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A hundred years of the hepatotrophic controversy

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Abstract Venous blood returning from the splanchnic viscera has liver-supporting (hepatotrophic) qualities not found to the same degree in other kinds of arterial or venous blood. The effects of portal blood have been noted in animals with two livers (or a differential portal blood supply to different regions of one liver) to include hypertrophy, glycogen storage, hyperplasia, capacity for regeneration, increase of several synthetic functions, and maintenance of normal structure. The main splanchnic venous hepatotrophic factors are endogenous hormones of which the single most important is insulin. Thus, the foregoing portal hepatotrophic effects are largely eliminated with the diabetes produced by alloxan or total pancreatectomy.

The injury of portacaval shunt is caused by the diversion of the hormones around the liver. Accordingly, the atrophy, injury to the organelles, and loss of the capacity for cell renewal is minimized if insulin is infused into the portally deprived liver. In these and other experiments, exogenous glucagon alone or the addition of glucagon to insulin has had no effect, but this may be because of the masking presence of gut glucagon and other hormonal or non-hormonal substances in our models.

At present, the effects on the liver of exogenous insulin, glucagon, epidermal growth factor, and numerous other hormones are being determined by their intraportal infusion into eviscerated dogs in which other endogenous splanchnic factors have been eliminated.

In this paper we shall be summarizing an array of evidence from our centre about how hormones released by the splanchnic organs into the portal venous system can influence the morphology, regenerative capacity, and function of the liver in ways that were not even suspected until quite recently. It may be well to state in advance our conclusion that insulin is the most important of these portal blood constituents which we have collectively termed portal

hepatotrophic substances but in addition to emphasize our belief that many other less important factors are also involved.

Although we shall be focusing for the most part on our own observations, we would be remiss not to acknowledge the supporting data and/or new information coming from other laboratories. Recent summaries have been published of the important work done with *in vivo* preparations at Columbia University (Price 1976; Whittemore *et al.* 1975), the University of California, San Diego (Broelsch *et al*, 1974; Duguay & Orloff 1976), Harvard University (Bucher & Swaffield 1975; Farivar *et al.* 1976), and the University of Pittsburgh (Fisher *et al.* 1971). Within the past several years, Gerschenson *et al.* (1972), Benzo & De La Haba (1972), Wagle *et al.* (1973), Leffert (1974; Leffert *et al.* 1976), and Richman *et al.* (1976) have used hepatocyte culture preparations to test the role of insulin and other hormones in controlling the growth, replication, and function of liver cells.

ECK FISTULA

Much of the evidence supporting the importance of insulin as a hepatotrophic factor has to do with what happens when the liver is deprived of portal venous blood or that portion of portal blood that emanates from the pancreas. The most extreme portaprival state is when all the splanchnic venous return is diverted around the liver via a portacaval shunt, leaving the liver with only an arterial supply. Portacaval shunt is also called Eck's fistula after the Russian military surgeon who described it in dogs 100 years ago (Eck 1877). Few articles have led to such prolonged controversy as this one.

On the basis of the short-term survival of one of his eight dogs, Eck thought that a completely diverting portacaval shunt was compatible with prolonged good health. This conclusion was refuted by Hahn *et al.* (1893) whose dogs with Eck fistula developed weight loss, liver atrophy and hepatic encephalopathy. The inability in ensuing years to explain these consequences caused Bollman (1961) of the Mayo Clinic to write: 'In the 83 years since it was first reported, the Eck fistula has been reasonably successful in hiding its secrets as well as in giving rise to may additional questions fundamental to an understanding of the functions of the intestine, liver, and brain'.

The light microscopic changes in the liver caused by portal blood deprivation include atrophy, fatty infiltration, and deglycogenation. Ultrastructurally, the most striking and specific changes are depletion and disruption of the rough endoplasmic reticulum, and reduction in the membrane-bound ribosomes. We now realize that all these events occur with surprising speed, being about 90 % complete within four days (Starzl *et al.* 1976). We also know from other

work recently summarized by Putnam *et al.* (1976) that the same general light and electron microscopic changes have been seen after portal diversion in the livers of rats, dogs, swine, baboons, and humans with some variations in degree. Thus, the hepatic injury of Eck fistula is common to all species so far studied. The metabolic consequences have been the mildest in rats and man.

When Bollman (1961) summarized the situation of Eck fistula (quoted above), it was widely accepted in what was called the flow hypothesis that the Eck fistula syndrome was caused by a suboptimal volume, as opposed to quality, of hepatic blood flow. This conclusion had what looked like incontrovertible support from the classic paper by Child *et al.* (1953) entitled 'Liver regeneration following portacaval transposition in dogs'. With portacaval transposition the splanchnic venous blood is diverted by an end-to-end anastomosis to the transected upper vena cava but the lost portal blood is replaced with an inflow to the hilar portal vein from the transected distal inferior vena cava. With this portal blood replacement, Child avoided in dogs most of the adverse effects of Eck fistula. Fisher *et al.* (1954) had similar results using arterial blood for portal flow replacement. Thus, portal blood seemed to possess no physiologically important special qualities.

THE UNMASKING ROLE OF AUXILIARY LIVER TRANSPLANTATION

The flow hypothesis began to fall apart about 13 years ago with experiments done in dogs to define the necessary conditions for auxiliary liver transplantation. If an extra canine liver was not given splanchnic blood, it promptly underwent severe shrinkage (Starzl *et al.* 1964) even though the lost portal flow was replaced with equal volumes of systemic blood (Fig. 1). Conversely, if the graft was given the splanchnic venous return, the acute atrophy now affected the native liver (Marchioro *et al.* 1965*a*). The organ with first access to the splanchnic venous blood apparently was efficiently extracting something (subsequent work has shown this to be mainly insulin; see later), the absence of which was profoundly damaging to the second organ.

PARTIAL (SPLIT) PORTACAVAL TRANSPOSITION

The transplant preparations which had made apparent the foregoing physiological effect had a flaw which prevented complete acceptance of the hepatotrophic concept. There was, in addition to different kinds of portal vascularization, an inherent inequality of the two organs since the homograft (or auxiliary liver) was often under immunological attack despite host immunosuppression, whereas the animal's own liver was not. Consequently, we under-

FIG. 1. An auxiliary homograft (*right*) and the recipient dog's own liver (*left*), 45 days after transplantation. The graft portal vein was vascularized with systemic venous return. Normal splanchnic inflow was retained for the host liver. (From Starzl *et al.* 1964, by permission of *Annals of Surgery*.)

took other experiments which were designed to eliminate this objection.

The key step was the introduction of what has been termed a split or partial transposition which in effect divided the animal's own liver into two fragments (Marchioro *et al.* 1965*b*, 1967). With this operation, splanchnic venous blood is provided for one portal branch of the liver, whereas the other portal branch is detached and supplied with blood from the inferior vena cava (Fig. 2A). The quantity of flow was measured in many of these experiments and found to be generally greater on the side perfused by vena caval blood.

The results from this work were clear (Marchioro *et al.* 1965*b*, 1967). The lobes receiving splanchnic blood always were hypertrophic relative to the hepatic lobes nourished by systemic venous inflow. Furthermore, the splanchnic-fed lobes always had more hepatocyte mitoses than the liver tissue on the other side, indicating an influence of portal blood on cell renewal and presumably regeneration.

The two liver sides after partial transposition have been demonstrated to have other easily quantifiable differences using experiments in which the splanchnic venous blood went to the right lobes for 60 days (Starzl *et al.* 1973, 1975*a*).

