PORTACAVAL TRANSPOSITION AND SUBSEQUENT PARTIAL HEPATECTOMY IN THE RAT

A STUDY OF LIVER REGENERATION

by

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"But thanks to my friends for their care in my breeding,
Who taught me betimes to love work and reading."

From Lazy Student,

Dr. Isaac Watt (Devine Songs for Children, 1715)

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DEFINITIONS

Liver Regeneration

Liver regeneration is the restoration of normal liver weight by a process of hyperplasia and hypertrophy of liver cells (Ponfic 1895).

The term "regeneration" has been used in the past indisciminately to cover restoration of liver weight without knowledge of cellular activity. This has confused true regeneration with infiltration by fat, congestion with blood and oedema.

Hepatotrophic Factors

Twenty-four eminent researchers on liver disease met in London in 1978. The meeting is recorded in a book running to 396 pages of text and references. No definitiion was obtained of hepatotrophic factors (Ciba Foundation Symposium 55, 1978).

However, hepatotrophic factors may be categorised into the following general sub-groups:

i) factors initiating regeneratiion

ii) factors promoting regeneration

iii) factors protecting against injury.

No agreement has been possible about the placing of any of the known "hepatotrophic" factors in any of the above groups.

INTRODUCTION

The normal mammalian liver has a "stable" population of hepatocytes and there is a continuous process of cell death and replacemnt at a rate of approximately one new cell in every 20,000 mature cells. There is also a continuous remodelling of the lobular structure of the liver. The propensity of the liver to maintain a "stable" cell population is most dramatically shown in a process which has become known as "liver regeneration"; a response which is best seen after a substantial proportion of the liver has been removed or killed in situ.

The process of liver regeneration was acurately described first by Emil Ponfic, a German pathologist, who presented his work in the 1890's. He showed that in the rabbit, unlike enlargment of the heart due to strain, enlargement of the liver after resection of part of its mass was due to both hypertrophy and hyperplasia of the remaining cells. In man, this same process occurs after liver resection necessitated by trauma, and full restoration of liver size is apparent by between 1 and 3 months (Blumgart et al 1971).

The process of liver regeneration has been studied most closely in the rat, an animal with a conveniently lobulated liver, an ability to survive surgery and inexpensive requirements. This animal has been used to establish the basic morphological and biochemical changes of regeneration (Higgins and Anderson 1931).

The dog has also been used for the study of liver regeneration and the mechanism by which it was brought about (Mann 1940). This animal has been more useful for measurement of blood flow to the liver because of its larger size.

As yet the mechanism by which liver regeneration is initiated after resection of liver tissue or destruction of hepatocytes is unclear. Of the main variables which may affect the liver, two have been studied in detail. Firstly, the quantity of blood received by the liver, and secondly the quality of that blood. Unfortunately in in vivo experiments, alteration of one of these variables will of necessity produce changes in the other, by an imbalance in the perfusion ratio between arterial blood and portal venous blood. Often profound changes will be found in the hepatocytes themselves, e.g. diversion of portal blood from the liver produces atrophy, which is a stimulus to regeneration (Weinbren 1955).

Hepatic tissue perfusion alters considerably after surgical resection in that the portal blood flow is forced through the remaining tissue (Rabinovici and Wiener 1963). In fact, it has been suggested that this effect per se is responsible for restoration liver mass (Mann 1940).

Portal blood caries nutrients, toxins and antigens from the gastrointestinal tract but also contains very high concentrations of the hormones glucagon and insulin. Insulin is one of the most important anabolic hormones and approximately 30% of portal blood insulin is cleared by the liver (McIntyre 1978).

The purpose of the present study is to examine the hypothesis that after surgical resection, liver regeneration will occur independently of either the source or the total liver blood flow, and that this regeneration will occur with a normal periodicity. This has been studied by producing a liver deprived of intestinal venous blood but with an adequate and stable blood supply from both

the arterial and venous systems. Under controlled conditions the effect of partial hepatic resection on the regenerative process has been studied.

The model used is the portacavally transposed rat subjected to a two-thirds partial hepatectomy. The nutritional blood flow in this model has been measured and the alterations in blood flow and regeneration will be discussed.

REVIEW OF LITERATURE

Historical

Prometheus was sentenced to be chained to a rock and have his liver eaten each day by vultures. In this punishment for the theft of fire from the Gods, Zeus revealed his divine knowledge of liver regeneration, which occurred overnight.

The first mortals to note scientifically the restoration of liver mass are quoted by Fishback (1929) as Cruveilhier and Andral. However, according to Florey (1970), the German pathologist Emil Ponfic (1895) laid the basis for the understanding of liver regeneration by demonstrating the restoration of liver mass which followed partial hepatic resection in the rabbit, the mass being restored by a process of both hypertrophy and hyperplasia of the remaining cells. Maximum mitotic activity was noted at the end of the first week after partial hepatectomy. In similar experiments on dogs Ponfic also describes a similar process, though less clearly visible (Ponfic 1895).

Fishback (1929) reviewed other early work which concentrated on regeneration following acute liver atrophy induced by inhaled or parenteral chemicals. New hepatocytes were thought to be produced by the proliferation of bile ducts. However, Milne (1909) suggested that proliferative activity of the bile ducts was to reconnect the regenerated hepatocytes to the main bile ducts. Mall (1906) recognised that regeneration occured principally in the area of the portal triad and that new and apparently normal lobules were formed.

Reporting his own work, Fishback noted that only technical difficulties of resecting further tissue prevented the infinite process of regeneratiion from sequentially replacing removed canine liver tissue, assuming that time was allowed for regeneration to occur.

One further paper of historical importance is the description by Eck in 1877 of an experiment to explore the treatment of ascites. Eck developed a technique in the dog for shunting portal blood into the vena cava (the translation was made by Child in 1953). This work stimulated Mann to experiment with portal diversion as a means of elucidating the stimulus to liver regeneration (Mann 1931).

Liver Anatomy and Physiology

The liver develops as a cellular proliferation, in the ventral mesogastria, of the blind ends of a tubular outgrowth from the junction of foregut and midgut. The surface anatomy has little to do with function and is a result of the peritoneal folds on which it has developed and the presence of surrounding organs and vessels. The liver receives a blood supply from two distinct sources, the hepatic branch of the coeliac artery and the hepatic "portal" vein which carries all the effluent blood from the viscera. The hepatic veins drain into the vena cava.

The microscopic structure is based on the liver lobule which is described as a plate of cells radiating from a central hepatic vein

with artery, portal vein and bile ducts (the portal triad) at the periphery. Embryologically the hepatic cell plates develop from the portal triad towards the hepatic vein (Rappaport 1973).

The liver has a stable population of hepatocytes. There is a continuous process of cell death, replacement and remodelling of the lobular structure. There is approximately one mitotic figure for every 20,000 mature cells (Florey 1970).

The liver functions as a short-term energy store (utilising glycogen) and it is the major synthetic organ for the body. It is capable of utilising proteins, fats and carbohydrates as an energy source and it is capable of utilising any one to synthesise another. It is the principal organ of detoxification of compounds produced within the body and also of compounds absorbed from the gastro-intestinal tract. The liver also acts as an immunological filter of blood, and in particular portal blood, this being a function of its reticulo-endothelial cells. The metabolic activity of the liver can also be used independently to generate heat to maintain body temperature.

Liver Regeneration

Morphological and Biochemical Changes

The macroscopic changes in the regenerating liver are characteristic, "at first the surface was glistening, moist and tense ..... the edges were rounded, blunt and devoid of notches. The surface became flattened and was yellowish brown instead of

normal reddish brown." These changes were noted in the regenerating dog liver by Fishback (1929), but similar changes were noted by Ponfic (1895) in the rabbit and by Higgins and Anderson (1931) in the rat. These early changes were apparent within 24 hours of resection and are due to extensive temporary infiltration of neutral fat, especially triglycerides (Gurd et al 1948; Bucher 1967; Bengmark 1969).

Ponfic (1895) noted mitotic activity as early as 45 hours after partial hepatectomy in the rabbit. In the dog the peak of mitotic activity occurs on the fourth day after resection (Sigel 1969), but in the rat this peak of cell division occurs between 18 and 20 hours after partial hepatectomy (Higgins and Anderson 1931).

Light microscopic changes are apparent within one hour in the rat. The basophilic bodies start to disintegrate and this occurs first in the cells nearest the portal triad and later in those nearer the hepatic veins. In 2-3 hours cytoplasmic inclusion bodies or vacuoles, as well as fat globules begin to accumulate. The fat later becomes more conspicuous and some cells are left with only a rim of cytoplasm. Glycogen also rapidly disappears from the hepatocytes falling to a very low level by 10 hours, and thereafter it begins to reappear. By 6-12 hours the cells, their nuclei and nucleoli start to enlarge, doubling in size by 24 hours, at which time mitotic activity becomes apparent, again starting in the region of the portal triad (Grisham 1962; Bucher 1963).

Hepatocyte replication occurs before sinusoidal and littoral cells have started to divide resulting in crowding of the lobules and duplication of the cells of the hepatic cell plates. Duct and

littoral cell mitotic activity commences about 6 hours after hepatocyte mitotic activity. There is no evidence that the bile ducts give rise to hepatocytes, as was suggested by earlier workers (see Fishback 1929). However, it is possible that littoral cell restoration is aided by recuitment from cells circulating in the blood (Harkness 1957)

Collagen replacement takes approximately 6 months to complete in the rat (Bucher 1967). Little information is published in relation to lymphatic restoration but a normal anatomical relationship is restored (Florey 1970).

Unlike the hyperplasia of the hepatocyte there appears to be no directional component of mitotic activity in the littoral cells and connective tissue in the liver (Bucher 1967).

In experiments using autotransplants of liver with reversed blood flow Sigel (1968) was able to demonstrate that the direction of the wave of mitosis in the hepatocytes could also be reversed, suggesting some direct relationship to nutrient flow.

Electron microscopy has been used to detail further the early changes in the hepatocytes. The basophilic bodies of light microscopy have been identified as rough endoplasmic reticulum. This cytoplasmic organelle is associated with the production of proteins for export from cells. The membranes of the endoplasmic reticulum break up during the first 4-12 hours after partial hepatectomy to form rosettes of ribosomes, reorganisation occurs by about 72 hours and the endoplasmic reticulum has returned to normal by between 96-120 hours (Stenger and Confer 1966). Swelling of the mitochondria with reduction in numbers to normal by the end of the first day has

also been noted. Activation of lysosymes to autophagosomes, which digest parts of the endoplasmic reticulum, glycogen granules and mitochondria, were also noted. The hepatocyte itself was noted to swell and separate from its neighbours and loss of microvilli from the surface was also noted. These changes were corrected after 24 hours (Bucher 1957). It was noted by Bassi and Bernelli-Zagzera (1964) that these changes were similar to those seen after toxic and ischaemic injury.

More recently Grisham et al (1973) used intravenous and intra-arterial perfusion of the liver with fixative to improve the fixation of blocks of tissue for electron microscopy. They demonstrated that the rough endoplasmic reticulum, far from disintegrating, showed an increase in quantity per cell before mitosis. They also noted no alteration in mitochondria or changes in lysosymes. Apart from cells undergoing mitosis, there were no changes in the microvilli and no tendency for the cells to pull apart. Mitotic cells were noted to have a smooth surface on electron microscopy. It would therefore appear that earlier reported changes were related to poor fixation of tissue.

The cycle of activity in normal stable cells can be conveniently divided into five stages which occur in the following sequence in almost all mammalian cells, including the liver:

G.0 A phase of normal functional activity of the cell with no evidence of cell growth (this is sometimes known as arrested stage of G.1).

G.1 Ribonucleic acid (R.N.A.) and proteins are synthesised in

this period during which growth in cell volume may be apparent. This phase may be prolonged or as short as 20 hours as in the liver cell in the rat.

S. This is the synthetic phase for deoxyribonucleic acid (D.N.A.) and in the rat liver lasts from 7-8 hours.

G.2 During this phase D.N.A. synthesis ceases but R.N.A. and protein synthesis continues.

M. Mitosis in the liver takes from 1.49-3.17 hours to complete (this is relative to the distance away from the portal triad). At the end of this cycle the cell reverts to G.1 (or G.0) and may then repeat the process (Rabes 1975).

The above cycle has been elucidated using radioactive isotopes of the precursors of the various materials synthesised. For example, thymidine, a D.N.A. precursor, can be labeled with tritium (^{3}H) an isotope of hydrogen. Thymidine is principally taken up by replicating D.N.A. In this way it is possible to demonstrate by light microscopy those cells which have entered a synthetic phase of their cycle during the time of exposure to the radioactive isotope. This is the principle of auto-radiography and has been used to demonstrate the wave of synthesis (and later mitosis) which tends to start in the peri-portal triad area where it is most intense. This wave then passes towards the hepatic vein.

Using auto-radiography as a marker of D.N.A. synthesis, Grisham (1962) documented that hepatocytes began to synthesise D.N.A. as soon as 6 hours after partial hepatectomy, but that the peak value of nearly 30% of all hepatocytes were synthesising D.N.A. by

approximately 20 hours. This was followed by a peak of mitotic activity about 6 hours later in the hepatocytes. Duct and littoral cells were beginning D.N.A. synthesis approximately 8-12 hours later than the hepatocytes.

Rabes (1975) in a very detailed paper has demonstrated that in the three zones of the liver, zone 1 (peri-portal), zone 2 (intermediate), and zone 3 (peri-venous), there are varations in the probability of cells passing from G.1 to S. In zone 1 the probability is 18 times more likely than in zone 3.

The S. phase varies little, taking around 7 hours, but G.2 can vary from 2.8 hours (zone 1) to 4.5 hours (zone 3). If zone 1 cells are chemically destroyed, zone 2 cells still do not respond with the rapidity of cells in zone 1.

Cells in zone 1 would therefore have a higher probability of replication and Grisham (1962) suggested that 80% of all new hepatocytes are formed in zone 1 and zone 2.

Auto-radiography results must be treated with some caution as the technique has inherent errors. The assumption that the ^{3}H label is associated with D.N.A. may not be entirely valid and Schneider and Greco (1971) have shown that the ^{3}H label was associated not only with microsomes but also with lipids and proteins.

Chemical assessment of D.N.A. synthesis has been developed using a radio-isotope tracer technique by Bucher (1964) and Weinbren and Woodward (1964). Bucher used ^{14}C labelled thymidine and Weinbren and Woodward used ^{32}P labelled orthophosphate. Both these compounds are incorporated into D.N.A. during its synthesis. A comparison of the amount of labelled D.N.A. to total D.N.A. will then give a

measure of D.N.A. synthesis during the time of exposure to the labelled precursor.

Using slightly differing techniques in the rat and giving the labelled precursor 2 hours (Bucher 1964) and 3 hours (Weinbren and Woodward 1964) before killing the rats, both workers found a peak of D.N.A. synthesis after two-thirds hepatectomy in the young adult rat. There is a lag period of minimal uptake of label for 12-15 hours after partial hepatectomy and in the young adult rats a subsidiary peak with a gradual tail-off. In weanlings there is a second peak at 33-35 hours and the primary peak is at 20-22 hours (Bucher 1964).

Weinbren et al (1969) later modified the technique to use ^{3}H labelled thymidine and a reproducible peak was shown to occur between 18-24 hours post-partial hepatectomy under standard experimental conditions (e.g. Bradbrook et al 1974). This method depends on giving a saturating dose of the labelled precursor which is possible in the rat and mouse but is difficult to produce economically in the dog and in other large animals. D.N.A. synthetic studies have therefore been concentrated on the rat and mouse livers. The method, however, does not differentiate between the types of cell involved in replication, viz hepatocytes and ductal cells. Comparison of auto-radiography and mitotic timing with D.N.A. synthesis by chemical uptake reveals that the 18-24 hour peak of activity is due almost entirely to hepatocyte replication (Bucher 1967).

In 1931 Higgins and Anderson in a very detailed paper compared the alteration in body weight in the rat subjected to a two-thirds

partial hepatectomy to that ocuring after simple laparotomy. They found little difference, each group losing 12-15% of their original weight and returning to normal within 30 days.

However, a biochemical imbalance does arise. This imbalance was said by Weber (1975) to "confer selective biological advantage to the regenerating tissue and to the host". In the rat he studied the biochemical pattern of regenerating liver cells and suggested that gene expression does not alter as far as carbohydrate metabolism is concerned, but shows a marked change towards synthesis and away from catabolism of nucleic acids. There is also a decrease in the production of cyclic A.M.P. at the plasma membrane.

In an attempt to explain the remarkable growth rate of 20% per day in regenerating mouse liver, Scornick (1975) examined protein metabolism and found that the only substantial change was decreased protein catabolism in the liver and that this was sufficient in itself to account for the net protein gain. He also noted that the proportion of proteins exported (as plasma proteins) was the same as in normal animals.

After two-thirds partial hepatectomy in the rat, mild hypoglycaemia occurs (Weinbren and Dowling 1972) within the first few hours. A rise in bile pigment occurs and plasma free cholesterol also rises. A fall has been recorded in plasma proteins (Harkness 1957). These changes last several days.

Liver function tests reveal a fall in serum albumin, a rise in alanine transferase and serum bilirubin. Bromsulphthalein (B.S.P.) and Rose Bengal retention are increased immediately after partial hepatectomy and more so at 24 hours (Wood et al 1973). Partial

hepatectomy increases blood flow through the remaining portal bed (Benacerraf et al 1957) and physically reduces the number of hepatocytes.

B.S.P. and Rose Bengal retention tests typify the complexity of examining liver function as they are dependent on clearance of the dye from blood passing through the liver, active transport of the dye and conjugation within hepatocytes, and excretion into the biliary system. Clearance itself is dependent on blood flow and total retention depends on total cell numbers. Similarly, alterations in the serum levels of substances normally metabolised or cleared by the liver may be a result of partial hepatectomy and not a response to resection.

Morley et al (1975) examined changes in various hormones after partial hepatectomy and showed that the levels of glucagon rose eleven-fold while insulin levels remained unchanged and growth-hormone and thyroxine levels fell. These hormones were measured in peripheral blood. They concluded that insulin could not function as a primary stimulant in liver regeneration and that glucagon may modify the process. It is perhaps more interesting that the peripheral blood levels of insulin (a known anabolic hormone) did not rise, as a large proportion of this hormone is cleared on passage through the liver (McIntyre 1978), and indeed the liver is the principal organ of insulin homeostasis (Izzo et al 1967).

Desser-Weist (1975) suggested that corticosterone, the principal glucocorticoid in the rat, had a peak serum level 15-18 hours after partial hepatectomy. Unfortunately, she gives no control values and goes on to claim that cortisone may synchronise the

mitotic activity of the liver as it had been shown previously that glucocorticoids and A.C.T.H. suppress mitotic activity in the regenerating liver (Guzek 1964; Hyde and Davis 1965; Stevens and Docherty 1968).

The regenerative process can be altered by changes in the rat's environment such as alterations in its day/night cycle and its feeding cycle (Shulte-Hermann 1975). A diurnal periodicity in the mitotic activity of the regenerating liver was first noted in a control experiment by Jaffe (1954). He showed that in the 12-hour intervals after partial hepatectomy, mitotic activity was greatest between 6 a.m. and 10 a.m. and lowest between 6 p.m. and 10 p.m. He suggested that this might be related to the feeding cycle and therefore to carbohydrate metabolism. However, it must be remembered that the rat's circadian rhythm is based on night activity with plasma glucocorticoid levels which are minimal in the morning and maximal at night (Barbason and Van Cantford 1975).

Regenerative activity can also be modified but not abolished by alterations in diet. Calorie-rich protein-deficient diets reduce mitotic activity, while semi-starvation delays the peak of mitotic activity (Brues et al 1936; Doyle et al 1968; Stirling et al 1975). Hypoxia and even increase in gravity will also delay the onset of mitosis after partial hepatectomy. Decreasing gravity increases mitotic activity (Feller et al 1967; Talarico et al 1968).

Most of the experiments described above have been in young adult rats with a two-thirds partial hepatectomy, as described by Higgins and Anderson (1931). This has become the standard model for

study, in that resection is easily performed and induces a uniform response. If younger rats, such as weanlings, are chosen the mitotic activity in the liver is more dramatic, occurs earlier and is biphasic. Older rats show a smaller peak (Bucher et al 1964).

Resection of less than 30% of hepatic mass does not produce a synchronised peak of mitotic activity (Bucher and Swaffield 1964) and resection of over 80% of the liver produces a profound hypoglycaemia and delays the peak of mitotic activity (Weinbren and Woodward 1964). This delay is not reversed by the administration of glucose (Weinbren and Dowling 1972).

The Stimulus to Regeneration

In 1940 Mann reported the results of an experiment designed specifically to investigate the stimulus to liver regeneration which was already known to occur in the dog. He suggested that the "proportion of liver left (after partial hepatectomy) should have its normal supply of portal blood" and to provide an overflow he performed an Eck fistula prior to hepatectomy (Mann 1940). Necropsy was carried out a minimum of 15 days after partial hepatectomy and only liver weight was used as an index of regeneration. Prior performance of an Eck fistula abolished liver regeneration and he concluded that in dogs with an adequate side-to-side fistula, no regeneration occurred and that therefore the "necessity for regeneration of vascular channels appears greater than for the restoration of function in hepatic tissue .... and the fact that the matrix happens to be hepatic tissue may be only a fortuitous

circumstance". In the above work Mann ignored previous work of his own which showed that liver atrophy occurred after portacaval shunting (Mann and Magath 1922; Mann et al 1931). Despite the lack of data regarding the total blood flow through the liver or fistula, the concept of portal per se being a stimulus to regeneration was developed by Mann at this time (Mann 1944).

A second concept developed around this time suggested that there was a circulating humoral agent responsible for initiating liver regeneration. In an experiment performed by Christensen and Jacobsen (1949) cross-circulation was established between a normal and a partially hepatectomised rat. Increased mitotic activity was found in the liver of the normal rat and was thought to have been induced by a circulating agent.

In 1951 Bucher et al established parabiosis between twin or triplet rats and found that partial hepatectomy of one of the rats produced increased mitotic activity in the liver of other rats in cross-circulation. This work was confirmed by some workers (Islami et al 1959), but others (Fisher et al 1963) were unable to repeat the work. The problem with parabiosis experiments is that it is not possible to measure the amount of cross-circulation ocurring.

Child et al (1953) performed supra-adrenal portacaval transposition in the dog. They demonstrated 50% regeneration in portacavally transposed dogs, compared to 75% regeneration after partial hepatectomy in controls. They compared this to the apparent lack of regeneration in portacaval shunt animals and concluded that portal blood in itself was not necessary for regeneration. They had again in fact no way of knowing total blood flow through the

regenerating liver and glucocorticoids were allowed to pass directly to the liver. Again only liver weight was measured as an index of liver regeneration.

Support for Mann's theory came in 1954 with work by Fisher et al who showed that while regeneration did not occur in portacavally shunted dogs as compared to controls, that if the portal vein was arterialised after portacaval shunt, then regeneration as measured by liver weight, was greater than in control animals. Once again these experiments took no account of liver atrophy subsequent on portacaval shunt nor of mitotic activity, and indeed Clarke et al (1968) showed no significant rise in mitotic activity in this preparation.

