

Evidence that Host Size Determines Liver Size: Studies in Dogs Receiving Orthotopic Liver Transplants

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Orthotopic liver transplantation was performed in two groups of dogs; Group I animals consisted of large dogs that served as recipients of livers obtained from smaller dogs while Group II animals consisted of dogs that received liver from donor dogs of nearly the same size. The small-for-size livers transplanted into the Group I dogs rapidly increased in size over the course of 2 weeks until they achieved a size equal to that originally present in the larger recipient dogs. In contrast, the livers transplanted into dogs of the same size as the donors underwent some degree of atrophy. In both groups of animals, plasma levels of insulin and glucagon and hepatic (graft) activities of thymidine kinase and ornithine decarboxylase were followed serially. The only difference between the two groups of animals for these measures was that the ornithine decarboxylase activity rose to a greater degree in the liver that underwent graft enlargement. These data suggest that recipient size determines, at least in part, liver graft size once it is transplanted. These data also suggest that of the parameters followed, only ornithine decarboxylase activity parallels the finding of growth of the transplanted liver.

Occasionally, in clinical liver transplantation, a liver from a small donor is transplanted into a much larger recipient because of urgent need and inability of the transplanting surgeons to identify a more appropriate donor. In such cases, we have noted that the intact transplanted organ rapidly increases in size to achieve a size comparable to that of a normal liver within a normal individual of the size and age as the recipient over a period of 2 weeks (1). To investigate the mechanisms responsible for this phenomenon, the following studies were performed.

MATERIALS AND METHODS

Animals. Orthotopic liver transplantation (OLT_x) was performed in two groups of young adult mongrel dogs using a modification of the procedure originally described by Starzl et

al. (2). The first group of experimental animals consisted of seven large dogs weighing 23.7 ± 0.8 kg (S.E.). All dogs in this group underwent hepatectomy and were given allograft organs obtained from smaller dogs (mixed breed young adults) that weighed 13.2 ± 0.4 kg. A second group of animals acting as controls underwent hepatectomy and was transplanted with organs obtained from young adult animals of the same size (recipient animal's weight = 18.7 ± 4.6 kg; donor animal's weight = 19.5 ± 4.5 kg). Prior to OLT_x, all dogs had a computed tomography (CT) scan of their liver to determine their hepatic volume using a recently described technique (3).

Experimental Design. At the time of hepatectomy and immediately upon removal from the animal, the weight of the livers of all donors and recipient animals was determined by weighing the organs to the nearest gram. Following OLT_x, serial liver volume determinations were obtained by repeat CT scanning procedures. In addition, blood samples were obtained at predetermined regular intervals for measurement of total bilirubin, ALT (or SGPT), glucagon and insulin levels at 0, 2, 4, 6, 8, 10, 12, 14 and 30 days. At termination of the experiment, the weight of the allograft liver to the nearest gram was determined immediately upon sacrifice, for all of the animals, by reweighing the removed allograft organ. All animals were treated with cyclosporine A at a dose of 20 mg per kg per day to prevent allograft rejection.

The entire protocol was repeated in an additional group of separate animals for the determination of the hepatic content of both ornithine decarboxylase and thymidine kinase and an examination of the histopathologic consequences of liver transplantation.

Liver CT Scanning. CT scans of the liver for volume determinations were obtained on a General Electric model 9800 CT scanner. Serial transverse scans at 1 cm intervals from the dome of the liver through the most inferior portion of the organ were obtained with the dog's respiration suspended in full expiration. Using a track ball device, the perimeter of each slice of the liver was outlined, and the enclosed area was calculated electronically. Total hepatic volume was obtained by summing the volumes of individual slices. Intraobserver error for measuring a single slice or for measuring the total liver volume was $\leq 5\%$.

Measurements

1. Serum total bilirubin and ALT levels were measured in the clinical laboratories of Presbyterian-University Hospital using standard methods.
2. Plasma insulin levels were determined by radioimmunoassay using an insulin kit obtained from Serono Diagnostics (Boston, Mass.). The detection limit of the assay used was $5 \mu\text{U}$ per ml. The intra- and interassay variations

