The Ripple Effect of Liver Transplantation

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DURING the last 2 decades there has been a revolution in the understanding of hepatic pathophysiology and in the treatment of liver disease. Liver transplantation per se has been a major factor in this broad movement, particularly as increasing numbers of chronic survivors have inched out to, and now beyond, the first decade of posttransplantation life. The dangers of liver transplantation are moving into the acceptable range for the first time. This year we have performed six liver replacements. All six recipients are well. Well over 50% of last year's liver recipients are well. The ancillary research permitted by these patients has been fundamental, as for example, observations on correction of inborn errors of metabolism[1].

However, liver transplantation has influenced hepatology in such broad and more subtle ways that instead of saying more about this procedure, its past, or its future, I will talk about the fall-out benefits that have been coincidental from research and development in liver transplantation. An obvious example is the perfection of techniques for major hepatic resections, such as right trisegmentectomy (extended right hepatic lobectomy). Historically, this operation, in which 75%–90% of hepatic parenchyma is removed, has had an operative mortality averaging almost 40%. By applying technical and management principles from our liver transplantation experience, the operative mortality has been reduced to 3% or less.[2,3]

Such an achievement is of interest mainly to surgeons. In contrast, an important area of broadly applicable basic research in portal physiology has derived from efforts at liver transplantation.

HEPATOTROPHIC FACTORS

The notion that portal venous blood possessed qualities not found in other kinds of blood repeatedly surfaced in and receded from the literature during the nearly 100 years before 1965. However, the hypothesis of portal blood specificity was not established until the early 1960s, when it was found that auxiliary liver homografts placed in an ectopic location and doubly revascularized with arterial plus systemic venous blood underwent striking atrophy and other abnormalities within a few days.[4,5] The small graft shown in Fig. 1 had been the same size as the native liver at the time of transplantation a few weeks earlier.

Such acute atrophy also was found in nontransplant models, such as the so-called split transposition.[6] With this preparation, one liver fraction was nourished with splanchnic venous blood via a portal branch. It remained healthy. The other liver fraction supplied with equal volumes of or greater systemic venous inflow underwent atrophy and tissue damage.

The hypothetical ingredients in venous blood returning from the splanchnic organs were termed hepatotrophic factors. But what were they? The first solid clues were not unearthed until 1972 and 1973. Then, using different kinds of split liver techniques in dogs, circumstantial evidence was obtained that the hepatotrophic factors were endoge-

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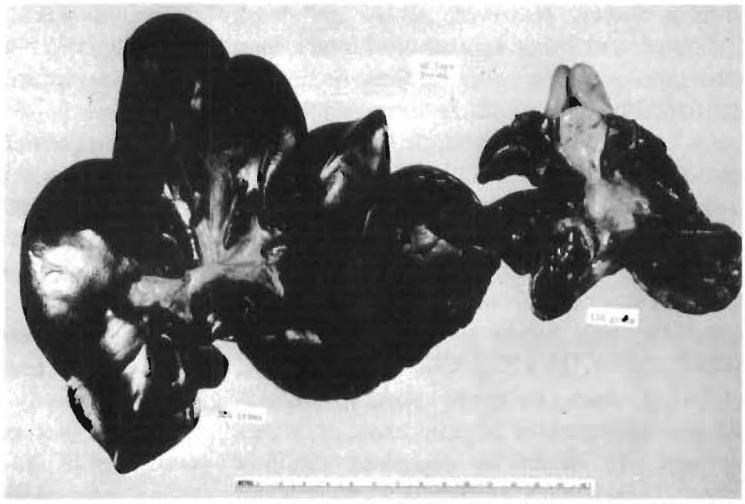

Fig. 1. The auxiliary homograft (right) and the recipient dog's own liver (left) in an experiment in which the host and transplanted organs had originally been about the same size. Postoperative immunosuppression was with azathioprine. Note the well preserved but dimensionally reduced general structure of the homograft. The gallbladder did not shrink proportionately. The specimens were obtained 45 days after transplantation. (Reproduced by permission of *Annals of Surgery*.[4])

nous hormones.[7] One particularly useful preparation was splanchnic division in which venous return from the pancreas and other upper abdominal organs was given to part of the liver, whereas the other part was given intestinal venous return (Fig. 2). The liver region nourished with venous blood from the pancreas and duodenum had large, glycogen-rich, and ultrastructurally healthy hepatocytes compared to the atrophic hepatocytes nourished with venous return from the intestines. Pancreatectomy or the creation of alloxan diabetes (Fig. 2) eliminated or diminished the regional differences.[8]

