Survival from Hepatic Transplantation

Relationship of Protein Synthesis to Histological Abnormalities in Patient Selection and Postoperative Management

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Forty-one patients, all in end stage hepatic failure, underwent 46 liver transplantations with a long-term survival rate of 63%. Six patients died of uncontrollable bleeding due to primary graft malfunction at or immediately after operation. Nine died early or late with overwhelming infection. In addition to clinical assessment, needle liver biopsy, central plasma clearance rate of amino acids (CPCR-AA), and routine "liver function tests" were employed to aid in selection of patients for transplantation and for guidance in postoperative management. Although liver biopsies usually afforded an exact diagnosis, neither they nor the routine liver function tests quantitated the extent to which hepatocyte function was impaired. CPCR-AA, which measures the rate of amino acid uptake by the liver and other central tissues for oxidation, gluconeogenesis, and protein synthesis was 91 ± 9 ml/M²/min in the preoperative transplant group. This compares with a value of 97 ± 16 in a previously studied series of cirrhotics who died following other forms of surgery and a CPCR-AA of 220 ± 26 ml/m²/min in those who survived. In addition, the preoperative CPCR-AA was found to correlate with the *in vitro* hepatic protein synthetic rate of slices from the resected recipient liver ($r = 0.72$, $p < 0.02$). After operation, serial hepatic needle biopsies were classified by histology into four grades of injury, ranging from normal liver transplant (Grade I) to mild hypoxic or rejection injury (Grade II), viral hepatitis (Grade III), and severe hypoxic or rejection injury (Grade IV). Significant relationships of the histological grades to ultimate mortality, CPCR-AA, and prothrombin times were found. CPCR-AA and prothrombin time correlate inversely ($r = 0.57$, $p < 0.001$), further demonstrating the relationship of CPCR-AA to protein synthesis of clotting factors. These patterns of posttransplant response were delineated by serial CPCR-AA values. "Early" responders had values over 290 ml/M²/min and all survived. Twelve patients with delayed response were characterized by values of 150 ± 12, rising to over 200 ml/M²/min after 2 weeks. Two who failed to increase CPCR-AA died. In six "poor" responders, CPCR-AA with Grade IV injury remained below 110 ml/M²/min. All died except for one whose CPCR-AA subsequently rose following retransplantation. It is concluded that percutaneous hepatic needle biopsies and CPCR-AA measurements in combination are of proven value, not only in understanding the nature of injury and functional impairment of the liver, but are also important as guides to selection of patients and for their posttransplant management.

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CLINICAL TRANSPLANTATION of the liver, made possible by advances in surgical techniques[1,2] and improved methods for the control of rejection,[3,4] is becoming an effective means for treating end stage acute or chronic liver failure. Although hepatic insufficiency is characterized clinically by protein depletion, jaundice, ascites, and bleeding, specific measurements of important liver functions are needed both to aid in selection of patients for the irreversible transplant procedure and for guidance in their postoperative management. Among the more important metabolic roles of hepatocytes in daily existance or in the pattern of survival from trauma and infection is protein synthesis. In the liver and other central tissues, amino acids are employed, not only for gluconeogenesis and oxidation to produce energy but, more importantly, for accelerated synthesis of "acute phase" and numerous regulatory proteins required for immunocompetence and the maintenance of cellular function. Inability to mount this response, whether from liver failure or other causes, usually results in overwhelming infection, multisystem failure, and death.[5,6]

To examine this function, the central plasma clearance rate of amino acids (CPCR-AA) was developed to assess the uptake of amino acids by the liver and other central tissues including spleen, lymph nodes, bone marrow, and wound, which participate in this metabolic reaction to

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TABLE 1. *Preoperative Diagnoses and Clinical Data of Hepatic Transplant Recipients**

Diagnosis	Primary Biliary Cirrhosis	Chronic Active Hepatitis	Submassive Hepatic Necrosis	Sclerosing Cholangitis	Alcoholic Cirrhosis	Miscellaneous† Hepatic Disease	Total All Diagnoses
Number of Patients	17	8	6	6	2	2	41
Age	43.9 ± 4	29.4 ± 7	43.8 ± 7	35.2 ± 6	44.5 ± 3	49.5 ± 0.7	43.6 ± 2.2
Male/female	4/13	5/3	0/6	5/1	2/0	0/2	16/25
White cell count (10^3)	7.5 ± 1	7.6 ± 0.8	8.9 ± 2.2	11.0 ± 3.5	8.1 ± 4.3	9.5 ± 0.1	8.5 ± 0.7
Serum albumin (g/dl)	2.9 ± 0.2	2.7 ± 0.2	2.7 ± 0.2	2.2 ± 0.2	2.5 ± 0.1	2.5 ± 0.4	2.7 ± 0.1
Total bilirubin (mg/dl)	11.8 ± 1.9	19 ± 6	29 ± 4	27 ± 6	18 ± 4	10 ± 13	19 ± 2
Prothrombin Time (seconds)	14 ± 0.4	18 ± 2	28 ± 5	15 ± 1	21 ± 3	14 ± 1	17 ± 1
SGOT (units)	136 ± 2	126 ± 27	251 ± 137	220 ± 65	56 ± 10	33 ± 28	154 ± 23
Alkaline phosphatase (units)	737 ± 130	320 ± 76	141 ± 20	697 ± 237	76 ± 11	214 ± 72	481 ± 72
Encephalopathy present	35%	50%	100%	33%	50%	0%	46%
Infection present	12%	38%	50%	17%	50%	50%	37%
CPCR-AA ($ml/M^2/min$)	88 ± 11 (14)	113 ± 26 (5)	78 ± 60 (3)	92 ± 19 (3)	173 (1)	84 ± 3 (2)	91 ± 9 (28)

* Mean ± standard error (number of patients if different from line 1).