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- of the assay method for normal level samples were 6 and 10%, respectively.
- Plasma glucagon levels were determined by radioimmunoassay using a glucagon kit obtained from Serono Diagnostics (Boston, Mass.). All samples for glucagon measurements were collected in chilled tubes containing 500 units trasylol and 1.2 mg sodium EDTA per ml blood collected. The detection limit for this assay was 15 pg per ml. The intra- and interassay variations for normal samples were 7 and 12%, respectively.
 - Ornithine decarboxylase activity was determined utilizing the method of McGowan and Fausto (4). For this assay, 1 gm liver tissue was homogenized immediately in 4 ml buffer consisting of 0.25 M sucrose, 1.5 mM Na₂EDTA, 10 mM mercaptoethanol and 10 mM Tris-HCl buffer (pH 7.4). The resultant homogenate was centrifuged for 50 min at 105,000 × g at 4°C. An aliquot of the cytosolic supernatant solution (0.4 ml) was added to 0.5 ml ornithine decarboxylase incubation buffer [10 mM Tris-HCl (pH 8.0), 0.2 mM pyridoxal phosphate, 5 mM dithiothreitol and 1.5 mM L-ornithine] and preincubated for 5 min at 37°C, at which time 100 μl (0.5 μCi) [1-¹⁴C]ornithine was added to the reaction vessel. The vessel was capped, and the reaction was maintained at 37°C for 60 min until termination by the addition of 100 μl concentrated trichloroacetic acid. The CO₂ released from the ornithine was trapped in ethanolamine/ethylene glycol (2/1) and subsequently measured in 10 ml of ACS scintillation fluid by counting radioactivity on a Tricarb 460 liquid scintillation counter. The detection limit for this assay is 40 cpm per hr per mg protein. The coefficient of variation for identical samples assayed on different days is <9% (n = 10).
 - Thymidine kinase activity was determined utilizing the method of Kahn et al. (5). Specifically, 0.5 gm liver was homogenized immediately in 5 ml 0.1 M Tris-HCl buffer (pH 8.0), and the resultant homogenate was centrifuged for 50 min at 105,000 × g at 4°C. The cytosolic supernatant (0.2 ml) was incubated with 800 μl thymidine kinase incubation buffer consisting of 50 mM Tris-HCl (pH 8.0), 5 mM ATP, 3.6 mM MgCl₂ and 0.01 mM [³H]thymidine for 10 min at 37°C. The reaction was stopped by immersion of the reaction vessel in boiling water for 2 min. Control assays consisting of incubation mixtures which had been immediately placed in boiling water were performed for all samples. Denatured protein was removed by centrifugation at 100 × g for 10 min and 100 μl of the resultant supernatant were spotted on DEAE cellulose paper. The paper was then washed consecutively in 1 mM ammonium formate (5 min), distilled water (3 min) and the sequence was repeated again. The washed paper was placed in a scintillation vial to which 1 ml 0.1 M HCl and 0.2 M KCl was added. The vial was capped and shaken for 15 min. ACS (10 ml) was then added and the radioactivity counted on a Tricarb 460 liquid scintillation counter. The detection limit for this assay is 15 fmoles per mg per min. The coefficient of variation for similar samples assayed as different days is <11% (n = 10).
 - Protein determinations were performed using the method of Lowry (6) using bovine serum albumin as the standard.

Histological Preparation and Examination of the Liver. At the time of sacrifice, specimens of liver tissue were obtained and fixed in 10% formalin and then embedded in paraffin. Sections 4 μm thick were cut and stained with hematoxylin-eosin. The sections were examined for evidence of regeneration as evidenced by the numbers of mitoses and multinucleated hepatocytes present within representative sections of Zones I and III.

Statistical Analysis. All results are reported as mean values ± S.E. All statistical analyses were performed using a two-tailed Student's t test. A p value < 0.05 was considered to be significant.

RESULTS

In the first group of seven large dogs having a mean body weight of 23.7 ± 0.8 kg and a mean hepatic weight of 767 ± 48 gm, a liver obtained from 1 of 7 smaller dogs having a mean body weight of 13.2 ± 0.4 kg and a mean liver weight of 363 ± 20 gm was engrafted. The subsequent growth of the small-for-size liver in the larger recipient animals was followed for various lengths of time. Three dogs were allowed to live for a full 30 days following OLTx, at which time they were killed and the liver grafts were removed and reweighed. The four other large dogs transplanted either were sacrificed or died at earlier time points (10, 11, 13 and 31 days). In each case, however, the graft liver was removed and reweighed. Figure 1 (Parts 1 and 2) demonstrates the serial liver volumes determined by CT scanning in two of the 3- to 30-day surviving dogs which received a small-for-size organ graft. The four animals that survived for shorter periods showed similar changes in liver volume over time and were examined after intervals of 10, 11, 13 and 31 days as noted earlier. Table 1 shows the growth of the transplanted small-for-size organs presented as per cent volume change over time for all seven of the animals studied.