Liver atrophy produced by portacaval shunting was examined in detail by Weinbren (1955) who showed that while ligation of a branch of the portal vein produced reduction in size of that lobe, and reduction in cell size within that lobe, the lobe itself was still capable of regenerative activity with increase in weight, cell and lobular size. This regenerative activity was also reflected by an increase in mitotic activity. Regeneration occurred in the absence of portal blood after partial hepatectomy in the lobe deprived of portal blood flow (Weinbren et al 1972; Marks et al 1975).

Saetren (1956) attempting to define mechanisms controlling liver regeneration showed that injection of macerated liver and kidney into the peritoneal cavity inhibited mitosis in both liver and kidney after resection of part of these organs, but there was no cross-reactivity and therefore the inhibitor was organ-specific and not a general depressant of mitotic activity.

Other workers investigated the possibility that the hepatotrophic factor was not portal blood per se but a constituent of portal blood. Harkness (1957) in a review citing the portal deprivation experiment of Weinbren (1955) and the portacaval transposition experiment carried out by Child et al (1953) suggested that available blood supply determines equilibrium liver size and this may affect liver regeneration. He went on to quote Glinos and Gey (1952) as suggesting that since plasmapheresis in vivo produces mitosis in the liver, plasma albumin concentration may be the factor controlling liver regeneration.

Sigel (1963) again defining regeneration in terms of weight only, utilised a model in which portions of liver were autotransplanted to sites in the small intestine, observed the effects of subsequent partial hepatectomy and showed that after initial atrophy the grafts were stimulated by partial hepatectomy to regenerative activity. This suggested a humoraly mediated response.

Bullough (1962) suggested that mitotic activity in organs was controlled by a "chalone" a chemical messenger which had a depressant effect. He suggested that chalones are released by an organ into the circulation, metabolised by the rest of the body and that the residual concentration acted as a depressant on the organ in question.

Fisher et al (1963) were unable to show any regenerative response in the cross-circulation experiments beween partially hepatectomised and normal rats. They were also unable to show a response in the livers of animals infused intravenously or intraperitoneally with sera from partially hepatectomised rats. This

re-opened the debate about the presence of a humoral agent. A number of workers showed that autografts of rats or dogs in non-portal sites still showed regenerative activity after partial hepatectomy (Leong et al 1964; Virolainen 1964; Sigel et al 1967). Moolton and Bucher (1967) reported a further series of cross-circulation experiments supporting previous work (Bucher 1951) and defining that the circulation between animals must be at least 2 mls/minute for a minimum of 7 hours after partial hepatectomy in the rat. This work was supported by an aorto-aorto cross-circulation experiment carried out by Saki (1970). Bradbrook et al (1974) showed that after partial hepatectomy the intraportal infusion of normal rat sera inhibited mitotic activity, compared with contols and this was not shown by the infusion of serum from partially hepatectomised rats.

Mann and his co-workers' earlier study (Higgins, Mann and Priestley 1932) suggested that an increase in blood flow to the liver after partial hepatectomy in the chicken increased the apparent regeneration. No measurements of blood flow were actually made and once again liver size and weight were used as an index of regeneration. Thomson and Clark (1965) repeated this work in the fowl and dog and they showed that there was no rise in the mitotic index after a reversed Eck fistula (end-to-side caval-portal anastomosis).

Separation of the hypertrophic and hyperplastic response of the liver was achieved by Sigel (1967) in an experiment with small autotransplants of liver. If an Eck fistula was performed in animals bearing an autotransplant, the transplant increased in mass but showed no increase in total D.N.A. However, if partial hepatectomy

was performed, the autotransplant both increased in size and the total D.N.A. increased. This work was carried out after allowing time for initial transplant atrophy.

Awareness of the effects of total liver blood flow was reflected in a paper by Mizumoto et al (1970) showing that the drop in portal pressure produced by hepatic artery ligation after partial hepatectomy does not prevent the completion of regeneration. Further examination of this area was carried out by Weinbren et al (1972) who noted atrophy as distinct from lack of regeneration in the rats with inadequate total liver blood flow. Mann's thesis that liver regeneration was a result of blood flow *per se* was based on animal experiments which produced atrophy and in which hyperplasia was not measured. It has been shown by workers subsequently that even in the presence of atrophy, liver regeneration does occur and that it is not abolished by the absence of arterial or portal blood supply. Further, increasing the blood supply to the liver does not produce an increase in replicative response. Indeed, the theory that a humoral agent was responsible for liver regeneration was becoming increasingly evident (*vide infra*).

In a series of experiments by Fisher et al (1971a) using first a cross-circulation and then adding a portacaval shunt to the partially hepatectomised rat, they suggested that the humoral agent not only originated in the portal circulation but was also cleared by the liver. Subsequently, Fisher et al (1971b) using a technique of transplant with full vascular reconnection (hepatic artery and portal veins), went on to suggest that only liver receiving portal venous blood regenerates. Both of these experiments may only

demonstrate flow dependent regeneration as no hepatic blood flow information was available and both involved considerable alteration in hepatic perfusion.

Various operative procedures were used to try to isolate the humoral factor which was thought by Price et al (1967) and Starzl et al (1973) to originate in the viscera drained by the portal vein. Max et al (1972) demonstrated an increased hyperplastic response to liver resection in dogs after removal of all other splanchnic organs, and suggested that factors of portal origin have a role in the control of hepatic hypertrophy and, in their absence, there is an increased hyperplasia after hepatic resection. Price et al (1972) went on to suggest that glucagon was a modifying factor of liver regeneration and had an inhibitory action.

Using a synthetic graft to carry blood from the inferior mesenteric vein to the liver, Starzl et al (1973) directed pancreatico-duodenal blood to one half of the liver and the rest of the portal venous blood to the other half. That part of the liver receiving blood from the pancreas showed an increase in weight, glycogenation and hyperplasia, whereas the part receiving blood of non-pancreatic portal origin showed no increase in these parameters. They suggested that both insulin and glucagon played an important part, but that the principal actions of insulin are anabolic while those of glucagon are mainly catabolic. In subsequent experiments Starzl et al (1975) showed that following partial hepatectomy in this model, liver regeneration occurred mainly in the lobes fed with pancreatic-derived portal blood. In this article they suggest that the most important specific hepatotrophic factor in portal blood is

insulin and that no prominent hepatotrophic role for glucagon could be identified.

In both the above experiments the authors assume blood flow to both lobes is equal, despite the use of a graft to carry blood from the non-pancreatic portal circulation. Leiberman et al (1976) has shown that blood flow within the two lobes is similar.

The accuracy of the measurement of mitotic activity after partial hepatectomy depends on achieving a saturation dose of tritiated thymidine, the dog requires a dose of 1 mCi/gm (Price et al 1972). In Starzl's 1975 paper doses ranged from 0.05-0.41 mCi/gm and the time of sacrifice varies between 2-5 days. This unfortunatey casts doubt on any quantitative measurements of regeneration in this experiment. However, it would appear that in diabetic dogs and in livers receiving no insulin the regenerative response was remarkably diminished.

Bucher and Swaffield (1973) abdominally eviscerated rats leaving only the liver. The animals were maintained on intravenous infusions of glucose with insulin for 48 hours. Partial hepatectomy was performed at the time of evisceration and resulted in D.N.A. synthesis and mitosis in the liver. The regenerative response was delayed and diminished in comparison with controls. In a further experiment (Bucher and Swaffield 1975) full activity was restored by infusions of insulin plus glucagon but not by either hormone alone. This second experiment also included control animals receiving no insulin and they too showed an increase in D.N.A. activity and mitosis after partial hepatectomy and evisceration. Bucher and Swaffield conclude that the synergistic action of insulin and

glucagon may regulate the rate and perhaps the extent of the regenerative process in the liver. However, they also feel that additional factors are important in initiation of the regenerative response.

In Vitro Experiments

Since hepatocytes remain capable of replication, they have been used to study the process of cell division in vitro. The growth potential of hepatocytes under standard conditions is inversely proportional to the age of the animal at the time of harvest. If rat liver cells are harvested within one month of partial hepatectomy, these hepatocytes behave like those from a young rat (Glinos and Bartlett 1951). However, cells cultured from liver of an adult rat subjected to a partial hepatectomy 24-48 hours beforehand do not survive. At least 72 hours must elapse after partial hepatectomy before a proportion of the cells survive in culture (Hays et al 1968 and 1971).

The conditions under which cells are cultured can be varied by the addition of various sera to the basic culture media. Using hepatic fibroblasts, Glinos and Gey (1952) showed that normal sera had a greater inhibitory effect than serum subjected to plasma-pheresis. However, Grisham et al (1967) took sera from partially hepatectomised rats and potentiated the in vitro replication of hepatocytes. This work was confirmed by Paul et al (1972) and Rabes (1963). This effect is not species specific as serum from calves after partial hepatectomy also stimulates the

growth of rat liver cells whereas normal calf serum did not (Hays et al 1969).

Levi and Zeppa (1971) established an isolated in vitro preparation of the liver with a cross perfusion in the rat. Their experiments suggested that there was a factor present between 18-24 hours after partial hepatectomy which would stimulate normal liver to increased D.N.A. activity. They suggested that the delay of release of this factor is due to the fact that the liver must experience a metabolic overload to produce a regenerating factor. However, account must also be taken of the known length of G.1 and S. phases of the hepatocytes. Levi and Zeppa (1971) proposed that in their closed loop system a regenerative factor must come from the liver. In a subsequent paper (Levi and Zeppa 1972), they described the collection of this factor by continuous perfusion of an isolated liver at 24 hours post-partial hepatectomy. They then assayed the agent in normal liver looking for an increase in the D.N.A. in the liver. Once again this experiment does not eliminate the possibility of an inhibitory factor. Perfusion of a normal liver will extract a normal quantity of inhibitory factor, while perfusion of one-third of a liver would only produce one-third of this quantity. Any inhibitory factor may not be released by mitotically active cells and this would further reduce the amount of such a factor released.

The isolation and characterisation of a specific initiating factor in liver regeneration was attempted by Scaife (1970) who suggested that a high molecular weight chalone controlled in vitro growth of rat liver embryonic foetoblasts. This inhibitory chalone

was found in normal rat serum and to a lesser extent in serum from regenerating rats.

Verily (1973) subjected regenerating liver slices to a chalone derived from rabbit livers and also produced inhibition of D.N.A. synthesis. This molecule was described as a small polypeptide with a molecular weight of 2,000-5,000 and showed organ specificity but no species specificity. Rabbit liver had the highest concentration of the chalone. Later work by Vinet and Verily (1976) using hepatocyte derived cells (Novikoff) suggested a 40,000 molecular weight protein as an inhibitor of D.N.A. synthesis. They also noted an inhibitory peak with a protein in the 2,000-5000 range. The larger protein appears to have little effect on regenerating liver. Also in 1973, Morley and Kingdom described a stimulating humoral agent of molecular weight 26,000 which was a specific stimulator of D.N.A. synthesis in the liver. This agent was heat stable and boiling in fact increased its effectiveness. It may be that an inhibitory factor was destroyed by this process. Other workers (Demetriou et al 1974a,b) suggested a stimulating factor of molecular weight 12,000 in serum from partially hepatectomised rats and also in serum from partially hepatectomised rats after pancreatectomy.

in vitro evidence of an alpha-1 globulin having an inhibitory effect on regenerating liver when injected intraperitoneally. They did not rule out the possibility of this being a toxic effect and could not show any organ specificity. They point out that the epidermal chalone, initially described by Bullough (1973), is a protein or glycoprotein and the high molecular weight inhibitors described by Vinet and Verily may well be alpha-1 globulins.

Portacaval Transposition
(Fig. 1)

In 1952 Child published a translation of the original paper by Eck describing the technique for a portacaval shunt. In the same year he published a paper on liver regeneration in the dog in which he tried to refute the then current opinion that liver regeneration was dependent on portal blood flow per se. He used a dog model with complete portal diversion and at the same time perfused the liver with systemic blood. This was called a portacaval transposition. He allowed these animals to recover and after one month performed partial hepatectomy and monitored the patency of the shunt and the regenerative activity using venography and change in liver weight. He showed that regeneration averaged 50% in portacavally transposed dogs compared with 75% in control animals, whereas no regeneration was apparent in dogs bearing an Eck fistula. He concluded that portal blood in itself was not necessary for regeneration, but that total hepatic blood flow, which he had assumed to be normal in the portacavally transposed animals, was the most important factor.

Silen et al (1957) compared hepatic function in dogs subjected to portacaval transposition and Eck fistulae with that of normal dogs. Dogs with Eck fistulae showed weight loss, reduced B.S.P. clearance, reduced serum albumin, raised peripheral ammonium nitrogen and reduced tolerance to oral ammonium lactate. The liver became atrophic and infiltrated with fat. In contrast, function in portacaval transposition dogs was similar to that of control animals, except that they had an increased B.S.P. clearance. This increased B.S.P. clearance may be related to total liver blood flow.

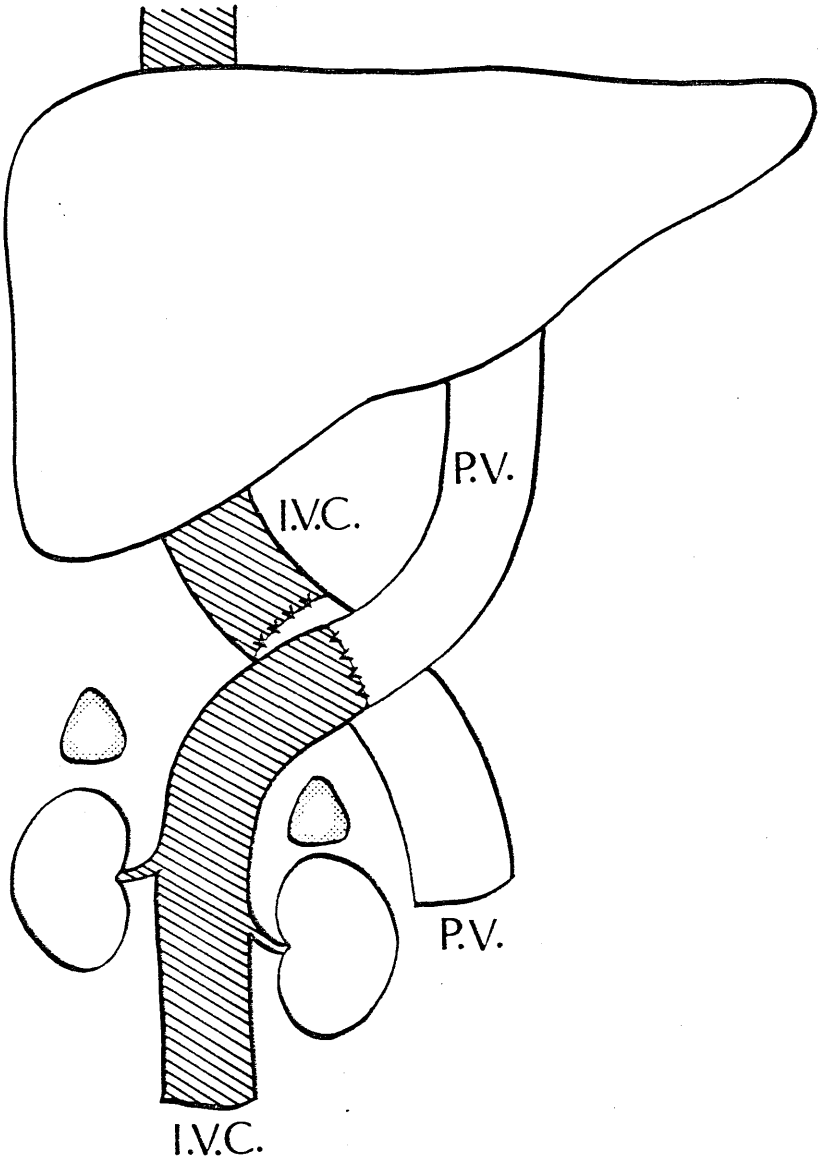


FIGURE 1

Portacaval transposition.

IVC - inferior vena cava.

PV - portal vein.

In the rat, the technical problems of producing a portacaval shunt were not satisfactorily mastered until Lee and Fisher were successful in 1961. In a review article in 1974, Lee details both the technique and the effects on the rat of a portacaval shunt. These effects included loss of weight, raised peripheral ammonia levels, alteration in liver enzyme activity (correctable by insulin in part), altered glucose tolerance and insulin response curves, and changes in neuroglial cells. A rise in gamma-globulin levels was also noted by Benjamin et al (1976).

Portacaval shunting produces marked liver atrophy but Weinbren had demonstrated in 1955 that hepatocyte replication could take place in the portally deprived rat liver. In 1962 Fisher et al examined liver regeneration in rats bearing a portacaval shunt using non-hepatectomised portacaval shunt animals as controls. They were thus able to eliminate the atrophic effect on the liver of the shunt alone from the regenerative activity in the liver.

Portacaval transposition in the rat was first described by Le Compte et al in 1970 using an end-to-side portacaval anastomosis with anastomosis of the left renal vein to the portal vein at the hepatic hilus. The intervening inferior vena cava was ligated. End-to-end portacaval transposition was first described by Ryan et al (1974) and they compared the body weight and growth curves of rats with portacaval transposition and portacaval shunt. Young rats with portacaval transposition followed similar body weight curves to sham controls. Portacaval shunt animals lost more weight initially and did not return to the normal growth curve. Liver weight was also better maintained after portacaval transposition when compared to portacaval shunts.

Lee et al (1974) used the rat portacaval transposition model to study liver regeneration. They first assumed that the portal diversion would not produce an alteration in total liver blood flow, and endeavoured to confirm the existence of a portal blood factor which affected liver regeneration after partial hepatectomy. Portacaval transposition was followed 3 days later by partial hepatectomy and the rats were killed after a further 7 days. Liver regeneration was measured by D.N.A. activity and liver size. Lee et al showed no difference between regeneration in portacaval shunt and portacaval transposition animals, and they concluded that the magnitude of hepatic blood flow was not significant. Sham-operated animals showed significantly more evidence of regeneration than the experimental groups (in terms of labelled nuclei and protein increase) and they suggested that portacaval shunt and portacaval transposition animals were subjected to a slight diluting effect of the portal blood factor. These results are at odds with Child's original work presented in 1953 and there are two major defects in Lee's paper. Firstly, partial hepatectomy was performed only 3 days after portacaval transposition or portacaval shunt, allowing insufficient time for the atrophy which is known to occur after portal deviation (Weinbren 1955). Secondly, livers were examined biochemically and histologically for evidence of regeneration some 7 days after partial hepatectomy while the peaks of regenerative activity in hepatocytes in the rat are known to occur 18-30 hours after resection (Bucher et al 1967; Harkness et al 1972). Their conclusion that the magnitude of hepatic blood flow was not significant is also unproven, as they in fact have no record of

blood flow through the liver after portacaval transposition in the rat.

Liver Blood Flow

The hepatic microcirculation is based on the "complex acinus". This "complex acinus" is made up of three or more simple acini which are irregular masses of liver cells surrounding a central axis terminal portal venule, terminal hepatic arteriole along with bile ductules, lymphatic vesels and nerves. The acinus lies between two or more terminal hepatic venules (so-called central veins). As noted above, the hepatic unit is sub-divided into three zones of cells from central to peripheral.

Flow into the sinusoid is from the terminal portal venule and from hepatic arterioles, either directly emptying into the sinusoids in zone 1, or via the peri-ductal arteriolar plexus and capillaries to join the sinusoid. There is direct neuronal control of arterioles and of part of the capillaries. Large endothelial cells at the inlet and outlet of the sinusoids may produce a fine adjustment of portal blood flow. Flow out of the sinusoids is by the terminal hepatic venules (Rappaport 1973).

Appart from the sympathetic nervous regulation of flow via the arterioles, the portal flow may also be lowered by the effect of adrenergic hormones on the terminal portal venules (Daniel et al 1951). Glycogenolytic hormones increase blood flow via the arterioles (McClusky 1966). Local vasoactive chemicals may also control blood flow through the arterioles and it would appear that

bile salts may increase flow via the peri-ductal arteriolar plexus (Rappaport 1973).

Measurement of liver blood flow may be made by the following methods:

1. Clearance of dyes by the liver, e.g. bromsulphthalein, indocyanine green, Rose Bengal, which occurs by the action of functioning hepatocytes in the liver. This technique requires normal cellular activity which may not exist in pathological states.
2. Clearance of particulate matter, e.g. colloidal gold or ^{131}I denatured albumin, by the reticulo-endothelial system of the liver. This method is rendered inaccurate in conditions where portasystemic shunting of blood takes place.
3. Clearance of highly diffusible material from the liver, e.g. radioactive inert gases such as $^{85}Krypton$ and $^{133}Xenon$. This technique will measure hepatic tissue perfusion but not total liver blood flow.
4. Indicator dye dilution which requires that the indicator substance (usually ^{131}I or ^{51}Cr labelled red blood cells) remains within the circulation. This does not allow us to differentiate between tissue perfusion and physiological or pathological shunting.
5. Electromagnetic flow probes which again measure total liver blood flow and not tissue perfusion. Flow probes also require manipulation of all sources of inflow, viz cannulation or encircling of hepatic artery and vein.

Blood flow in regenerating rat liver has been measured using the phagocytic activity of the reticulo-endothelial system for ^{131}I labelled denatured albumin. Benacerraf and his colleagues (1957) found that first the efficiency of clearance fell from 84% to 62.5% and that although liver blood flow fell, blood flow per gram of tissue rose from 1.44 mls/minute to 2.5 mls/minute, and that this was restored to normal after 5 days. They suggested that rate of liver blood flow may be the determining factor in regeneration of the liver after partial hepatectomy. These changes in liver blood flow after partial hepatectomy were confirmed by Rabinovici and Wiener (1963) using the same clearance technique and they too noted a persistant small rise in portal venous pressure.

Aronsen et al (1969) measured liver blood flow in dogs after 50% hepatic resection. They used the radioactive gas ^{133}Xenon which is highly soluble in all lipids. Disappearance of the gas from the liver was assessed after bolus injection into the portal vein. They demonstrated a rise in liver blood flow per gram of tissue immediately after partial hepatectomy with a return to normal after 3 days. This was seen despite a fall in total cardiac output during the period of measurement. After partial hepatectomy the percentage of fat in the liver rapidly increases. This alters the partition coefficient of ^{133}Xenon in the liver and so affects the calculation of liver blood flow from the disappearance rates. After correction of liver blood flow for this alteration, Aronsen et al found that the rise in liver blood flow was in fact sustained for 7 days.

In our own laboratories Rice et al (1977) were able to miniaturise the gas clearance technique using ^{85}Krypton, and were

thus able to measure hepatic tissue perfusion before and after two-thirds hepatectomy in the rat. They confirmed the findings of Benacerraf's group, and of Rabinovici and Wiener, of increased perfusion in the liver remnant after partial hepatectomy (Rice et al 1966; Rice et al 1977).

