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portacaval shunt operation 2 weeks before (group 1: n=13). Cell-free medium was used for the control group instead of cells (group 2: n=10). The recipient rats were fed regular diet and AsA-containing water (3g/l) for the following 4 weeks and thereafter AsA-poor diet and tap water. In the organ transplantation groups, an auxiliary heterotopic partial liver transplantation (HLT) was performed, where approx. 20% of the whole liver was transplanted with the portal venous blood diverted to the graft. The experimental rat (group 3: n=6) and the control rat (group 4: n=6) were transplanted with +/+ and od/od donor livers, respectively, and the external AsA supplementation was ceased immediately after the transplantation. No other stimulation such as hepatectomy or bile duct ligation was added in any group. At 0, 2 and 4 weeks after the cessation of AsA supplementation, the AsA concentration in the plasma was measured by spectrophotometric method. Results are shown as means ± SEM and the Mann-Whitney *U* test was used for analysis.

Results. [HTX] The histological examination revealed that each PGA matrix contained numerous layers of healthy hepatocytes at 8 weeks after the transplantation. After the cessation of the external AsA supplementation, the plasma AsA concentrations were 7.65±0.43 and 7.04±0.55 μg/ml at week 0 (N.S.), and 0.90±0.11 and 0.43±0.10 ($p<0.01$) at week 4, in group 1 and 2, respectively. The ratios of the body weight at week 4 to the weight at day 0 were 1.12±0.03 and 0.92±0.02 in group 1 and 2, respectively ($p<0.05$). However, all of them began to show the physical signs of scurvy. [HLT] The rats in group 4 showed a rapid loss of the body weight (0.76±0.04 at week 3). On the other hand, all group 3 rats maintained normal appearances and grew normally. The body weight ratio was 1.16±0.08 at week 4 and 1.52±0.17 at week 9. The plasma AsA concentration at week 4 was 2.96±0.36 μg/ml, which was not different from the value by an orthotopic whole liver transplantation (2.67±0.42).

Conclusions. The ODS rat model is useful to evaluate the effective-ness of HTX. Hepatocytes were successfully implanted in the biodegradable PGA matrices using only portacaval shunt as a hepatotrophic stimulation. HTX succeeded in maintaining a significantly higher plasma AsA concentration compared to the control. HLT was able to normalize the plasma AsA concentration with a 20% of normal hepatic mass. Additional studies are necessary to improve our system of HTX to achieve comparable results to the whole or partial organ transplantation.

82 Effects of Cotransplanted Pancreatic Islets on hepatocyte Transplantation in the Rat

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Introduction. It is well known that diverting portal venous blood flow from the liver with a subsequent inadequate supply of pancreatic hormones causes liver atrophy and dysfunction of hepatocellular metabolism. Recent studies in our laboratory demonstrated the adequate response of the hepatocytes transplanted to the spleen when the animals were subjected to several regenerative stimuli (partial hepatectomy -PH- and cyclosporine A -CsA-). Several authors have shown that clusters of hepatocytes transplanted under the kidney capsule of rats quickly degenerate if transplanted alone (HCT), while they thrive when transplanted in conjunction with islets (IHCT). In cases of great functional demand -like extended hepatic resections and acute liver failure- the metabolic capacity of the hepatocytes is submitted to a "limit" situation. Therefore, we have examined whether intrasplenic islets isografts do have a beneficial effect on the regenerative capacity of the hepatocyte graft under several functional stimuli.

Methods. Male Wag rats weighing 250 g were used as donors and recipients for both islet and hepatocyte transplants. The cotransplant is composed by 20 million of hepatocytes and 300 islets. The hepatocytes were obtained according to the technique of Seglen. The pancreatic islets were isolated by collagenase digestion followed by centrifugation on a discontinuous Dextran gradient and hand picked while stained with Dithizone. The presence of islets in the spleen was verified by an immunohystochemical procedure using an anti-insulin monoclonal antibody. Eight groups of animals were considered: HCT (n=5), IHCT (n=5), HCT+PH (n=10), IHCT+PH (n=10), HCT+CsA (n=5), IHCT+CsA (n=5), HCT+PH+CsA (n=10), IHCT+PH+CsA (n=10). The hepatocytes' regeneration rate 24 hours after the transplants was assessed by quantifying DNA content. The results were analyzed with the "rank sum test".