In the second group of three animals having a mean body weight of 18.7 ± 4.6 kg and a mean liver volume of 482 ± 114 gm, the livers obtained from similar-sized dogs

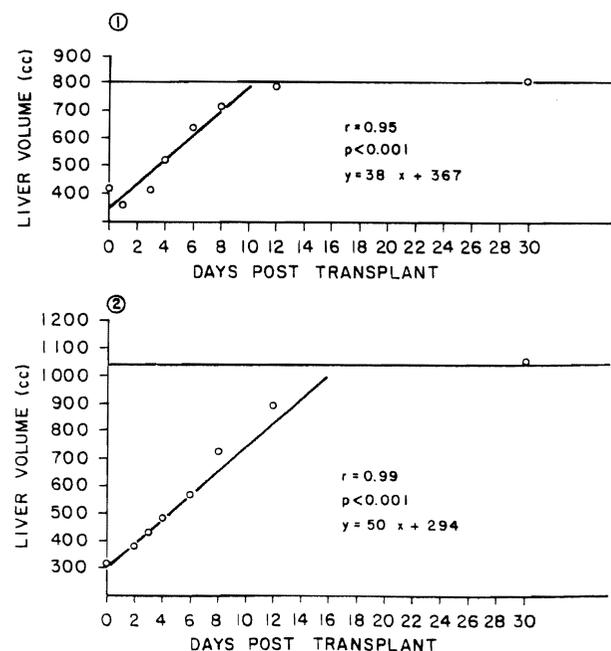


FIG. 1. Growth of a small-for-size liver transplanted into a larger recipient. Each component part identified as 1 and 2 shows the data for a separate experiment. The ordinate for each graph shows liver volume. The abscissa shows time postliver transplantation in days. The solid horizontal line represents the size of the recipient's own liver prior to hepatectomy. The open circles represent the liver volume of the small-for-size organ transplanted into the larger recipient. The oblique line represents the best line obtained using least squares analysis for fit of the data obtained.

TABLE 1. Growth of an intact small-for-size organ in a larger recipient animal

Day posttransplant	% increase in liver volume	n
2	33 ± 6	7
4	58 ± 8	7
6	125 ± 15	7
8	142 ± 12	6
10	164 ± 4	5
12	182 ± 8	4
14	233 ± 10	3
20	221 ± 12	3
30	208 ± 9	3

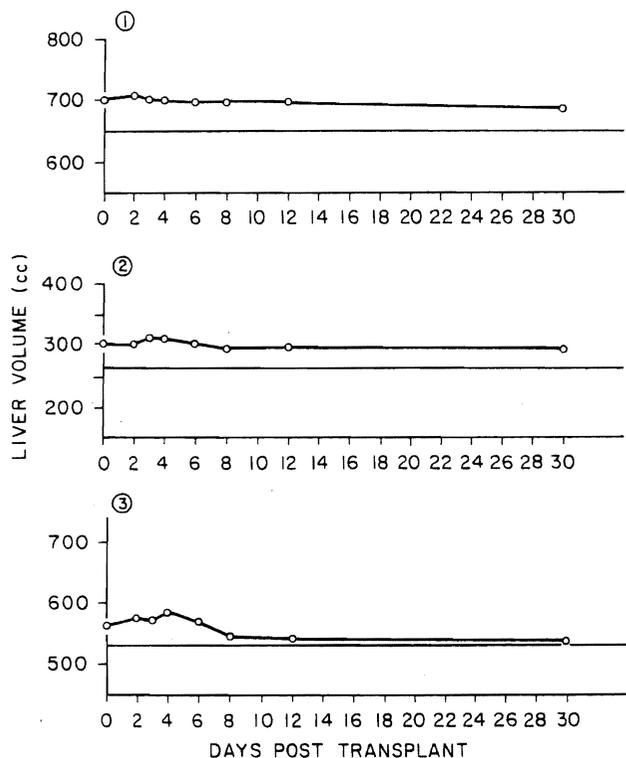


FIG. 2. Change in size of a normal liver transplanted into a dog of similar size. Each component part identified as 1, 2 or 3 represents the data for a separate experiment. The ordinate shows liver volume. The abscissa shows time in days following transplantation. The open circles represent consecutive values obtained for liver volume after transplantation. Note that in each case the transplanted liver atrophies slightly. The solid horizontal line represents the recipient animal's liver volume prior to transplantation.

having a mean body weight of 19.5 ± 4.5 kg and a liver weight of 523 ± 116 gm were used as the donor organs for OLTx. As can be seen from Figure 2 (Parts 1 to 3), none of the transplanted organs in the same-sized recipients increased in size. In fact, in the recipient, they all decreased slightly in size and weight with time.