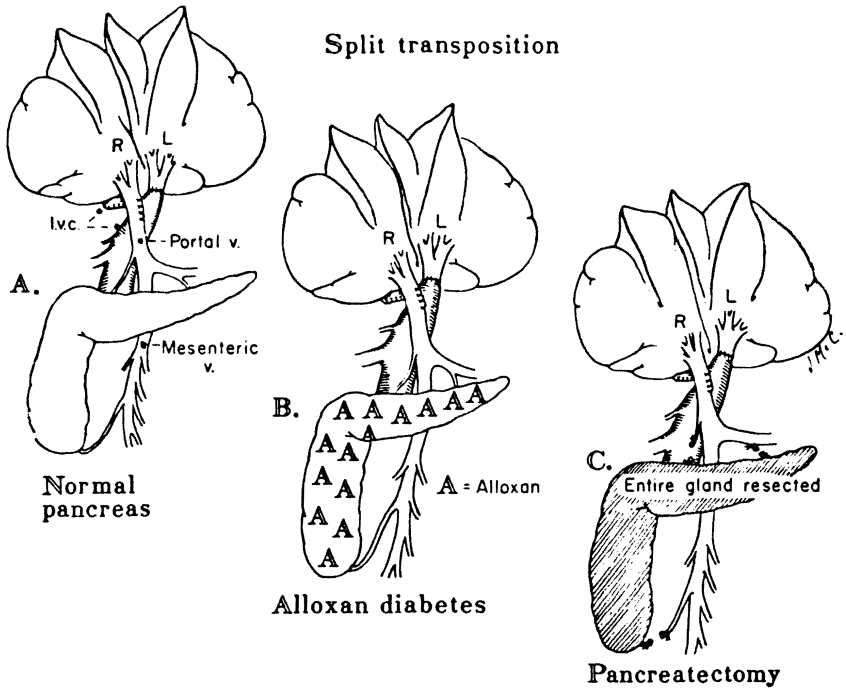


FIG. 2. Partial or split portacaval transposition experiments in which the non-hepatic splanchnic venous return was directed in its entirety to the right lobes, and the inferior vena caval blood was used to perfuse the left liver lobes by anastomosing the supra-adrenal inferior vena cava to the detached left portal branch. A. Non-diabetic dogs. B. Dogs with alloxan-induced diabetes. C. Dogs with total pancreatectomy. (From Starzl *et al.* 1975*a*, by permission of *Surgery, Gynecology & Obstetrics*.)

The splanchnic-fed lobes had more glycogen and glucokinase, lower concentrations of cyclic 3′ : 5′-adenosine monophosphate (cyclic AMP) and active phosphorylase, increased deoxyribonucleic acid (DNA) synthesis and higher cholesterol synthesis. The biochemical dissociation was shown in many other ways that will not be detailed here. But a reasonable generalization was that the two liver sides were living in different metabolic worlds in which hormone control, especially that by insulin, played the dominant role.

The significance of the pancreatic hormones in these differential effects was further studied in partial transposition experiments in which some of the dogs were made diabetic with alloxan (Fig. 2B) or by total pancreatectomy (Fig. 2C) and then treated with subcutaneous insulin (Starzl *et al.* 1975*a*, *b*). The exogenous insulin now was expected to be distributed without obvious preference

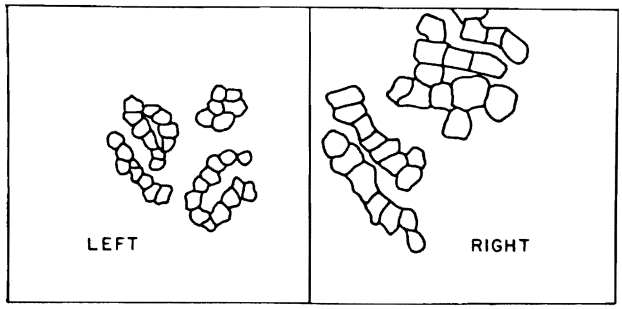


FIG. 3. Hepatocyte shadows traced during histopathological examination, cut out on standard paper and weighed as an index of hepatocyte size. (From Starzl *et al.* 1973, by permission of *Surgery, Gynecology & Obstetrics.*)

to both sides. The right lobes were receiving the total splanchnic venous return and the left had systemic blood.

A previously developed and exquisitely accurate way to measure liver cell atrophy was exploited for such experiments. With light microscope tracing hepatocytes were drawn on a standard thickness paper and weighed (Fig. 3). The weights were called size units. These measures correlated well with the true size of single cells as measured directly with planimetry and other techniques. The cell size data could then be summarized in graphs.

In split transposition experiments which ran for 60 days, the hepatocytes in the right lobes receiving the total splanchnic venous return of non-diabetic dogs were twice as large as their left-sided companions receiving vena cava blood. The cell size advantage was lost by the superimposition of alloxan diabetes, or of total pancreatectomy (Starzl *et al.* 1975*b*).

In non-diabetic dogs these same right lobes receiving the total splanchnic blood also had a higher rate of cell mitosis as measured by autoradiography, and the rates on both sides were higher than normal. The right-sided advantage was only partly removed by alloxan and pancreatectomy diabetes (Starzl *et al.* 1975*b*). These dogs were being treated with subcutaneous insulin which was distributed to both sides. We think the residual difference in right and left hepatocyte proliferation with retention of some right-sided advantage even after diabetes represented an influence on cell renewal of splanchnic factors other than insulin, a point to which we shall return later.

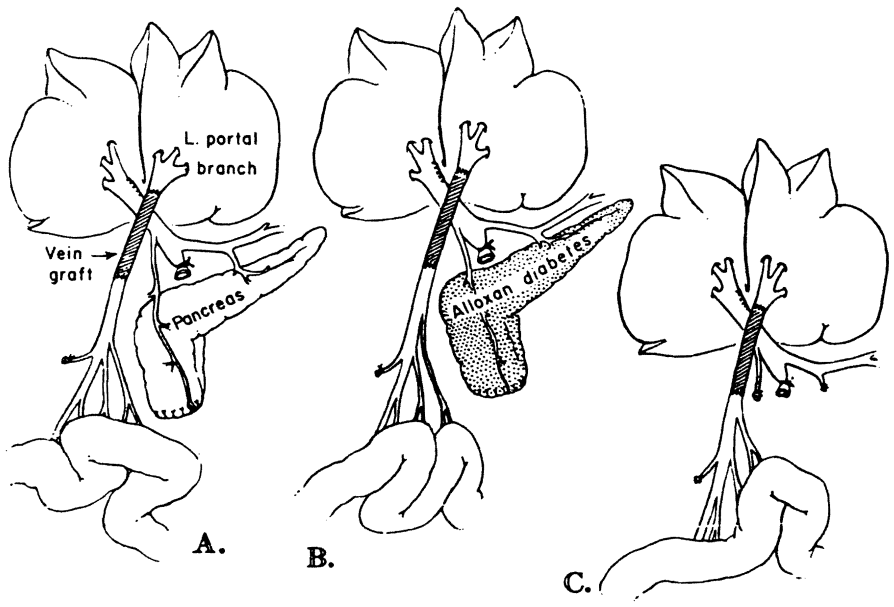
Diverse other measures including DNA synthesis, lipid synthesis, and the ability to regenerate were affected by the diabetic state (Starzl *et al.* 1975*a*, *b*, *c*).

