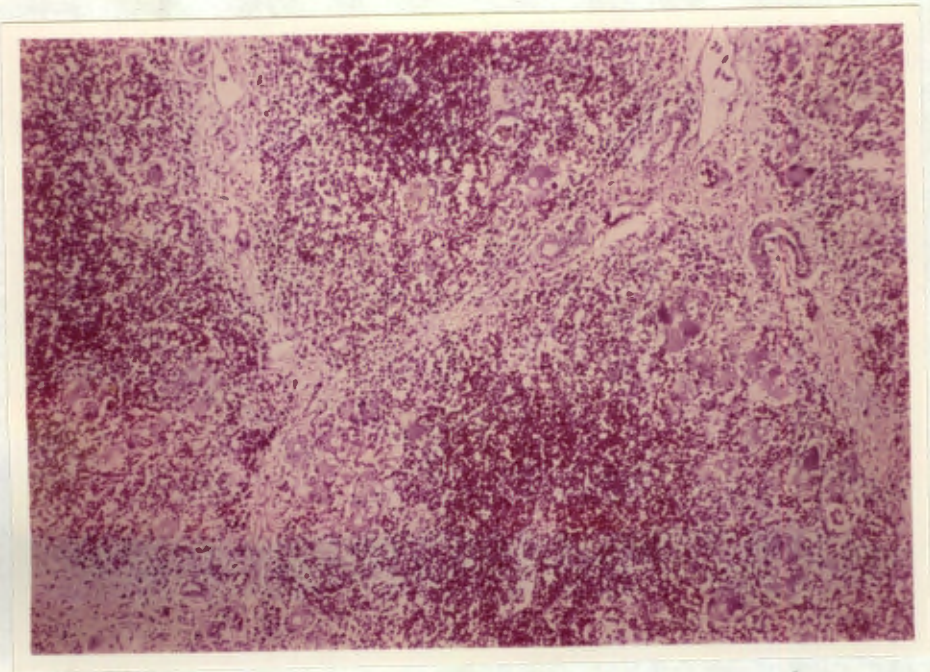


Fig. 37 Mag. x 25

	DONOR	RECIPIENT
	Landrace	Landrace
	19.2 Kg.	20.4 Kg.
	Female	Female
ISCHAEMIC TIME		12 Hours 22 Minutes
DURATION OF SURVIVAL		3 Days
HYPERBARIC PRESSURE		1.5 ATA
PRE STORAGE p.02		215 mm.Hg.
POST STORAGE p.02		610 mm.Hg.
DECOMPRESSION TIME		10 minutes

POST OPERATIVE COURSE

Three hours post operatively oozing of blood was noted at the lower end of the abdominal incision. This was packed and clotted 24 hours later. Haemoglobin on the first post operative day was 7 Gm% and the animal appeared to be maintaining its general condition. On the 2nd day, however, the haemoglobin was found to be 4 Gm%. A unit of packed cells was transfused but the animal died the next day.

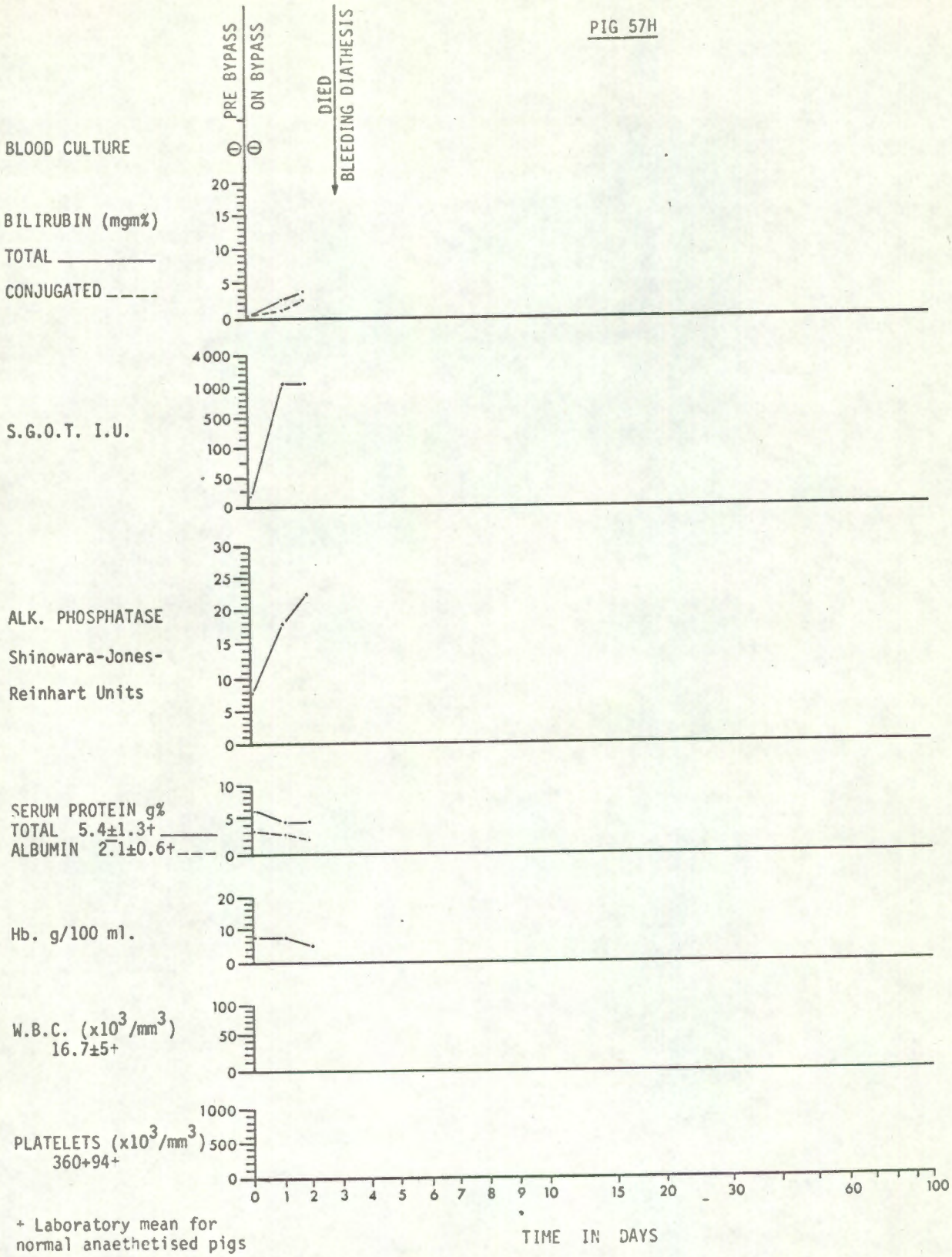
BIOCHEMISTRY

This clearly demonstrates the progressive liver cell damage occurring on the 2nd day with further elevation of the serum bilirubin, alkaline phosphatase and S.G.O.T.

POSTMORTEM

A 900 ml. haemoperitoneum with a haemoglobin concentration of 5 Gm% was found.

PIG 57H



+ Laboratory mean for normal anaethetised pigs

POSTMORTEM LIVER HISTOLOGY (Figs. 38 & 39)

The sections showed evidence of all phases of necrosis (massive, zonal, focal). In addition, one area showed infarction with a line of demarcation between the necrotic and viable liver tissue.