As can be seen in Figure 3 (A and B), little or no changes in total bilirubin and a transient increase in ALT levels occurred in all of the animals studied. It should be noted that, although the bilirubin levels in the experimental group are uniformly greater than those in the control group for the first 12 days of the experiment, none of these differences are significant.

Similarly, plasma insulin and glucagon levels did not differ between the two groups, with insulin levels not changing over time and the glucagon level increasing 5-

fold within 48 hr in both groups but then returning to normal levels by 96 hr after transplantation (Figure 4, A and B).

Hepatic levels of thymidine kinase increased in both groups of transplanted dogs reaching peak levels in 3 to 4 days. No difference was seen between the two groups of transplanted animals, however, either basally or at peak levels following transplantation (Figure 5).

In contrast, although no difference was seen in the pretransplant basal levels of ornithine decarboxylase, the peak levels observed at 24 hr after transplantation were much greater in the experimental Group I animals than they were in the control Group II animals (Figure 6).

Histologically, the livers of the Group I animals all showed evidence of panlobular hepatic regeneration characterized by double and triple cell thick plates, numerous mitotic figures and moderate numbers of triple-nucleated hepatocytes. In contrast, the livers of the Group II animals showed less evidence for regeneration which was limited to the area of Zone III (Table 2).

DISCUSSION

The results of the present studies in dogs clearly demonstrate that the recipient's size determines the size of a transplanted liver in its new host. Moreover, they highlight the difference between the terms growth and

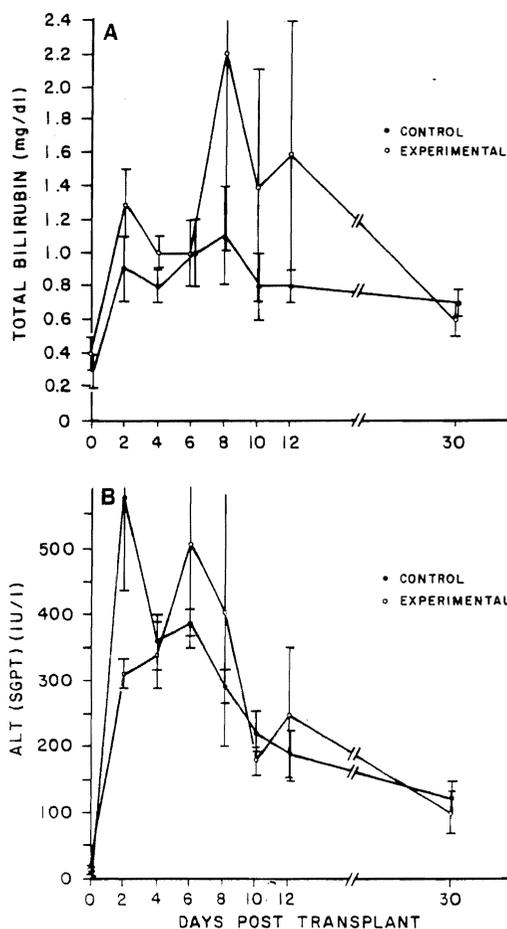


FIG. 3. Total bilirubin (A) and ALT (SGPT) levels (B) in serum of the two groups of animals studied. The experimental group received a small-for-size liver. The control group received a normal-sized liver. The abscissa shows time since transplantation.