Heer et al (1960) measured liver blood flow in the normal dog and the dog subjected to portacaval shunt and portacaval transposition. They used a radioactive colloidal gold clearance method and found the liver blood flow to be 46.4 mls/kg body weight/minute in the normal dog. Side-to-side shunted dogs had a liver blood flow of 23.7 mls/kg/minute and end-to-side shunts showed a liver blood flow of 19.3 mls/kg/minute. Portacaval transposition dogs maintained their liver blood flow at 43.6 mls/kg/minute. Starzl et al (1962) using the clearance of B.S.P. found very similar results in unanaesthetised trained dogs. With electromagnetic flow probes in the anaesthetised dog, Kreuzer and Schenk (1971) were able to measure both the hepatic artery and portal vein components of the liver blood flow of normal dogs and of dogs subjected to portacaval transposition. They showed that by 6 weeks after portacaval transposition, there was a sustained small rise in total liver blood flow which was not statistically significant. However, there was a signigicant fall (32%) in the hepatic arterial flow and a significant rise (50%) in the venous inflow. Their figures for total liver blood flow after portacaval transposition are remarkably similar to those of Heer et al with a mean value of 40.86 mls/kg/minute.

A STUDY OF LIVER REGENERATION

The initiation and control of the process of regenerative hyperplasia which follows partial hepatectomy has been extensively studied in laboratory animals including dogs, but particularly in the rat. An increasing body of evidence has reinforced the view that liver regeneration may be initiated and controlled by circulating humoral agents. There is disagreement and lack of conclusive evidence firstly as to the existence of such a humoral mechanism and secondly as to whether any such mechanism is stimulatory or inhibitory in nature.

Cross-circulation techniques have yielded conflicting results and the systemic or portal administration of blood or serum during the period of hyperplasia has failed to clarify the situation.

Experiments demonstrating a proliferative response in auto-transplants of liver in host animals subjected to partial hepatectomy supports the humoral control hypothesis, as does the perfusion of hepatocytes in culture by serum from partially hepatectomised and normal rats.

It has been suggested that portal venous blood flow plays an important role, either by maintaining liver perfusion or by virtue of its content of possible specific hepatotrophic factors, and deprivation of portal venous blood results in atrophy but does not prevent a regenerative response.

Child studied regenerative activity after partial hepatectomy in dogs with a portacaval shunt and a portacaval transposition. He concluded that deprivation of hepatic portal venous perfusion depressed liver regeneration. This work was open to criticism as the atrophy which follows portal deprivation was not allowed to occur and measurements of regeneration were based on liver weight.

Lee et al studied the response to partial hepatectomy carried out 3 days after portacaval transposition in the rat and concluded that the magnitude of hepatic blood flow is not a major determinant of liver regeneration. Unfortunately their work gives no evidence of hepatic blood flow measurement and further criticisms of this work relate to the short interval of time between the operation of portacaval transposition and partial hepatectomy, and also to the fact that measurements of uptake of tritiated thymidine into the liver were made one week after partial hepatectomy, although it is well accepted that the D.N.A. synthetic activity occurs within the first 3 days. Further, they allowed the effluent from the left adrenal to flow directly into the liver. This may be of considerable importance since corticosterone and other adrenal hormones may considerably influence mitotic activity.

HYPOTHESIS

In the rat, liver regeneration in response to surgical resection will proceed until the weight of the liver is commensurate with that of the animal. In the presence of adequate oxygenation and a supply of metabolites this effect is independent of either the source of portal blood inflow (intestinal or systemic venous blood) and the total liver blood flow (portal venous plus arterial). In addition, since the timing of the events in cellular replication are genetically determined, the various phases of cell reduplication will occur with a normal periodicity.

Therefore if alteration in the quality or quantity of liver blood flow alters the timing or extent of liver regeneration then there must exist a specific hepatotrophic factor in the intestinal effluent which modifies gene expression in the hepatocyte. Portacaval transposition will result in an alteration in both quantity and quality of liver blood flow.

While it is possible that an intestinal "hepatotrophic" factor may exist which is not affected by first passage through the systemic circulation, with its attendant dilutional effect, and is not metabolised but has its full effect after reaching the liver by recirculation via the arterial and systemic venous inflow, it is more likely that such an hepatotrophic agent is released by the liver remnant and returns to produce its inhibitory or stimulatory effect by its natural route via the hepatic artery on first passage and via the venous inflow as it passes through the "portal" bed.

The work presented in this thesis was designed to study under controlled conditions the effect of hepatic resection on the process of liver regeneration in a liver deprived of intestinal venous effluent with a stable and adequate flow of both arterial and venous blood.

The model used to examine the hypothesis is the portacavally transposed rat subjected to a two-thirds hepatectomy. Firstly, the model is itself examined in a study of growth in body weight and liver weight after transposition, and their relationship demonstrated. Secondly, a validated measurement of regional liver blood flow is used to demonstrate alterations in blood flow through the liver of the model before and after portacaval transposition and, after subsequent partial hepatectomy.

These changes in blood flow and the effects on the regeneration produced by hepatic resection are then discused.

EXPERIMENTS

STATISTICAL METHODS

In the results presented from the following experimental studies all figures quoted represent the mean values plus or minus one standard deviation unless otherwise stated.

Parametric analysis of body and liver weight, blood flow and pressure was performed using the Student's t-test with an incorporated Fisher's F-test for comparison of variance. The paired t-test was used where applicable.

Student's t-distribution was developed by W.S. Gosset ('Student' 1907) for the comparison of means when there were less than 30 in each sample. The t-distribution does not quite follow a normal distribution (Fisher R.A. 1925).

Fisher's F-test is a comparison of variance between the two groups tested. The size of the ratio of the standard deviation of the compared groups (F) can be used to dictate whether the t-test is suitable for the degrees of freedom applicable.

Non-parametric analysis of the D.N.A. specific activity results was performed by the Mann-Whitney Rank test which is a powerful comparison of distributions of ordinal measurement. The test is also designed for small numbers in each group and it results in a Wilcoxon's U-statistic. Tables have been drawn up for this U-statistic to be assessed in terms of probability (p) with small numbers in each group (Mann and Whitney 1947). Formulae used are presented in the Appendix.

Regression analysis for the liver weight/body weight comparison was carried out on the Royal Postgraduate Medical School mainframe computer (Perkin Elmer 3220) utilising the "Minitab" statistical package developed at Pennsylvania State University (Ryan et al 1976).

Correlation was by the Pearson product moment correlation coeficient, and line fitting by the least squares method. A t-value is calculated from the ratio of the estimated standard deviation of the coefficient along the X-axis and the coefficient itself. From this value p can be calculated to give an estimate of usefulness of prediction of results.

EXPERIMENTS

LIVER REGENERATION

Experimental Design

The following groups of animals were utilised:

1. Experimental Group - Portacaval transposition plus left adrenalectomy (PCT+A).
2. Control Group - Sham portacaval transposition plus left adrenalectomy (Sham+A)
   - Left adrenalectomy alone (Ad).
   - Ether control (Eth).

Experiment 1

A group of 5 rats within the selected weight of 200-220 gms were killed immediately after partial hepatectomy had been performed. This experiment was designed to give mean figures for total liver weight and percentage of resection in the normal rat.

Experiment 2

Animals in each of the experimental and control groups were allowed to survive for 3 and 6 weeks after the initial operation to study the body and liver weight changes occuring. In addition using the results from Experiment 1 the consistancy of the ratio of liver weight to body weight could be examined over the weight range of the animals used throughout the study.

The group killed at 3 weeks was also used to measure the extent of resection of partial hepatectomy in the experimental and control groups.

Experiment 3

Groups of experimental and control animals were killed at 12, 18, 21, 24, 30, 36, 48, and 72 hours, and at 3 weeks after partial hepatectomy, which was performed 3 weeks after the initial procedure. This experiment was designed to study alterations in liver weight, histology and D.N.A. activity in regenerating liver. At each time point 6 or 7 of the experimental group, 6 or 7 of the sham group, 4 adrenalectomised and 2-3 ether control animals were killed with duplicated groups at 18, 21, and 24 hours to cover the known peak of D.N.A. activity.

The ether control groups are made up as follows:

Ether 1 had a sham partial hepatectomy (to act as a control for laparotomy and mobilisation of the liver lobes).

Ether 2 had no operation (to act as a control for the anaesthetic alone).

Ether 3 had a partial hepatectomy (these animals were used as occasional methodological checks).

Animals, Surgical Techniques, Materials and Methods

Statistical Methods: see page 39.

Animals

Male Sprague Dawley rats were used throughout. The animals were selected for a starting weight of between 200-220 gms, were fed on a standard pellet diet (41B cube diet, Oxoid Ltd.) and were allowed water ad libitum. The animals were caged in groups of three to encourage similar feeding habits, one member of the experimental group and two control group animals in each cage. The animals were maintained in a constant warm temperature environment with lighting control producing a 12 hour daylight period.

Surgical Methods

All operations were carried out under open ether anaesthesia in a clean but non-sterile environment.

Incision

All incisions were ventral midline and extended from the xiphisternum to approximately 1 cm above the symphysis pubis. This incision is through the linea alba and is almost bloodless. At the end of the operation the muscle layer was closed with 3/0 catgut (Ethicon) and the skin closed with 9 mm autoclips which were left in situ until the next operation or sacrifice. The animals recovered from the operation in a warmed box with water ad libitum. Occasional wound abscesses in earlier groups of animals were later eliminated by the use of Rikospray antibiotic (Rikospray Ltd.) to the muscle layer before skin closure.

Operations

Microsurgical procedures were carried out under a Zeiss operating microscope or a Nanchette operating microscope with stereoscopic lenses and a facility to magnify from x4 to x16.

Portacaval Transposition plus Left Adrenalectomy (PCT+A)

Portacaval transposition was carried out after the technique described by Ryan et al (1974) with the addition of left adrenalectomy.

Through a standard laparotomy incision the anterior two lobes of the liver were mobilised by dividing the three ligaments attached thereto. The anterior lobes were delivered onto the thorax and covered with a moist swab. The spleen was retracted to the right of the rat exposing the left adrenal which was picked up in forceps. The adrenal pedicle was then ligated with 3/0 silk and the gland excised and kept for histology. The spleen was returned to the abdomen and the bowel retracted to the left exposing the inferior vena cava (IVC) and the portal vein (PV). The IVC was dissected free from above and below the right renal vein with special care being taken of the adherent right renal artery which was carefully teased off the posterior aspect of the IVC. The portal vein was freed from the splenic vein to the portahepatis and the right gastric (or gastroduodenal) branch divided between ligatures.

The IVC was then divided between clamps above the right renal vein, the open ends were flushed with water. The portal vein was similarly divided between clamps, caudal to the ligated right gastric branch, and the open ends flushed.

The distal portal vein and proximal IVC were approximated by two stay sutures of 7/0 silk (Ethicon), taking care not to twist the vessels. The posterior wall of the anastomosis was closed by a single through-and-through continuous stitch and the anterior wall similarly closed. Tension was maintained on the stay sutures to prevent contracture of the anastomosis. The occluding clamps were removed and the vessel pumped while covered with a cotton wool pledget to encourage blood flow and to break up small clots. The average time for this anastomosis is between 10 and 12 minutes.

The distal IVC and proximal portal vein were then similarly anastomosed end-to-end. The total occlusion time for the distal IVC was approximately 22 minutes.

After blood flow through the anastomosis was assured, the liver was returned into the abdomen and the wound closed (Figs. 1, 2).

Sham Portacaval Transposition plus Left Adrenalectomy (Sham+A)

Through a standard laparotomy incision the liver was delivered onto the thorax as in portacaval transposition and the left adrenal removed. The IVC and portal vein were similarly dissected free but the right gastric vein was not ligated nor divided.

Clamps were placed on the supra-renal IVC and on the portal vein cephalad to the right gastric for 22 and 12 minutes respectively. When the clamps were removed, the vessels were pumped with cotton wool pledgets. After the second clamp had been removed the liver was returned to the abdomen and the wound closed.

It is not in fact possible to produce "sham" occlusion of each vessel for the correct period relative to both portal deprivation of

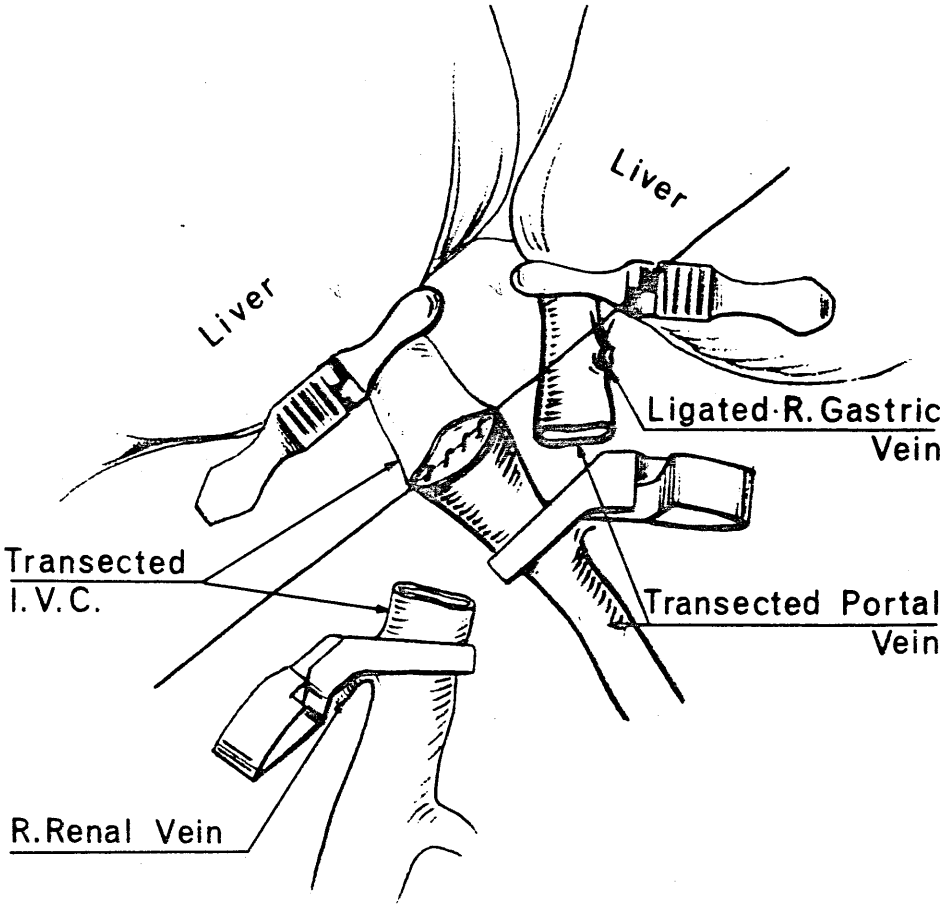


FIGURE 2

Portacaval transposition.

Completion of posterior wall of first anastomosis.

the liver and to portal congestion of the bowel. As congestion of the bowel for 20 minutes or more produces a markedly greater operative mortality and as the principal organ of interest is the liver, it was decided to clamp the portal vein for 12 minutes to simulate the portal deprivation to the liver produced by the operation of portacaval transposition.

Left Adrenalectomy (Ad)

The left adrenal gland was exposed and removed through a standard incision with minimal disturbance of abdominal contents. Particular care was taken not to disturb the liver. The wound was then closed.

Partial Hepatectomy (PH)

Through a standard laparotomy incision the anterior lobes of the liver were delivered onto the thorax. In adrenalectomy alone animals, this required only simple division of the three ligaments attached to the anterior two lobes. However, in animals which had been subjected to either portacaval transposition or sham portacaval transposition, adhesions had to be carefully divided between liver and omentum and small bowel. When the anterior lobes were delivered, the pedicle was ligated with 3/0 silk and the anterior lobes excised leaving only a small stump (Higgins and Anderson 1931). All partial hepatectomies were carried out between 0900 and 1100 hours to minimise the effect of diurnal variation on mitotic activity in the liver (Jaffe 1954).

Sham Partial Hepatectomy

Through a standard incision the liver was mobilised and a ligature passed around the pedicle of the anterior lobes but not tied. The ligature was then removed and the liver returned to the abdomen. The wound was then closed.

Intavenous Injection of Thymidine

An intravenous injection of 100uCi of ^{3}H thymidine was carried out one hour before sacrifice. Under ether anaesthesia, a small transverse incision was made lateral to the base of the neck and the external jugular vein exposed. Injection was then made by a 25 gauge needle directly into the vein.

Sacrifice

Through the standard incision the portahepatis was dissected bluntly to display the portal vein or IVC-portal anastomosis. Patency of the anastomosis was first checked, then the venous inflow to the liver was interrupted by clamping and the liver removed, blotted and cleaned of fat and other extraneous tissue. After careful weighing, the liver was divided into a specimen for histological examination and a specimen for determination of D.N.A. specific activity. The remaining adrenal or adrenals were also removed for histology.

Liver Weight

At the time of sacrifice the liver was excised and all non-hepatic tissue removed. The liver was then blotted dry to remove

any surface moisture and blood. The liver was weighed by myself, or by one of two technicians under my supervision, on a Sartorius top pan precision balance (accuracy of 0.001mg) (Avery Ltd.) and the weight noted. The anterior lobes were then removed and seperately weighed as were the caudate and right-posterior lobes.

Relative Liver Weight

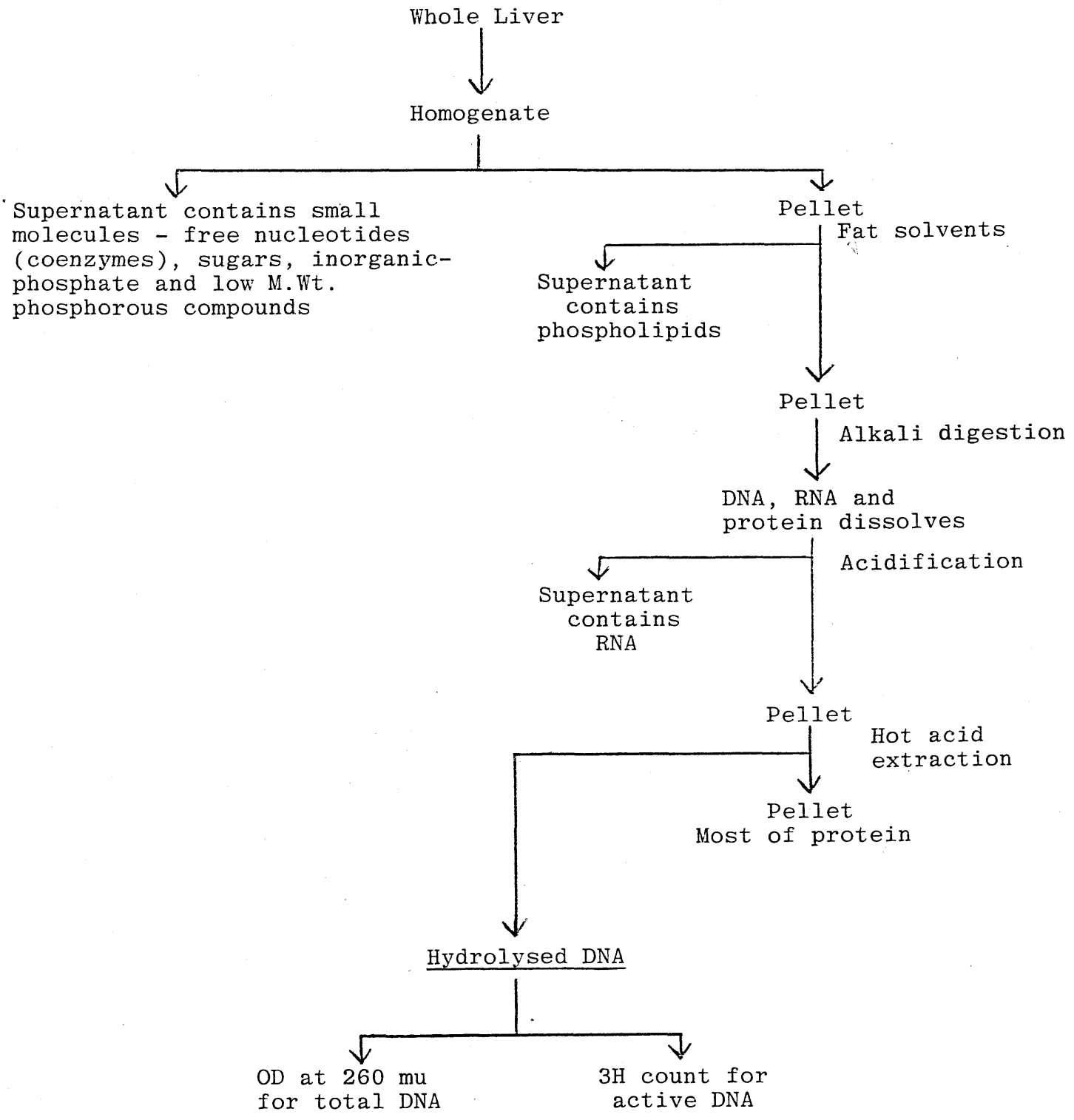
Alterations in body weight, by reduced food and fluid intake, are produced as a result of the operative procedures in the immediate post-operative period. Comparison of absolute liver weight will reflect these alterations, but by using the ratio of liver weight to body weight (expressed as a percentage), this additional factor can be eliminated in comparison between groups. This ratio has been called the relative liver weight.

The measurement of body weight was taken at the time of partial hepatectomy in those animals sacrificed within the first 72 hours. The liver weight 3 weeks after partial hepatectomy is related to the sacrifice body weight. As the tissue fluids and intracellular fluids may be reduced at the time of sacrifice, the measurement of relative liver weight may tend to underestimate liver regeneration.

D.N.A. Specific Activity

From all animals receiving ^{3}H thymidine at the time of sacrifice, 700-800 mgs of liver was taken from the right posterior lobe and frozen to $-10^{o}C$ for later estimation of the ratio of radioactive D.N.A. to that of total D.N.A. (specific activity). This ratio was measured as follows (see flow chart).

FLOW CHART OF DNA ASSAY



A 700-800 mg sample of liver was homogenised in 10 mls of normal saline and kept at 4°C. The homogenate was then divided into two and the rest of the estimation performed in duplicate.

0.2 mls of 10 N $HClO_4$ was added to the homogenate which was stirred, centrifuged at 2,000 rpm for 3 minutes and the supernatant discarded. The pellet was then rinsed with distilled water.

5 mls of ethanol ether (3 : 1) was added to the pellet and stirred before centrifugation as above with the supernatant again being discarded.

5 mls of ethanol chloroform (3 : 1) was added to the pellet and stirred before centrifugation as above and again the supernatant was discarded.

The pellet at this stage contains D.N.A., R.N.A. and protein. The pellet was then incubated in a water bath at 37°C with 5 mls of 0.3 N KOH for 16 hours, at the end of which time the sample was chilled to 4°C and 0.2 mls of 10 N $HClO_4$ was added. The sample was then centrifuged and the supernatant containing R.N.A. discarded. The pellet at this stage now contains D.N.A. and protein only.

The pellet was rinsed twice with distiled water before 6.5 mls of 0.5 N $HClO_4$ was mixed with it. The sample was kept in a water bath at 70°C for 20 minutes. At the end of this time the tubes were chilled to 4°C, centrifuged for 30 minutes and the supernatant (containing hydrolysed D.N.A.) divided into two samples. 1 ml of the supernatant was added to 9 mls of distilled water for estimation of optical density at 260 micron wavelength in a SP500 spectrophotometer using 1 ml of 0.5 N $HClO_4$ as a blank for each sample.