The unmasking of these effects in split liver models had a simple explanation. It appeared that the splanchnic hepatotrophic substances were largely cleared by one passage through hepatic tissue. Therefore, hepatotrophic factors passing through one liver fragment became unavailable for the other competing fragment, which suffered accordingly. From the evidence just cited, endogenous insulin seemed to be the most important hepato-

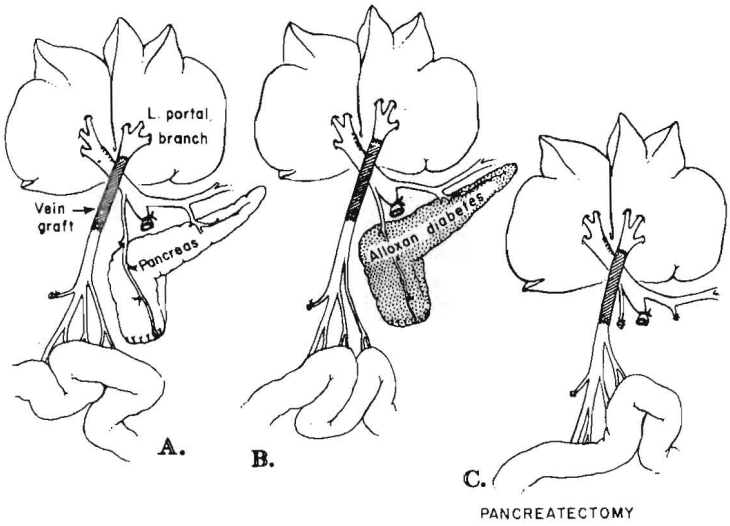

Fig. 2. Splanchnic division experiments. In these dogs, the right liver lobes received venous return from the pancreaticogastroduodenosplenic region, and the left liver lobes received venous blood from the intestines. In other experiments the intestinal blood was directed into the right lobes with pancreatic flow to the left side. (A) Nondiabetic dogs; (B) alloxan-induced diabetic dogs; (C) dogs with total pancreatectomy. (Reproduced by permission of *Surgery, Gynecology and Obstetrics*.[6])

trophic factor. However, all of our work indicated that other splanchnic factors were also important. The nature of these noninsulin contributory splanchnic factors, which have been assumed to be multiple, has not been accurately determined. Other hormones (such as glucagon) and nutrients absorbed from the intestinal tract have been suggested.

A crucial experiment to test the hepatotrophic role of insulin was carried out in 1975, using the Eck fistula (complete portacaval shunt) model. In all species so far studied, including man, complete portal diversion causes characteristic hepatic changes, which in dogs are essentially complete within 4 days.[9] The changes are identical to those in the portaprival liver fragments of split liver experiments that I just described. Canine hepatocytes shrink to about half their original size within 4 days and become infiltrated with fat. Various organelles become abnormal, the most specific change being disruption and quantitative loss of rough endoplasmic reticulum. There is an increase in thymidine incorporation and mitotic activity to about three times the previous level.[9]

Insulin was tested in such dogs in the following way. At the time of Eck fistula, a continuous infusion of nonhypoglycemic doses of insulin (with or without glucagon) was given into the tied off left portal vein (Fig. 3). The left lobes could then be compared to the preshunt control tissues as well as to the nontreated right lobar tissues, and to the findings in control untreated animals after Eck fistula.

The results were clear.[9] Insulin prevented most of the acute damage caused by Eck fistula, but only in the directly infused left lobes. Atrophy was largely prevented and so were most of the expected light and electron-microscopic changes. Glucagon, with insulin or by itself, had no demonstrable effect. In addition to the foregoing effects, insulin treatment in Eck fistula dogs influenced the rate of hepatocyte cell renewal. Thymidine incorporation and actual mitoses were increased about four times in the infused lobes but not on the other side.