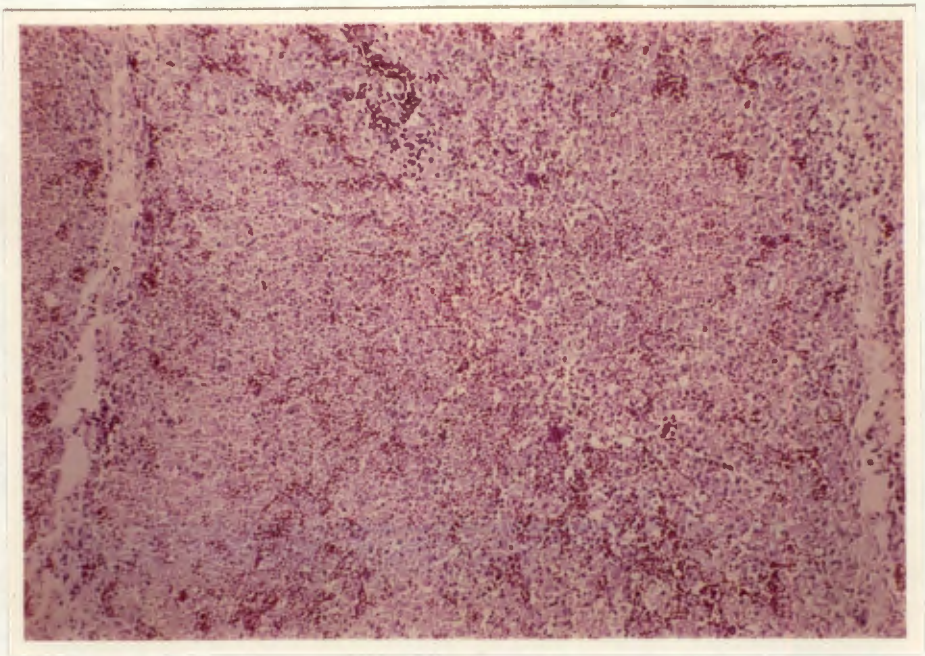


Fig. 38 Mag. x 25

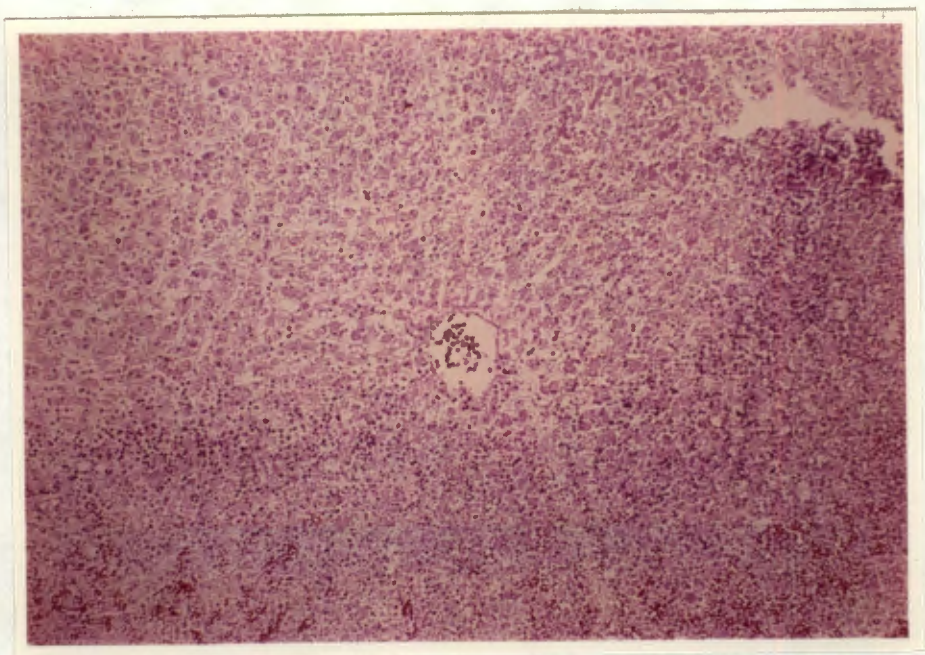


Fig. 39 Mag. x 25

DISCUSSION

Hyperbaric oxygen combined with hypothermia was unsuccessfully used for 23-24 hours storage in only two experiments. The optimum period of preservation by this method was also not ascertained. However, the undoubted efficacy of hyperbaric oxygen was clearly demonstrated. Whereas previously, successful storage for periods of 12 hours at 2-5^{o}C was not possible by simple hypothermia, the addition of hyperbaric oxygen at 3 ATA resulted in four consecutively successful experiments. In the only failure, a leak in the chamber resulted in incomplete pressurisation (1.5 ATA). This was reflected in the lower value of the post storage pO_2.

The immediate post operative values of the serum bilirubin, alkaline phosphatase and S.G.O.T. were significantly elevated, suggesting a more severe degree of ischaemic damage. These parameters, however, returned to normal within 4 days demonstrating the remarkable regenerative ability of the hepatocyte.

An inexplicable phenomenon was the persistent depression of the platelet count during the first post operative week. This may be of importance in the pathogenesis of the bleeding diathesis encountered in unsuccessful liver storage.

CONCLUSION

Hyperbaric oxygen at 3 ATA combined with simple hypothermia (2-5^{o}C) successfully extended the period of preservation of the pig liver to 12 hours.

GROUP IV

PERFUSION STORAGE

PERFUSION STORAGE (TABLE 6)

1. PERFUSION STORAGE 6 - 7 HOURS AT 37°C

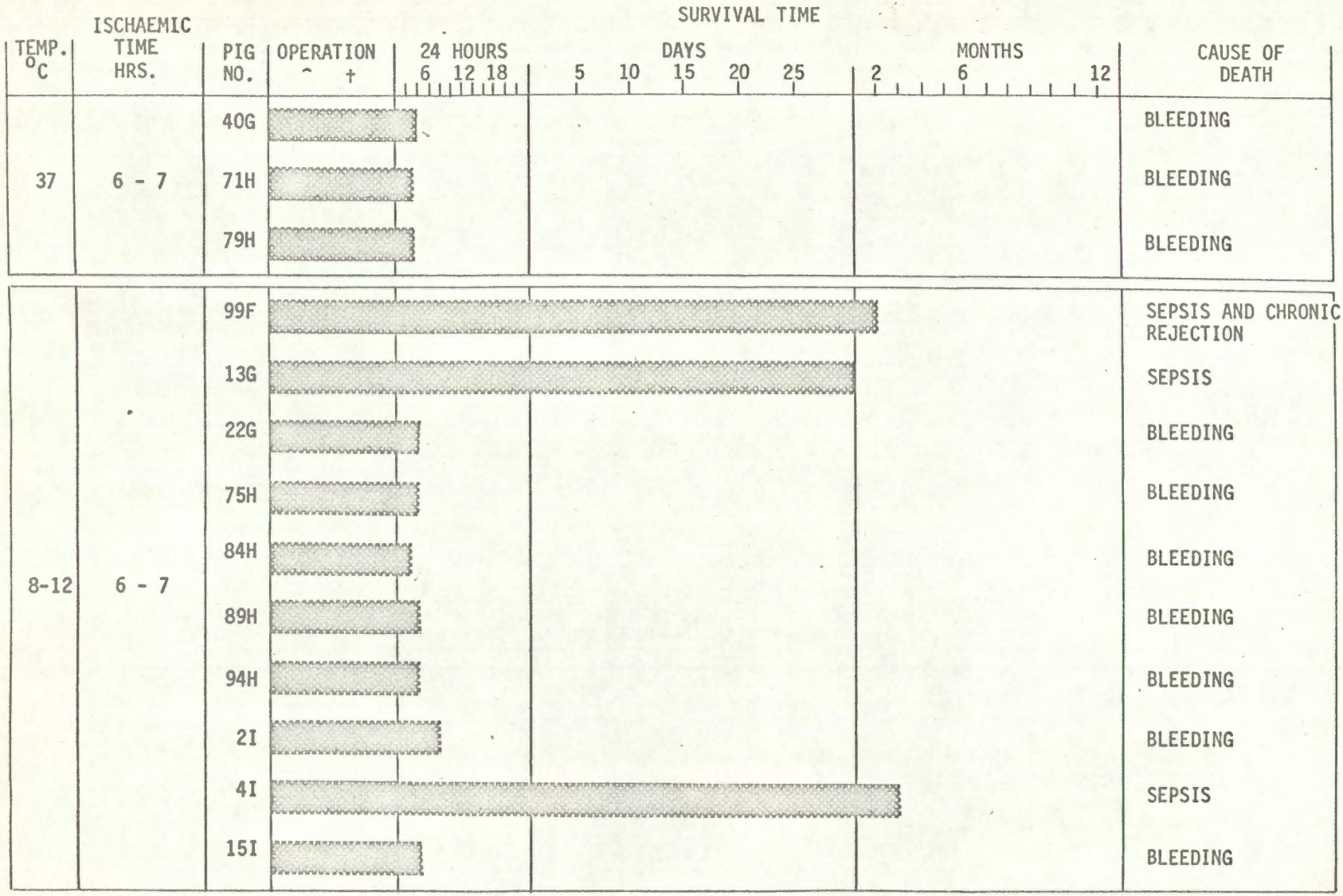
All three animals survived the operation and recovered from the anaesthetic; but died within a few hours from a generalised bleeding diathesis.

2. PERFUSION STORAGE 6 - 7 HOURS AT 8-12°C

In only three of the ten animals was storage successful. The remaining seven animals all recovered from the anaesthetic but died within a few hours from a generalised bleeding diathesis.