SPLANCHNIC DIVISION EXPERIMENTS

Eventually, another kind of double fragment model provided much more decisive information about the nature and action of splanchnic hepatotrophic factors (Starzl *et al.* 1973). In these experiments, one portion of the liver was fed by the effluent of hormone-rich blood returning from the pancreas, duodenum, stomach and spleen. The opposite lobes were perfused via a graft with nutrition-rich blood returning from the intestine (Fig. 4A).

The morphological results in 60-day experiments were dramatic. The hepatic lobules receiving pancreaticoduodenal venous effluent became large compared to those perfused with nutrient-rich intestinal blood. The individual hepatocytes on that side were strikingly bigger, had evidence of hyperplasia, and contained much glucogen compared with the cells on the other side. Other differences in chemical composition were also noted to which we shall return later.

The probability that insulin was the major cause for the differences between



Splanchnic division

FIG. 4. Splanchnic division experiments in which the right liver lobes received venous return from the pancreaticogastroduodenosplenic region and the left liver lobes received venous blood from the intestines. A. Non-diabetic dogs. B. Alloxan-induced diabetic dogs. C. Dogs with total pancreatectomy. (From Starzl *et al.* 1975*b*, by permission of *Surgery, Gynecology & Obstetrics*.)

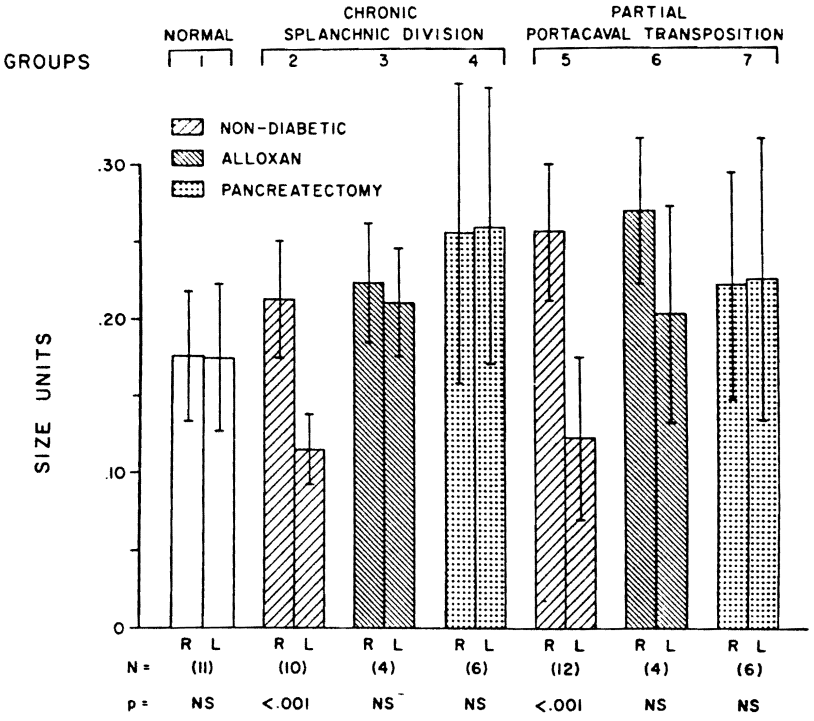


FIG. 5. The effect on hepatocyte size of alloxan-induced diabetes and the diabetes of total pancreatectomy in dogs with splanchnic division. The splanchnic division diverted pancreaticogastroduodenosplenic blood to the right lobes (R), whereas the left lobes (L) received intestinal venous blood.

the two sides was strengthened by additional 60-day experiments in which alloxan diabetes and pancreatectomy (Fig. 4B, C) were superimposed upon splanchnic division (Starzl *et al.* 1975*a*, *b*). In these dogs, pancreaticoduodenogastrosplenic blood was directed to the right lobes and intestinal blood to the left lobes. In control non-diabetic dogs after splanchnic division, the liver cells in the hormone-enriched right lobes became hypertrophic as expected; the left lobes atrophied. These effects were cancelled about equally by alloxan diabetes or pancreatectomy (Fig. 5). In all such experiments, the nearly equal effect of alloxan poisoning and pancreatectomy tended to minimize any major role of glucagon as a hepatotrophic factor (Starzl *et al.* 1975*b*), contrary to an earlier hypothesis we had advanced (Starzl *et al.* 1973) that insulin and glucagon would be synergistic. We emphasize again that these diabetic animals had to be treated with insulin which was delivered to both hepatic sides.

The effect of insulin on cell proliferation was also convincingly demonstrated

TABLE 1

Number of labelled hepatocytes per 1000 hepatocytes in dogs

Group	*Description*	*No. dogs*	*Right lobes (mean ±S.D.)*	*Left lobes (mean ± S.D.)*
1	Normal	11	1.6±0.5	1.5±04
2	Non-diabetic[a]	6	17.3±3.8	4.0±1.0
3	Alloxan[a]	4	4.9±0.4	17.8±3.6
4	Pancreatectomy[a]	5	5.1±1.0	17.5±3.9

[a]Animals had splanchnic division.

(Starzl *et al.* 1975*b*). In non-diabetic animals with splanchnic division, the right liver lobes receiving pancreatic blood had unequivocal autoradiographic evidence of hepatocyte hyperplasia relative to the left lobes, although both sides had greater cell renewal than normal. The right lobar dominance was eliminated and indeed was transferred to the left side by either alloxan or pancreatectomy diabetes in the insulin-treated animals (Table 1).

Shifts of DNA synthesis were also brought about by diabetes (Starzl *et al.* 1975*b*). In non-diabetic dogs after splanchnic division, the right lobes which were perfused with pancreatic blood had the dominant DNA synthesis. This dominance changed over to the left with the addition of either treated alloxan or pancreatectomy diabetes (Fig. 6).

The effects of these various manipulations on lipid metabolism were also marked (Starzl *et al.* 1975*a*). In normal unaltered dogs, cholesterol synthesis was equal on both liver sides. After splanchnic division in non-diabetic animals, the liver portion being perfused with blood from the pancreas and upper splanchnic organs had much greater cholesterol synthesis than the other liver portion being perfused with venous return from the intestine. This dominance of cholesterol synthesis was eliminated and reversed, both by alloxan diabetes and total pancreatectomy, in insulin-treated dogs. Triglyceride synthesis followed the same pattern.

Although the foregoing remarks are based on 60-day splanchnic division experiments, it should be added that most of the described changes have now been shown in this same model (and also after partial transposition) to be nearly complete within four days (Starzl *et al.* 1975*c*).