At long last, the explanation for the mysterious Eck fistula syndromes was at hand. In essence, these syndromes seemed to

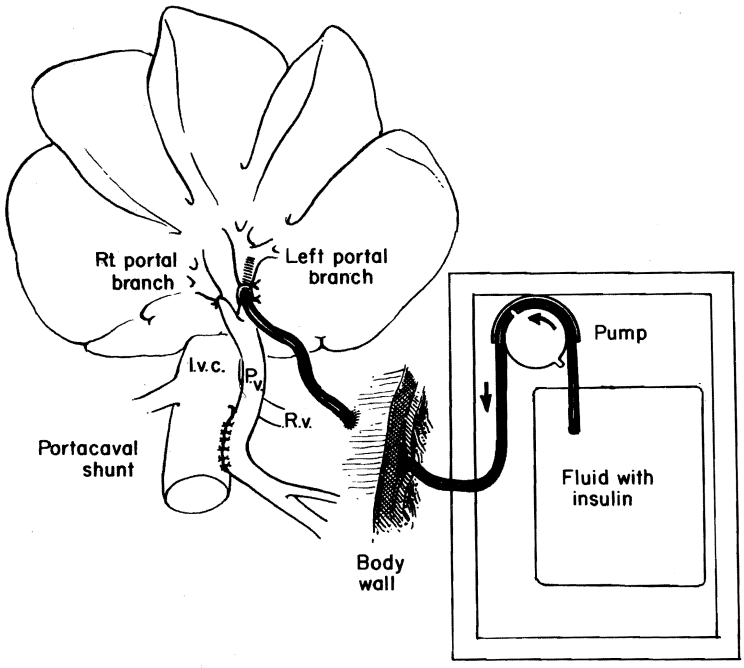

Fig. 3. Experiments in which Eck's fistula is performed and postoperative infusions are made into the left portal vein. (Reproduced by permission of *Lancet*.[9])

be caused largely by the loss to the liver of direct exposure to hormones (especially insulin), which were being diverted from their natural transhepatic route, even though they were returned in diluted form through the arterial circulation. The secondary complications of Eck fistula, such as encephalopathy and weight loss, were presumably derivative from the liver changes. With the liver changes came other subtle but profound metabolic consequences. For example, there was a fall in serum lipid components, such as cholesterol and phospholipids,[10] which was at least partly due to a reduction in hepatic cholesterol synthesis.

Quite understandably, the morphological effects of Eck fistula in dogs can be duplicated by another straightforward procedure, namely, removal of all nonhepatic splanchnic organs.[11] Here also, the structural and ultrastructural changes could be greatly reduced or even prevented by the continuous infusion of insulin into the now virtually nonexistent portal circulation.[11]

There are broad implications to the concept that the liver is more than a way-station for splanchnic hormones and that a functional interplay exists between the splanchnic hormone sources and the liver. Although the clearest example is with insulin, the same potential exists with polypeptides, nutrients, and other substances of splanchnic origin that are normally brought to the liver in high concentration on first pass. The role of this splanchnic–hepatic axis in understanding liver physiology and liver disease is a task to which we will be addressing ourselves for a long time to come. The subject is germane to an important question in pancreatic transplantation, namely: Where is the optimal place to put pancreatic grafts?

HEPATIC REGENERATION AND HEPATOTROPHIC FACTORS

The suspicion has been entertained by us[7,12] and by others that the foregoing splanchnic factors influence the hepatic regeneration that follows liver injury or partial hepatectomy. That hormones such as insulin can affect the rate of hepatocyte proliferation has been clearly established in many experimental models, including the insulin infusion experiments described earlier.[9] The work of Bucher at Harvard[13] and the San Diego investigators directed by Orloff[14] has been particularly influential.

As would be predicted, evisceration changes the normal course of regeneration.[15] If total pancreatectomy is performed at the time of or before partial hepatectomy, regeneration is delayed and diminished but by no means eliminated. Retention of the pancreas but removal of all other nonhepatic splanchnic organs has a similar or even greater but still incomplete inhibiting effect on regeneration.[15] However, when all the nonhepatic splanchnic organs are excised, regeneration is brought to a dead standstill, and it cannot be restored to normal with insulin or insulin/glucagon therapy.[15]

What is the role of hepatotrophic factors in liver regeneration? The hypothesis that hormone interactions create an initiating climate for regeneration is compatible with data on secondary messenger systems obtained in our laboratories[15,16] and elsewhere. After liver resection in rats and dogs, early rises and later declines in cyclic adenosine monophosphate (AMP), adenyl cyclase, hormone binding, and polyamine concentration precede regeneration by many hours. These orderly changes[16] are perturbed by all of the evisceration procedures that inhibit or abolish regeneration,[15] but it is not known if such associations and iatrogenically caused disassociations represent cause or effect.