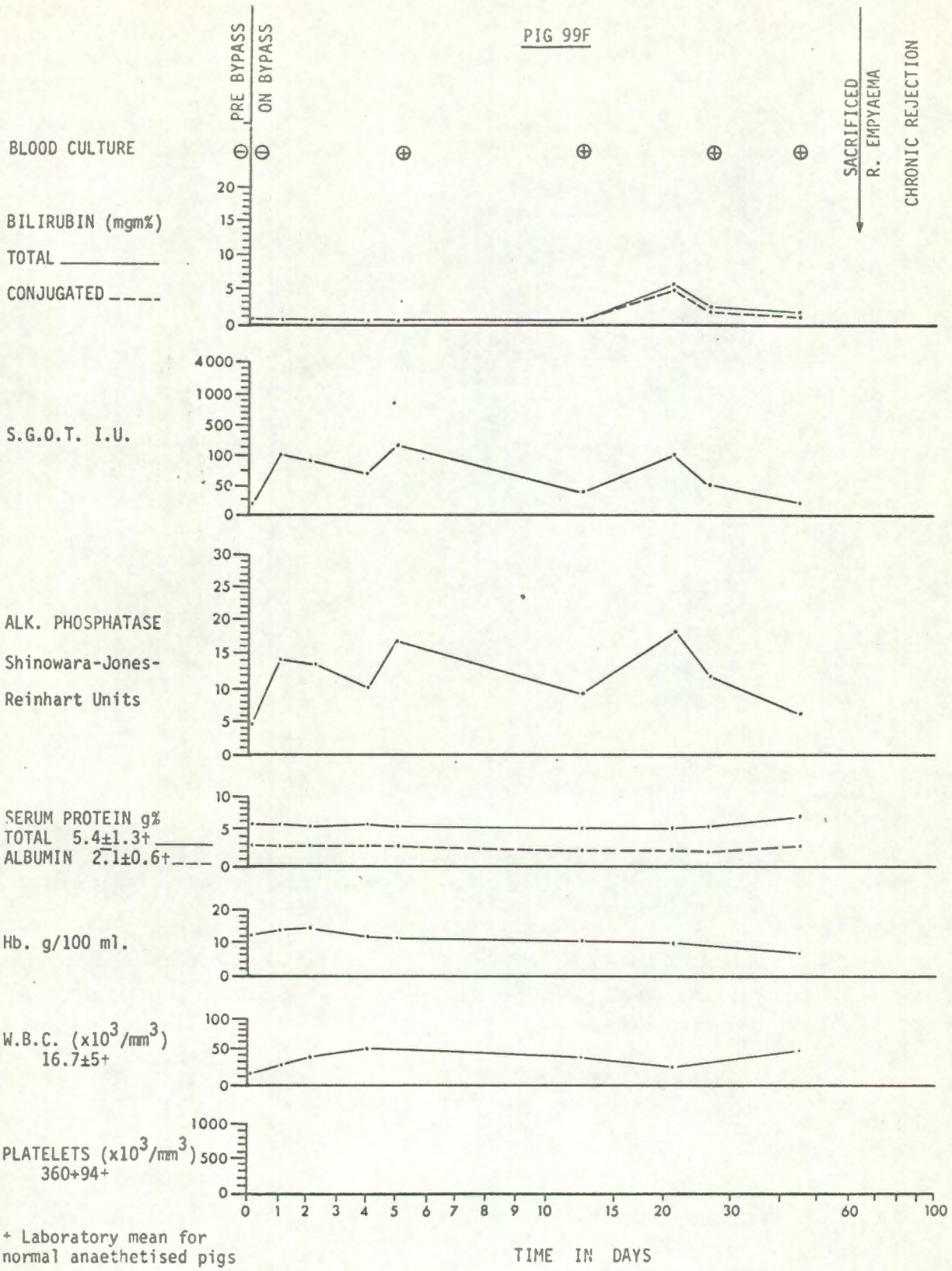
TABLE 6

PERFUSION STORAGE



^ PORTAL VEIN REVASCULARISATION
† HEPATIC ARTERY REVASCULARISATION

	DONOR	RECIPIENT
	Landrace	Landrace
	16 Kg.	20.4 Kg.
	Female	Female
ISCHAEMIC TIME		6 Hours 20 Minutes
DURATION OF SURVIVAL		60 Days (sacrificed)
POST OPERATIVE COURSE		This animal was well until the 3rd week post transplantation; thereafter it became anorexic, jaundiced and failed to thrive. When sacrificed, it weighed 13.6 Kg.
BIOCHEMISTRY		The biochemical changes during the first and third week were suggestive of rejection.
BACTERIOLOGY		Blood cultures were positive throughout and a variety of organisms were grown (staph aureus, proteus, enterococci, Beta haemolytic strep).
POSTMORTEM		There was an empyaema of the right chest (proteus, achromobacter, Beta haemolytic streptococci). The liver, macroscopically, was normal in appearance.



+ Laboratory mean for normal anaethetised pigs

POSTMORTEM LIVER HISTOLOGY (Figs. 40 & 41)

There were areas of focal necrosis of hepatocytes, the sinusoids were dilated and Kupffer cells were prominent. The portal tracts and interlobular septa were widened and there was fibroelastic thickening of the hepatic artery. There was also a prominent infiltration with mononuclear cells. These features were suggestive of chronic rejection and probably contributed to the animal's failure to thrive.

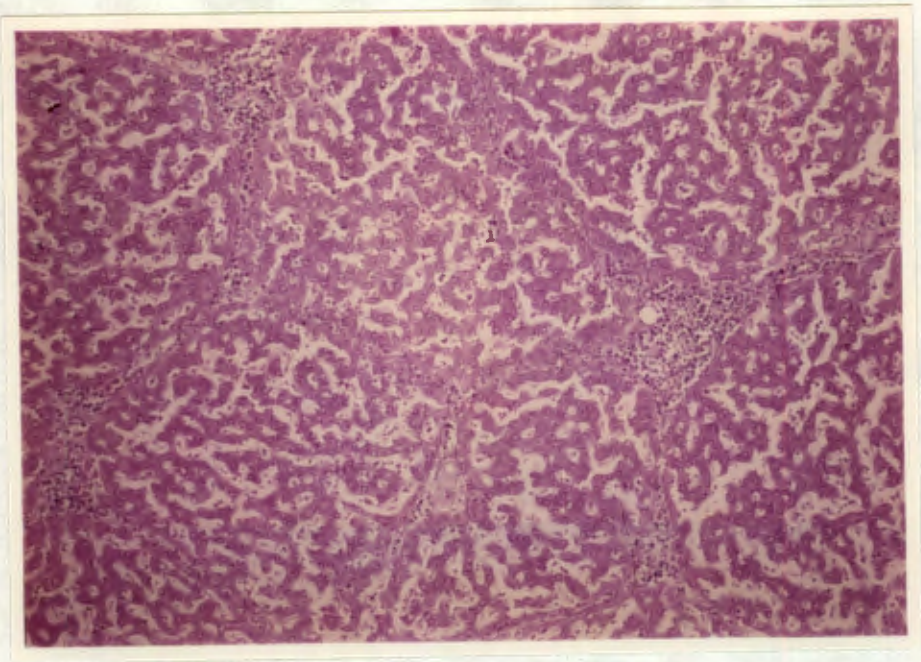


Fig. 40 Mag. x 25.

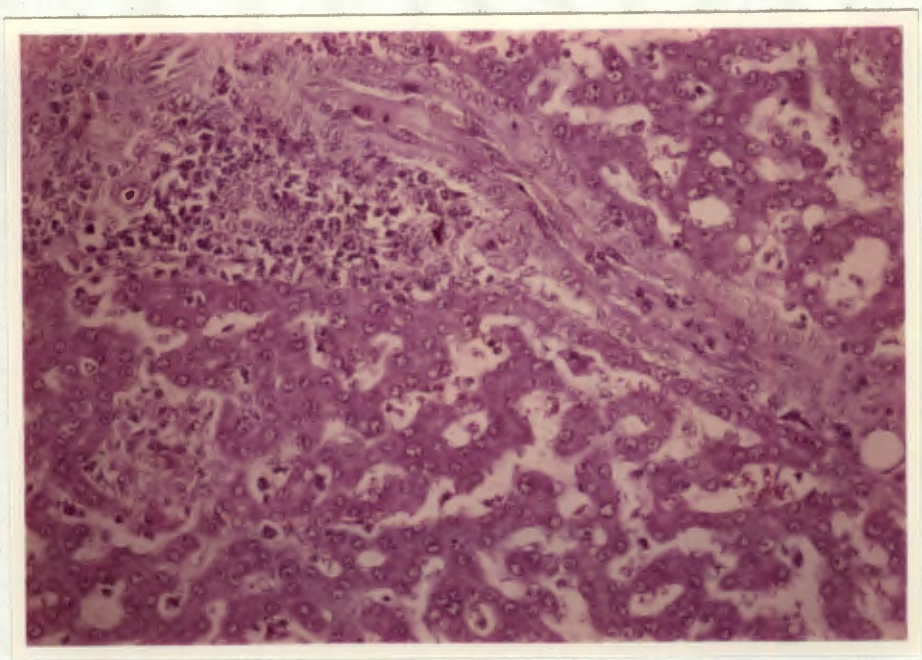
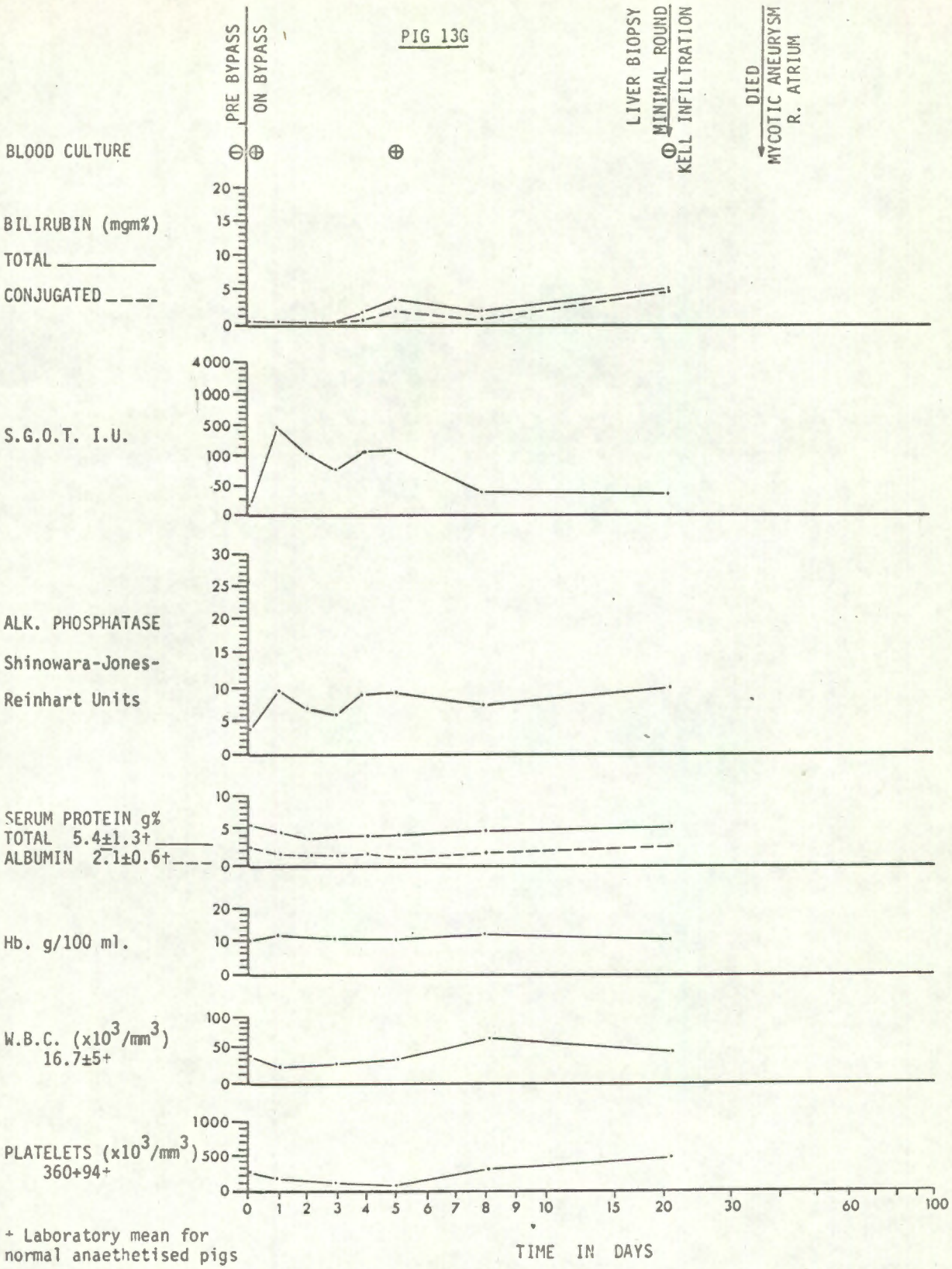