Results. PH has increased the hepatocytic regeneration in the liver (42%) and in the spleen (27%). CsA has shown a similar effect (20%; 38%). The combination of both regenerative stimuli showed an additive effect in the liver (54%), but not in the

spleen (35%). However, the addition of islets to the HCT neither has increased the regenerative response following regenerative stimuli in the liver, nor in the spleen. It is remarkable that in the spleen the regenerative rate was nearly the same irrespective to the stimuli induced, which didn't happen in the liver.

Conclusions.

1. The cotransplantation of islets and hepatocytes in the spleen has not affected the regenerative response of the hepatocytes.
2. We have not found a synergistic effect of both regenerative stimuli in the spleen. The threshold of this response seems to be reached by the splenic hepatocytes and there is no demonstrable beneficial effect exerted by the islets of Langerhans.

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83 Improvement of Biocompatibility and Graft Function of Alginate-Polylysine Microencapsulated Islet Grafts Applying Purified Alginates

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Microencapsulation allows for successful transplantation of allo- or xenogeneic islet grafts without rejection. However, the graft functions only for a limited period of time. Usually, graft failure is interpreted as the consequence of a non-specific foreign body reaction against the microcapsules resulting in fibrotic overgrowth of the capsules and necrosis of the islets. It has often been suggested that the foreign body reaction is initiated by impurities present in crude alginate preparations. In this study we investigate if the purity of the alginate applied improves the biocompatibility of alginate-polylysine microcapsules. Alginates were purified by filtration, extraction and precipitation followed by several washing steps. Microcapsules were prepared and implanted in the peritoneal cavity of normoglycemic AO-rats and retrieved 1,2,3,6,9,12 months after implantation. With crude alginates all capsules were overgrown within one month after implantation. However, with purified alginates only a small portion (2-10%) of the capsules was overgrown even at 12 months after implantation. Next, we studied the function of islet allografts in capsules prepared of purified alginates. Recipients were subjected to intravenous (IVGTT) and oral glucose tolerance tests (OGTT) 4 to 6 weeks after implantation. All became normoglycemic within 5 days after implantation, but became hyperglycemic after 6 to 18 weeks. During IVGTT and OGTT recipients were found to be glucose intolerant with maximal blood glucose of, respectively, 11.7 ± 0.4 mM and 8.8 ± 0.4 mM, while no elevation of plasma insulin levels were observed. After graft failure, capsules were retrieved (90-100%) from the peritoneal cavity by peritoneal lavage and examined histologically. A small portion of 8-31% of the capsules proved to be overgrown by fibrotic tissue. Our results indicate that purification of the alginate improves the biocompatibility of alginate-polylysine microcapsules. However, purification of the alginate does as such not result in indefinite survival of the islet graft.

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84 Newly Developed Xenogeneic Pig Liver Perfusion System for Utilization as an Extracorporeal Liver Assist Device

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Introduction. Xenogeneic liver perfusion has been re-evaluated as a bridge to liver transplantation for fulminant hepatic failure. In order to achieve long-duration perfusions, a new and simplified xenogeneic pig liver perfusion system was developed in view of simulating physiological conditions. In this study, we examined the suitability of this perfusion system as an extracorporeal liver assist device.

Material and Methods. Several procedures were manipulated in our newly designed xenogeneic pig liver perfusion system;

1) complete in situ isolation of the liver and flushing with ice-cold Ringer's solution via portal vein (PV) and hepatic artery (HA) at procurement
2) short cold ischemic time (27.4±5.6 minutes)
3) reperfusion with fresh human blood through both PV and HA by two separate roller pumps
4) immersing the liver in a 37ºC water bath filled with colloidal solution and maintaining portal and arterial oxygen partial pressures within 80mmHg and 150mmHg, respectively, for physiological conditioning