INTRAHEPATIC GROWTH CONTROL FACTORS

About 2 years ago, we concluded that our investigations of hepatic growth control needed a new direction. We began a search for factors within the liver itself that could influence liver growth. Although this concept had important historical roots going back 20 years, it has been controversial because of conflicting and inconsistent experimental results.

Our approach was simple. By ultracentrifugation at 140,000 *g*, we made canine hepatic cytosol extracts that were free of cell membranes, organelles, and viruses. Such cytosol contains proteins and other constituents that are soluble but no insulin and very little glucagon. The cytosol was tested in the same Eck fistula model described earlier (Fig. 3) by injecting it as a 4–6-hr bolus into the tied off left portal vein, just after completion of the portacaval shunt.

The results were remarkable.[17] Cytosol from normal adult livers had no effect on the events that follow Eck fistula, nor did the cytosol prepared from liver fragments remaining after 72% hepatectomy performed 1 day earlier. However, 2-day regenerating liver changed the pattern of results, and 3-day regenerating liver was even more potent. In the directly infused left liver lobes these latter cytosol extracts inititiated a burst of regeneration that had a delayed onset (Fig. 4). The increased mitoses were not clearly seen until 2 days after the pulse of cytosol had been given, with a response that was doubled again at 3 days and almost completely confined to the directly treated lobes.

Proliferation was not the only cytosol effect. The atrophy characteristic of Eck fistula proceeded for 2 days *after* the delivery of the active liver extracts, but between the second and third days, there was a dramatic reversal (Fig. 5). Now the hepatocytes returned to normal size at the same time as other structural and ultrastructural features of these liver cells were restored to or toward normal. The result at 3 days was a liver in which healthy hepatocytes in the left, but not the right, liver lobes were undergoing changes indistinguishable from vigorous regeneration.

Such observations create a new dimension for research in regeneration. The findings will have to be reconciled with the earlier work on hepatotrophic factors. The appearance of the growth factor in regenerating livers is dependent on portal blood input, since no stimulating substance can be found in liver fragments

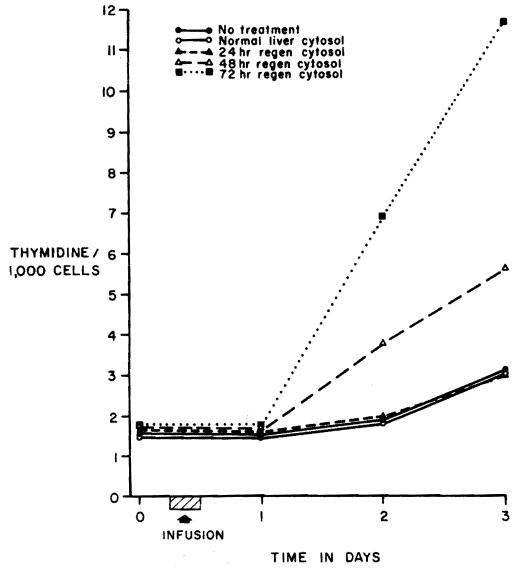

Fig. 4. Number of thymidine-labeled hepatocytes per 1000 hepatocytes. Cytosol from normal and 24-hr regenerating livers does not alter the pattern that occurs after portacaval shunt (no treatment group). The 48-hr and particularly the 72-hr regenerating liver cytosol has a marked stimulatory effect at 2 and 3 days. (Reproduced by permission of *Viewpoints on Digestive Disease* 11:1–4, 1979.)

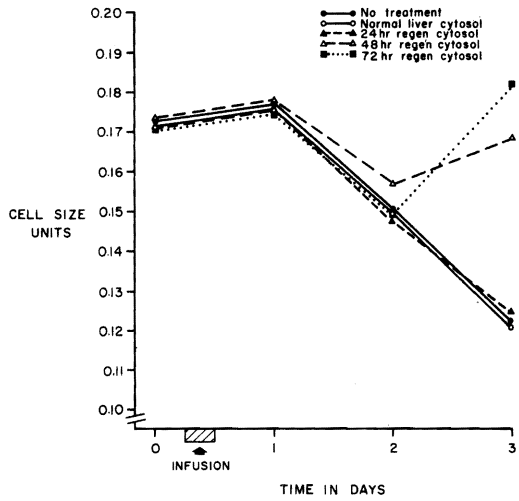

Fig. 5. Cytosol from normal and 24-hr regenerating livers does not alter the atrophy that occurs after portacaval shunt (no treatment group). The 48-hr and particularly the 72-hr regenerating liver cytosol causes reversal of these changes between the second and third days. (Reproduced by permission of *Viewpoints on Digestive Disease* 11:1–4, 1979.)