Fig. 41 Mag. x 64

	DONOR	RECIPIENT
	Landrace	Landrace
	22.7 Kg.	24.9 Kg.
	Female	Male
ISCHAEMIC TIME		6 Hours 30 Minutes
DURATION OF SURVIVAL		34 Days
POST OPERATIVE COURSE		The animal was well until the 4th day then became jaundiced and remained so during the remaining post operative period. It died suddenly 34 days post transplantation.
BIOCHEMISTRY		The biochemical changes on the 5th day were probably due to rejection but thereafter were in keeping with a septicaemia.
POSTMORTEM		A ruptured mycotic aneurysm was present in the right atrium with an associated haemopericardium. The pericardium was covered with a fibrinous exudate and Cl. Welchii and Klebsiella Aerogenes were cultured from both the wall of the aneurysm and the pericardium. The cut surface of the liver had a classical nutmeg appearance.

PIG 13G



+ Laboratory mean for normal anaethetised pigs

POSTMORTEM LIVER HISTOLOGY (Fig. 42)

This showed both centrilibular and focal necrosis with polymorphonuclear leucocyte infiltration due probably to anoxia. Bile lakes were present suggesting extrahepatic obstruction which was, however, not demonstrated at post-mortem.

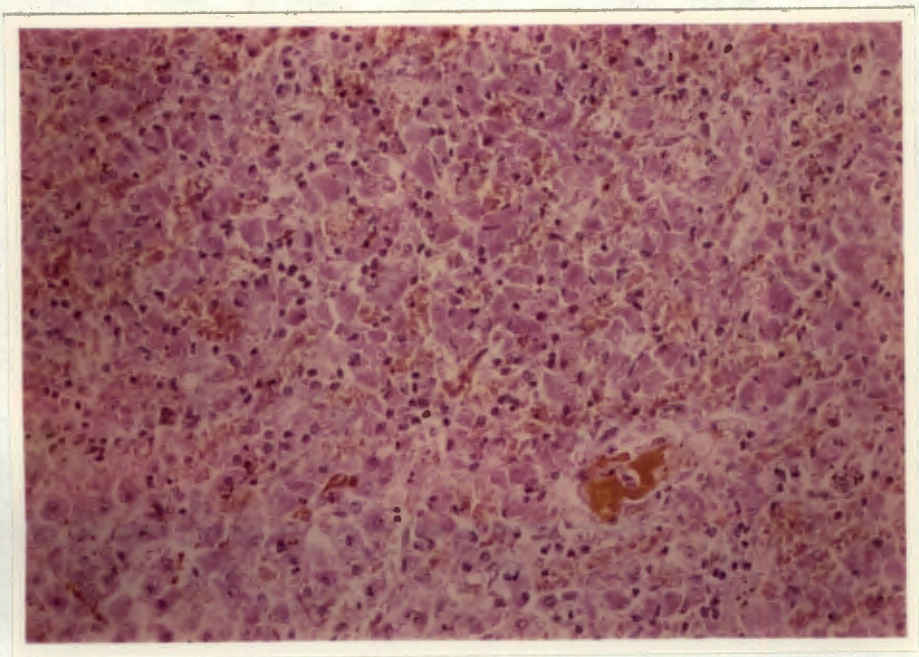
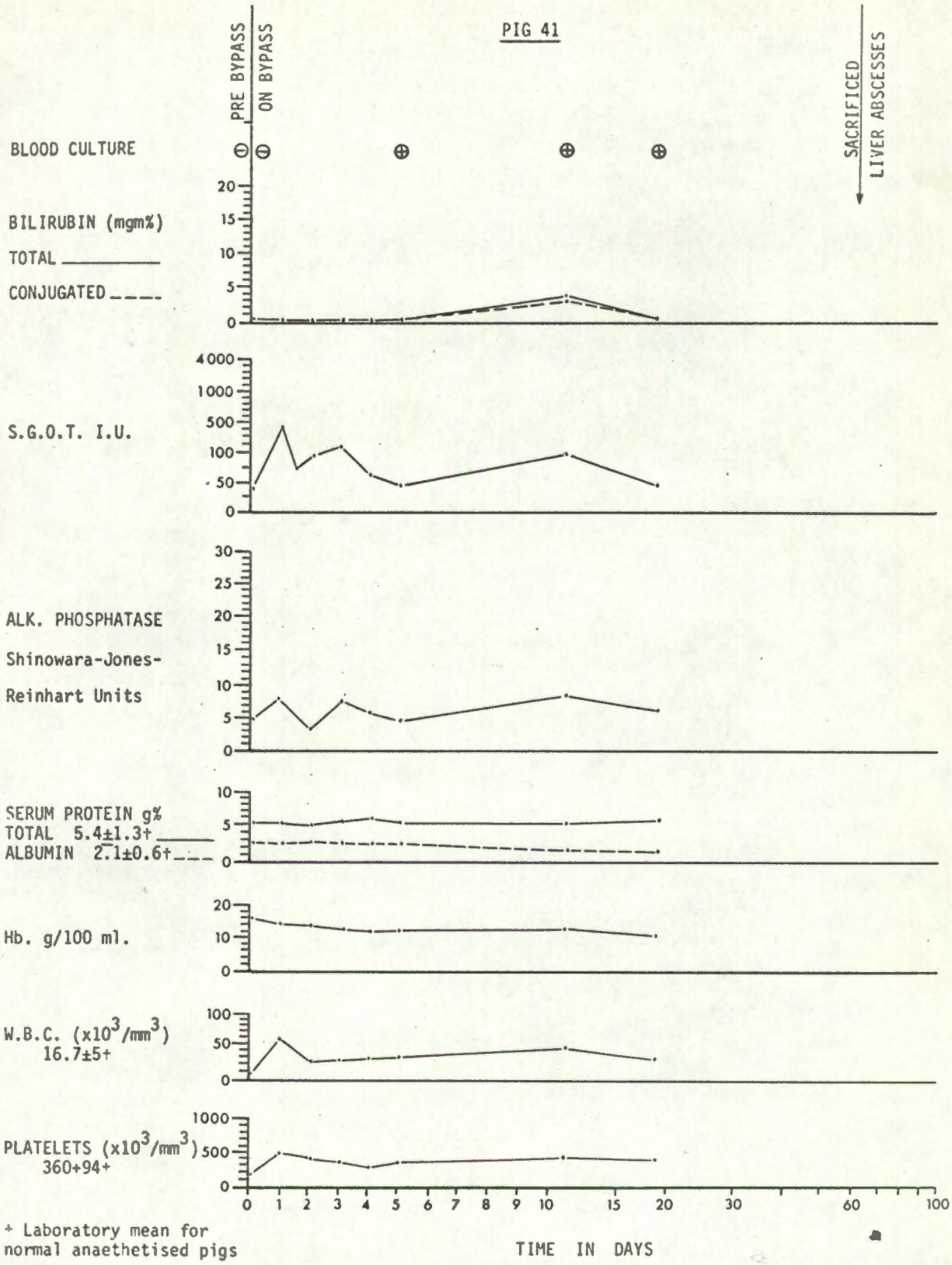


Fig. 42 Mag. x 25.

	DONOR	RECIPIENT
	Landrace	Landrace
	15.9 Kg.	19.5 Kg.
	Male	Female
ISCHAEMIC TIME		6 Hours 45 Minutes
DURATION OF SURVIVAL		60 Days (sacrificed)
POST OPERATIVE COURSE		This animal failed to thrive ab initio. It had a transient episode of jaundice during the 2nd week which remitted spontaneously. Prior to sacrifice it had persistent diarrhoea and weighed 11.3 Kg.
BIOCHEMISTRY		Biochemical changes during the 2nd week (elevated serum bilirubin, alkaline phosphatase and S.G.O.T.) were suggestive of rejection.
BACTERIOLOGY		Blood cultures were positive throughout the post operative course (pseudomonas and klebsiella species).
POSTMORTEM		The liver contained multiple abscesses in all the lobes.

PIG 41



+ Laboratory mean for normal anaethetised pigs

POSTMORTEM LIVER HISTOLOGY (Fig. 43)

Shows a liver abscess.