The four-day experiments convincingly confirmed the role of insulin in controlling both cell size and cell proliferation in the acute regeneration that followed 30% or 60% hepatic resections. The absence or paucity of insulin caused by alloxan diabetes or total pancreatectomy was associated with defective regeneration (Starzl *et al.* 1975*c*). A prominent role could not be identified for glu-

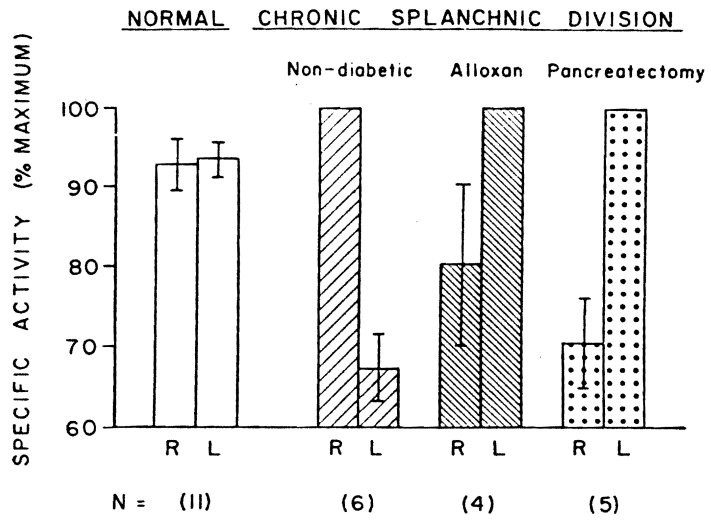


FIG. 6. Deoxyribonucleic acid synthesis in the right (R) and left (L) liver lobes of normal dogs and of non-diabetic or diabetic dogs approximately two months after splanchnic division. The actual values for [CH_3-^{3}H]thymidine uptake were converted to percentages in which the side with the greater thymidine uptake was accorded 100 %. (From Starzl *et al.* 1975*b*, by permission of *Surgery, Gynecology & Obstetrics*.)

cagon in these four-day experiments any more than in the 60-day ones. However, the possible compensatory action of gut glucagon after pancreatectomy in the double liver fragment preparations of Figs. 2C and 4C made it impossible in these models to rule in or out a hepatotrophic action of glucagon.

PORTAL INFUSION EXPERIMENTS

We return full circle to the Eck fistula. If insulin was the most vital portal hepatotrophic factor, the reason for its unmasking by the double liver fragment experiments became understandable. The well-known efficiency of insulin's removal during a first pass through hepatic tissue made the insulin relatively unavailable for a second liver or liver fragment. At the same time the benefit after portal diversion from flow augmentation procedures such as Child's portacaval transposition was explained. If insulin and other hepatotrophic substances were bypassed around a single liver, they would be returned to it in diluted form in direct relation to the total hepatic blood flow which these procedures increased.

If the secrets of the Eck fistula were caused by deprivation of the liver of direct access to endogenous insulin, the experiment shown in Fig. 7 should

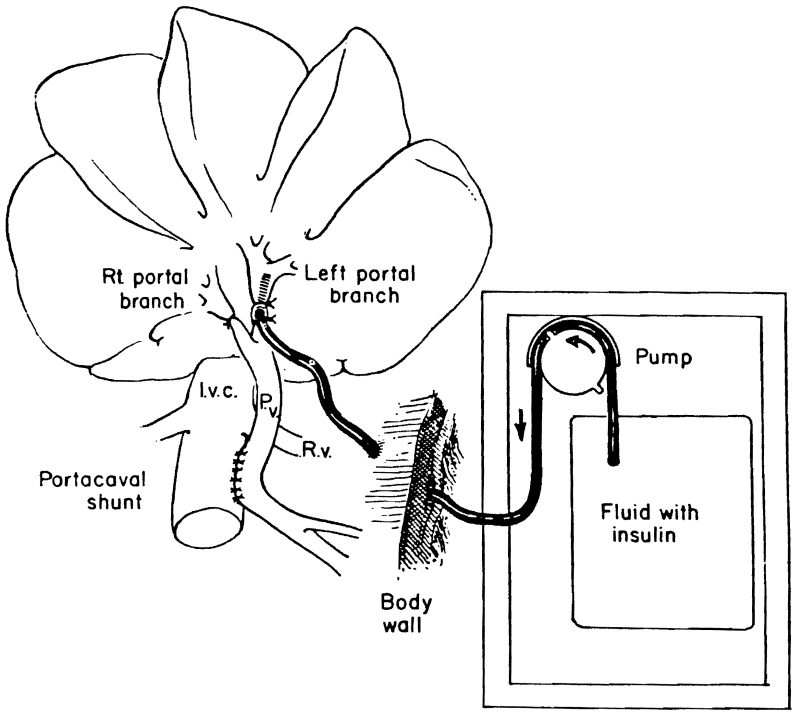


FIG. 7. Experiments in which Eck fistula is performed and postoperative infusions are made into the left portal vein. (From Starzl *et al.* 1976, by permission of *The Lancet*.)

have been a direct test of that hypothesis. Non-hypoglycaemic infusions of insulin and other substances were made for four days into the tied-off left portal vein after Eck fistula. The design of the experiment allowed an evaluation of any direct protective effect on the left lobar hepatic tissues as well as a judgement of whether there was a spillover effect on the right lobes after recirculation.

The results were unequivocal (Starzl *et al.* 1975*d*, 1976). Insulin greatly reduced the acute atrophy that otherwise halved the size of the cells (Table 2), and it preserved hepatocyte ultrastructure. In small doses, glucagon did not potentiate the action of insulin and, in large doses, it may have reduced the insulin benefit. Glucagon alone in either small or large doses had no effect under the conditions of this experimental model.

The effect of insulin on hepatocyte proliferation was also striking (Table 3). After Eck fistula, the mitotic rate was increased to about three times normal, from 1.5 to 4.5–5 per 1000 cells. Insulin more than tripled this cell renewal, with no spillover to the contralateral lobes. Glucagon alone had no effect, nor

TABLE 2

Effects of infusion of hormones into the portal vein of dogs after Eck fistula: experimental groups and hormone doses

Group no.	*No of experiments*	*Type of portal infusion*[a]	*Insulin dose (units/kg/day mean ±S.D.)*	*Glucagon dose (mg/kg/day, mean ±S.D.)*	*Cell size units (mean ±S.D.)*			*P values*[b]
					L0	*L4*	*R4*	*(L4 versus R4)*
1	11	No treatment	0	0	0.197±0.04	0.108±0.02	0.108±0.02	N.S.
2	6	Heparinized saline	0	0	0.200±0.04	0.105±0.02	0.104±0.01	N.S.
3	13	Large dose insulin	0.43±0.05	0	0.189±0.03	0.160±0.02	0.100±0.02	< 0.001
4	4	Small dose insulin	0.16±0.11	0	0.194±0.04	0.143±0.02	0.094±0.01	< 0.05
5	8	Purified insulin	0.42±9.08	0	0.196±0.04	0.158±0.01	0.095±0.02	< 0.001
6	8	Small dose glucagon	0	0.0053±0.0011	0.189±0.01	0.103±0.01	0.103±0.01	N.S.
7	6	Large dose glucagon	0	0.60 ±0.10	0.165±0.03	0.085±0.01	0.082±0.01	N.S.
8	7	2/1 insulin/glucagon	0.45±0.03	0.0053±0.0005	0.211±0.05	0.156±0.05	0.094±0.02	< 0.05
9	7	2/100 insulin/glucagon	0.42±0.01	0.05 ±0.02	0.215±0.04	0.114±0.03	0.073±0.02	< 0.05

[a]All insulin was commercial regular insulin, except in Group 5.
[b]Comparisons were by Student's *t* test.