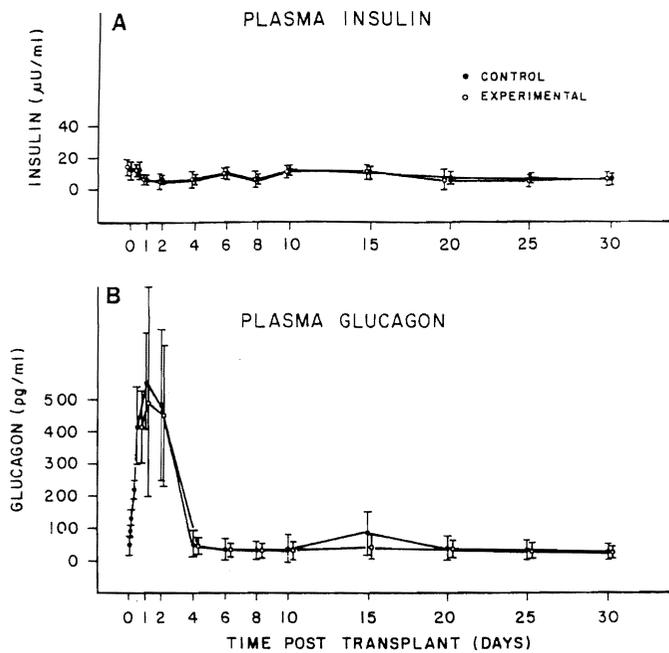


FIG. 4. Plasma insulin (A) and glucagon (B) levels in the two groups of animals studied. The *abscissa* shows time since transplantation.

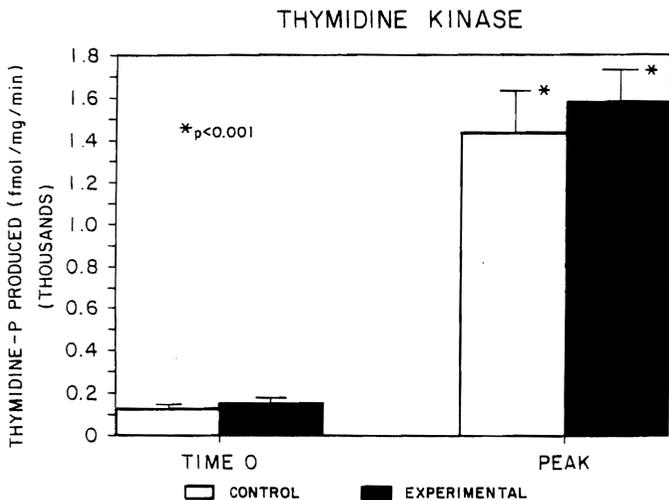


FIG. 5. Thymidine kinase levels in the liver of dogs receiving normal size liver at time of transplantation (controls) and small-for-size organs at time of transplantation (experimental group). Both basal pretransplant and peak values after transplantation are shown. The bars represent mean values and the brackets represent S.E. The asterisks denote that the peak levels of both groups are significantly greater than the respective basal levels ($p < 0.001$).

regeneration. In animals that receive a small-for-size donor organ, the liver rapidly increases in size or grows until it achieves a size nearly identical to that of the recipient's own liver prior to hepatectomy (Figures 1 and 7). In contrast, livers grafted into animals of the same size as the recipient actually atrophy slightly, although regeneration is evident in these livers (Figures 2 and 7). These quite different changes in graft liver size or growth occur despite similar changes in serum total bilirubin and ALT levels and plasma levels of glucagon and insulin. Moreover, they occur despite similar changes in the hepatic content of thymidine kinase activity, the rate-limiting enzyme in DNA synthesis, over time. The only parameter that distinguished between the two groups of animals, those receiving small-for-size livers that in-

creased in size and those that received normal-sized livers that atrophied, was the hepatic content of ornithine decarboxylase which increased in both groups of animals studied but more so in those given small-for-size donor organs. Thus, these data suggest that thymidine kinase may be a marker for regeneration but that growth

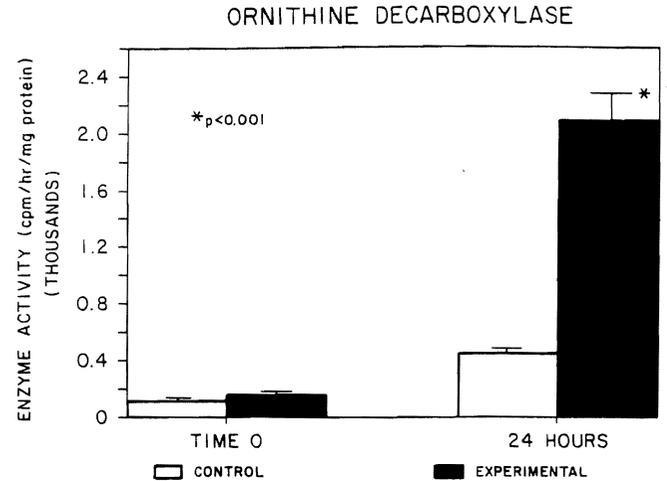


FIG. 6. Ornithine decarboxylase levels in the liver of dogs receiving normal size livers at the time of transplantation (controls) and small-for-size organs at the time of transplantation (experimental group). Both basal pretransplant and peak values after transplantation are shown. The bars represent mean values and the brackets represent S.E.