of dogs subjected to concomitant evisceration.[18] There are other lines of inquiry. Isolation and identification of the growth factor should be possible by standard biochemical techniques, and it should be tested for species and organ specificity.

CLINICAL IMPLICATIONS

General Considerations

Implications of the hepatotrophic concept have already been mentioned or implied, including: the planning of optimum vascularization of liver homografts; the possibility of the provision of an hepatic renewal stimulus in patients with acute hepatic disease who exhibit poor regeneration; the better understanding of a variety of liver diseases as these are influenced or even caused by pertubations of the hepatic–splanchnic axis; and a better understanding of nonhepatic diseases (diabetes mellitus, for example) the courses of which are probably effected by interorgan relationships.

In addition, the hepatotrophic concept should be part of the consciousness in choosing portal-systemic shunt procedures for control of hemorrhage from esophageal varices. In principle, the Warren-type procedure should be the best, since it can decompress the dangerous gastroesophageal venous collaterals with minimum acute loss of residual hepatopetal flow.[19]

Portal Diversion and Inborn Errors of Metabolism

Glycogen Storage Disease

Historically, portacaval shunt has been used clinically for mechanical objectives—decompression of the portal venous system and relief of ascites. More recently, portal diversion has been performed for metabolic purposes. The first such application was for glycogen storage disease.[20–22] We have treated 10 such patients who now have potential follow-ups of 3.5–16 years. Their liver enzyme deficiencies were of glucose-6-phosphatase (type 1, 6 examples), amylo-1-6-glucosidase (type 3, 3 examples), and phosphorylase (type 6, 1 example). All 10 children had growth retardation, and 9 had recurrent hypoglycemia and acidosis. Secondary hyperlipidemia, coagulation disorders, hyperuricemia, and bone dysplasia were common.

After complete portacaval shunt, the insulin rise in systemic blood after a glucose meal was markedly increased compared to preoperatively.[21,22] All metabolic abnormalities were immediately ameliorated or fully corrected except for nocturnal hypoglycemia. All the children had striking growth spurts, at an average rate of more than 0.5 cm/month for the first year. Later biopsies of the livers showed no decrease in the glycogen concentration and no change in the enzymes. However, the hepatocytes had undergone the kind of atrophy described earlier in animals. This, plus the increased total body growth, resulted in a relative decrease in the hepatomegaly, which was a common preoperative complaint. Bone growth and maturation were striking (Fig. 6).

The subsequent important investigations of Folkman et al. of Harvard[23] have shown that many of these benefits can be achieved by continuous alimentation with glucose or an elemental diet delivered through a gastrostomy or oral feeding tube. The relative roles of portal diversion versus continuous alimentation (singly or together) for the treatment of glycogen storage disease will undoubtedly be clarified in the next few years.

Hyperlipidemia

In a second metabolic disease, namely, homozygous type II hyperlipidemia, a reasonable nonsurgical option is not available. Patients with this disorder have serum cholesterol concentrations of 600–1200 mg/dl. They progressively develop xanthomatous deposits in numerous external contact areas. Such deposits internally in the coronary arteries and heart valves inevitably lead to lethal cardiovascular complications, usually by the teen years. Medical and dietary treatment are ineffective.