0.5 mls of the supernatant was added to 16 mls of liquid scintillant (Instagel, Packard Ltd.) for counting in a Packard Tricarb. Liquid Scintillation Counter for 20 minutes. 0.5 mls of 0.5 N $HClO_4$ was used as a control blank.

The number of counts per minute (cpm) for each sample was then collected in two channels and from a quench calibration curve of efficiency against channels ratio, the total disintegrations per minute (dpm) was calculated. The disintegrations per minute were then divided by the optical density of the sample to give a D.N.A. specific activity.

Histology

Tissue from adrenals and liver were fixed in formol saline and stained with haematoxylin and eosin. After routine microscopy, photomicrographs were taken from representative slides.

LIVER REGENERATION

RESULTS

Overall Mortality

A total of 259 animals were used in this study; 254 in survival experiments. The overall mortality figures are shown in Table I. There was no difference in survival between PCT+A and Sham+A groups.

Experiment 1

In the 5 animals of the selected weight range of 200-220 gms (mean 209.8 $\pm$ 5.8 gms), the mean liver weight was 8.9 $\pm$ 0.7 gms. This represents a relative liver weight of 4.2 $\pm$ 0.3.

Experiment 2

The changes in body weight are shown in figure 3 for the experimental group (PCT+A) and for each of the control groups (Sham+A and Ad) over the 6 weeks subsequent to the procedure. As can be seen the body weight 3 weeks after PCT+A was significantly less than the control groups ($0.01>p>0.001$). However, by 6 weeks there was no significant difference between PCT+A and the controls.

Figure A is a plot of liver weight against body weight of control animals from the time of selection, through to 3 and 6 weeks after control procedure. The grouping of weights at 200 gms represents the selection of animals, subsequent weights are unselected and are as a result of weight changes after the various procedures. Over the weight range examined there is a linear

TABLE I

OVERALL MORTALITY FIGURES

Group	Type of Experiment	No. Surviving Operation	No. Surviving to Sacrifice & %	
PCT + A	Survival	86	63	73%
Sham + A	Survival	85	64	75%
Ad.	Survival	56	54	96%
Eth.	Survival	27	26	96%
	TOTAL	254	TOTAL 207	MEAN 81%
Others	Acute	5		
		259		

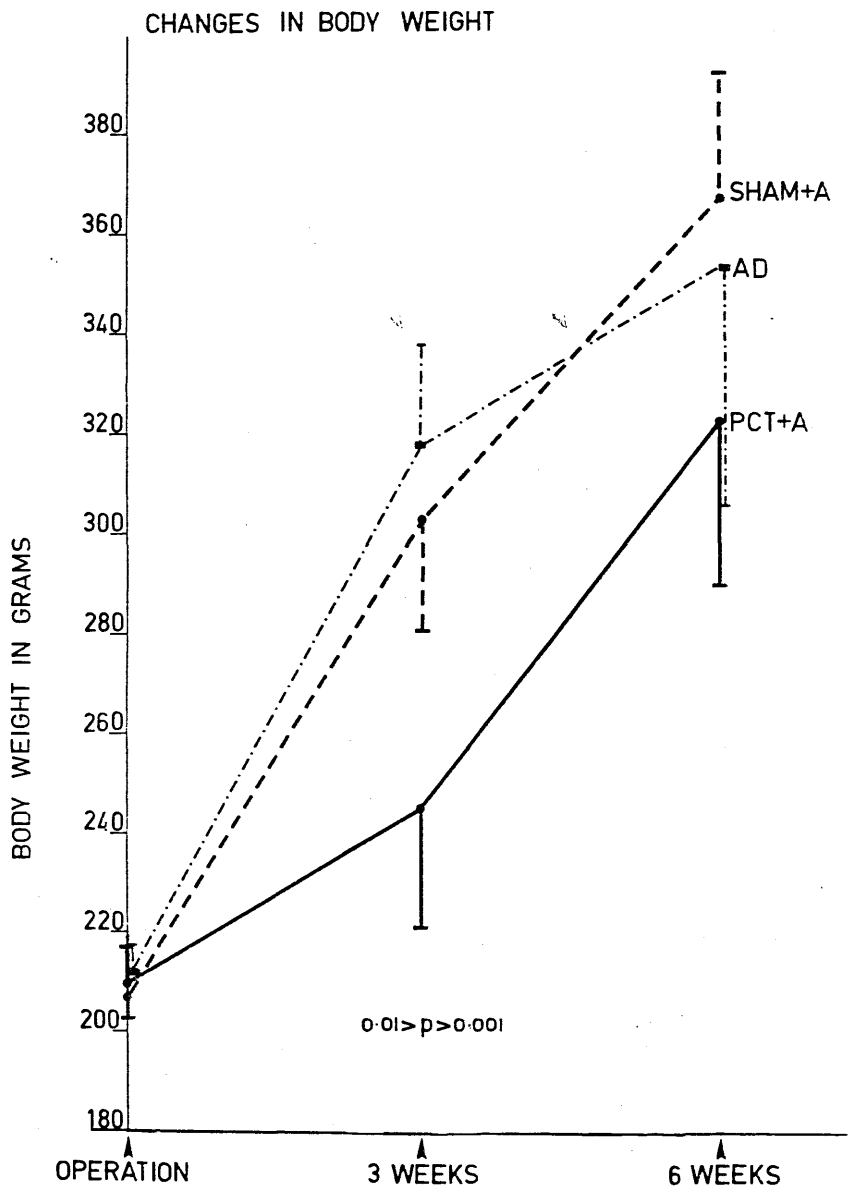


FIGURE 3

Changes in body weight (mean and one standard deviation). PCT + A body weight significantly less than controls ($0.01 > p > 0.001$).

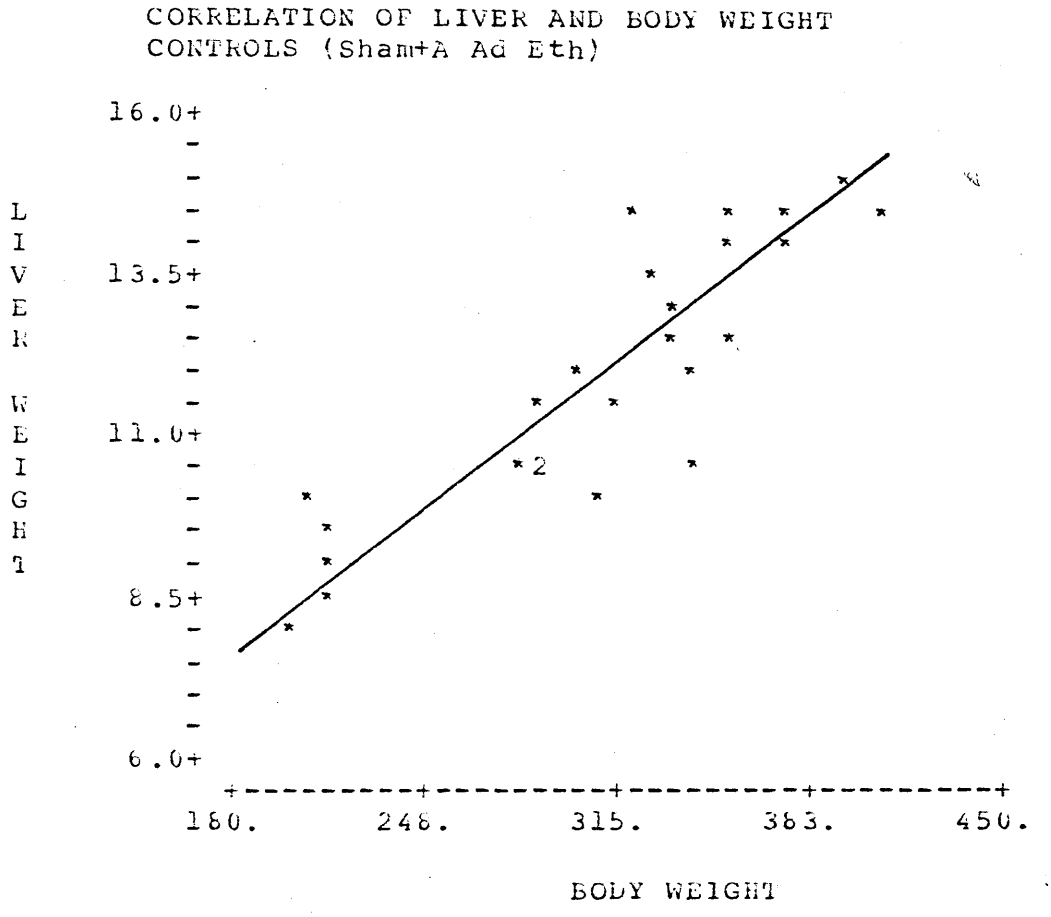


FIGURE A

CORRELATION COEF(r) = 0.887

t = 9.21 p<0.001

Y = 2.02+0.0317 x X

ST. DEV. OF Y ABOUT REGRESSION LINE = 1.013

correlation between body weight and liver weight ($r = 0.887$). The t-value derived from the ratio of coefficient to the standard deviation gives a $p<0.001$ indicating that body weight is a good predictor of liver weight (standard deviation of liver weight residual was 1.013).

A simalar plot (Fig. B) can be generated for the experimental group (PCT+A). This also shows a linear correlation between liver weight and body weight ($r = 0.840$; $0.005>p>0.001$). The slopes of the two regression lines are very similar, although the Y intercept is less in the PCT+A group. This difference in intercepts demonstrates the fall in liver weight after PCT+A.

Figure 4 represents the fall in relative liver weight which occurs in each group after the initial procedure. This fall was most marked in the PCT+A group where the relative liver weight fell to 2.9 ± 0.2. This is significantly less than the control groups ($p<0.001$).

By carrying out a partial hepatectomy at the time of the 3 week kill and carefully measuring the weights of the individual lobes including the ligated stump, the mean value of a two-thirds partial hepatectomy can be calculated for the experimental group (PCT+A) and for the control groups.

It would appear that there is a relatively greater atrophy in the anterior lobes after PCT+A than after control procedures. Three weeks after the procedure a partial hepatectomy in the PCT+A group produces a 59.4 ± 3.6 % resection, as compared to 61.2 ± 2.4 % in Sham+A and 64.2 ± 2.4 % in Ad. There is, however, no significant difference between any of these figures. These percentage resection

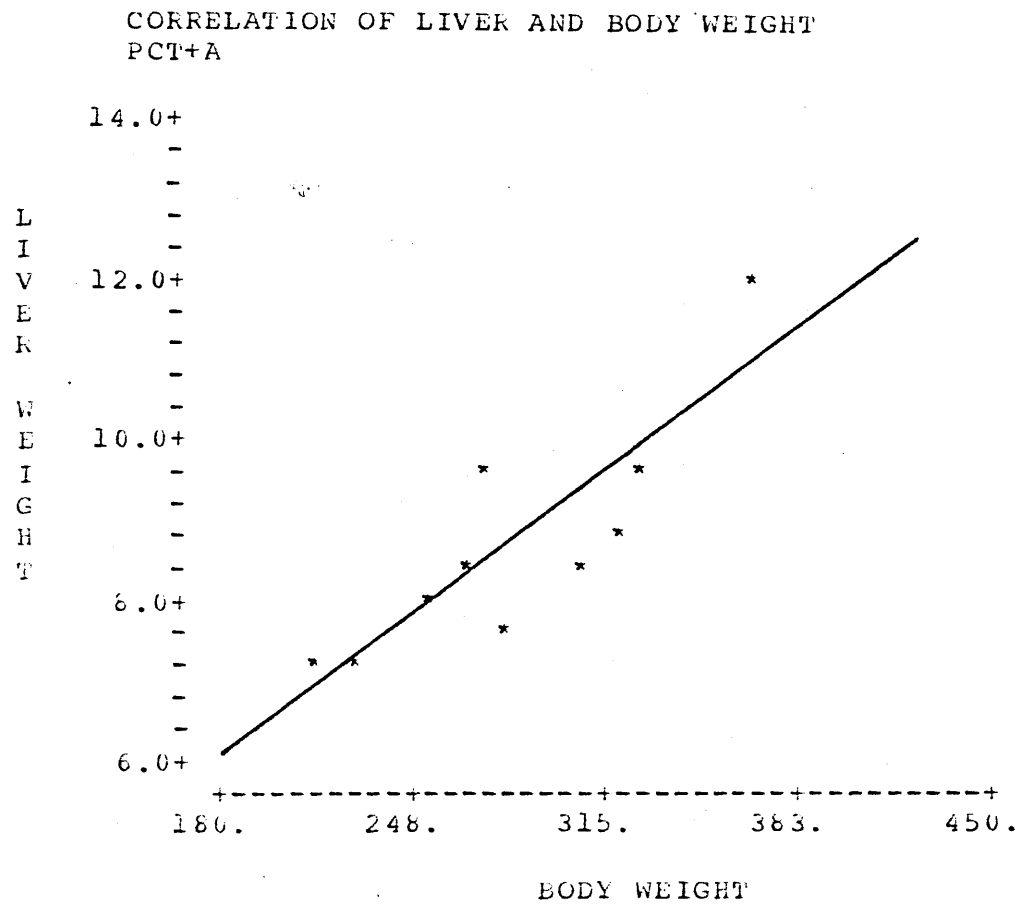


FIGURE B

CORRELATION COEF(r) = 0.840

t = 4.37 0.005>p>0.001

Y = 1.42 + 0.0257 x X

ST. DEV. OF Y ABOUT REGRESSION LINE = 0.8546

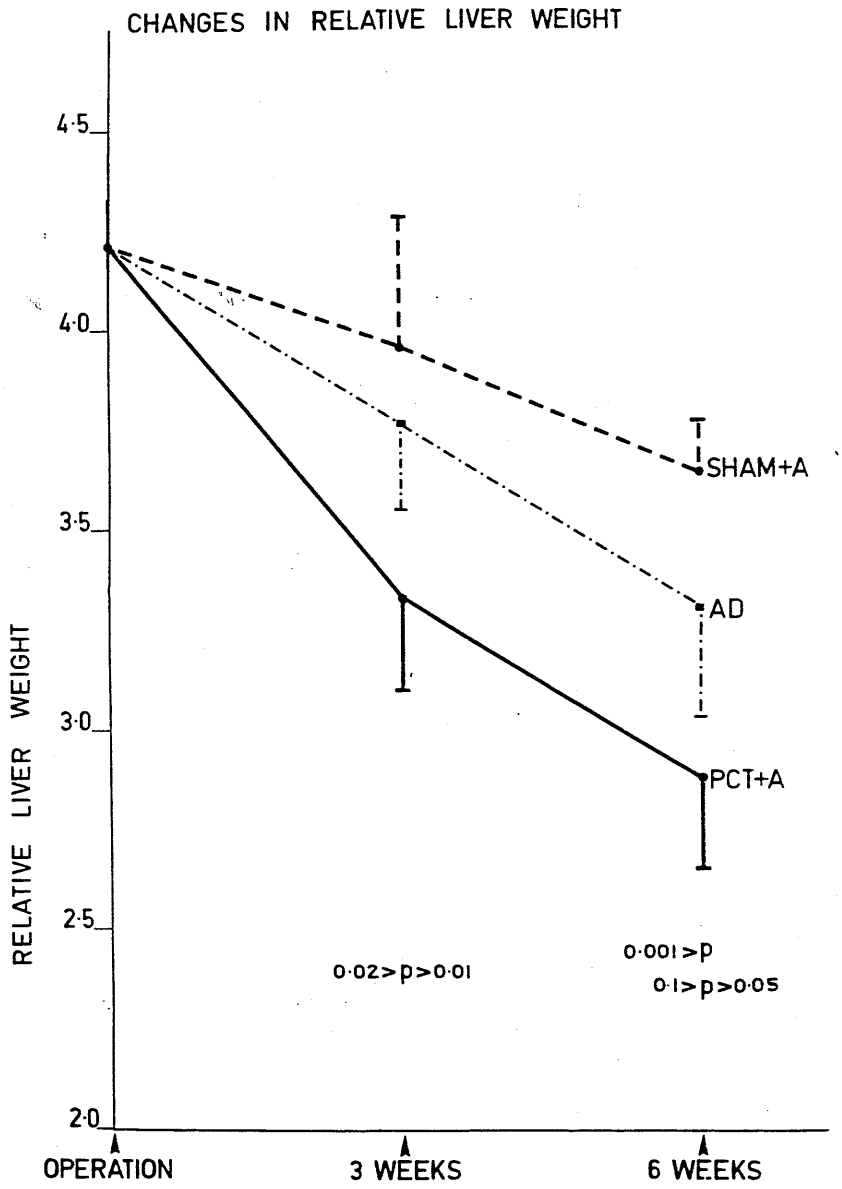


FIGURE 4

Changes in relative liver weight (mean and one standard deviation).

PCT + A relative liver weight significantly less than Sham + A at 3 weeks ($0.02 > p > 0.01$), and at 6 weeks ($p < 0.001$).

values are used in subsequent experiments to determine the total liver weight from the weight of the resected specimen at the time of partial hepatectomy.

The posterior lobes represented a remarkably constant proportion of the total liver weight at 3 and 6 weeks, although there is a significant fall ($p<0.001$) between the first operation and 3 weeks (Table II).

Experiment 3

The body weight curves of animals subjected to experimental and control procedures followed 3 weeks later by partial hepatectomy are shown in figure 5 and can be compared with figure 3 of the body weights of animals not undergoing partial hepatectomy. By 3 weeks after partial hepatectomy Sham+A and Ad groups had passed their weight at the time of partial hepatectomy. The PCT+A group did not re-attain their starting weight by the time of partial hepatectomy at 3 weeks.

If body weight is not taken into account in the experimental group (PCT+A) and the total liver weight only examined (Table III), then the posterior lobes grow from 3.3 ± 0.7 gms at the time of partial hepatectomy to only 3.4 ± 1.1 gms by 72 hours. By 3 weeks after partial hepatectomy they had reached 6.1 ± 1.6 gms. These posterior lobes represent the total liver weight and are significantly less ($0.01>p>0.001$) than the weight of liver at the time of the initial operation. Figures for Sham+A and Ad groups are also given in Table III and they show that the posterior lobes have regenerated to a total liver weight at 3 weeks after partial

TABLE II

POSTERIOR LOBES : RELATIVE LIVER WEIGHT

	1st Operation	p	3 weeks	p	6 weeks
PCT + A	2.99 ± 0.26 (5)		1.26 ± 0.19 (5)		1.10 ± 0.13 (5)
Sham + A	2.99 ± 0.26 (5)	<0.001	1.45 ± 0.17 (6)	No significance	1.33 ± 0.10 (4)
Ad.	2.99 ± 0.26 (5)		1.26 ± 0.12 (4)		1.08 ± 0.16 (3)

TABLE III

TOTAL LIVER WEIGHT (GMS) AFTER PROCEDURE FOLLOWED BY PARTIAL HEPATECTOMY

	First Operation	PH (3 weeks after 1st op.)	3 weeks after PH
PCT + A	8.87 ± 0.74 (209.8 ± 5.7) (5)	3.33 ± 0.69* (247.4 ± 26.3) (5)	6.1 ± 1.6^{+} (245.6 ± 39.5) (5)
Sham + A	8.87 ± 0.74 (209.8 ± 5.7) (5)	4.66 ± 0.69 (302.2 ± 21.6) (6)	11.4 ± 2.2 (324.2 ± 32.9) (5)
Ad.	8.87 ± 0.74 (209.8 ± 5.7) (5)	4.32 ± 0.68 (317.5 ± 20.2) (4)	10.1 ± 1.1 (303.3 ± 20.8) (3)

(Body Weight Gms)

*$0.02 > p > 0.01$ versus Sham

$0.01 > p > 0.05$ versus Ad.

$^{+}0.01 > p > 0.001$ versus Controls

$0.01 > p > 0.001$ versus Pre-op.

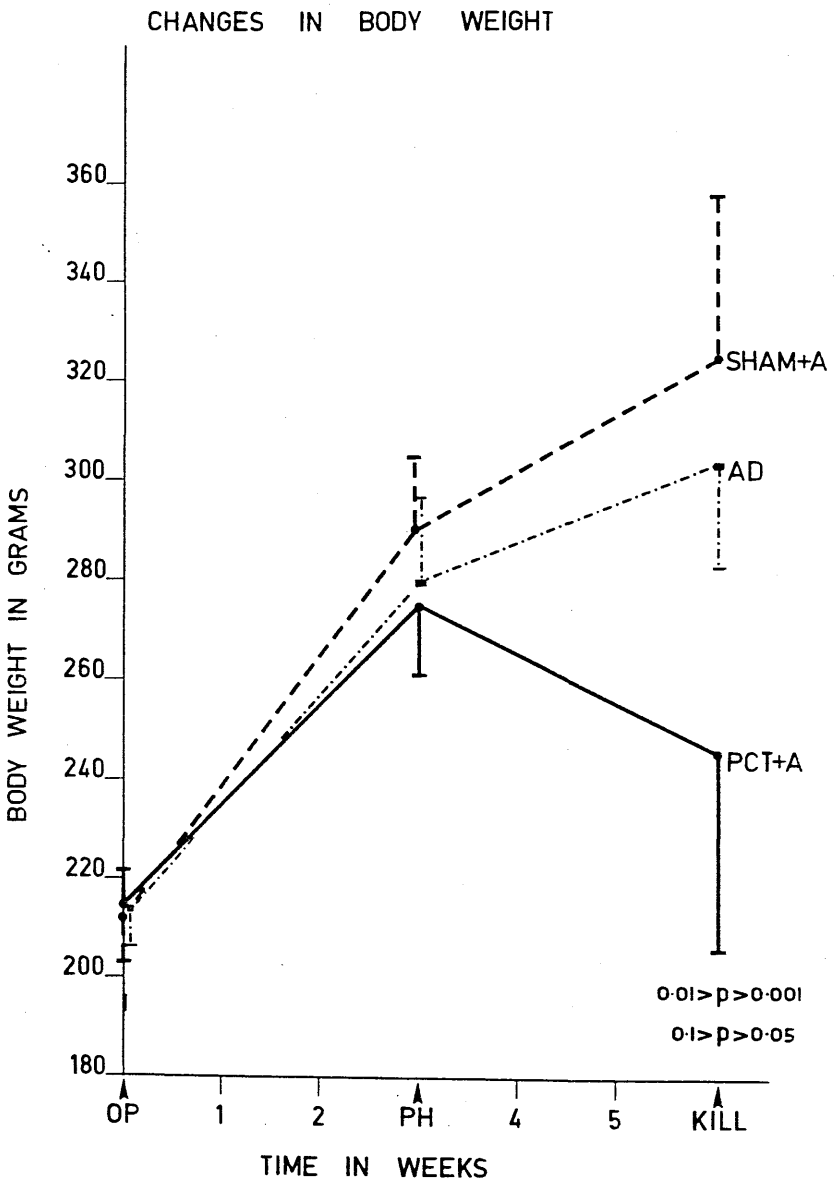


FIGURE 5

Change in body weight (mean and one standard deviation).

OP - operation (PCT + A, Sham + A, Ad.).