TABLE 2. Histologic evidence for regeneration in the liver grafts studied

	Small size donor group		Normal size donor group	
	Zone I	Zone III	Zone I	Zone III
Mitosis ^a	10 ± 1 ^{b,c}	15 ± 2 ^c	2 ± 1	5 ± 1
Multinucleated hepatocytes*	11 ± 1 ^{b,c}	24 ± 2 ^c	1 ± 0	3 ± 1

^a Per high power field.

^b $p < 0.001$ compared to Zone III within the same group.

^c $p < 0.001$ compared to the result for the same zone in the other group.

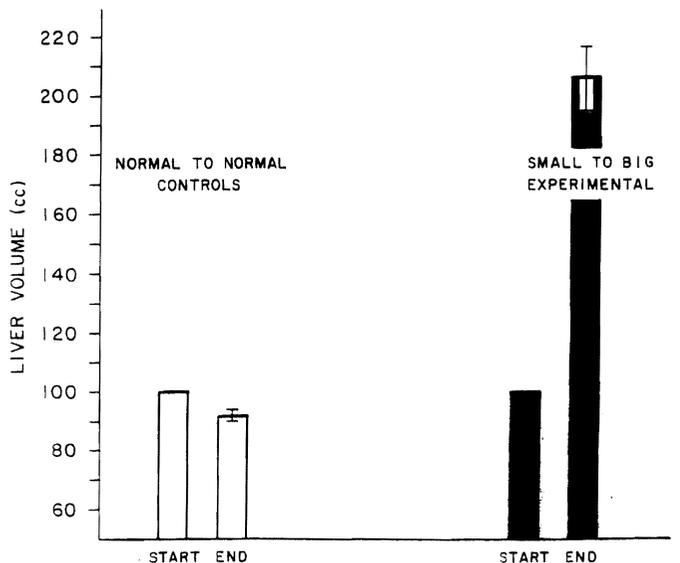


FIG. 7. This diagram shows the change in size of a transplanted liver either transplanted into a normal-sized animal (control) or into a larger recipient (experimental). The bars represent mean values and the brackets represent the S.E.

occurs only if ornithine decarboxylase is also increased above a certain level. Thus, ornithine decarboxylase which is known to be important in polyamine synthesis may be the putative initiator of growth. It is well-known that polyamines are involved in the regulation of rRNA synthesis and that blocking ornithine decarboxylase activity prevents regrowth of an injured organ presumably by preventing polyamine synthesis. (6).

These data suggest further that the recipient's size determines, in large measure, the change in size that a liver graft will experience following successful transplantation. Taken together, these data suggest rather strongly that the liver growth experienced by a transplanted organ is either signaled by or initiated by a marked increase in the hepatic content of ornithine decarboxylase and presumably, although not tested in the present study, by the polyamine content of the liver. In contrast, changes in the plasma levels of insulin and glucagon or changes in the hepatic content of thymidine kinase appear not to be important in this regard although the activity of this latter enzyme certainly increases and is required for hepatic regeneration.

These data do not negate the considerable data available suggesting that both insulin and glucagon, as well as a host of insulin-like growth factors, are necessary for or capable of initiating hepatic regeneration under certain circumstances (7-29). In contrast, the present data extend such data and demonstrate that should the hepatic mass be appropriate for the metabolic demands of the host that despite such signals hepatic regeneration leading to hepatic growth does not occur. In contrast, when the hepatic mass is too small (inadequate) to meet the metabolic demands of the host, signals such as insulin, glucagon and other putative growth factors initiate a cascade of intracellular events culminating in a dramatic increase in ornithine decarboxylase activity which is followed not only by hepatic regeneration replacing dead or dying hepatocytes as seen in the controls, but also by actual hepatic growth as evidenced by a marked increase in the usual signs of hepatic regeneration but also and more importantly by an actual increase in the hepatic mass as evidenced by hepatic volume and weight measures.

The specific mechanisms responsible for the marked increase in the hepatic content of ornithine decarboxylase in enlarging liver grafts is not as yet known. Moreover, the sequence of events that either initiates or allows hepatic growth to occur in response to an increase in hepatic content of ornithine decarboxylase in a transplanted liver are also as yet poorly understood. Nonetheless, it appears that an increase in ornithine decarboxylase and, presumably, polyamines within the liver occurs and acts as the signal initiating hepatic growth such that the liver graft enlarges to a size that approximates the size of a normal liver expected to be present in the recipient.

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