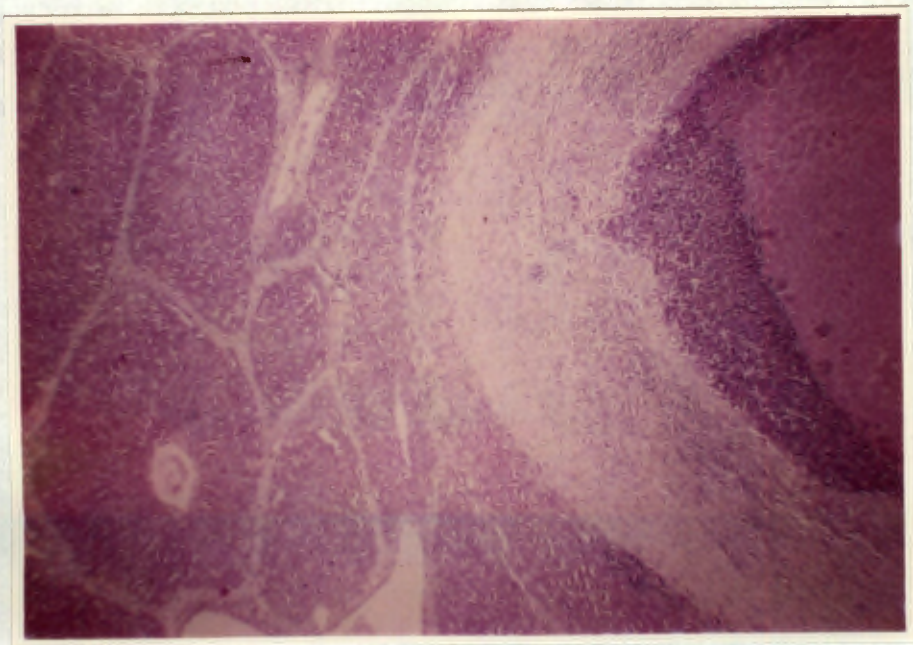


Fig. 43 Mag. x 12.

DISCUSSION

Perfusion storage under normothermic temperature was totally unsuccessful in providing adequately functioning livers. Macroscopically these livers were oedematous with obvious haemorrhage into some areas and even patches of necrosis. Histologically (Fig. 44) there was extensive centrizonal and peripheral necrosis with frank haemorrhage into the lobule. The sinusoids were congested with both red blood cells and polymorphonuclear leucocytes.

Hypothermic perfusion storage provided only three successfully stored livers in 10 experiments. These were less oedematous, but when completely revascularised, they appeared unevenly perfused. The macroscopic appearance of the 7 unsuccessfully preserved livers were extremely variable in respect to oedema, haemorrhage and patchy necrosis; however, in all cases a bleeding diathesis occurred shortly after complete revascularisation and this was noticeably aggravated by the administration of protamine. Although all these animals recovered from the anaesthetic, they all died within a few hours of the operation.

Histologically (Fig. 45) there was less extensive centrizonal necrosis and haemorrhage, but there was marked congestion of the sinusoids with red blood cells and infiltration of the lobule with polymorphonuclear leucocytes.

Of the three surviving animals, two died of gross infection which may have been related to the perfusion being carried out under clean but not sterile conditions and to the use of abattoir blood for the perfusate.

CONCLUSION:

In this study, isolated hypothermic perfusion storage for 6-7 hours provided an occasional adequately functioning liver.

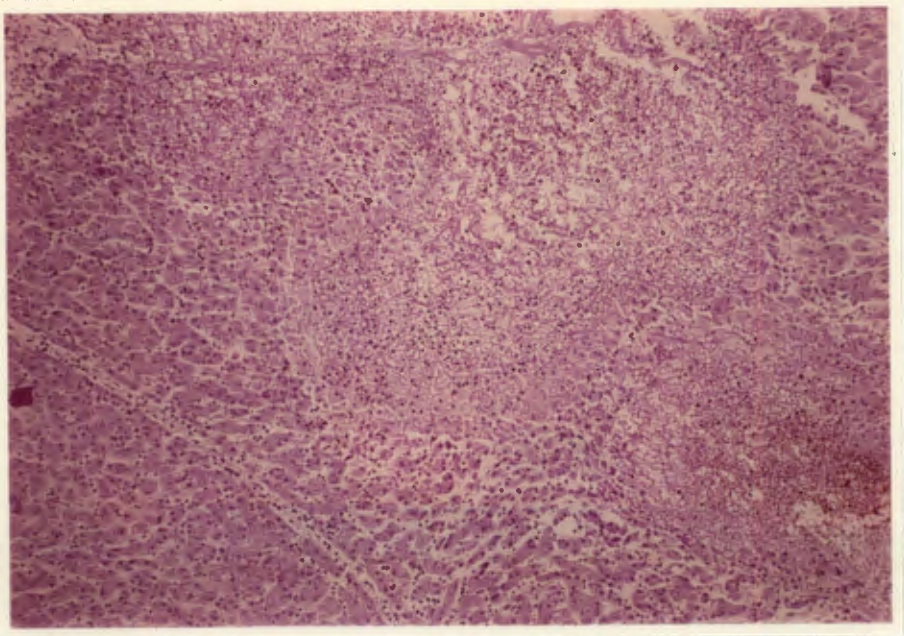


Fig.44 (mag.x 25)

NORMOTHERMIC PERFUSION STORAGE

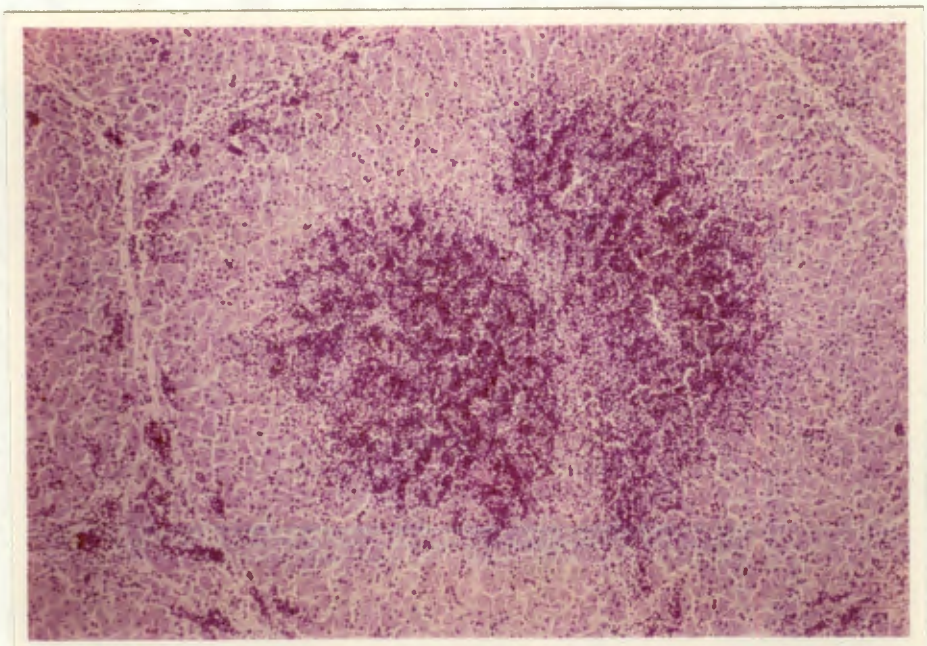


Fig. 45 (mag.x25)

HYPOTHERMIC PERFUSION STORAGE

CHAPTER SIX

CHAPTER SIX

DISCUSSION

In orthotopic liver transplantation the life of the recipient is both immediately and completely dependent on the transplanted liver. It is obvious therefore that any successful method of liver storage must preserve sufficient function for the organ to sustain life. In experimental work where normal organs are invariably used and the procedure performed under ideal conditions, this consideration assumes paramount importance: if parenchymatous damage caused from the storage procedure results in dysfunction incompatible with life, the clinical use of the technique cannot even be considered.

In these experiments consistently successful preservation of the pig liver was achieved by simple hypothermic immersion storage for a period of 6 to 8 hours and for 12 hours by the addition of hyperbaric oxygen.

This discussion concerns the factors considered to be of importance in the methodology, the complications encountered and the merits of the different procedures.

METHODOLOGY

CHOICE OF ANIMAL:

There is evidence that the dog is an unsuitable animal for experimental liver transplantation (Mieny et al. 1967(b), Terblanche et al. 1968(a), Mikaeloff and Calne 1969), which may account for the failure to preserve its liver beyond 3.5 hours using simple hypothermia (Starzl et al. 1960, Schalm 1968). This has resulted in the introduction of more complicated techniques (Brettschneider et al. 1968(b)) to store livers, without adequate assessment of simple methods in other species.

The remarkable anatomical and physiological similarity of the pig liver, its vessels and biliary tract to that of man and the resistance

of the / . . . .

of the porcine organ to the development of "outflow" block makes it a more suitable organ, though the primate liver which was not available for investigation would probably be the ideal.

Prior to this study, successful storage by either simple hypothermia or simple hypothermia combined with hyperbaric oxygen, was not reported using either a pig or a primate liver. Recently, Abouna et al. (1970) presented their results of successful pig liver storage for 6 hours and Slapak et al. (1970b) reported long term survival after 3.5 to 5 hours preservation of the rhesus monkey liver using simple hypothermia. These reports confirm the importance of species differences and the choice of animal in the experimental situation.