PH - partial hepatectomy.

PCT + A body weight significantly less than Sham + A 3 weeks after partial hepatectomy ($0.01 > p > 0.001$).

hepatectomy which is in excess of the initial liver weight.

Relative liver weight has been used to eliminate the variable of total body weight which is altered by the operation of partial hepatectomy as can be seen in Table III in parenthesis. For example, at the time of partial hepatectomy the PCT+A group weighed a mean of 247 $\pm$ 26 gms and 3 weeks later the mean weight was 246 $\pm$ 40 gms.

Partial hepatectomy 3 weeks after the initial procedure reduces the liver weight in the PCT+A group to 1.26 $\pm$ 0.19 gms (Table IV).

Regeneration in the experimental and control groups may be followed using relative liver weight (Table IV and Fig. 6) which shows parallel changes in each group until 72 hours when there is a significant fall ($p<0.001$) in the relative liver weight in PCT+A relative to controls.

If the data is examined in terms of regenerated liver weight as a percentage of liver weight estimated at the time of partial hepatectomy, which is also a method of eliminating the bias of body weight, there is still a fall at 72 hours which is significant ($p<0.001$).

However, if the final regenerated liver at 3 weeks after partial hepatectomy is compared with either the expected relative liver weights (Figs. 7,8,9) or the estimated liver weight at the time of partial hepatectomy, it can be seen that regeneration has in fact taken place.

There was no significant difference between the relative liver weight of PCT+A three weeks after partial hepatectomy, compared to PCT+A with no partial hepatectomy (Fig. 7). Similar figures are shown for Sham+A and Ad (Figs. 8,9).

TABLE IV

RELATIVE LIVER WEIGHT IN THE REGENERATING RAT LIVER

	PH	t = 12h	t = 18h	t = 21h	t = 24h
PCT + A	1.26 ± 0.19 (5)	1.06 ± 0.07* (5)	1.45 ± 0.17 (8)	1.37 ± 0.19 (10)	1.41 ± 0.27 (6)
Sham + A	1.45 ± 0.17 (6)	1.20 ± 0.10 (5)	1.43 ± 0.13 (12)	1.44 ± 0.12 (8)	1.65 ± 0.17 (11)
Ad.	1.26 ± 0.12 (4)	1.10 ± 0.04 (4)	1.39 ± 0.22 (8)	1.32 ± 0.12 (8)	1.47 ± 0.14 (8)

*$p < 0.05$ vs. Sham

	t = 30h	t = 36h	t = 48h	t = 72h	t = 3 weeks
PCT + A	1.49 ± 0.09* (5)	1.62 ± 0.17* (5)	2.02 ± 0.03 (3)	1.36 ± 0.19* (6)	2.47 ± 0.42* (5)
Sham + A	1.65 ± 0.11 (5)	2.01 ± 0.19 (6)	2.45 ± 0.40* (6)	2.71 ± 0.51 (4)	3.47 ± 0.37 (5)
Ad.	1.58 ± 0.07 (4)	1.82 ± 0.25 (4)	2.04 ± 0.08 (4)	2.68 ± 0.17 (4)	3.35 ± 0.46 (3)

*$p < 0.05$ vs. Sham

*$p < 0.01$ vs. Sham
< 0.1 vs. Ad.

*$0.05 > p > 0.02$ (F +ve)

*$p < 0.001$ vs. Control

*$p < 0.01$ vs. Sham
< 0.05 vs. Ad.

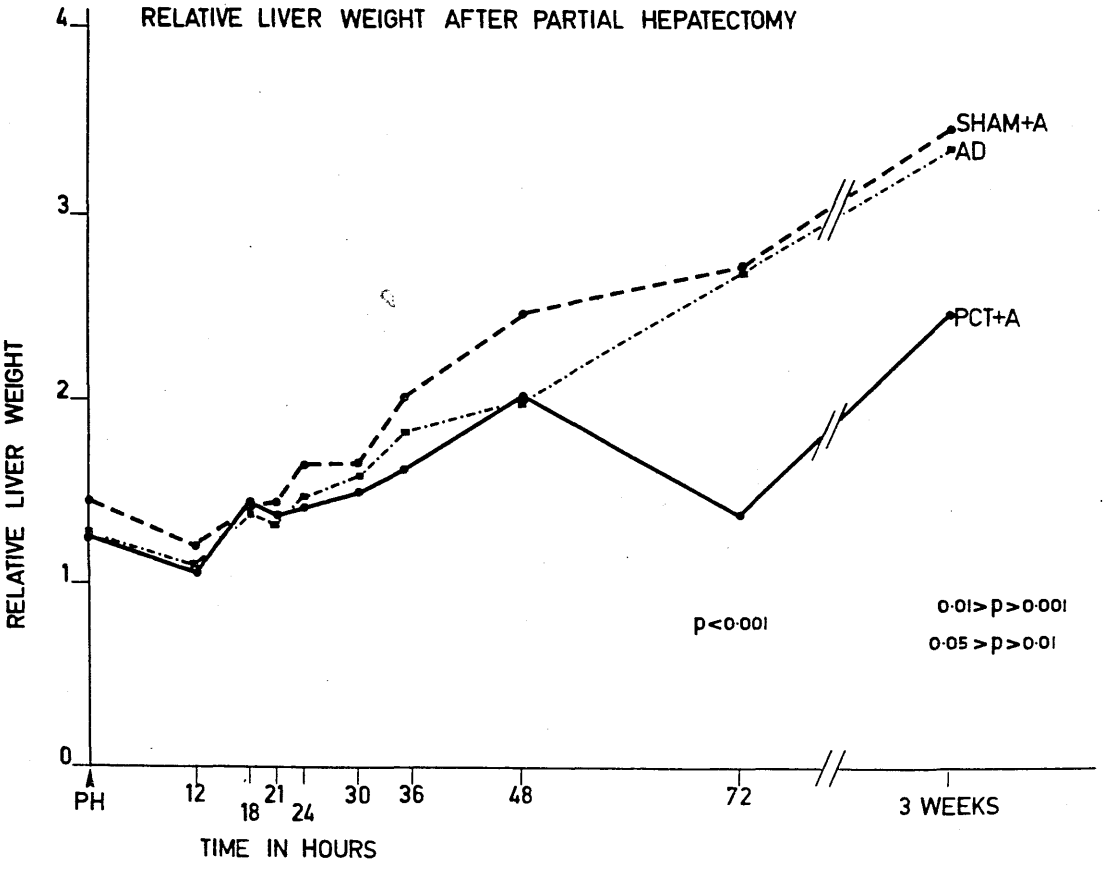


FIGURE 6

Relative liver weight after partial hepatectomy (mean values).

PH - partial hepatectomy.

PCT + A relative liver weight significantly less than controls at 72 hours and 3 weeks (see Table IV for detailed breakdown of p values).

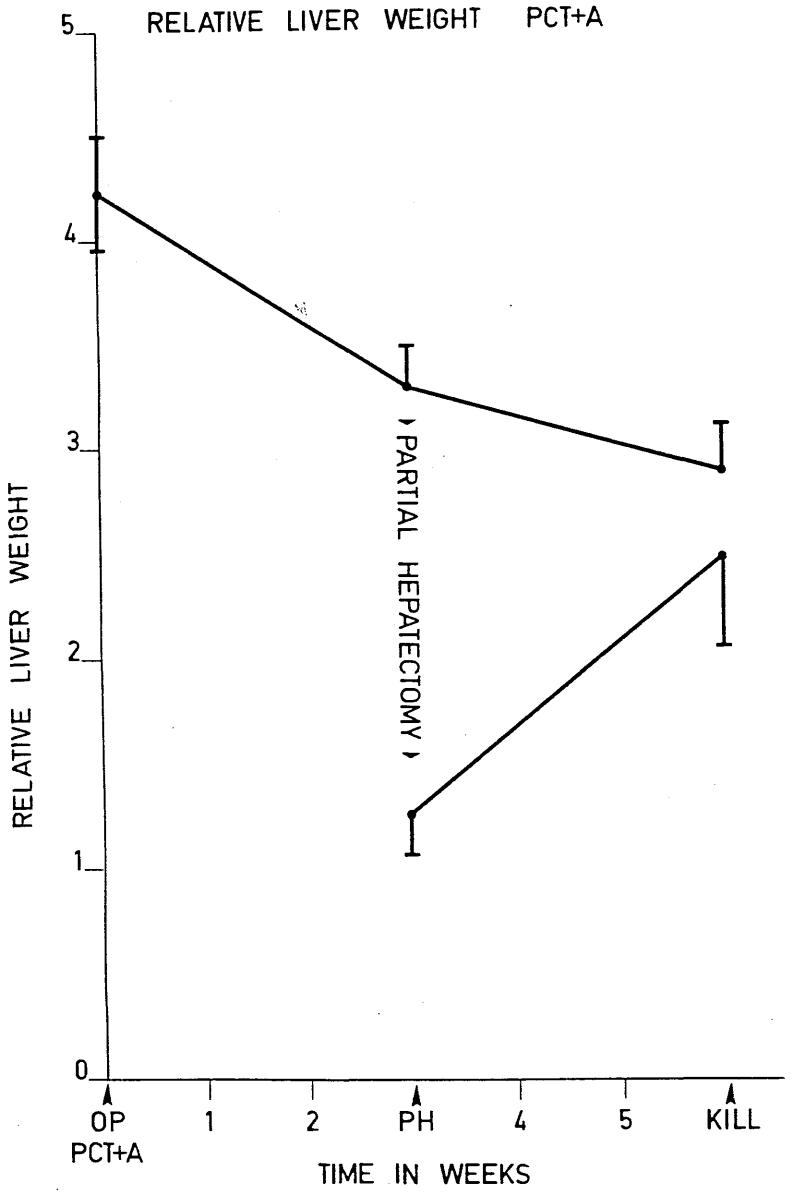


FIGURE 7

PCT + A relative liver weight (mean and one standard deviation).

OP - operation.

PH - partial hepatectomy.

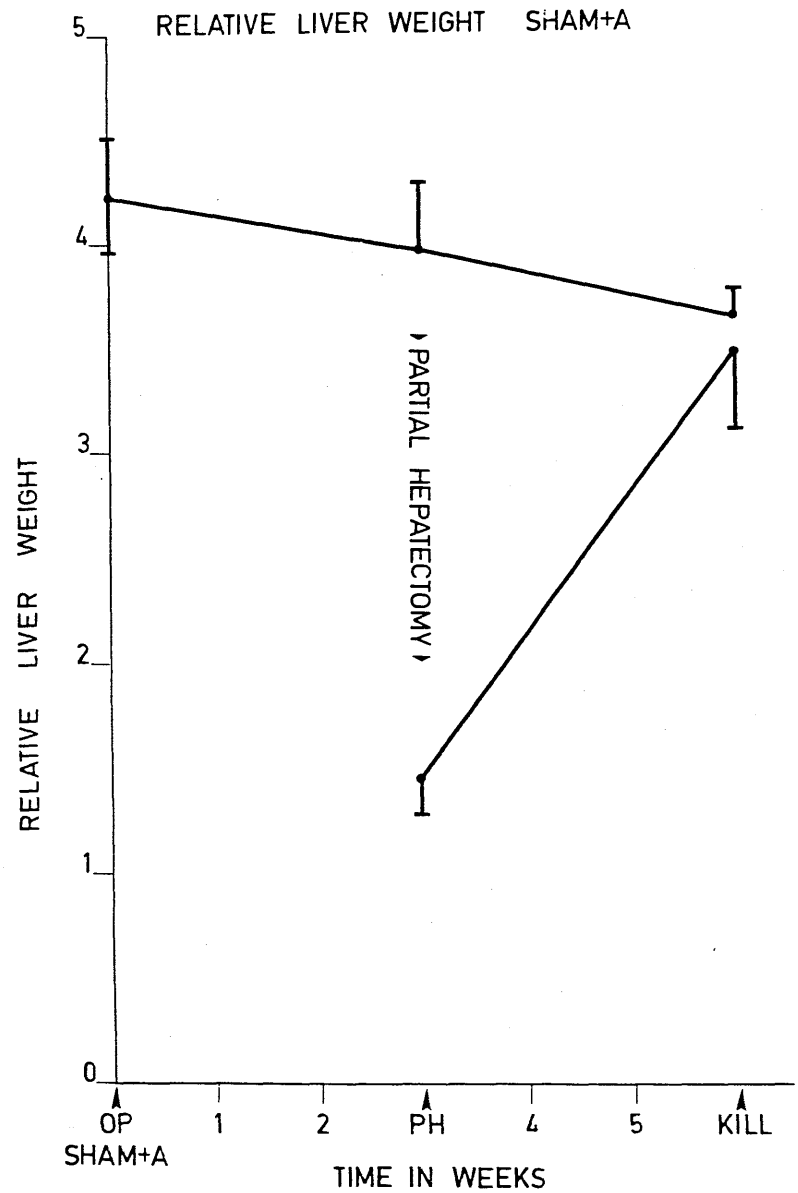


FIGURE 8

Sham + A relative liver weight (mean and one standard deviation).

OP - operation.

PH - partial hepatectomy.

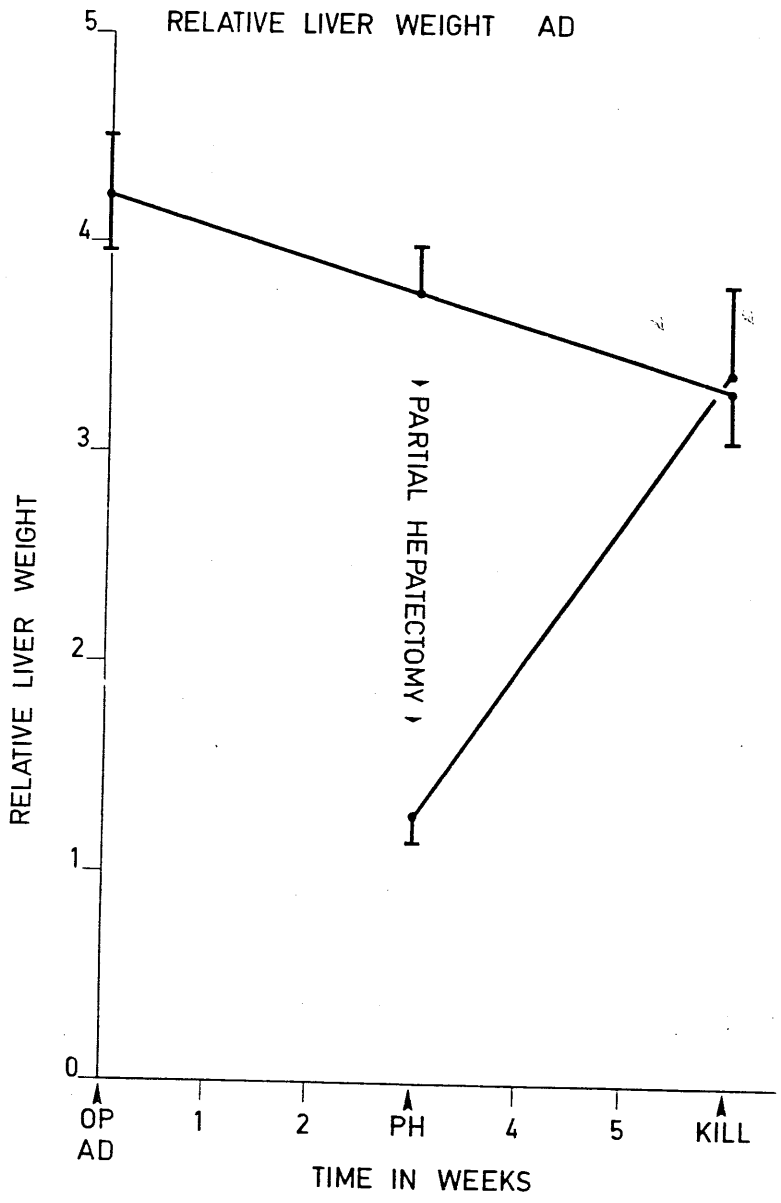


FIGURE 9

Ad. relative liver weight (mean and one standard deviation).

OP - operation.

PH - partial hepatectomy.

Regeneration of the liver of the rat subjected to partial hepatectomy after portacaval transposition plus left adrenalectomy occurs such that the relative liver weight does not differ significantly from the expected value.

D.N.A. Specific Activity

The D.N.A. specific activity for the ether control animals undergoing either anaesthesia alone or sham partial hepatectomy are shown for each time point in Table V. No reading is above 1,500 and only two above 1,000. It would appear that sham hepatectomy may inhibit any increased activity produced by the ether anaesthesia.

The results for the specific activity of D.N.A. in the experimental group and both Sham+A and Ad control groups are shown in figures 10, 11 and 12. The median results are shown in Table VI and the statistical analysis shown in Table VII.

As can be seen there is no significant difference by the Mann-Whitney test at any time in the first 36 hours after partial hepatectomy. At 48 hours D.N.A. specific activity in the PCT+A group is significantly less ($p<0.05$) than in both control groups. At 72 hours Sham+A is significantly less ($p<0.05$) than both PCT+A and Ad.

Regeneration of the liver of the rat subjected to partial hepatectomy after portacaval transposition plus left adrenalectomy occurs contemporaneously with control animals in terms of D.N.A. specific activity. There is no significant difference in the height of the peaks of activity within the first 36 hours after partial hepatectomy.

TABLE V

DNA SPECIFIC ACTIVITY FOR ETHER CONTROLS (RANKED)

	Sham PH	Ether Only
	467	1485
	378	1125
	354	824
	286	572
	271	481
	247	444
	226	433
	218	312
	202	260
	183	245
Median	269	462.5

TABLE VI

DNA SPECIFIC ACTIVITY AFTER PH

	Time in Hours							
	12	18	21	24	30	36	48	72
PCT + A	345	10461	23101	14592	4981	8116	3533	8540
	(5)	(8)	(10)	(5)	(4)	(5)	(3)	(4)
Sham + A	290	10590	21635	24079	11281	10547	12717	2778
	(5)	(12)	(8)	(11)	(5)	(6)	(6)	(6)
Ad.	517	34096	32324	26495	8059	17689	11216	8797
	(3)	(7)	(8)	(7)	(4)	(4)	(4)	(4)

Median Results

(n)

TABLE VII

COMPARISON OF DNA SPECIFIC ACTIVITY p VALUE

(MANN-WHITNEY RANK TEST WITH WILCOXON'S 'U' STATISTICS

			Time in Hours							
			12	18	21	24	30	36	48	72
PCT + A	v	Sham + A	NS	NS	NS	NS	NS	NS	0.05	0.05
PCT + A	v	Ad.	NS	NS	NS	NS	NS	NS	0.05	NS
Sham + A	v	AD.	NS	NS	NS	NS	NS	NS	NS	0.05

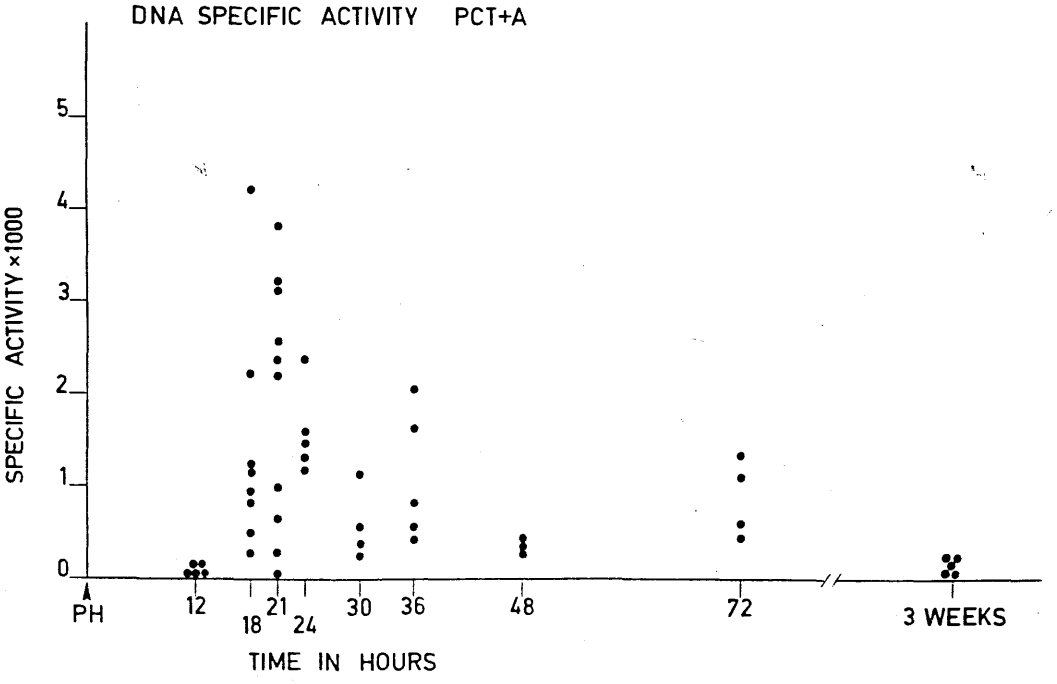


FIGURE 10

PCT + A D.N.A. specific activity.

Each point represents a single animal.

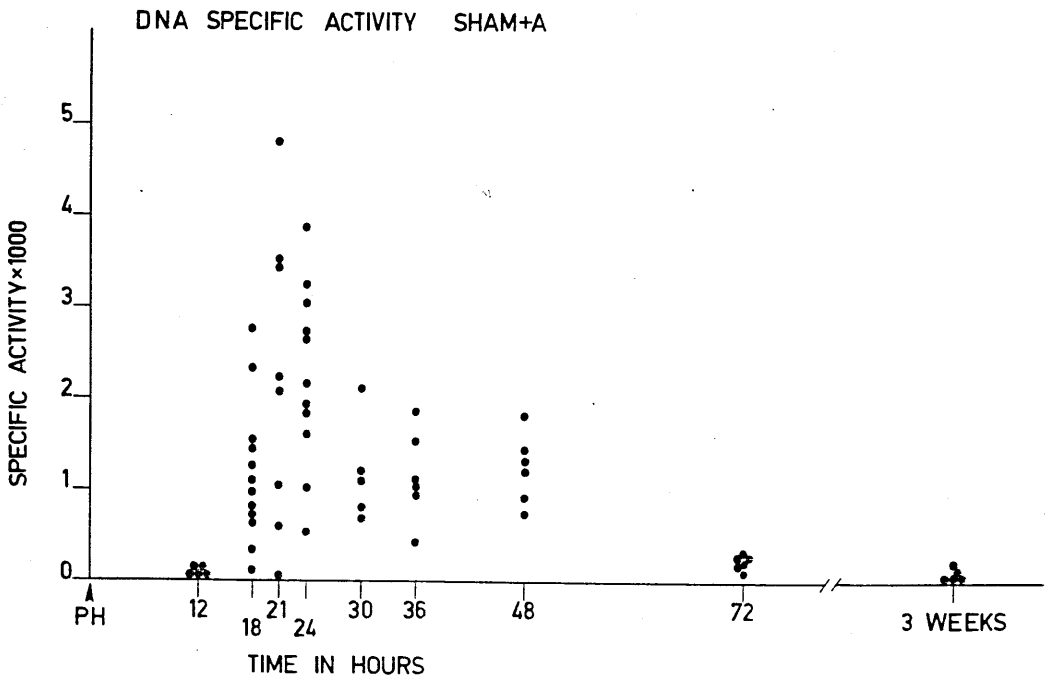


FIGURE 11

Sham + A D.N.A. specific activity.