TABLE 3

Cell division in the li

Group no.	*No ex*
1	8
2	6
3	9
4	4
5	8
6	8
7	6
8	7
9	7

[a]Comparisons were by
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TABLE 3

Cell division in the livers of 63 dogs (groups as in Table 2)

Group no.	*No. of experiments*	*No. of labelled hepatocytes per 1000 hepatocytes (mean ±S.D.)*		*P values[a] (L4 versus R4)*
		Left	*Right*	
1	8	4.9±1.0	4.7±0.9	N.S.
2	6	4.6±0.8	4.7±0.9	N.S.
3	9	13.0±3.9	4.6±0.9	< 0.001
4	4	15.6±2.0	5.3±1.0	< 0.001
5	8	14.4±1.1	4.8±1.0	< 0.001
6	8	4.9±0.9	4.3±0.6	< 0.05
7	6	4.2±1.5	4.3±1.1	N.S.
8	7	11.8±1.2	4.5±0.8	< 0.001
9	7	14.8±1.0	4.5±0.9	< 0.001

[a]Comparisons were by Student's *t* test.
In unaltered or sham-operated dogs the left and right lobar values respectively were 1.6±0.4 and 1.6±0.5. The increase caused by Eck fistula is significant ($P < 0.001$).

did it potentiate the action of insulin.

Thus, it has been established that relative 'hepatic insulinopenia' is the most important element in the liver injury of Eck fistula. It would be regrettable now if the very clarity with which insulin has emerged as a principal hepatotrophic substance were to obscure the search for contributory factors. The observation that the insulin protection in our infusion experiments was not complete may be a reflection of missing ancillary substances.

PORTAL INFUSION IN EVISCERATED DOGS

The problem has been to find appropriate *in vivo* models with which to examine factors other than insulin. A final preparation which we are currently using in dogs should permit more quantitative studies of 'minor' hepatotrophic hormones than had previously been possible. In essence, the splanchnic organs are removed, except the liver (Fig. 8). In some experiments, the colon is retained and anastomosed to the oesophagus, leaving a source of an estimated 20% of gut glucagon. In other experiments, the colon and rectum are also excised. The base of the mesentery with an arterial inflow plus the portal vein, which thus has some residual flow returning from the mesentery, are retained (Fig. 8).

Endogenous insulin and pancreatic glucagon levels postoperatively reach low levels in 48 hours. The portal infusion of exogenous hormones in this model

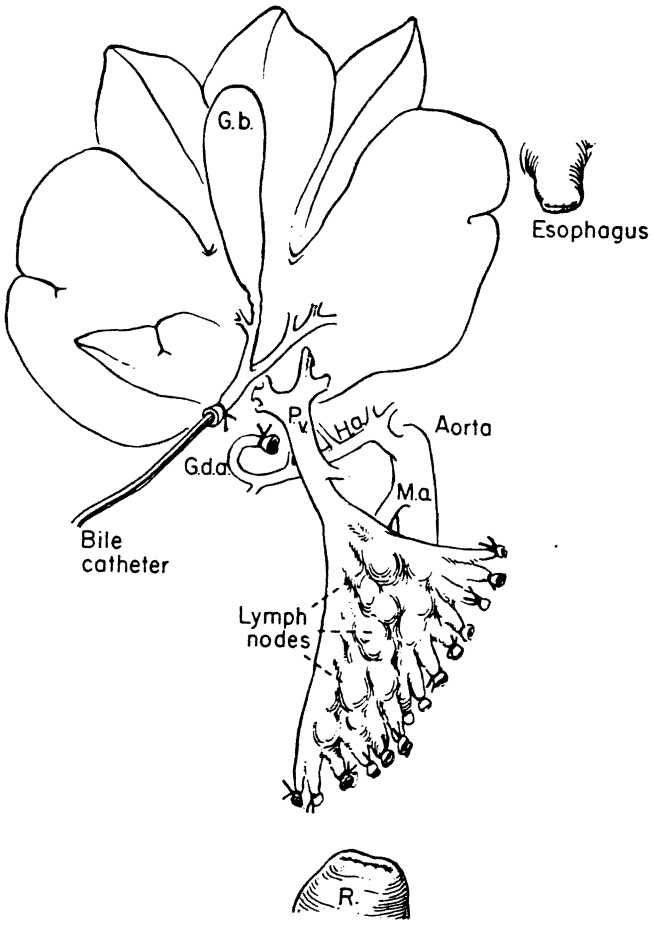

FIG. 8. Evisceration procedure under investigation.

is under way, including insulin, glucagon, secretin, cholecystokinin, vasoactive intestinal polypeptide, somatostatin, growth hormone, and epidermal growth factor.

BIOCHEMICAL QUESTIONS

Although we have learned a lot about the so-called hepatotrophic influences of insulin and other hormones, the cellular mechanics of these effects remain obscure. In this final section, we shall discuss one of our earlier observations (Starzl *et al.* 1973) which illustrated the interdependence of hormone factors,

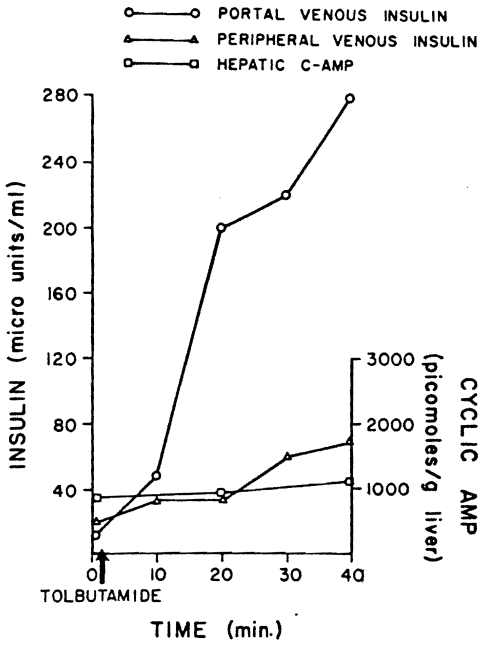

FIG. 9. Changes in peripheral and portal venous insulin and hepatic cyclic AMP, occurring in a normal dog infused with tolbutamide. (From Starzl *et al.* 1973, by permission of *Surgery, Gynecology & Obstetrics.*)

as also will be developed in several other papers in this symposium. As a first step, the insulin response to 40 mg/kg tolbutamide was shown to be substantially greater in the portal than in the systemic venous blood of normal dogs at the same time as little change occurred in the concentration of hepatic cyclic AMP (Fig. 9). Then, in dogs with the double liver fragment preparations shown in Figs. 2A and 4A, the same tolbutamide conditioning was carried out 25 minutes before systemic venous infusions of 1.4 μg/kg of glucagon given over eight minutes.

There were modest increases of cyclic AMP in the liver tissue that was perfused with insulin-rich total splanchnic return (Fig. 10) or splanchnic return from the pancreas and other upper abdominal organs. In contrast, there were spectacular increases in cyclic AMP in the liver tissue that was not 'protected' by endogenous insulin (Fig. 10). The results indicated in a dynamic test system not only the importance of insulin *per se* but also the role of insulin in modifying the response to glucagon. By extrapolation from the work of Bataille *et al.* (1974) in Freychet's laboratory, some insulin modification is a possibility for gut glucagon and vasoactive intestinal polypeptide (VIP), which stimulate