SURGICAL TECHNIQUE

The technique of orthotopic liver transplantation used was evolved from those described by Moore et al. (1960) and Starzl et al. (1960) in the dog and Mieny et al (1967b), Terblanche et al. (1968b) and Calne et al. (1968a) in the pig. The surgical technique throughout must be impeccable to ensure minimal trauma to the organ which is to be stored and transplanted. In this way, post storage assessment will reflect deviations from the normal which may be attributed only to the ischaemic or anoxic damage caused by the storage procedure.

DONOR OPERATION

(i) HEPARINISATION OF THE DONOR:

Cromwell et al. (1955), demonstrated the protective effects of systemic heparinisation in dogs subjected to acute circulatory failure. Hardaway and McKay (1959) confirmed this by administering incompatible blood transfusions or injecting amniotic fluid into dogs: only unheparinised animals died and microscopic examination revealed numerous

thrombi / . . .

thrombi in the capillaries and small vessels of the lung and liver. Van Wyk et al. (1965b), in assessing the function of the excised cadaver pig liver at room temperature, found that the majority of liver functions survived almost undiminished in efficiency for 3 hours without perfusion. The only protection afforded the liver against ischaemic damage being premortem heparinisation. In this study, although the donor was heparinised to facilitate the collection of blood, its use was considered to be of primary importance in the preliminary preparation of the donor liver by preventing the formation of microthrombi.

(ii) EXSANGUINATION

The donor was exsanguinated to obtain blood for the recipient operation, as abbatoir blood was invariably contaminated with micro-organisms. The delay between exsanguination and intraportal cooling was introduced to simulate clinical circumstances. It is possible, however, that preliminary exsanguination enhances perfusion by removing blood from the organ - a factor considered to be of major importance in successful preservation of kidneys (Manax et al. 1965c).

(iii) COMPOSITION OF THE PERFUSION SOLUTION

It is now well recognised that the method of preparing the donor organ and the choice of perfusate are important factors in organ storage (Humphries 1967, Abouna 1968b). There are two indispensible prerequisites in the preparation: prevention of anoxic damage and intravascular coagulation.

The liver can be protected from anoxic damage by rapid core cooling. In principle, core cooling is carried out by hypothermic perfusion through the main artery of the organ, this being superior to simple immersion in a cold medium (Sicular and Moore, 1961). In the case of the liver, intraportal perfusion alone with a suitable solution produces rapid cooling within 8 to 10 minutes. If the donor has been heparinised prior to hepatectomy the prevention of intravascular coagulation is no longer urgent, though preliminary irrigation with a balanced salt solution containing low molecular weight dextran provides additional

protection / . . .

protection against this hazard (Manax et al. 1965c). The anti-sludging and oncontic properties of low molecular weight dextran appears to account for its beneficial action.

The solution used in this study to irrigate the liver (Solution I) was a balanced salt solution with low molecular weight dextran autoclaved and then refrigerated at 4°C. Three litres ensured that the final perfusate draining the liver was virtually free of haemoglobin.

(iv) PRESERVING SOLUTIONS

The use of a specific preserving solution presumes that the constituents of the solution provides additional protection to the isolated hypothermic liver either by supplying essential substrate or by slowing the process of cell death.

Four basic solutions were investigated (a balanced salt solution, plasma, plasma with cell membrane stabilising drugs and a solution similar in electrolyte composition to intracellular fluid).

Schalm (1968) showed that plasma was superior to Ringer Lactate for storing dog livers and Fonkalsrud et al. (1969) demonstrated that pre-treatment of donor and recipient with chlorpromazine and phenoxybenzamine were effective in prolonging tolerance to hepatic ischaemia in situ. Preservation without perfusion using a solution resembling intracellular fluid has been confined to the kidney (Collins et al. 1969; Collste et al. 1970).

It is concluded from this study that of these four solutions, plasma is the most suitable media for preserving pig livers by simple hypothermia.

RECIPIENT OPERATION

(i) PORTO SYSTEMIC SHUNT

In the original descriptions of the technique of orthotopic liver transplantation in the pig (Mieny et al. 1967b; Terblanche et al. 1968b;

Calne et al. 1968a) both the portal and the lower inferior vena cava circulations were bypassed. It has since been found unnecessary to bypass the inferior vena cava (Starzl and Putnam 1969; Calne et al. 1969b; Dent et al. 1971a) but porto systemic bypass is still mandatory and was used in all experiments with a mean bypass time of 30 minutes.

(ii) BILIARY DRAINAGE

A complication encountered early in the experience of pig liver transplantation was ascending cholangitis in association with biliary drainage by cholecystoduodenostomy. Calne et al. (1967b) suggested that this may be prevented by a direct bile duct to bile duct anastomosis to retain the sphincter of Oddi mechanism. The superiority of this form of biliary drainage has been confirmed in both the dog (Brettschneider et al. 1968a) and the pig (Calne 1969; Dent et al. 1971a) and was used throughout this study.

(iii) HEPATIC ARTERY ANASTOMOSIS

The arterial blood supply of an orthotopic liver transplant is crucial (Moore et al. 1964; Kashiwagi et al. 1968) and many types of arterial anastomoses have been tried to produce an efficient flow through the liver (Moore et al. 1960; Starzl et al. 1960; Calne et al. 1968a; Fonkalsrud et al. 1968; Terblanche et al. 1968b). Better results have been obtained with small vessel anastomosis than with hepatic artery aortic pedicle grafts. Starzl et al. (1967) reported a 70% incidence of hepatic artery thrombosis using the latter, yet in this study this complication was not encountered in a single animal. This is attributed to the extreme care taken to avoid damage to the vessel wall during hepatic artery dissection in the donor.

(iv) SPLENECTOMY

Removal of the spleen reduces the amount of lymphoid tissue in the recipient and should therefore favour the survival of the graft. Calne et al. (1969a) however, found that longterm survival occurred independently of whether the animals were splenectomised or not.

(v) / . . .

(v) GASTRODUODENOSTOMY

In the first publication on orthotopic liver transplantation to unmodified dogs (Moore et al 1960), a high incidence of acute gastroduodenal ulceration was reported and the immediate cause of death in many animals was due to a complication of these lesions. It was postulated that the ulcer diathesis was generated by poor hepatic function due to rejection of the organ. When rejection in dogs was mitigated by the use of Azathioprine, complicated gastric ulcers still occurred (Starzl et al. 1965; Farris et al. 1965). Subsequently, Stuart et al. (1967b) found that a 70% gastrectomy was the only effective measure to eliminate gastric ulceration complicating liver transplantation in dogs.

In the pig naturally occurring gastric ulcers have been widely documented and the incidence and aetiological factors reviewed (Kowalczyk 1969). Terblanche et al. (1968a) first reported gastric ulcers in the pars oesophagea of the porcine stomach complicating orthotopic liver transplantation and Dent et al. (1971b) found that irrespective of the type of biliary drainage used, 60% of their animals succumbed from complications of these ulcers. Calne et al. (1967b) were able to eliminate gastric ulcers in the pig with liver transplants by prophylactic vagotomy and gastroduodenostomy but Peacock et al. (1969) found that vagotomy in liver transplantation introduced a number of serious additional complications. A preliminary study by the author (unpublished data) demonstrated the efficacy of gastroduodenostomy without vagotomy. In this study, no animal died from a complication of a gastric ulcer, nor is there evidence to suggest that any of the surviving animals are harbouring an active ulcer.

The aetiology of these ulcers remains unexplained, though their control by an adequate drainage procedure suggests that stasis may be the precipitating factor. It is postulated that division of the pyloric branches of the anterior vagus nerve during the recipient hepatectomy, results in delayed emptying of the stomach, stasis follows which stimulates the release of gastrin. A gastroduodenostomy provides adequate emptying and therefore eliminates stasis and ulceration. Further experimental work, however, will be required to substantiate this hypothesis.

In / . . . .

In man a prophylactic gastric acid controlling procedure has not been found necessary; though patients with protracted survival are not exempt from gastrointestinal haemorrhage many months after the operation (Starzl and Putnam, 1969).

(vi) SIMULTANEOUS SKIN ALLOGRAFTS:

These were performed to obtain additional information of the immunosuppressive effects of the allografted liver on other tissues and organs (Calne et al. 1969a) and is not pertinent to this thesis.

COMPLICATIONS

(i) BLEEDING DIATHESIS

This was the single most important complication limiting successful storage and confirmed the experience of Brettschneider et al. (1968b), Belzer et al. (1970) and Mieny (1970). It is undoubtedly related to the fact that the liver produces most of the haemostatic factors responsible for blood coagulation and that it is also involved in the clearance of several substances active in coagulation and fibrinolysis.

In the first quantitative study of the bleeding diathesis, Von Kaulla et al. (1966) placed considerable emphasis on the role of the explosive intra operative fibrinolysis which occurred during liver transplantation and speculated on the dangers of the succeeding intra vascular coagulopathy.

Subsequently, it was shown that in the pig (Blecher et al. 1968; Perkins et al. 1970), in the dog (Hutchison et al. 1968; Pechet et al. 1969) and also in humans (Groth et al. 1969; Williams, 1970) there was a simultaneous decline of other measured clotting factors (fibrinogen, prothrombin and antihaemophylic factor) associated with the increase in fibrinolytic activity. This in the presence of thrombocytopaenia suggests extensive intravascular coagulation (Rodriguez - Erdman, 1965) as the primary phenomenon with fibrinolysis as a secondary event (Abildgaard, 1969). Pechet et al. (1969) related the extent of these

changes to the magnitude of the liver injury. Despite this strong evidence of a disseminated intravascular coagulopathy (DIC) a search of canine homografts as well as of all the recipient tissues failed to reveal evidence of microthrombi (Hutchison et al. 1968).