Each point represents a single animal.

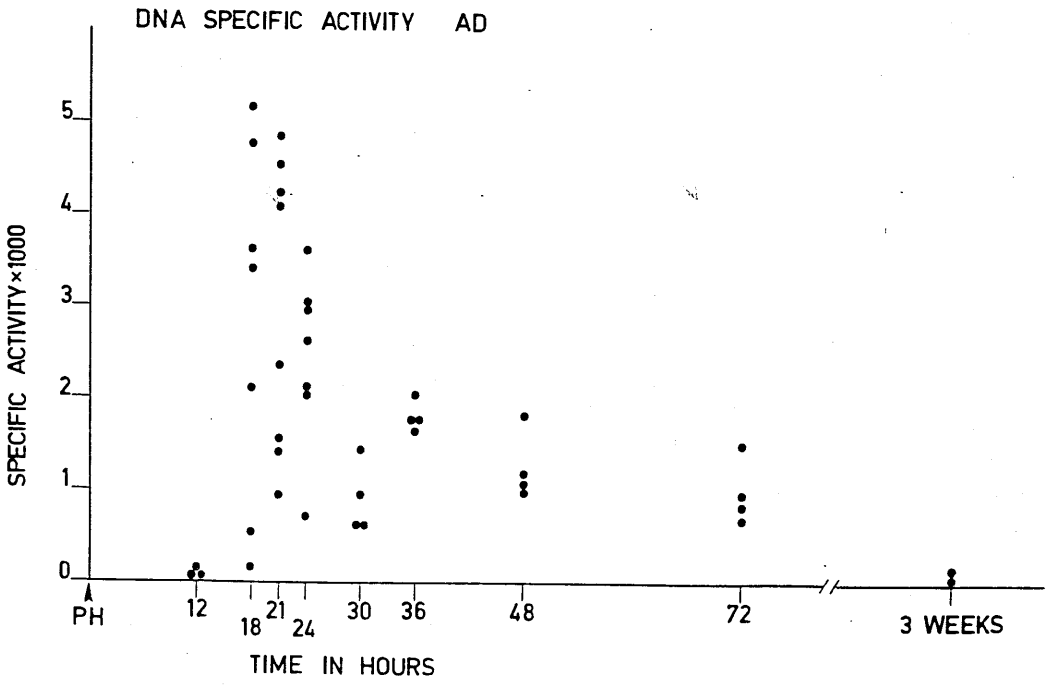


FIGURE 12

Ad. D.N.A. specific activity.

Each point represents a single animal.

Pathology

Macroscopically within 24 hours of partial hepatectomy in all groups, the residual hepatic lobes appeared enlarged with a tense capsule and distinct yellowish colour. The liver was also much more friable.

Histology of the normal rat liver is shown in Plate 1. Fatty infiltration is apparent in both experimental and control groups at 18 hours, and as can be seen in Plate 2 is much more extensive in PCT+A animals at 21 hours. This fatty infiltration in the PCT+A group persisted for much longer and was still clearly identifiable at 36 hours (Plate 3), but was diminished by 72 hours (Plate 4).

Mitotic activity was first seen in substantial numbers at 24 hours in the livers of control animals undergoing partial hepatectomy. Although at this time there were mitotic figures in the PCT+A group and a considerable number of pyknotic nuclei, there appeared to be some delay in mitotic activity (Plate 5) when compared with control groups. The mitotic activity started in the peri-portal area and thus conformed to the usual pattern seen in regenerating liver after partial hepatectomy. There was also more mitotic activity in the PCT+A group than in the control groups at 36 hours (Plate 6).

At 72 hours in the PCT+A group there were still some mitotic figures present (Plate 4) and double cell plates can be seen. Apart from these double cell plates, there was no other gross abnormality by 3 weeks after partial hepatectomy (Plate 7). Lymphocytic infiltration was seen in the livers of two rats. Plate 8 shows infiltration in the liver of a rat subjected to a partial hepatectomy after adrenalectomy alone. The other rat with focal

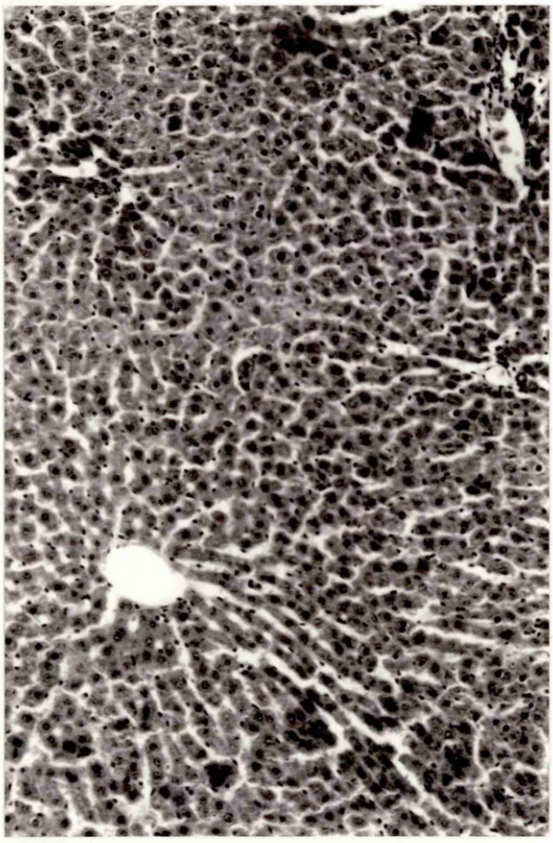


PLATE 1

Ether control 18 hours after sham partial hepatectomy.

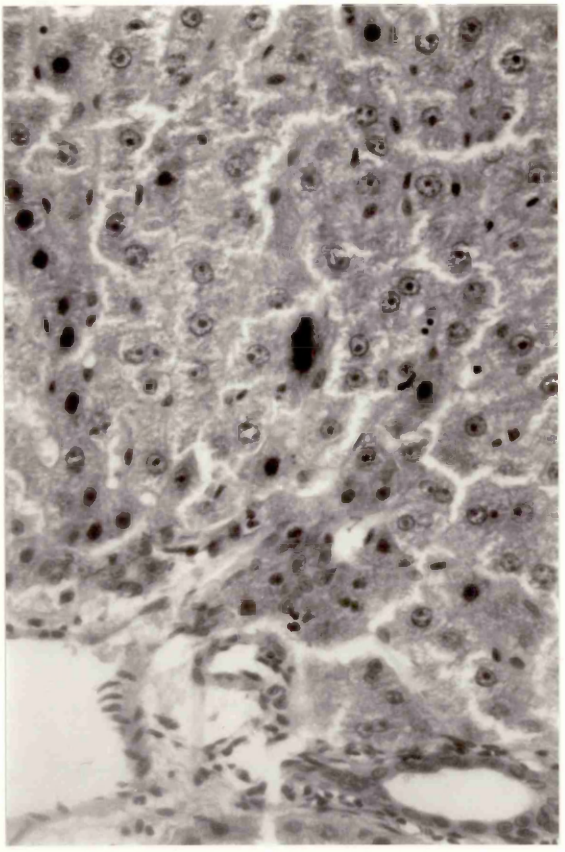


PLATE 2

PCT + A 21 hours after partial hepatectomy showing increased fat deposition and pyknotic nuclei.

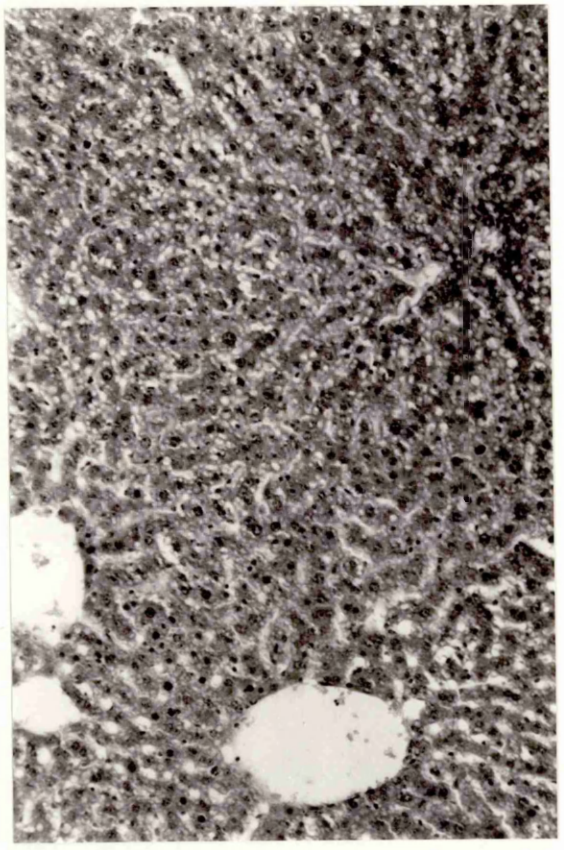


PLATE 3

PCT + A 36 hours after partial hepatectomy showing gross fatty infiltration and mitotic activity.

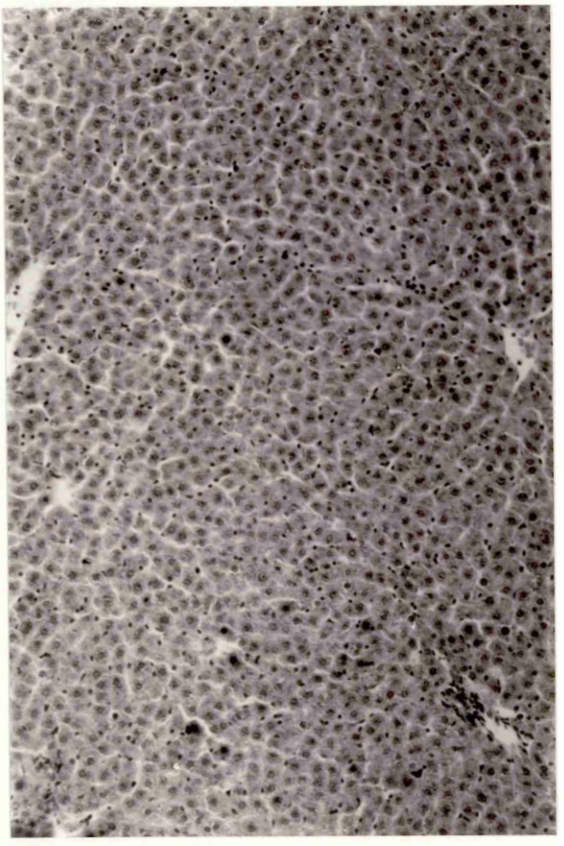


PLATE 4

PCT + A 72 hours after partial hepatectomy showing double cell plates and continuing mitotic activity with diminished fatty infiltration.

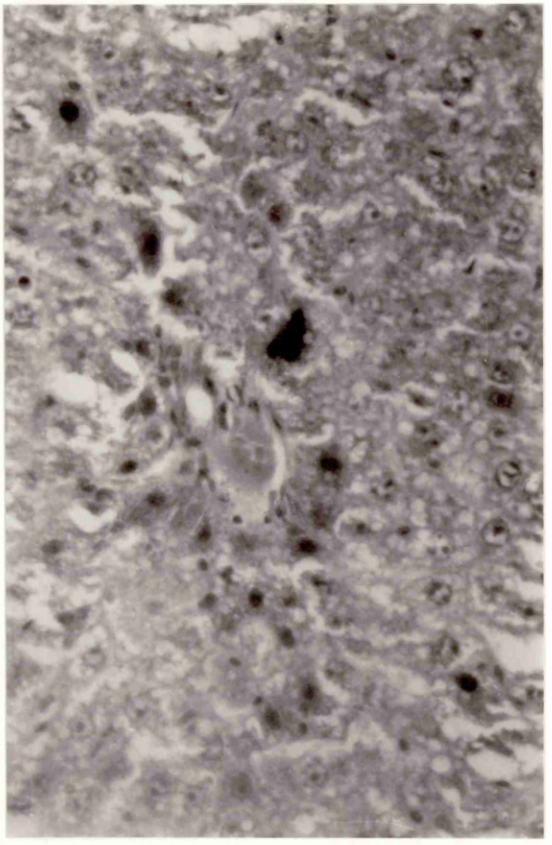


PLATE 5

PCT + A 24 hours after partial hepatectomy. Higher magnification of portal triad with periportal mitotic activity.

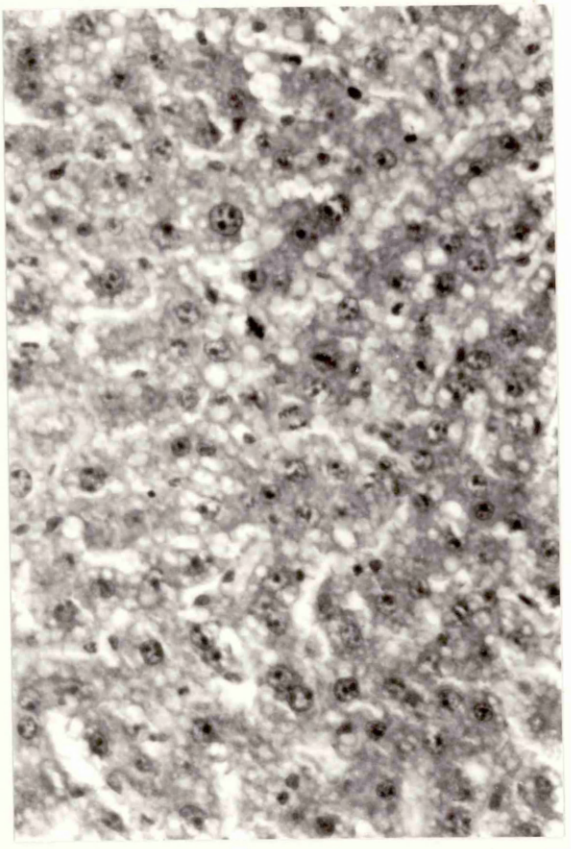


PLATE 6

PCT + A 36 hours after partial hepatectomy. Higher magnification to show mitotic figures - centre field.

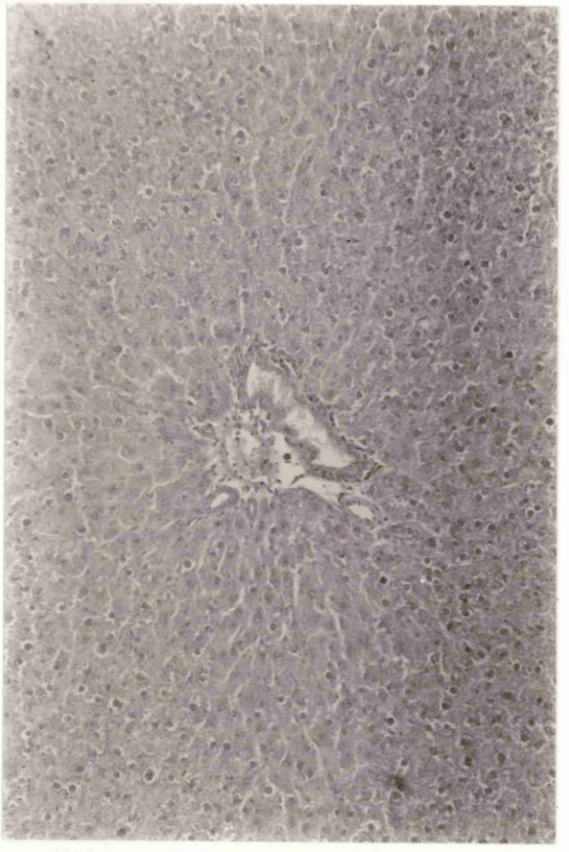


PLATE 7

PCT + A 3 weeks after partial hepatectomy. Portal triad centre field; double cell plates characteristic of the regenerated liver after partial hepatectomy.

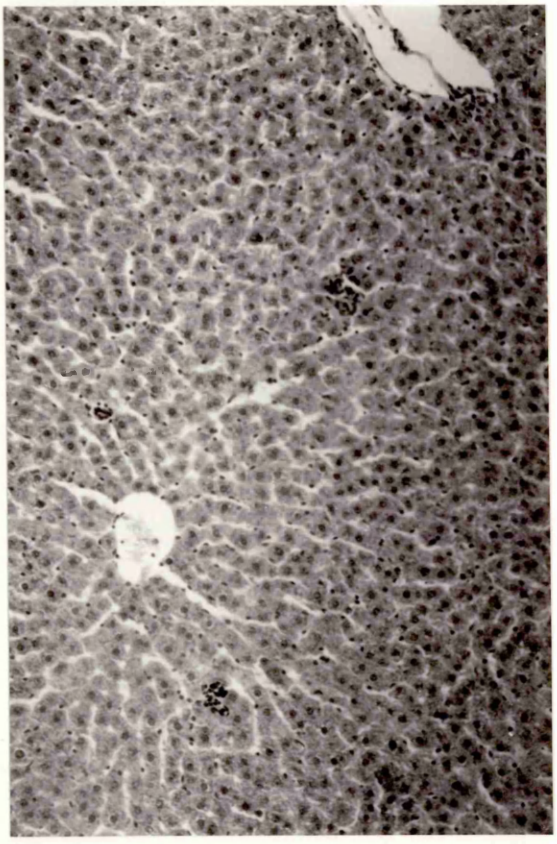


PLATE 8

Adrenalectomy control 72 hours after partial hepatectomy showing focal lymphocytic infiltration.

infiltration of lymphocytes was an ether control animal not subjected to partial hepatectomy.

LIVER REGENERATION

DISCUSSION

In this study I have examined the regeneration of the liver of the rat subjected to a partial hepatectomy carried out 3 weeks after portacaval transposition. The work confirms the depressed somatic growth rate after portacaval transposition compared to sham operated controls (Ryan et al 1974) and this is even more marked after partial hepatectomy in the portacavally transposed rat.

Liver weight has been shown to correlate closely with body weight and relative liver weight can therefore be used to compare similar groups of animals directly. PCT+A results not only in a reduction in total liver weight but also in a reduction in relative liver weight. It is also apparent that the anterior lobes bear the brunt of this atrophy, but to a degree, this may also be the case in sham portacaval transposition and may be related to the reflection of the anterior lobes onto the thorax during these procedures.

Partial hepatectomy was invariably more difficult in portacavally transposed rats than in sham operated controls. This was due to small bowel adhesions in the area of the anastomosis and to the undersurface of the liver. It was, however, always possible to ensure the patency of the anastomosis at the time of partial hepatectomy.

The histology of the regenerating liver of the portacavally transposed rat revealed some differences from the control animals. Particularly much more extensive fat infiltration of the liver,

which also persisted for a longer period. There appeared to be a slight delay in the appearance of mitotic figures, but this latter observation must be compared with the results of the D.N.A. specific activity.

Measurement of D.N.A. synthetic activity was carried out biochemically by utilising the uptake of ^{3}H thymidine given intravenously one hour before sacrifice. The activity related to the operative procedure can be measured by the specific activity in ether control rats and in rats subjected to a sham partial hepatectomy. It is interesting to note (Table V) that while the median results for these two groups did not differ significantly, the sham partial hepatectomy produced lower results and it may be that this procedure tended to inhibit the mitotic activity due to increased stress.

For all groups undergoing partial hepatectomy there was a peak of D.N.A. activity between 21 and 24 hours after partial hepatectomy. During the first 36 hours after partial hepatectomy there was no significant difference either in magnitude or timing between rats previously subjected to portacaval transposition and those subjected to control procedures. This is the period that has been associated with hepatocyte replication in the rat (Bucher et al 1967). At 48 hours the specific activity in portacavally transposed animals was significantly lower than both sham portacaval transposition and the adrenalectomy group, but at 72 hours it was significantly higher than sham portacaval transposition. Further measurements of groups killed before and after these time points would be nessesary to ensure that these values do not represent differing timings of a secondary peak.

Measurement of relative liver weight during the first 72 hours after partial hepatectomy shows that for the first 48 hours the experimental and control groups follow very similar patterns (Fig. 6). The fall at 12 hours may be associated with relative dehydration and also deglycogenation. The fall occurring in the portacaval transposition group at 72 hours, which is highly significant, is unexplained at present and will require further examination. However, if the final relative liver weight at 3 weeks after partial hepatectomy in portacavally transposed rats is compared with the expected relative liver weight 6 weeks after the initial operation of portacaval transposition, it does not differ significantly.

Partial hepatectomy carried out 3 weeks after portacaval transposition and left adrenalectomy in the rat results in a hyperplastic response of the hepatocytes which is similar in both timing and magnitude to controls. The relative liver weight 3 weeks after partial hepatectomy does not differ significantly from that which would be expected.

EXPERIMENTS

LIVER BLOOD FLOW

Experimental Design

A total of 25 animals were studied in four groups, three liver blood flow measurements were made in each animal at 5 minute intervals before transposition and thereafter three measurements were made at intervals from the median segment of the anterior lobe when present and from the right lateral segment of the of the posterior lobe of the liver.

Experiment 1

In 13 animals liver blood flow was measured pre-operatively and at one hour after transposition. Five of these animals then also had liver blood flow measurements 2, 3, 4 and 5 hours after portacaval transposition.

Experiment 2

In 4 animals, portacaval transposition was completed under ether anaesthesia and the animals allowed to recover. Liver blood flow measurements were carried out 24 hours after transposition.

Experiment 3

In 4 animals, portacaval transposition plus left adrenalectomy was carried out and the animals allowed to recover. Liver blood flow measurements were carried out 3 weeks after transposition.

Experiment 4

In 4 animals, portacaval transposition plus left adrenalectomy was performed and the animals allowed to recover. Three weeks post-operatively liver blood flow was measured, a partial hepatectomy performed and the liver blood flow measured at hourly intervals until 4 hours after partial hepatectomy.

Animals, Surgical Techniques, Materials and Methods

Statistical Methods: see page 39.

Animals

Male Sprague Dawley rats were used throughout. The rats were selected for a starting weight between 198 and 235 gms and housed and fed as described in the regeneration experiment. As each animal was starved for 16 hours before blood flow measurements the weight at the time of the procedure was more variable.

Surgical Methods

Portacaval transposition was carried out as described on page 44 with the addition of left adrenalectomy as required. Partial hepatectomy was carried out as described on page 46. Anaesthesia was achieved by open ether for portacaval transposition and partial hepatectomy, when there was not to be a sequential measurement of liver blood flow immediately after. Anaesthesia for liver blood flow measurements was induced using an inhalation of 4%

halothane in pure oxygen, and maintained by intravenous chloralose (70 mg/kg body weight).

Measurement of Liver Blood Flow (LBF)

A tracheostomy was performed through a vertical midline incision and the animal allowed to breath 100% oxygen in open circuit through a Y-piece. The secretions from the tube were aspirated frequently and the rat was "sighed" every 20 minutes. The right carotid artery was cannulated with a nylon catheter 1.02 mm in external diameter (Figs. 13,14). The nylon catheter was connected via a strain gauge pressure transducer to a two-channel pen recorder (Devices Ltd.) to monitor systemic blood pressure.