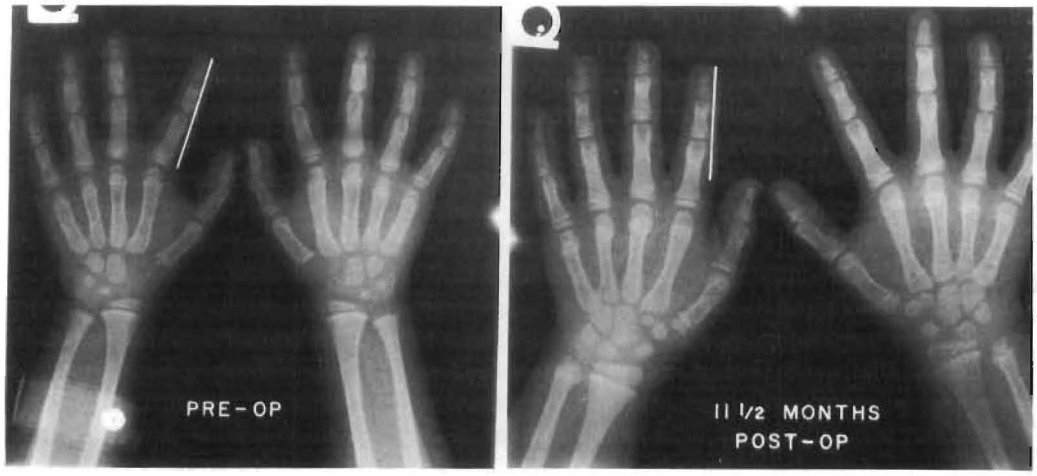

Fig. 6. The dramatic wrist and hand bone growth and mineralization in the first 11.5 postoperative months. The bracket on the left index finger is 5 cm in length. (Reproduced by permission of *Annals of Surgery*.[21])

In 1973, we performed end-to-side portacaval shunt on a patient whose serum cholesterol concentrations fell from about 800 mg/dl preoperatively to about 350 mg/dl. At the time, visible xanthomatous deposits disappeared and a moderate aortic stenosis regressed.[23–25] Unfortunately, she had suffered a major myocardial infarction a few weeks before the portal diversion, leading to a myocardial aneurysm. Nineteen months after the portal diversion she died suddenly while walking from school. The cause of death probably was a cardiac arrhythmia.

We have treated five other patients with type II hyperlipidemia.[21,26] One subsequently had an aortic valve replacement and triple coronary artery bypass. The two oldest of these patients have follow-ups now of 4.5–5.5 years. All five of the surviving patients have had major cholesterol declines as well as resorption of xanthomas. Careful metabolic studies on one of these patients revealed a substantial decline in hepatic cholesterol synthesis[27] analagous to that observed in normal dogs.[10] It is likely that decreased hepatic lipid synthesis is only one of multiple factors accounting for the antilipidemic effect of portacaval shunt. In any event, the antilipidemic effect of portal diversion has been confirmed in many centers throughout the world.

It is interesting that pathologic changes caused by portal diversion in the seemingly normal human liver are indistinguishable from those produced in animals. The changes include atrophy, organelle disruption, fatty infiltration, and especially loss of rough endoplasmic reticulum. Despite this, the human (like the rat) has been resistant to encephalopathy and other devastating complications of Eck fistula that regularly kill dogs, swine, and baboons within a few months.[28]

Alpha$_1$-Antitrypsin Deficiency

It seems likely that there are still some spirits left in the Pandora's box I have been discussing. Some preliminary observations in three patients with alpha$_1$-antitrypsin deficiency have suggested that this third inborn error of metabolism also can be ameliorated by portacaval shunt. These three children with deteriorating liver function had portacaval anastomosis 4, 2.5, and 0.5 years ago. The hepatic disease has not progressed since then. One of the patients had a repeat biopsy more than a year after the operative procedure. The hepatocyte atrophy and other changes of portacaval shunt were found. In

addition, intrahepatic granules, which are thought to be abnormal alpha$_1$-antitrypsin, were quantitated by a morphometric technique. The intrahepatic granules had decreased in number. Since these deposits have been thought to be the cause of the cirrhosis of this disease, a rational explanation is at hand for the clinical benefit. Presumably, the portacaval shunt diminished the synthesis by the liver of the abnormal glycoprotein without commensurately reducing its transport out of the liver.

Within a month or two, we will be rebiopsying a second of these three patients. If the aforementioned histopathologic observations are confirmed, we will consider expanding this clinical trial. Even if portal diversion fails in some cases, it has been established that the inborn error can be cured by the drastic final step of liver replacement.[29]

SUMMARY

Apart from its own intrinsic importance, liver transplantation has been the stimulus for development of better techniques of hepatic surgery and for a better understanding of hepatic physiology. A particularly important off-shoot of transplantation has been the recognition that factors (called hepatotrophic) are present in the portal blood. The nature of these factors and their influence on the liver have been discussed, as well as the clinical implications of the hepatotrophic concept.

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