Although a simultaneous investigation of the clotting mechanism could not be included in this study, an observation encountered in group 3 (immersion storage with hyperbaric oxygen) may be pertinent to the pathogenesis of the bleeding diathesis.

It was noted that in this group the platelets remained persistently depressed until the 5th or 6th post operative day. This may be explained by one of two mechanisms.

(a) That the platelets were trapped within the liver adhering to the damaged vessel wall or to the wall of the sinusoids. This would be in keeping with the hypothesis of DIC.

(b) That the damaged liver produces a humoral factor which lysis platelets or specifically depresses the bone marrow until normal liver function is recovered. This mechanism would more readily explain the persistent failure to demonstrate microvascular thrombi and may account for the thrombocytopaenia encountered clinically in liver disease associated with extensive hepatocyte destruction.

In a recent report, Slapak et al. (1970b) extensively investigated the clotting mechanism during preservation and orthotopic autotransplantation of primate livers. These authors demonstrated intravascular coagulation during the anhepatic phase which diminished after transplantation with recovery of fibrinogen, Factor II, VII and IX. However, platelet function as indicated by serum prothrombin and platelet adhesiveness was found to be diminished. These observations require further investigation and elaboration.

(ii) / . . . . .

(ii) ASCITES

Only two deaths occurred due to massive ascites and in both cases were associated with the clinical picture of acute hypovolaemic shock. Mieny et al. (1968a) showed that with marginally preserved livers, once the bleeding diathesis had been controlled with epsilon amino caproic acid (EACA) the second limiting complication to successful storage was the formation of ascites.

The pathogenesis of this was elegantly demonstrated by Kashiwagi et al. (1968). They showed that a damaged liver was associated with striking changes in the constituents of the serum proteins and that some discrete fractions disappeared completely. This produced the "stripped tree" appearance of the recipient's serum on immuno electrophoresis. These abnormalities were totally reversible if the hepatic injury was moderate, but when severe, the missing electrophoretic bands never returned to normal.

(iii) REJECTION

Cordier, Garnier and Clot (1966) first reported the observation that pigs can tolerate a liver allograft for prolonged periods without immunosuppression. This interesting and biologically significant phenomenon was soon confirmed by Peacock and Terblanche (1967) whose studies were carried out without the knowledge of previous work in this field. Since then further proof of the blandness of hepatic allograft rejection in pigs has been contributed to by Calne et al. (1967b), Mieny (1968), Starzl and Putnam (1969), Garnier et al. (1970), Belzer et al. (1970), Huguet et al. (1970) and Dent et al. (1971a). In the experience of these various groups the frequency and severity of rejection varied significantly ranging from minimal cellular infiltration with no deaths by the Cambridge group (Calne et al. 1969a) to an 80% mortality reported by Belzer et al. (1970).

In this study despite the lack of immunosuppression only two of the animals died of rejection at a time when death from rejection would have been expected in an unprotected animal with a liver allograft. The diagnosis was based on the clinical picture in conjunction with the biochemical, haematological and histological findings. Clinically, the

animals / . . .

animals were anorexic and jaundiced. The biochemical profile was similar to that seen in the unimmunosuppressed dog with partial biochemical recovery from the operation, followed by enzyme release, progressive jaundice and elevation of the alkaline phosphatase. Haematologically there was a significant decrease in the platelet count (Starzl et al. 1968a; Calne et al. 1968b) and histologically there was dense round cell infiltration, haemorrhage, cellular dissolution and necrosis.

In the remaining animals, rejection did not appear to play a significant role in causing mortality, but a wide spectrum of morphological changes was encountered. In some animals these were associated with clinical or biochemical changes; but in others no associated changes were found. In an attempt to correlate these findings, the following classification of rejection in the unimmunosuppressed pig is proposed.

(a) FATAL REJECTION

The association of clinical, biochemical, haematological and histological evidence of rejection with a fatal outcome.

(b) SEVERE REJECTION

When clinical, biochemical and histological rejection are present but the animal survives the episode and remits spontaneously.

(c) MODERATE REJECTION

There are no clinical manifestations of rejection, but biochemical and histological changes are present.

(d) MILD REJECTION

The histological changes of round cell infiltration are unassociated with clinical or biochemical evidence of rejection.

Two of the long term survivors showed an increased amount of fibrous tissue in the portal tracts and interlobular septa. One of these (Fig.41)

had / . . . . .

had associated fibro elastic thickening of some of the hepatic arteries and infiltration of the portal tracts with immunocytes. These changes are thought to be due to low grade but continuous intravascular coagulation (Williams et al. 1969) and indicates chronic rejection.

Although previous studies have shown that clinical and biochemical evidence of rejection can remit spontaneously in the unimmunosuppressed pig, histological evidence of spontaneous cellular remission has not been documented. Fig. 19 to 21 illustrates this phenomenon. This is a challenging observation. Do these animals demonstrate enhancement, or is this true immunological tolerance? The answer carries important clinical implications and intensive research is required in this direction.

(iv) INFECTION

Obviously, one cannot provide all the niceties of an intensive care ward to the environment of an animal laboratory, but adequate vetting pre-operatively, meticulous aseptic technique in the course of the operative and storage procedure and adequate antibiotic therapy post operatively would help to eliminate this hazard.

(a) Vetting

Young piglets of the size used in these experiments are particularly susceptible to parasitic infestation with round worm Ascaris Suum and lung worm Metastrongylus spp (Taffs 1969). The migration of the larvae of the round worm through the tissues produces "milk spots" in the liver, a granulomatous lesion which may predispose to micro abscess formation. The characteristic lesions of the lung worm are nodular wedge shaped areas of emphysema (Fig. 46). Any pre-existing parasitic infection will enhance the severity of pneumonic process (McKenzie 1969).

Since the common sites of infection in this study were the lungs and the liver, adequate pre operative vetting and anti helminthic treatment are essential.

Fig. 46 / . . . .

Fig. 46

LUNGWORM INFESTATION WITH METASTRONGYLUS SPP.

(b) / . . . .

(b) Aseptic Technique

Perfusion storage was carried out under clean but not sterile conditions. Non sterile abattoir blood was used and the presence of a toxic substance, e.g. bacterial endotoxin (Eiseman et al. 1963), may have contributed to perfusion failure. This probably also accounted for the higher incidence of sepsis in the survivors of this particular group. A striking histological feature of the unsuccessful perfusion stored liver was a profuse infiltration of the hepatic lobule with polymorphonuclear leucocytes (Fig. 45).

(c) Antibiotics

Blood cultures taken from the splenojugular bypass prior to its removal were positive in over 50% of the animals; whereas the peripheral blood cultures taken prior to the insertion of the external shunt were only positive in 13%. By the 5th post operative day, 66% of the peripheral blood cultures were positive. The predominent organisms were gram negative (pseudomonas, E. coli, proteus, klebsiella and enterococci with occasional Cl. welchii and staph. aureus). In spite of continuous intravenous antibiotics, 40% of the peripheral blood cultures were still positive on the 10th day (Table 7). This study has demonstrated the need to continue antibiotic therapy into the 3rd post operative week following liver transplantation and emphasises the role of the porto systemic shunt in the pathogenesis of the post operative septicaemia. There is, however, a strong belief that the transplanted liver itself occupies a central role in the high incidence of infection (Brettschneider et al. 1968a; McSween 1969; Fulginiti et al. 1968; Starzl et al. 1968b). The most likely source of the majority of the organisms cultured is the gastrointestinal tract and the persistent septicaemia in spite of antibiotics reflects the temporary inability of the liver to phagocytose organisms passing through it.

In humans increased susceptibility to infection is a problem common to the transplantation of all organs as a result of the immunosuppressive measures taken to prevent repudiation of the grafts, but the consequent risk appears to be disproportionately greater after hepatic transplantation (Fulginiti et al. 1968).

Table 7 / . . .

TABLE 7

Pig No.	Peripheral Blood	Portal Blood	5th Day Post Op.	10th Day Post Op.	3rd Week Post Op.	Duration of Survival	Cause of Death
17H	+ve	+ve	-ve			Alive (7/12)	
54H	-ve	-ve	+ve			10 days	Rejection
40H	+ve	-ve	+ve	-ve	-ve	44 days	Strangulated Hernia
26H	-ve	+ve	+ve	+ve		20 days	Subphrenic Abscesses
73H	-ve	+ve	-ve	-ve		Alive (4/12)	
81H	-ve	+ve	+ve	+ve	-ve	Alive (4/12)	
84I	-ve	-ve	-ve			Alive (6/52)	
86I	-ve	-ve	+ve	-ve		Alive (6/52)	
27F	-ve	+ve	+ve	+ve	+ve	74 days	Lung Abscesses
56F	-ve	+ve	+ve	+ve	-ve	Alive (1 year)	
86F	-ve	-ve	-ve	+ve	+ve	30 days	Lung Abscesses
63G	-ve	+ve	+ve	+ve	+ve	38 days	Liver Abscesses
24H	-ve	+ve	-ve	-ve		88 days	Volvulus
28H	-ve	-ve	+ve	-ve		44 days	Pulm. Embolus
12H	-ve	-ve	+ve	-ve	-ve	Alive (7/12)	

Porter (1969) has shown that injury to the intra hepatic portal veins with consequent increased "porosity" is a hallmark of immunological injury. Alternatively, the alteration which occurs may not be visible through a microscope, but represents a subtle decline of a particular kind of reticulo-endothelial activity leading in turn to a loss of the normal function of bacterial filtration. This would not only account for the greater permeability to micro organisms, but could in a more general way undermine the total host defences against infection of other organ systems.