The abdomen was opened through a midline incision and the ligamentous attachments of the liver divided. The liver was delivered onto the thorax and covered with a moist saline soaked swab. The right gastric vein was cannnulated with a nylon cannula 0.63 mm in external diameter. The tip of this cannula was positioned at the entrance to the main portal vein so that it did not impair portal venous blood flow. This cannula was used to record portal pressure through a pressure transducer and also to inject ^{85}Krypton for flow measurements prior to portacaval transposition.

In rats being subjected to portacaval transposition an 0.63 mm external diameter nylon tube was also inserted into the right lumbar vein until its tip was in the inferior vena cava. Again, care was taken not to impede caval blood flow and this cannula was used to record inferior vena cava pressure prior to portacaval transposition and pre-hepatic "portal" pressure after portacaval transposition.

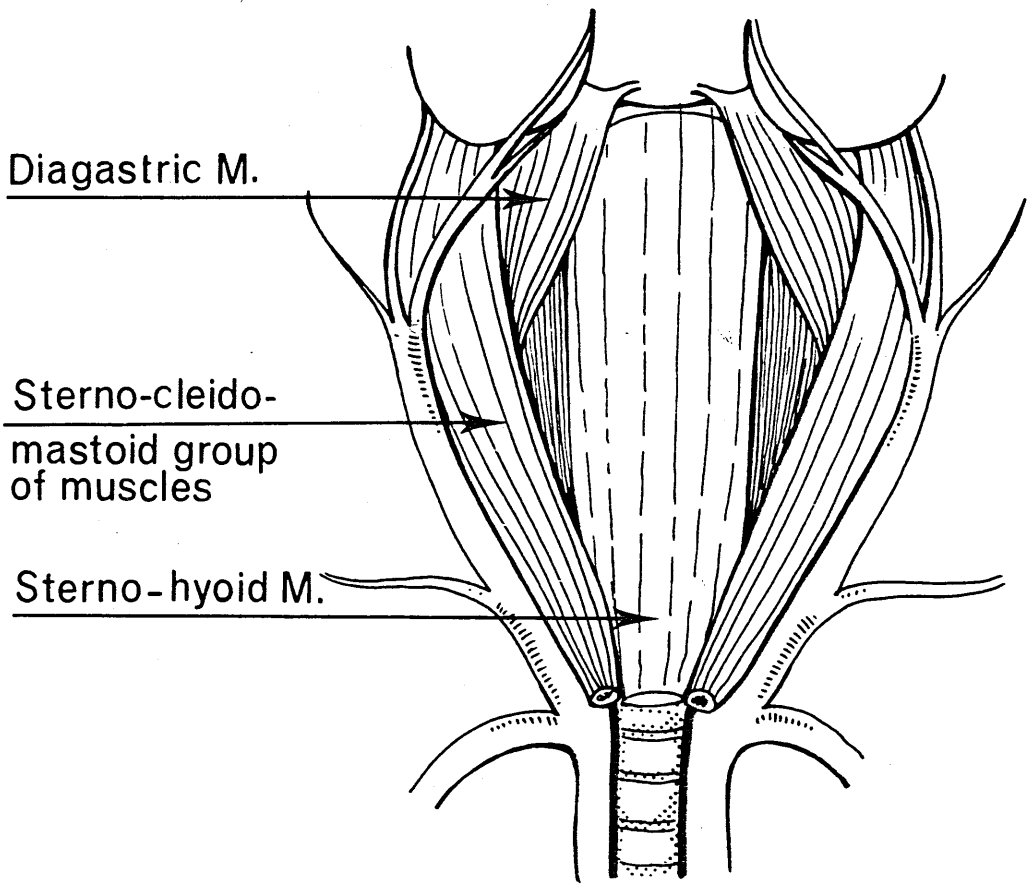


FIGURE 13

Anatomy of the neck I.

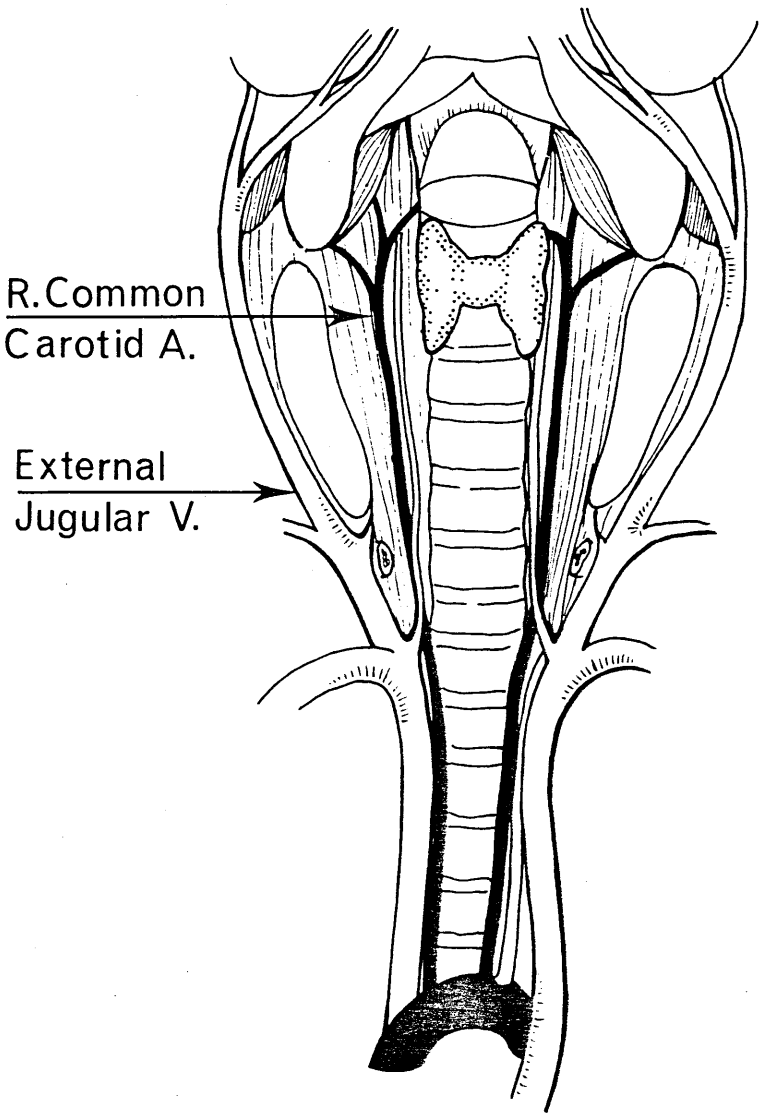


FIGURE 14

Anatomy of the neck II.

It was also used for the injection of ^{85}Krypton for flow measurements in portacavally transposed rats (Figs. 15,16).

All canulae were pre-filled with heparinised saline (325 IU/100 mls). Dissection and cannulation of vessels was performed using a Zeiss operating microscope.

After cannulation the liver was replaced in its normal position and its surface covered with a layer of 6 micron polyester film (Melanex, ICI Ltd.) to prevent desiccation of the superficial layers of the liver. The abdominal wound was then partially closed leaving a small window through which the liver surface was visible. Arterial pressure and pre-hepatic portal venous pressure were measured continuously. At intervals throughout the experiment PaO_2 and $PaCO_2$ were measured using a gas analyser (International Lab. Model 213), as was the packed cell volume. Rectal temperature was measured with a mercury thermometer and the temperature maintained at 37^{o}C by external heat lamps.

Theory of Inert Gas Clearance Method

The rate of clearance from an organ of a gas molecule in solution is proportional to the rate at which blood perfuses the tissue if the gas is not metabolised. ^{85}Krypton is a radioiosotope of Krypton gas emitting a beta radiation. It is chemically inert.

When a bolus of ^{85}Krypton solution is introduced into the blood supply of an organ, it diffuses very rapidly throughout the tissue. If no further ^{85}Krypton reaches the organ, the rate of disappearance of the gas is determined only by the blood flow through the tissue and by the relative solubility of ^{85}Krypton between the tissue and

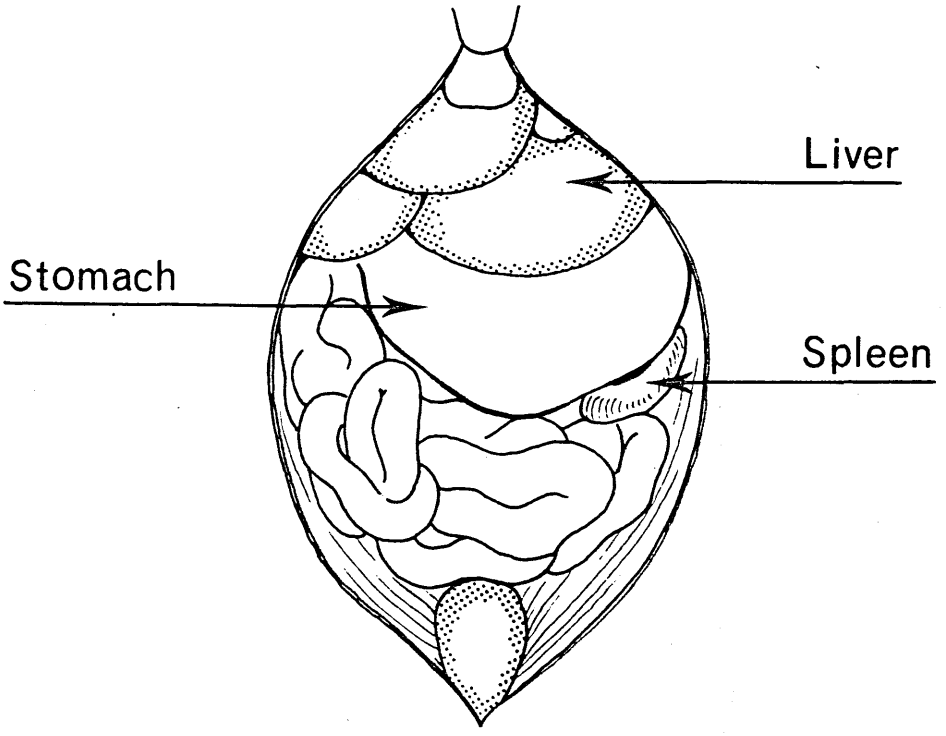


FIGURE 15

The contents of the abdomen exposed through a midline incision.

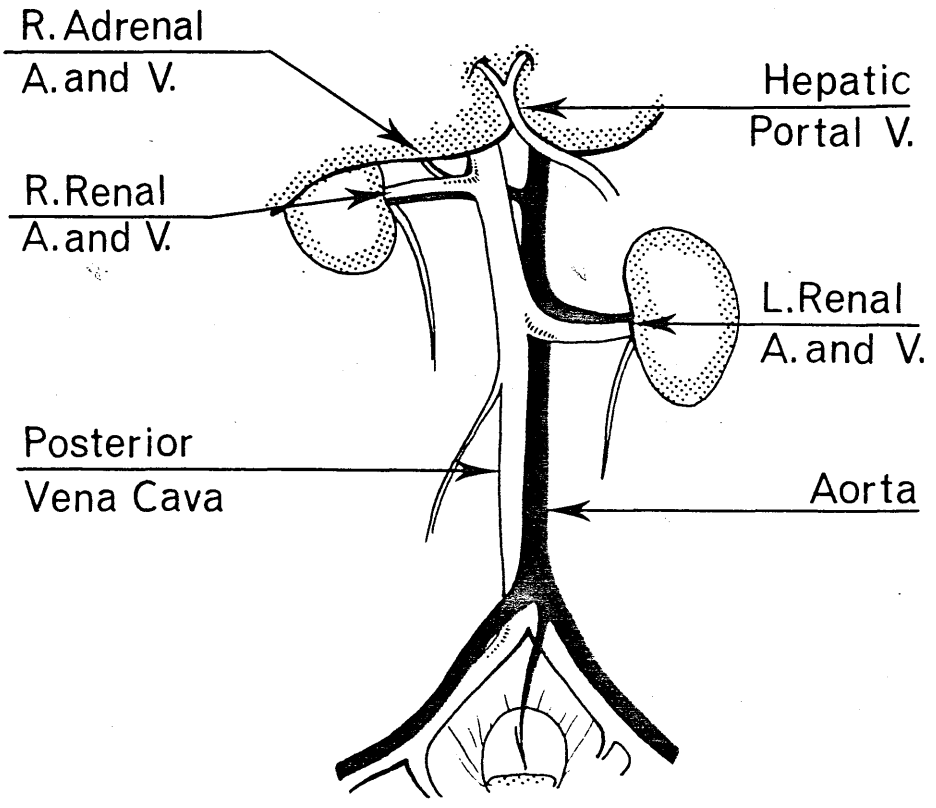


FIGURE 16

The vessels of the posterior abdominal wall.

the blood (the partition coefficient). Thus the clearance of ^{85}Krypton reflects the tissue perfusion and is unaffected by such factors as arteriovenous shunting which may modify the organ's total blood flow.

^{85}Krypton is markedly more soluble in air than in tissue or blood and is therefore almost completely cleared from the body in one passage through the lungs (Chidsey et al 1959). There is thus negligable recirculation of the isotope and repeated sequential measurements can be made in the same animal.

The beta emissions of ^{85}Krypton have an energy of 0.695 MeV and a mean range in tissue of 0.7 mm with a maximum of 2.6 mm (Glass et al 1962). A Geiger-Muller tube, which records only beta emissions, when positioned over the liver can therefore only record clearance of ^{85}Krypton from that organ. It is assumed that the perfusion is uniformly distributed throughout the liver and that the record obtained from the superficial layer represents the perfusion rate of the liver as a whole.

The clearance recorded by the Geiger-Muller tube is plotted on a semi-logarithmic arithmetic scale and results in a straight line from which can be calculated a value for T1/2. This value represents the time in seconds for the ^{85}Krypton activity to fall to half its peak value. Liver tissue blood flow in mls/gms/minute is calculated using the following formula:

$$LBF = \frac{\log_e 2 \times 60 \times \lambda}{T1/2}$$

where the variable λ denotes the partition coefficient. The

partition coefficient between liver and blood for the normal rat has been determined in our laboratory by Mathie et al (1977) to be 0.95.

Flow Recordings

In order to maintain fluid balance 2 mls of normal saline was given to each animal after each surgical procedure. An additional 2 mls was given before portacaval transposition and at hourly intervals thereafter. Aliquots of arterial blood (0.35 ml) were withdrawn at intervals throughout the experimental period for the estimation of PCV, PaO_2 and $PaCO_2$. An equal volume of normal saline was given after withdrawal of each blood sample.

For each measurement of liver blood flow a bolus of ^{85}Krypton, dissolved in saline, was injected rapidly into the portal system followed by a flush of saline bringing the total volume of fluid injected per flow estimation to 0.2 mls. Flow measurements were carried out by injection into the portal venous catheter before transposition. When this cannula had been removed following transposition, subsequent liver blood flow measurements were carried out via the lumbar vein below the site on the inferior vena cava portal anastomosis. Three estimations were made in each hour. Estimations were separated by about 5 minutes.

The dose of ^{85}Krypton necessary to produce a peak count of approximately 400-500 counts/second varied from animal to animal. On average approximately 0.8 mCi were required. For accurate results, peak levels recorded from the liver had to be greater than 150 counts/second.

Prior to partial hepatectomy measurements were made over the medial segment of the anterior lobe and over the right lateral segment of the posterior lobe. These measurements were carried out sequentially. After partial hepatectomy measurements were made over the right lateral segment of the posterior lobe only.

A small lead-sheilded Geiger-Muller tube with an end window 9 mm in diameter (20th Century Electronics Ltd., Type MB4H) was mounted in a specially designed assembly platform which allowed the tube to be positioned accurately within 5 mm of the liver surface. Care was taken during each flow estimation to ensure that there was no contact between the tube and the liver surface as this could have affected the local tissue perfusion rate. Previous work (Rice et al 1977) had shown that the Melanex covering produced a biexponential clearance when left in situ. It was therefore removed before each flow measurement and replaced immediately afterwards.

The Geiger-Muller tube was connected to a ratemeter (Nuclear Enterprizes 7070) and the ratemeter output fed to a pen chart recorder (Smiths RE511.20).

The clearance curve inscribed by the pen recorder after a bolus injection was replotted manually on semi-logarithmic paper and the T1/2 measured from a straight line graphical plot. Blood flow in mls/gms/minute was then calculated.

LIVER BLOOD FLOW

RESULTS

Gas Tensions and Pressures

All animals tolerated the procedures well. During liver blood flow measurements, body temperature was maintained at 37°C and PCV at a mean of 38%.

Blood gases remained within normal limits throughout each experiment with a tendency for $PaCO_2$ to fall following PCT (40 mmHg to 35 mmHg) and following partial hepatectomy (41 mmHg to 35 mmHg). PaO_2 remained above 100 mmHg throughout each experiment.

The mean arterial blood pressure before portacaval transposition was 116 ± 22 mmHg falling to 78.2 ± 15 mmHg by 5 hours after operation (Table VIII). Blood pressure at 24 hours and 3 weeks later did not significantly differ from pre-transposition values (paired t-test).

Mean portal pressure before transposition was 3.92 ± 1.53 mmHg. Following portacaval transposition, the IVC pressure did not differ significantly from portal venous pressure (paired t-test)(Table IX).

Experiment 1

Table X shows the mean liver blood flow before portacaval transposition and at 1, 2, 3, 4 and 5 hours after transposition. Liver blood flow before PCT in 13 rats was 3.48 ± 0.68 mls/gm/min. in the anterior lobe and 4.38 ± 0.75 mls/gm/min. in the posterior lobe. Although there was a variation in liver blood flow between rats, the coefficient of variation (CV) remained below 11% for all

TABLE VIII

MEAN ARTERIAL BP BEFORE AND AFTER PCT

	Pre-PCT	Post-PCT					
		1h	2h	3h	4h	5h	24h
BP	116 ± 22	105 ± 14	91.6 ± 6.15	86.8 ± 7.1	73.4 ± 17.3	78.2 ± 15.4	95.5 ± 14.6
(n)	(13)	(13)	(5)	(5)	(5)	(8)	(4)
p vs. Pre-PCT		$0.05 > p > 0.001$	$0.01 > p > 0.001$ F +ve	$p < 0.001$	$p < 0.001$	$p < 0.001$	$0.05 > p > 0.02$

TABLE IX

PRE-HEPATIC VENOUS BLOOD PRESSURE (mean ± S.D. in mm of Hg (n))

Pre-PCT (Portal)	IVC Pressure Post-PCT					
	1h	2h	3h	4h	5h	24h
3.92 ± 1.53	4.66 ± 1.47	5.07 ± 1.02	4.97 ± 1.20	4.77 ± 1.45	5.02 ± 1.46	4.20 ± 1.25
(13)	(13)	(5)	(5)	(5)	(5)	(4)

No significant difference between any time point

TABLE X

LIVER BLOOD FLOW BEFORE AND AFTER PCT

	Pre-PCT	Post-PCT					
		1h	2h	3h	4h	5h	24h
Ant. lobe	3.48 ± 0.68	3.68 ± 1.10	4.01 ± 1.10	4.79 ± 0.87*	4.16 ± 0.93	4.80 ± 1.0+	4.50 ±
p Ant. v Post.	0.01 p 0.001	NS	NS	NS	NS	NS	NS
Post. lobe	4.38 ± 0.75	4.39 ± 1.15	4.45 ± 0.83	4.82 ± 0.88	4.03 ± 0.67	4.38 ± 1.22	5.78 ±
(n)	(13)	(13)	(5)	(5)	(5)	(5)	(4)

*Compared with preop. values $0.01 > p > 0.001$

\+ " " " " $0.02 > p > 0.01$

time points. Liver blood flow was significantly greater in the posterior lobe than in the anterior lobe ($p<0.001$)(paired t-test). After portacaval transposition the liver blood flow increased in the anterior lobes reaching significance at 3 hours and abolishing the significant anterior/posterior difference. There was no significant increase in flow in the posterior lobe.

Experiment 2

Four animals were subjected to portacaval transposition and allowed to recover. Liver blood flow at 24 hours was significantly higher than the pre-operative values seen in Experiment 1 above ($p<0.001$)(t-test). The anterior/posterior differential in flow was maintained, though again there was no significant difference between the flows (Table X).

Experiment 3

Four animals were subjected to portacaval transposition plus left adrenalectomy and allowed to recover. Liver blood flow measurements were carried out 3 weeks later and the results are shown in Table XI. Basal flows were compared with those in the normal rat (from Experiment 1).

Greater variation in the anterior lobe flow was apparent 3 weeks after portacaval transposition plus left adrenalectomy (CV 12.34%). There was, however, no significant difference in liver blood flow 3 weeks after portacaval transposition when compared to normal rats (t-test).

TABLE XI

LIVER BLOOD FLOW IN ML/GM/MIN 3 WEEKS AFTER PCT + A

	Pre-PCT + A	3 weeks after PCT + A
Anterior Lobe	3.48 ± 0.67	3.10 ± 0.42
Posterior Lobe	4.38 ± 0.75	4.05 ± 0.43
(n)	(13)	(4)

No significant difference in blood flow through same lobe

Experiment 4

Portacaval transposition plus left adrenalectomy was performed and the rats allowed to recover. Three weeks later liver blood flow was measured immediately before partial hepatectomy and at 1, 2, 3 and 4 hours after partial hepatectomy (Table XII). During the first 3 hours after partial hepatectomy, there was no significant change in liver blood flow. At 4 hours after partial hepatectomy there was a small but significant increase in flow (paired t-test).

During the same period the mean arterial blood pressure fell from a pre-operative value of 124.5 $\pm$ 8.7 mmHg to 52.8 $\pm$ 3.5 mmHg. This is a significant fall ($0.01>p>0.001$) (Table XIII). However, the pre-hepatic portal venous pressure was maintained at a constant value (Table XIV).

Liver blood flow is normal 3 weeks after portacaval transposition plus left adrenalectomy. It does not alter significantly in the first 3 hours after partial hepatectomy carried out at this time, but there in a 17% increase in flow at 4 hours.