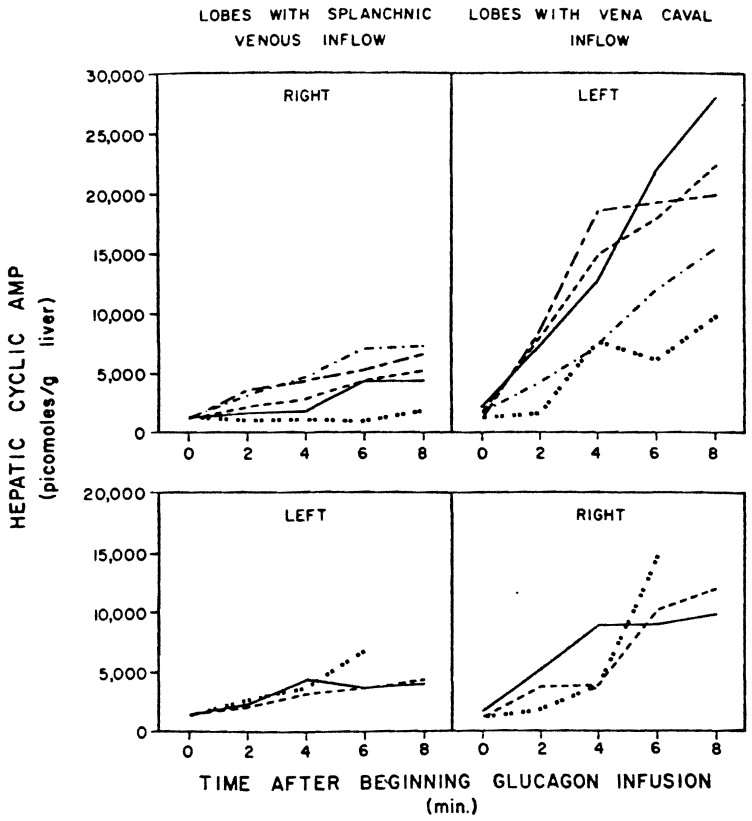

FIG. 10. Results of tolbutamide-glucagon tests in eight dogs with partial portacaval transposition, demonstrating that endogenous insulin in the lobes receiving splanchnic venous blood had a restraining influence on the cyclic AMP response to the exogenous glucagon whereas the response in the other lobes was uninhibited. (From Starzl *et al.* 1973, by permission of *Surgery, Gynecology & Obstetrics.*)

adenylate cyclase and glycogenolytic activity.

Although the foregoing principle of hormone interaction seems established the final picture, as shown by more recent studies of the evisceration preparations (Fig. 8, p. 124), is not going to be such a simple one as having anabolic hormones depress adenylate cyclase and catabolic hormones cause elevations. If all the non-hepatic splanchnic organs are removed, the adenylate cyclase activity in these livers is decreased about 50 % under basal conditions as well as with either glucagon or sodium fluoride stimulation (Fig. 11). The administration of purified insulin into the portal vein of such animals restores adenylate cyclase to normal (Fig. 11) and heightens the DNA synthesis. These ex-

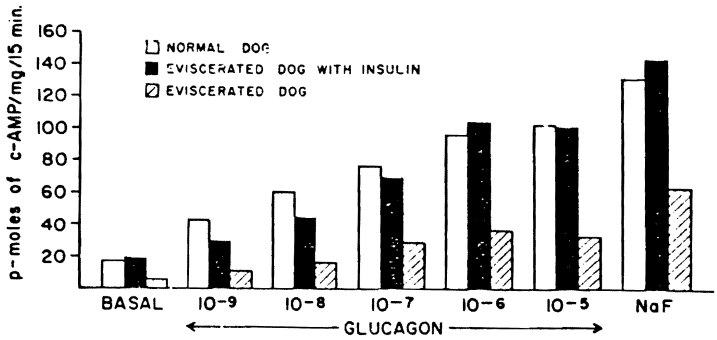

FIG. 11. Adenylate cyclase activity in dog liver homogenate, measured by the method of Salomon *et al.* (1974).

periments have confirmed the power of insulin in the absence of all other splanchnic factors, and they have shown that insulin controls adenylate cyclase in the appropriate circumstances.

Another example of how conditions may be changed by an experimental modification is shown in Table 4. These normal dogs had been subjected to 65 % hepatectomy two days before the experiment and the liver remnants showed a seven-fold increase in DNA synthesis. The basal adenylate cyclase activity was normal during this burst of regeneration and so was the total adenylate cyclase activity after sodium fluoride stimulation. However, the responsiveness to different concentrations of glucagon was greatly reduced. This was a direct demonstration that the cell membrane receptors for glucagon were less sensitive during regeneration, as Leffert *et al.* (1976) claimed earlier. Parenthetically, the observations tend to minimize the importance of glucagon in regeneration, but the evidence is not conclusive.

TABLE 4

Adenylate cyclase activity in dog liver during regeneration (48 hours after 65 % hepatectomy)*

	Basal activity	*Glucagon (moles)*					*Sodium fluoride stimulation*
		10^{-9}	10^{-8}	10^{-7}	10^{-6}	10^{-5}	
18 normal dogs	18.6±6	40±12	59±13	77±17	95±12	97±24	126±39
Regeneration:							
Dog 1	12	15.7	23.1	30.1	33.5	32.9	175.1
Dog 2	19	20	35	45.1	76.4	80.5	140.6

*Values are nmoles of cyclic AMP/mg protein homogenate per 15 min at 37 °C

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Discussion

McIntyre: Have you repeated the experiment of Rous & Larimore (1920) in which they damaged (by bile duct ligation) the side of the liver that should have hypertrophied? If so, did you prevent hypertrophy and did you stop or reduce atrophy of the side deprived of portal blood flow?

Starzl: If you damage the liver by any means, including bile duct ligation, you can reduce, if not prevent, hypertrophy. We have not done exactly the same experiment as Rous & Larimore, but we did something analogous with an auxiliary liver model. If one of two co-existing livers was given a portal splanchnic inflow, its expected advantage was partly eliminated if its duct system was ligated (Faris *et al.* 1966). Thus, I think that Rous & Larimore's conclusions were valid.

Weinbren: Rous & Larimore claimed that if you tied the bile duct to the advantaged side, you prevented both hypertrophy and atrophy, so they concluded that atrophy *depended* on hypertrophy. It is amazing that this is a riddle that has never been solved, because whenever you try to get differential function or differential flow into two sides of the liver, the disadvantaged side always atrophies in the rat, and when it atrophies the other side enlarges. That is the stumbling block in that experiment. It is difficult to say which comes first, which is why it is still a riddle. But we have confirmed their results in the rat. Ligating the bile duct has two effects. You create an increased intravisceral tension in the affected lobe, so the portal blood does not get into the lobe so easily. Also the bile ducts proliferate on that side. There is a relationship between the bile duct proliferation and atrophy of liver cells; the bile duct proliferates better if the liver cells atrophy.

Leffert: Dr Francavilla, have you any conclusions about the functional effect of the loss of adenylate cyclase? It might be premature to conclude that because glucagon activation is reduced, this indicates that the hormone's effects actually are being diminished.

Francavilla: Our data *in vitro* show that during the early stage of regeneration there are important changes in the responsiveness to glucagon of cell membrane adenylate cyclase. At 48 hours, physiological concentrations of glucagon (10^{-9}–10^{-8}M) are unable to stimulate *in vitro* adenylate cyclase activity. This finding, taken together with the specific hormonal receptor changes associated with liver regeneration (Leffert *et al.* 1975), supports the idea that glucagon is not important during all stages of liver regeneration, although it may be important at the beginning.

Leffert: These changes are rather late when one looks at regeneration; one doesn't see loss of responsiveness to the hormone until after 12 hours, as measured with isolated liver plasma membranes (H.L. Leffert & B. Rubalcava, in preparation). By then, many biochemical changes have occurred. Also, if glucagon is not important in liver regeneration,why is it obligatory in Nancy Bucher's regeneration model in which total pancreatectomy is accomplished (Bucher & Swaffield 1975)?