(v) THROMBOEMBOLIC COMPLICATIONS

The high incidence of thromboembolic complications encountered in this study was most disturbing. In three animals death was due to pulmonary embolisation. A bland thrombus was found in the superior vena cava of one of these animals. In addition, septic thrombi were found in the superior vena cava and the right atrium of two other animals which died from other causes. A number of factors may have contributed to this complication.

(a) Liver Transplantation

A hypercoagulable state occurring in the early post operative course of canine liver allografts was described by Von Kaulla et al. (1966) and may predispose to the thrombi forming particularly around retained intravenous catheters.

(b) Splenectomy

Hume et al. (1964), in comparing splenectomised kidney recipients with non splenectomised recipients, found a higher incidence of thromboembolic complications after splenectomy than in the controls.

(c) External Bypass

The potential source of thromboembolic complications resulting from the combined use of an external bypass and clot promoting factors (protamine, EACA) has been documented from the human experience (Starzl et al. 1963;

Von Kaulla et al. 1966; Starzl and Putnam 1969). The first three patients to survive the operation of orthotopic liver transplantation were found at autopsy from 6½ to 22 days later to have major pulmonary emboli. In these cases external plastic bypasses were used and it was suspected that the complication was related to the trauma caused by the insertion of the temporary shunt. Subsequently, it was found that the portal vein and the infrahepatic vena cava of the human can be safely crossclamped for long enough to remove the host liver and replace it with an allograft without the aid of a temporary bypass. Since then, the incidence of pulmonary embolisation has diminished. Unfortunately, the use of a porto systemic bypass is still mandatory in pig liver transplantation, but the incidence of thrombo embolic complication could probably be reduced by the omission of protamine after total revascularisation of the liver, or alternatively by the daily administration of small quantities of heparin. In this study, a catheter was maintained in the same neck vein for 10 days to allow continuous administration of antibiotics; the use of daily heparin under these circumstances is clearly indicated.

(vi) MISCELLANEOUS COMPLICATIONS

In two animals, death occurred due to imperfections of the operative procedure other than the transplantation of a poorly preserved liver. One animal died of a strangulated incisional hernia; the other from a volvulus of small bowel around the afferent and efferent limbs of the duodenal segment of the gastroduodenostomy.

MERITS OF THE VARIOUS STORAGE PROCEDURES

HYPOTHERMIC IMMERSION STORAGE

The ease of its application immediately recommends its use. The fact that it provides consistently successful results for up to 8 hours establishes this method on a par with more complicated techniques currently used both in experimental and human liver storage.

HYPOTHERMIC / . . .

HYPOTHERMIC IMMERSION STORAGE WITH HYPERBARIC OXYGEN

The efficacy of hyperbaric oxygen in extending hypothermic immersion storage of the liver has been proven beyond doubt in this study. However, its application does require special, yet simple, additional apparatus. The duration of storage achieved should allow adequate time for recipient operation, tissue typing and even intercity transportation.

The use of 3 atmospheres absolute of oxygen was empirical and was based on the experience of previous experimental and clinical results (Meinje et al. 1964; Manax et al. 1964; Hyperbaric Oxygen Symposium 1965; Ackerman and Barnard 1966b).

The livers were not weighed either pre or post storage; hence it was not possible to confirm the findings of Brettschneider et al. (1968b) and Huntley et al. (1968) that hyperbarism minimises the amount of oedema formation in the stored liver, though morphologically the two organs stored for 24 hours under hyperbaric oxygen showed minimal evidence of oedema or cell necrosis (Fig. 47).

PERFUSION STORAGE

Continuous in vitro perfusion with pumps and oxygenators was introduced because of the failure to preserve canine livers by simple cooling or by hypothermic cadaveric perfusion (Brettschneider et al. 1968b).

The perfusion system used in this study was inadequate for consistent liver storage and will require modification. However, even more efficient perfusion systems when used for longer than 10 hours (Mieny et al. 1968a; Brettschneider et al. 1968b; Belzer et al. 1970) have failed to provide livers from either the dog or the pig which could consistently sustain life in an anhepatic recipient.

CLINICAL BEHAVIOUR, BIOCHEMISTRY AND HISTOLOGY OF SUCCESSFULLY STORED LIVERS

It was not possible on clinical grounds to distinguish whether an animal received a stored or a non stored liver. All the animals recovered promptly from the anaesthetic and were moving around freely within a few hours. By the third day all were receiving a standard diet. Long term

Fig. 47 / . . .

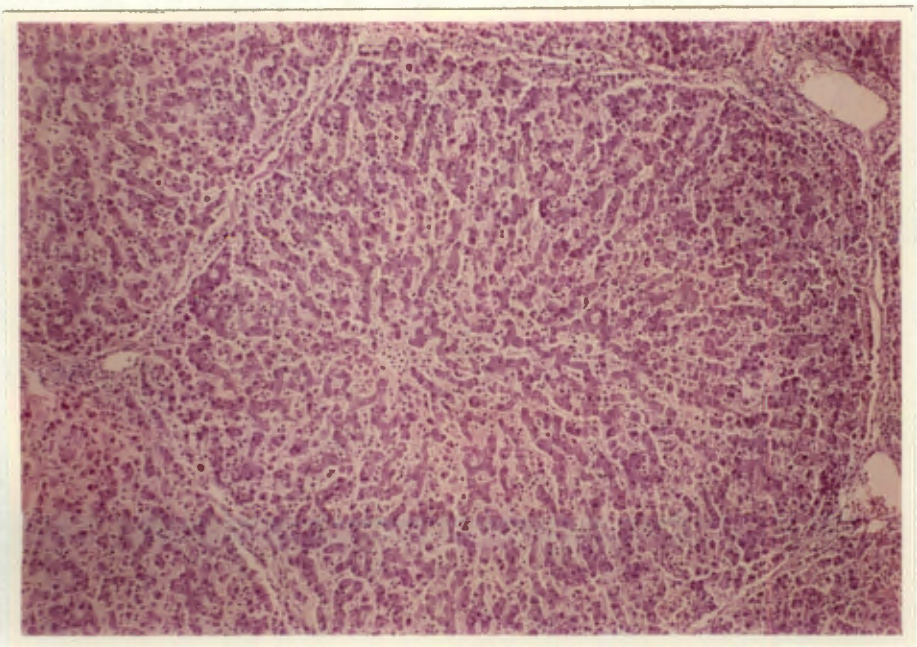


Fig. 47

24 HOUR STORAGE BY HYPOTHERMIC IMMERSION STORAGE AND HYPERBARIC OXYGEN (3ATA).

survivors / . . .

survivors developed at the same rate as pigs receiving non stored livers.

Biochemically the slight elevation in serum alkaline phosphatase and S.G.O.T. noted in the controls after immediate transplantation, rose appreciably following 6-8 hours storage and maximally after 12 hours perservation. These values returned to a normal level by the 5th day.

Sections of liver stained with haematoxylin and eosin failed to reveal any permanent morphological changes which could be attributed to the storage procedure.

The remarkable similarity after orthotopic transplantation between the stored and the non stored organ confirms the success of the storage procedures.

CHAPTER SEVEN

CHAPTER SEVEN

CLINICAL APPLICATION

The human liver is extremely sensitive to warm ischaemia (Goodrich et al. 1956; Raffucci and Wangensteen 1950) and its physiological integrity is only maintained for a short period. Studies by Marchioro et al. (1963), Fonkalsrud et al. (1967) and Peacock et al. (1969) have stressed the difficulties encountered when cadaveric rather than living donors were used.

In human liver transplantation organs may be harvested from donors with established brain death in the presence of an intact circulation (Starzl and Putnam 1969; Fortner et al. 1970), or traditional death is awaited prior to the removal of organs (Williams 1970). In the latter situation, the liver can be cooled by intraportal infusion within four minutes of the diagnosis of death (Calne and Williams 1968). In this study animals were exsanguinated over a period of seven minutes before cooling the liver to simulate the clinical situation.

The importance of species differences has already been referred to. Here the various methods of storage were confined to the pig, whose liver is both anatomically and physiologically similar to the human liver and therefore more suitable for comparative study than the dog liver. Calne and Williams (1968) and Orr et al. (1969) have used a modification of a simple hypothermic immersion storage technique described by Schalm (1968). In the laboratory this method provided successful storage in the dog for a maximum of 3.5 hours, whilst clinically it has been used successfully for up to 5 hours.

It is contended, therefore, that the simple methods of storage described in this thesis can be applied clinically with equal success. These procedures merit preference for their simplicity and should provide a wider clinical application than currently used complex perfusion techniques.

SUMMARY / . . .

SUMMARY

This study was undertaken to determine whether a liver could be stored in vitro for longer than 6 hours without continuous perfusion. Prior to this study, successful liver storage without perfusion did not exceed 3.5 hours.

The literature was reviewed and it was concluded that the only valid test of successful storage was for the stored organ to sustain life in an anhepatic recipient.

The experimental animal used was the pig whose liver is known to be anatomically and physiologically similar to the human liver.

The results indicate that the pig liver can be consistently stored by simple hypothermia for 6-8 hours using a plasma preserving solution at 8-12^{o}C. Hyperbaric oxygen (3ATA) at 2-5^{o}C extended successful storage to 12 hours.

It is concluded that these simple methods of storage can be applied clinically with equal success and should provide a wider clinical application than currently used complex perfusion techniques.

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