TABLE XII

LIVER BLOOD FLOW (ML/GM/MIN) BEFORE & AFTER PH IN RATS SUBJECTED TO PCT + A 3 WEEKS PREVIOUSLY

	PCT + A	Hours After Partial Hepatectomy			
		1	2	3	4
Anterior Lobe	3.10 ± 0.38[+]	-	-	-	-
Posterior Lobe	4.05 ± 0.38	3.58 ± 0.42	3.58 ± 0.47	4.33 ± 0.35	4.74 ± 0.42*
(n)	(4)	(4)	(4)	(4)	(4)

*Significant increase compared to pre-hepatectomy value $0.05 > p > 0.02$ (Paired t-test)

[+]Significant difference between anterior and posterior lobe $0.01 > p > 0.001$ (Paired t-test)

TABLE XIII

ARTERIAL BP (MEAN ± S.D. IN MM HG) BEFORE AND AFTER PH IN RATS SUBJECTED TO PCT + A 3 WEEKS PREVIOUSLY (n)

PCT + A	Hours After Partial Hepatectomy			
	1	2	3	4
124.5 ± 8.7	109.5 ± 5.1^{+}	86.0 ± 11.2*	60.8 ± 11.6*	52.8 ± 13.5*
(4)	(4)	(4)	(4)	(4)

(Paired t-test pre.op. value)

$^{+}0.02 > p > 0.01$

$*0.01 > p > 0.001$

TABLE XIV

PRE-HEPATIC VENOUS PRESSURE (MEAN ± S.D. IN MM HG) BEFORE AND AFTER PH IN RATS SUBJECTED TO PCT + A 3 WEEKS PREVIOUSLY

Pre-Hepatectomy	Hours after Partial Hepatectomy			
PCT + A	1	2	3	4
4.77 ± 0.24	4.74 ± 0.20	4.69 ± 0.23	4.78 ± 0.26	4.69 ± 0.23
(4)	(4)	(4)	(4)	(4)

LIVER BLOOD FLOW

DISCUSSION

Portacaval transposition maintains liver blood flow as has been shown in the dog by Starzl et al (1962) and Heer et al (1960). It has also been noted that the venous component is increased by up to 50% and the arterial component reduced by 30% (Kreuzer and Shenk 1971). In this study we have measured liver blood flow in the rat by the clearance of the inert radioactive gas ^{85}Krypton. This measures tissue perfusion and takes no account of internal shunting. We have shown that liver blood flow immediately after, and at 3 weeks after, portacaval transposition in the rat does not differ significantly from controls.

Our values for liver blood flow are very similar to those found by Rice et al (1977a) but are considerably higher than those described by Benacerraf et al (1957) and, Rabinovici and Weiner (1963). These later groups used radioactive labelled colloids dependent on efficiency of clearance to measure liver blood flow. However, both groups did demonstrate the substantial rise in liver blood flow after partial hepatectomy in normal rats.

In our experiments, during the prolonged measurement of liver blood flow for up to 5 hours after portacaval transposition, although the systemic blood pressure fell significantly, the pre-hepatic inferior vena cava pressure was maintained, as was the liver blood flow

Liver blood flow represents 20-25% of cardiac output in the rat

(Rice et al 1977b; Mathie 1978). Measurements of liver blood flow 24 hours after portacaval transposition, during which time the animal had recovered from the initial operation, showed no significant change in liver blood flow.

Hepatic tissue perfusion 3 weeks after portacaval transposition was also shown not to differ significantly from normal control animals through each lobe measured. However, there is a higher perfusion in the posterior lobes initially which ceases to be significantly different from that through the anterior lobes by 3 weeks after portacaval transposition.

Again the prolonged measurements of liver blood flow after partial hepatectomy in the animals 3 weeks after portacaval transposition resulted in a significant fall in blood pressure, but again there was preservation of pre-hepatic inferior vena cava pressure and the liver blood flow through the remaining posterior lobe was maintained.

The immediate and dramatic rise in tissue perfusion seen after partial hepatectomy in the normal rat (Benacerraf et al 1957; Rabinovici and Weiner 1963; Rice et al 1977b) does not appear to occur after partial hepatectomy in the portacavally transposed rat (Table XII). These results must, however, be treated with some caution as the systemic blood pressure falls after partial hepatectomy in these animals, but it is unlikely that this would conceal the 300% increase in tissue perfusion which was noted in the normal rat by Rice and his colleagues.

Partial hepatectomy carried out 3 weeks after portacaval transposition plus left adrenalectomy in the rat results in a

hyperplastic response of the hepatocyte which is similar in both timing and magnitude to controls. The relative liver weight 3 weeks after partial hepatectomy does not differ from that expected. This response occurs despite the porta-prival state of the liver and without the dramatic rise in tissue perfusion seen in control animals.

CONCLUSIONS

"When one talks about hepatotrophic factors one might perhaps consider all substances that cause liver cells to grow, and all the conditions under which they grow. Somatic growth is a factor, because it is the sloping baseline against which all other experiments are done, and if we forget the sloping baseline we may observe a change which may be interpreted as an added growth response." (Weinbren 1978)

The work presented here shows that in the absence of direct portal perfusion by effluent from the intestine and in the absence of a rise in liver blood flow normally associated with partial hepatectomy, there is still a response of regeneration to partial hepatectomy. The expression of this response is modified by the atrophy consequent on portacaval transposition, which may result from altered interaction between humoral agents, liver blood flow, and the liver cell (a negative "sloping baseline").

Mann's theory (1940) of the effect of liver blood flow *per se* as the cause of liver regeneration has been shown to be invalid by subsequent work looking at the mitotic activity. However, before this technique was available, Child et al (1953) demonstrated that regeneration after partial hepatectomy could be shown both radiologically and by weight of regenerating liver in portacavally transposed dogs when compared to portacavally shunted dogs. Weinbren (1955) convincingly showed that regeneration occurred even after portal vein ligation in rats.

Lee's paper (Lee et al 1974) repeated in the rat the work of both Child and Mann, comparing portacaval shunt and portacaval transposition. They demonstrated a significant reduction (of 25-50% of the parameters measured) in liver regeneration after portacaval transposition and shunt compared to controls, although they described this as a "vigorous liver regeneration". Lee et al concluded that the hepatotrophic portal blood factors reached the liver by recirculation and that the magnitude of hepatic blood flow (unmeasured) is not a major determinent of liver regeneration.

The work carried out in the study presented in this thesis was designed to re-examine Lee's theory in the same model, but under properly controlled circumstances.

First a decision was made to remove the left adrenal both in the experimental and control groups. This adrenal, after portacaval transposition, would otherwise drain into the hepatic bed. Lee makes no mention in the paper of the left adrenal or any effects it may have had on liver regeneration, and I have already described papers which show that both cortisol and adrenaline suppress mitotic activity.

Experiments reported in this thesis have demonstrated the atrophy consequent upon portacaval transposition, which was not allowed for by Lee et al, and the regeneration which occurs subsequent to partial hepatectomy has been exposed.

Further, Lee et al, looked for mitotic activity in the liver at the wrong time point (7 days) after partial hepatectomy and this may have reinforced their wrong conclusions. Experiments described in this thesis were designed to look at the known mitotic peaks which

occur in the period 18-36 hours after partial hepatectomy with additional time points bracketing the major peak at about 20 hours. They demonstrate that partial hepatectomy in portacavally transposed rats produces a normal regenerative response.

In addition, the liver tissue perfusion in the rat after portacaval transposition was measured and this was shown to be within the normal range. By measuring tissue perfusion immediately after partial hepatectomy, it has been demonstrated that regeneration occurs without the 300% increase in liver blood flow which has been shown to occur in the normal rat subjected to partial hepatectomy (Benacerraf et al 1957; Rabinovici and Weiner 1963a and b; Rice et al 1977). This also adds a further nail to the coffin of Mann's blood flow theory.

These results support the hypothesis which is the basis of this thesis; that liver regeneration is independant of both liver blood flow and of portal blood or any portal blood factor. However, the question of a specific "chalone" remains open, and the importance of further research into hepatotrophic factors is emphasised below.

Liver resection in man is carried out for the control of bleeding after trauma and to remove localised tumours. The limiting factor is the amount of liver required to sustain life immediately after the operation and experience has shown that two-thirds of the liver may be resected. However, patients in fulminant hepatic failure, as a result of resection or more commonly massive necrosis secondary to viral or toxic damage, require artificial support by dialysis or haemoperfusion to prevent coma and death, untill regeneration can take place. In these cases liver regeneration may

be inhibited by toxins or an unfavourable hormonal balance, and indeed the very use of "artificial livers" may inhibit regeneration in the patient. The isolation of factors promoting or inhibiting liver regeneration would revolutionise the management in these patients.

The debate on regeneration of the mammalian liver centres around the question of whether there is a single factor affecting control or whether control is a result of the interaction between many humoral agents and the liver cell.

Subsequent work by Bucher and Swaffield (1973) has demonstrated that liver regeneration will take place in the abdominally eviserated rat. Whitmore et al (1975) have confirmed this finding but suggest that the delay in regeneration was due to one specific missing agent, viz. glucagon, and that the replacement of this hormone resulted in normal timing. In a later paper (Whitmore et al 1976) they also noted that if the portal vein was arterialised after eviseration, then D.N.A. activity as a result of partial hepatectomy was increased, as was the sensitivity to glucagon.

Chandler (1976) used the rabbit to examine selective blood flow from the pancreatic and duodenal area to the liver. He concludes that the insulin-containing pancreaticoduodenal component of portal blood is not adequate by itself to prevent liver atrophy induced by portacaval shunt. This work gives no evidence of tissue perfusion by the various components of portal blood and unfortunately it was based on liver weight and D.N.A. activity at 3, 7 and 14 days only. Chandler goes on to suggest that the hepatotrophic activity is in the non-pancreatic, non-duodenal blood. Duguay and Orloff in two

papers (1976 and 1977), using the dog as a model, suggest that after partial hepatectomy the pancreas is the source of a "hepatotrophic portal blood factor", and that pancreatectomy abolishes the regenerative response to partial hepatectomy. Insulin partially restored the D.N.A. activity but insulin and glucagon together restored the peak with a 24 hour delay.

In a series of experiments in the dog Starzl et al (1978a and b) examined the effect of eviseration on the liver and on liver regeneration. They found that eviseration produced an effect on the liver similar to that produced by portacaval shunting. These changes could be reversed by intra-portal insulin but not by glucagon or epithelial growth factor (E.G.F.). After partial hepatectomy in the totally eviserated animal regeneration was essentially halted. This was not reversed by insulin or glucagon. However, if only partial eviseration was carried out (pancreatectomy or non-pancreatic eviseration) regeneration was present but decreased. The dog may be more sensitive to deprivation of portal blood than the rat.

Specific hormone replacement has been widely used as a test of hepatotrophic activity (vide supra). Insulin is one of the principal anabolic hormones and has been frequently suggested as a hepatotrophic hormone. It is secreted into the portal circulation and there may be a self-regulating release factor other than glucose (McIntyre 1978). It would be expected that insulin would produce an increase in the mitotic activity in the normal rat, and not just in the liver of the diabetic rat (Steiner et al 1978). The metabolic impact of variations in insulin levels will also be modified by the ability of the liver to increase or decrease insulin-binding sites

(Fraychet 1978). Starzl has previously shown that infusion of intra-portal insulin protects the dog liver from the effects of portacaval shunt (Starzl et al 1975).

Morley et al (1975) showed that glucagon rose eleven-fold in peripheral blood, but insulin remained unchanged after partial hepatectomy in the rat. Bucher (1976) examined portal blood and found insulin levels fell while glucagon rose. These two results are not at variance as after partial hepatectomy less insulin would be extracted from the portal blood which normally has a higher level than the level in peripheral blood. Bloom (1978) has shown that glucagon is elevated in patients with hepatic cirrhosis and he has also noted no apparent effect on the liver in patients with glucagonomas, nor in patients with no glucagon measurable in their blood after pancreatectomy.

Bucher (1976) showed that insulin and glucagon stimulate regeneration after partial hepatectomy in the rat. She was unable to initiate regeneration with either hormone alone. Starzl et al (1976) found that in the dog insulin exerted a protective effect against the atrophy of portacaval shunt but this effect was not observed with glucagon, nor did glucagon potentiate the insulin effect.

The effect produced by insulin in the prevention of atrophy due to portal vein ligation has been shown by Ozawa et al (1974) to be due to the restoration of the phosphorylation activity of the mitochondria in the liver cell. This phosphorylation activity is reduced by about 50% after ligation. The restoration results in a normal production of ATP and thus of energy availability to the cell. Glucagon does not have this effect when used on its own.

Bucher et al (1978) have shown that the intra-peritoneal infusion of epidermal growth factor (E.G.F.) into rats produced a peak of D.N.A. activity after 12 hours lasting untill 20 hours. This was augmented by glucagon and insulin to a figure 10 times normal. (This can be compared with the 5-fold to 8-fold increase seen in the first cross-circulation experiments against partially hepatectomised rats.) E.G.F. did not alter the rate of D.N.A. synthesis in rats after partial hepatectomy. The stimulation by E.G.F. may be along the same pathway as the stimulation of regeneration after resection.

Starzl et al (1979) have recently produced an extract from regenerating liver at various time points. They suggest that the 48 hour and 72 hour extracts can protect portacaval shunt livers in the way that they have already shown with insulin. These extracts, however, contain no insulin or glucagon. They suggest a growth stimulating factor present in the regenerating liver, which is heat stable and consists of soluble proteins and other cytoplasmic constituents. The authors point out that this agent may have little to do with initiation of liver regeneration as it can only be isolated from liver 48-72 hours after regeneration has been initiated and this may in fact just be a nutrient-rich solution.

Demetriou and Levenson (1977) using normal rat hepatocyte cultures showed that culture medium from hepatocytes taken after partial hepatectomy had a stimulating effect on the normal hepatocyte as shown by the uptake of tritiated thymidine. This effect was not seen in culture medium from sham hepatectomised rats. The paper unfortunately gives no indication of how soon after partial hepatectomy the cells were harvested for culture.

Clofibrate, a drug used to lower serum triglyceride levels in man, has a known side-effect of producing non-fatty liver hypertrophy. Seifter et al (1977) has shown that this drug not only stimulates increased size of the liver in the normal rat, but also increases the size of liver after 30% partial hepatectomy at 5 days. This produced normal water, protein and fat ratios but a disproportionate increase in D.N.A. quantity.

The use of in vivo models has not materially advanced the answer to the question "What initiates liver regeneration?". In vitro studies with cell cultures start with a paradoxical problem that they must have a replicative process initially to establish the cell culture. They must then expose that culture to a new environment and observe any further replicative activity. It has been found that foetal liver cells and cells from various time points after partial hepatectomy are best for liver cell culture.

Richman et al (1976) have grown adult liver cells harvested 18-24 hours after partial hepatectomy in culture, and exposed them to insulin, E.G.F., insulin + E.G.F. and insulin + E.G.F. + glucagon. It should be noted that these cells are already at the stage of replication subsequent on partial hepatectomy and that these hormones are therefore only permitting continuation of a process already started in the animal. This process appears to be aborted in the control culture group. Richman and his colleagues found that insulin and epidermal growth factor alone caused increases in the labelling index of these cultures. Insulin, epidermal growth factor and glucagon all act synergistically to produce higher labelling indices.

Leffert and Weinstein (1976) used foetal rat liver cells and showed that very low density lipoprotein (V.L.D.L.) inhibited mitotic activity in the cells in culture. They also noted that in a mutant "fatty" rat with elevated V.L.D.L. levels, two-thirds partial hepatectomy showed a reduced mitotic rate which was inversely proportional to the elevation in the V.L.D.L.. V.L.D.L. fits the chalone theory as a material produced by the liver and metabolised by the rest of the body. It may then return to the liver and inhibit replicative activity.

Although it is now nearly 100 years since a good pathological description of liver regeneration by Emil Ponfic in Germany, we would appear no closer to the answer to the simplistic question "What stimulates liver regeneration?". Perhaps the question has been refined in too much detail, no longer do we ask what causes regeneration and accept the answer that was put forward by Mann in 1940 that portal blood per se caused liver regeneration, because we now know of the various important constituents of portal blood. The suggestion by Bucher that it was some humoral agent which controlled liver regeneration initiated the research into sources of this agent, and this has come down in two general areas.

Firstly, a splanchnic source and as time has passed increasing numbers of hormones have been isolated from the splanchnic bed, almost all of which were found to have some significant effect on liver regeneration but almost invariably only a permissive effect. These include insulin, glucagon, epidermal growth factor, non-pancreatic glucagon and others.

With the concept of a chalone theory supported in the main by

Bullough and his epidermal chalone, research has proceeded to define a liver chalone. Verily (1973) suggested that he had found such a chalone in the rabbit but his work has not been confirmed. The suggestion by Leffert and Weinstein (1976) that V.L.D.L. could be the liver chalone has yet to run the test of time. V.L.D.L. would certainly fulfill the requirements for a chalone but the finding that rats with an elevated V.L.D.L. have inhibited replication after partial hepatectomy may reflect more on the metabolic abnormality causing the V.L.D.L. elevation than on the fact that V.L.D.L. is in itself an inhibitor of regeneration in vivo.

"So while there is as yet no clear understanding of the factors controlling liver regeneration, it seems likely to be a multi-factorial process with hormone changes playing a major part." This statement in a leading article in The British Medical Journal (Understanding Hepatic Regeneration, 1981) supports the multi-factorial hormone control of liver regeneration. However, in the same paragraph it goes on "Purification of the hepatic regenerative stimulator substance ....." leaving the door wide open for a continuing search for the elusive Hepatotrophic Factor.

SUMMARY

The mammalian liver is capable of replacing cells lost by tissue damage or by resection, by a process of hypertrophy and replication which has become known as liver regeneration. This process was well described by Ponfic (1895) and has subsequently been studied and the control mechanisms examined.

In 1940 Mann et al suggested that portal blood *per se* was the stimulus to liver regeneration, which in itself was a fortuitous effect of replacement of the venous outflow bed. This was based on the examination of liver weight in dogs bearing a portacaval shunt subjected to a partial hepatectomy. Mann overlooked the atrophy which resulted from the portacaval shunt. Later work (Bucher 1951; Islami 1959; Christensen and Jacobsen 1949) suggested that there was a circulating humoral agent which was responsible for the initiation of liver regeneration.

Portacaval Transposition was first used experimentally in the dog (Child et al 1953). Child and his colleagues showed that liver regeneration did occur in the transposed dog after partial hepatectomy, although not to the same extent as in normal dogs. By comparing regeneration after partial hepatectomy in the transposed dog to that occurring in normal dogs, they obscured the alterations which were due to the transposition alone. They were, however, able to conclude that portal blood perfusing the liver was not an essential pre-requisite for regeneration after partial hepatectomy, as the portacaval transposition model created liver deprived of portal blood which was replaced with a systemic venous inflow.

In 1974 Lee et al carried out the same experiment in the rat. Partial hepatectomy was carried out 3 days after portacaval transposition and the liver studied at 7 days after partial hepatectomy. They found that there was no difference in the regenerative activity between portacaval transposition and portacaval shunt animals and showed that this response was deminished when compared to controls. They concluded that the magnitude of blood flow is not an important factor in liver regeneration, but that an hepatic portal blood factor is "necessary for liver regeneration" which reaches these livers by recirculation.

In the light of other experiments, this paper does not stand scrutiny. Liver regeneration is most active in the first 36 hours after partial hepatectomy in the rat (Bucher 1963, 1967) and is virtually complete by 7 days so that comparison of mitosis and D.N.A. activity at this time point is meaningless. Comparison of liver weight must be seen against the background of the effect of the initial procedure (portacaval transposition or shunt), both of which cause liver atrophy (Weinbren 1955; Ryan et al 1974) and dramatic alterations in body weight.

One further criticism the animal preparation used by Lee et al is that after portacaval transposition, the venous outflow from the left adrenal drained directly into the pre-anastomotic vena cava. Thus the hormone rich venous effluent drained directly into the hepatic portal bed. Both adrenocorticosteriods and adrenaline inhibit mitotic activity.

In the study presented in this thesis, portacaval transposition was carried out with in addition left adrenalectomy in the rat.

Control operations included sham portacaval transposition with left adrenalectomy, left adrenalectomy alone and ether anaesthetic controls. Each animal was allowed to recover and was in good health 3 weeks later, by which time they had again reached a stable state. At that time a partial hepatectomy was performed and liver regeneration monitored by measurement of liver weight and D.N.A. synthetic activity at multiple time points for the first 72 hours. Final liver weights were examined 3 weeks after partial hepatectomy and those values compared to a separate group of animals which were also a total of 6 weeks post-transposition or sham operation, and thus represented true controls for each procedure. We have shown from previous work (Ryan et al 1974) that the operation of portacaval transposition produces profound changes in the rat growth curve and also in relative liver weights.

The work presented confirms the depressed growth rate after portacaval transposition compared to sham operated controls and this is even more marked after partial hepatectomy in the portacavally transposed rat. A reduction in relative liver weight is also confirmed.

Hepatic tissue perfusion 3 weeks after portacaval transposition was shown to be not significantly different to controls. However, the 300% increase in liver blood flow seen after partial hepatectomy in the normal rat (Rice et al 1977) does not appear to occur after partial hepatectomy in the portacavally transposed rat.

The histology of the regenerating liver of the portacavally transposed rat revealed some differences from the control animals. Particularly, much more extensive fat infiltration of the liver which also persisted for a longer period.

For all groups undergoing partial hepatectomy there was a peak of D.N.A. activity 21-24 hours after partial hepatectomy. During the first 36 hours after partial hepatectomy there was no significant difference either in magnitude or timing between rats previously subjected to portacaval transposition and those subjected to control procedures. This is the period which has been associated with hepatocyte replication in the rat (Bucher et al 1967).

The alteration in relative liver weight after partial hepatectomy in the portacavally transposed animal was similar to controls during the first 48 hours, but was significantly lower at 72 hours. However, if the final relative liver weight at 3 weeks after partial hepatectomy in the portacavally transposed rat is compared with the expected relative liver weight 6 weeks after portacaval transposition, it does not differ significantly.

Partial hepatectomy carried out 3 weeks after portacaval transposition plus left adrenalectomy in the rat results in a hyperplastic response of the hepatocyte which is similar in both timing and magnitude to controls. The relative liver weight 3 weeks after partial hepatectomy does not differ significantly from that expected. This response occurs despite the porta-prival state of the liver and without the dramatic rise in tissue perfusion seen in control animals.

Any specific initiator of liver regeneration would, of course, reach the liver via the systemic circulation. If such a factor does exist, it is unaffected by passage through the general circulation.

Specific portal hepatotrophic factors may exist and under normal circumstances may maintain the liver cell size and stability. Portal blood *per se* is not required to perfuse directly the hepatic portal bed for adequate regeneration on liver to occur.

STATISTICAL APPENDIX

Definitions

Number of samples = n

Value of sample = x

Mean $\bar{x} = \frac{\Sigma x}{n}$

Standard deviation $\sigma = \sqrt{\frac{\Sigma x^2 - n\bar{x}^2}{n-1}}$

Student's t-test $t = \frac{\bar{x} - \bar{y}}{\sqrt{\frac{\sigma x^2}{nx} + \frac{\sigma y^2}{ny}}}$

Fisher's F test $F = \frac{\sigma x^2}{\sigma y^2}$ (when $\sigma x > \sigma y$)

Median - The point at which there are equal numbers of larger and smaller numbers.

Mann-Whitney Rank test - Significance test for a difference of medians.

Procedure - Samples are ranked from smallest to largest and totalled (Rx)

$Rx + Ry = \frac{1}{2} (nx + ny) (nx + ny + 1)$

$= \frac{1}{2} N (N + 1)$ where $N = nx + ny$

Wilcoxon's U statistic

$Ux = nx\ ny + \frac{1}{2} ny (ny + 1) - Ry$

$Uy = nx\ ny + \frac{1}{2} nx (nx + 1) - Rx$

(N.B. $Ux + Uy = nx\ ny$)

Final t and U result were read against standard statistical tables.

Programmes for the calculation of Student's 't' incorporating Fisher's F test, and for Mann-Whitney Rank test utilising Wilcoxon's 'U' statistic were written by the author for use on a Commodore'Pet' 2001 computer.

(Ref. Statistics for Biologists by R.C. Campbell, Cambridge University Press)

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