Bucher: It may be difficult to evaluate the importance of glucagon properly in work with dogs because of the abundance in this species of gastrointestinal glucagon, which of course is not eliminated by pancreatectomy (Unger & Orci 1976).

Leffert: Dr Starzl, could you comment on the elevated basal [^{3}H]thymidine labelling index (4.5, as against a control of 1.5)? Might this be due to chronically elevated blood glucagon levels? Secondly, did you correlate the size of the hepatocytes that were labelled with [^{3}H]thymidine with onset times? You

suggested that this was direct evidence for atrophy *not* being a causal factor in the increase in labelling.

Starzl: What I said was that this work (Starzl *et al.* 1976), studies by Weinbren *et al.* (1975), investigations by Rubin *et al.* (1965) and work we have reported with the split liver preparations (Starzl *et al.* 1975*a*, *b*, *c*) all indicate that atrophy and hypoplasia don't necessarily go together, nor do hypertrophy and hyperplasia.

Conceivably, the dissociation could be contributed to by hyperglucagonaemia, but we have been remarkably unsuccessful in demonstrating much effect of glucagon on hepatocyte proliferation. In speculating on the increased cell renewal after Eck fistula, Rubin *et al.* (1965) suggested that the damage or atrophy caused by the Eck fistula is, in itself, a stimulus for renewal.

Of course, it is not known what sets the level of basal cell renewal in such situations, but there are paradoxes such as that described by Younger *et al.* (1966) in diabetic rats. In the livers of these insulinoprival animals there were more cells than normal and higher basal cell renewal. Yet when insulin was given there was a further burst of mitoses.

Alberti: Dr Porter, did you look at glycogen in the cells exposed to insulin plus high levels of glucagon? (These were the disparate ones.) Could loss of glycogen in response to a high glucagon concentration have made any difference to cell size?

Porter: It probably was a factor, but not a major factor.

Alberti: Secondly, I wasn't clear whether adenylate cyclase still responded to other hormones and was just resistant to glucagon, or was resistant to many different hormones. Did you try noradrenaline?

Francavilla: No, we only tried glucagon.

Steiner: Several other studies have suggested that insulin either lowers or doesn't affect hepatic adenylate cyclase activity in experimental animals (Pilkis & Park 1974), and it is thus surprising that you find it increased, or protected, by insulin in this situation. Most commercial insulin preparations are contaminated with small amounts of glucagon and possibly there was enough glucagon in the insulin to protect or maintain adenylate cyclase activity.

Francavilla: We are using pure insulin (Lilly) which contains only 0.002 % glucagon.

Steiner: We are talking about the amount of glucagon that might be required to maintain an activity of an enzyme, however.

Blumgart: The estimates of hyperplasia are based on thymidine labelling. What is the curve of labelling after hepatectomy in the dog? Is it characterized sufficiently, and at what time did you make your measurements after the split operations?

Starzl: After hepatectomy, the greatest burst of labelling activity in the dog is at 2–3 days. The observations I mentioned after hepatic resection were made after 48 hours. As Dr Bucher said, the peak mitotic activity is later in the dog than in the rat.

The observations in the divided liver preparations were after four days or after two months. Studies in dogs with portacaval shunt with or without insulin infusion were after four days.

Weinbren: Your liver cells after alloxan seem to be the same size as normal, but I thought you stated that after alloxan the liver cells atrophy just as if you had deviated the portal flow away?

Porter: They are about normal size after alloxan, but they contain a lot of fat.

Alberti: I am becoming increasingly confused about species differences. Some people are using beef insulin in dogs or in rats, and there are species differences in insulin structure. We are also using very high glucagon concentrations. I should like to know whether glucagon concentrations have been measured in the blood flow to different parts of the liver, particularly because of the glucagon that may be coming from the gut in dogs; this is a very murky area. Has anyone used insulin from the species which is being investigated, like rat insulin or dog insulin, *in vivo* or in culture?

Leffert: Dr Freychet showed that there is not much difference in functional response if you change the species of hormone, except for guinea-pig (Freychet *et al.* 1971).

Alberti: In the rat you are using massive amounts. We do the same: we treat our rats with 2u/day of ox insulin per kg body weight.

Steiner: Actually, when we treated chronically diabetic rats with insulin subcutaneously over a long period in order to determine the correct dose to restore them to a normal growth rate, it turned out to be somewhere between 2 and 4 units of PZI per day (Steiner *et al.* 1961). Thus this is not such a large dose. In studies with T. L. Blundell and S. P. Wood (in preparation, 1977), we have noted that both rat insulins bind less well than other insulins to fat cells, at about the level of 40–50 % as compared with porcine insulin, and they also have a correspondingly lower biological activity in various *in vitro* test systems. Thus both rat insulins are *less* active in rats than porcine insulin. However, there are no apparent differences in the nature of their biological effects.

Freychet: I agree with that. In rat liver membranes, insulins from various animal species are effective in competing with the binding of ^{125}I-labelled porcine insulin, the only known exception (in mammals) being guinea-pig insulin. Like Dr Steiner, we too have observed that rat insulins I and II are

less potent than most other mammalian insulins in competing with the binding of ^{125}I-labelled insulin to rat liver membranes (P. Freychet, S. P. Wood & T. L. Blundell, unpublished work 1974).

Starzl: The dose we infused to get the effects we described after Eck fistula was about 0.5 u/kg per day, delivered intraportally, either pig or beef insulin. At this dose, the dogs did not become hypoglycaemic. Dr Porter noticed, in some accidents when the pumps weren't working properly, that we got most of the protective effect with 1/5 or 1/4 of that amount. Thus, protection against hepatic injury was possible with very much smaller doses than the ones we usually used.

Bloom: Dr Starzl, when systemic blood is going to one lobe (right) and portal blood to the other (left), there is some insulin entering the systemically supplied lobe, yet it atrophies. Obviously this is a very dose-dependent event. Have you given insulin into the systemic circulation, which would then go back to the right lobe, thus switching off the insulin going from the pancreas to the left lobe? Exactly how much insulin does one have to give to restore the balance?

Starzl: We haven't done this, but it would be a good experiment.

Leffert: We have found that under the appropriate *in vitro* conditions, DNA synthesis initiation can be stimulated with a few nanograms per millilitre of insulin (Fig. 1, p. 62; Leffert 1974*a*; Koch *et al.* 1976; Leffert & Koch 1977). These concentrations are within the physiological range. Richman *et al.* (1976) also reported stimulation of DNA synthesis by nanogram concentrations of insulin (together with epidermal growth factor and glucagon). They included serum in their medium; we didn't. Proliferation does not occur in their system; it does in ours.

Blumgart: We measured flow in a split splanchnic preparation in five dogs. We found essentially the same weight changes as Dr Starzl did. We measured flow by a krypton clearance technique in both halves of the preparation. There was no significant difference in liver blood flow preoperatively, immediately postoperatively or 60 days later between the right and left sides. Thus, in this dog preparation, the changes Dr Starzl has shown were not related to flow. However, in Dr Starzl's experiments the side that atrophied was always the side that had the vein graft carrying blood from the gut. Nevertheless, our results do not exclude the possibility that flow is important in other situations. For instance, in one of your experiments insulin was infused directly into one lobe and then glucagon was infused into a lobe with no portal flow. This had no effect, but with a completely ligated portal vein you would not expect to see an effect of glucagon in relation to flow.

Starzl: How does glucagon increase flow, then, if it cannot affect the remaining arterial supply?

Blumgart: I don't know whether the precise mechanism has been worked

out. We have started to work on this question.

Bloom: Glucagon is used for coeliac axis angiography because it gives massive coeliac artery dilatation.

Starzl: It shouldn't necessarily lose this effect, should it, just because it is infused into the tied-off central portal vein?

Blumgart: I agree that glucagon might have an effect on arterial flow and cause some increase. I am suggesting that while we can support your results in the split splanchnic situation, it is not right to exclude blood flow as a possible factor in other experiments.

Bloom: Glucagon might be useful to test in mice, with viral hepatitis for example, where it might keep blood flow going to areas that have become thrombosed and necrotic secondary to the infection.

Smith-Laing: Dr Starzl, in the split liver preparations combined with pancreatectomy or alloxan diabetes, splanchnic flow appeared to replace the effect of insulin on the liver. The amount of gut glucagon that might have been there would have been much less than in, say, Nancy Bucher's work. What is it in that insulin-free splanchnic flow that protects the liver?

Starzl: I don't know; Steve Bloom may have to tell us that! There are lots of candidate substances.

However, I do want to correct the impression I apparently left that the splanchnic venous blood was insulin-free in these diabetic dogs. Most of the animals were being treated with subcutaneous insulin, so that dilute exogenous insulin went to both sides. Thus, the advantage enjoyed by the liver tissue receiving splanchnic inflow was due to *other* factors but added to small amounts of insulin. The important conclusion from this kind of experiment is that insulin is not the only hepatotrophic factor.

Smith-Laing: It looks like an area to focus on, because at no other time have you shown a synergistic effect with insulin using other peptides which you might expect to find in splanchnic blood. But all the same there appears to be something in splanchnic flow that protects the liver.

Starzl: I think that is exactly right. The obvious candidate substances are other hormones, or perhaps nutrients absorbed from the intestine.

Smith-Laing: Could there be a 'pecking order' in responsiveness? If you don't have insulin, can the liver respond to something else to which it wouldn't normally respond in the presence of insulin?

Starzl: We think there probably is a pecking order and that insulin may determine that order. That would be the implication of some of Dr Francavilla's findings, such as the protective influence of insulin on adenylate cyclase activity, as was discussed earlier (p. 127).

Leffert: What is the likelihood that splanchnic blood supplies prostaglandins

from adipose stores in the splanchnic bed? We found, using the *in vitro* fetal liver system, that nanogram levels of PGE_1 stimulate DNA synthesis (Leffert *et al.* 1976). Dr L. Levine measured 'PGE_1' for us in portal blood and found concentrations to be elevated after 70 % hepatectomy about three-to four-fold.

Steiner: I shall be describing some work later (pp. 229–236) which suggests that there are factors or processes that 'down' regulate liver mass as well as 'up' regulate it. In Dr Starzl's experiments it may be that the atrophied side of the liver is being suppressed by the normal side. Then when you take away the stimulus that maintains the relative hypertrophy on the normal side, you create a new balance which now tends to bring up the atrophic side while the hypertrophic side slides back. The question is whether factors arriving in the intestinal blood are the signals for this, or whether there are more intrinsic mechanisms that sense liver size in addition.

Starzl: In that connection, I would like to point out a fundamental difference between the two split liver preparations which we used. In one (partial transposition), all the splanchnic blood goes to one liver side and all the vena caval flow goes to the other. With the second model (splanchnic division), insulin-rich blood goes to one side and nutrient-rich intestinal blood to the other.

After partial transposition, the presumably deficient qualities of systemic blood are devastating on the side receiving it, in that atrophy and other changes occur with great rapidity and completeness. Several years ago we suggested (Starzl *et al.* 1973) that there might also be 'down regulation' by something in the systemic venous blood (as opposed to deficiency of substances) to account for the accelerated involution. Because the transposition was done above the adrenal glands, an obvious possibility was that adrenaline had such a 'down regulating' influence.

Popper: I would like to raise the question of different effects on different hepatic cells. I believe your slides showed less reticulum framework in the non-atrophic area. This could be explained by an approximation of the reticulum fibres as a result of atrophy of the cells. Also, the portal tracts looked darker on the atrophic side, which suggests new formation of fibres stimulated by deprivation of hepatotrophic substances. This would raise the interesting possibility that active fibrogenesis is associated with atrophic hepatocytes.

Porter: I am sure you are right. In the silver preparations there is an increase in silver-staining fibres round the portal tract on the atrophic side.

Bloom: Clinically, one sees patients with livers which are large because they have acromegaly. As far as I know the livers really are enlarged. One wonders what happens when growth hormone is added to isolated liver cell preparations. If it is not particularly potent there and doesn't act directly on hepatocytes, is

this an indication that the *in vitro* preparations may not allow us to detect certain types of growth factor?

McIntyre: John Jenkins (personal communication 1977) and Mike Davis of St George's Hospital have been transplanting pituitary tumours in rats. The livers of the recipients became enormous with intense mitotic activity. The tumours secreted prolactin as well as growth hormone and the insulin levels in the recipient rats were 3–4 times normal. Apparently the livers were 3–4 times larger than normal.

Bloom: Prolactinaemia does not produce this effect in man. The high levels of insulin might be due to diabetes – for example, insulin resistance. If this were the hepatotrophic factor, I think you would expect to find big livers in obese people or those with Cushing's syndrome. Thus one might suspect that the observed effect was due to growth hormone alone.

Freychet: Did these transplantable tumours also produce ACTH? Some pituitary tumours in the rat secrete ACTH, prolactin and growth hormone.

McIntyre: No, and steroid levels in the tumour-bearing rats were normal.

Leffert: We reported (Leffert 1974*a*) that an NIH Study Section preparation of somatotropin enhanced the action of insulin and glucocorticoid in stimulating DNA synthesis initiation in the fetal liver culture system. We have since shown that this enhancement was due to a contaminant of the preparation (Leffert & Koch 1977). We were assured that it was pure; but when we obtained pure growth hormone (from Dr W. Vale) it was inactive in our assay. As far as I know, no one has found a convincing direct effect of growth hormone in stimulating DNA synthesis in animal cells *in vitro*.

Bloom: You can see it *in vivo* but not *in vitro*, perhaps?

Popper: In leukaemia the liver is enlarged even if leukaemic cells do not accumulate in the liver, for instance after antileukaemic therapy. The enlargement is explained by both hyperplasia and hypertrophy of the hepatocytes (Wolf & Klemperer 1955). This suggests the presence of growth factors in leukaemia. As a matter of fact, an enlarged liver with normal hepatocytes and without inflammatory cells suggests leukaemia.

Leffert: Many tumours produce what many people think are hepatomitogenic peptides (Morgan & Cameron 1973; Ibsen 1977).

Bloom: The results of Jenkins and Davis are in line with what happens clinically in man. Acromegalic patients have large livers which are not infiltrated or in any other way diseased.

Alberti: Did they measure somatomedins?

McIntyre: I don't know. Has anyone tested somatomedins?

Leffert: The C form of somatomedin promoted DNA synthesis initiation in the fetal liver system and it was additive with insulin (Leffert 1974*a*). We tested

somatomedin because of its insulin-like effects (Van Wyk *et al.* 1974) and for other experimental reasons described elsewhere (Leffert 1974*b*; Koch & Leffert 1974).

Discussion

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