† Polycystic disease (1), toxic drug (1).

stress. Previous studies revealed significantly higher values of CPCR-AA and hepatic protein synthesis in patients who survived trauma[16] or sepsis[8] than in those who subsequently died. The CPCR-AA of noninfected cirrhotic patients surviving surgery was also significantly elevated above normal in a fashion similar to that of infected patients with normal livers. Furthermore, the mean preoperative CPCR-AA of cirrhotic patients who survived portacaval shunts or other surgical procedures was found to be more than twice that of those who died after operation ($p < 0.001$).[7] The significance of CPCR-AA as an indicator of hepatic protein synthesis was established in trauma, sepsis, and liver failure by correlating the immediate preoperative CPCR-AA values in numerous patients with the rates of protein synthesis in slices of liver biopsies incubated *in vitro*.[7,8]

To assess the value of amino acid clearance, both in the selection of patients for liver transplantation and in their posttransplant management, serial studies were carried out in a consecutive series of 41 adult patients operated on since July 1983. This group, terminally ill with liver failure, underwent 46 hepatic transplants, with an overall mortality of 37%. After operation, CPCR-AA was compared at intervals with the histological alterations observed in percutaneous needle liver biopsies and with other more routine clinical measurements of the transplanted liver function. Clinical patterns of response emerged, differentiating transplanted livers functioning in a satisfactory manner from those damaged by the procurement/preservation process, rejection, or viral infection. These patterns of amino acid metabolism, coupled with histologic biopsy results, were further examined to determine their value as guides for retransplantation or for more aggressive treatment of rejection. The relationship of CPCR-AA to protein synthesis and the importance of each to defense against infection are also established in this seriously ill group of patients.

Methods and Materials

Patients

Liver transplantation has been carried out at the New England Deaconess Hospital since July 1983 as part of a city-wide consortium effort known as the BCLT (Boston Center for Liver Transplantation).[9] Based on histories, physical examinations, percutaneous needle biopsies of the liver, and serologic determinations in the clinical laboratories, the preoperative diagnoses in 41 adults who underwent liver transplantation are presented in Table 1.

Donor livers were harvested and transplanted into the recipients by the methods previously described.[1] The total cold ischemic time of the donor livers ranged from 2 hours and 45 minutes to 9 hours and 33 minutes (mean: +5 hours and 41 minutes). Venovenous bypass was employed routinely to avoid venous hypertension in the inferior and splanchnic portions of the body.

After operation, the patients were administered low dose methylprednisolone and cyclosporine daily,[10] with dosage adjustments guided by liver biopsy and plasma radioimmunoassay cyclosporine level determinations. Rejection episodes were managed by the addition of azathioprine (1–2 mg/kg daily) or pulses of 500–1000 mg of methylprednisolone for 1–3 consecutive days. Patients with severe or refractory rejection on biopsy were ran-

domized to receive either a 10-day course of OKT3 monoclonal antibody or an additional 3–5 days of bolus methylprednisolone.

In addition to vital signs and hemodynamic measurements, bile output and urine production were recorded at standard intervals. Daily hematological and chemical measurements included bilirubin, coagulation factors, hepatocellular enzymes, BUN, creatinine, total protein, and albumin.

Liver Biopsies

Before operation, needle biopsies were employed to aid in establishing the diagnosis and degree of liver impairment, but only when coagulation parameters made the risk of the procedure low. In every instance, the resected liver was examined by the Department of Pathology. The diagnoses are presented in Table 1.

In the postoperative period, percutaneous needle biopsy was performed through a portion of the abdominal wound left open for direct visual inspection. Biopsies of the allograft were obtained by protocol at intervals ranging from 3 to 10 days. A portion of the specimen was fixed in alcohol zinc formol solution and processed for routine histological examination using hematoxylin and eosin. Each specimen was examined by two of the authors (UK and RJ) to be classified into one of six categories according to previously described histological criteria,[14,15] examples of which are shown in Figure 1. For purposes of comparing the degree of functional impairment, various categories are combined to form four grades of liver injury.

Grade I: normal transplanted liver. Minimal portal cellular infiltrate and well preserved hepatic architecture.

Grade IIa: moderate ischemic injury. Moderate hepatocyte swelling and degeneration, vascular congestion, and lobular infiltration by polymorphonuclear leukocytes.

Grade IIb: moderate rejection. Lymphocyte infiltration in portal triads and centrilobular regions with centrilobular congestion and slight hepatocyte swelling and degeneration.

Grade III: viral hepatitis. Nuclear inclusion bodies associated with evidence of microabscess formation (CMV) or hepatic necrosis (HSV).

Grade IVa: severe ischemic injury. Extensive hepatocellular necrosis, diffuse infiltration by polymorphonuclear leukocytes, and thrombosis of microvasculature.

Grade IVb: severe rejection. Extensive portal and lobular lymphocytic infiltration associated with hepatocellular necrosis.

Amino Acid Metabolism

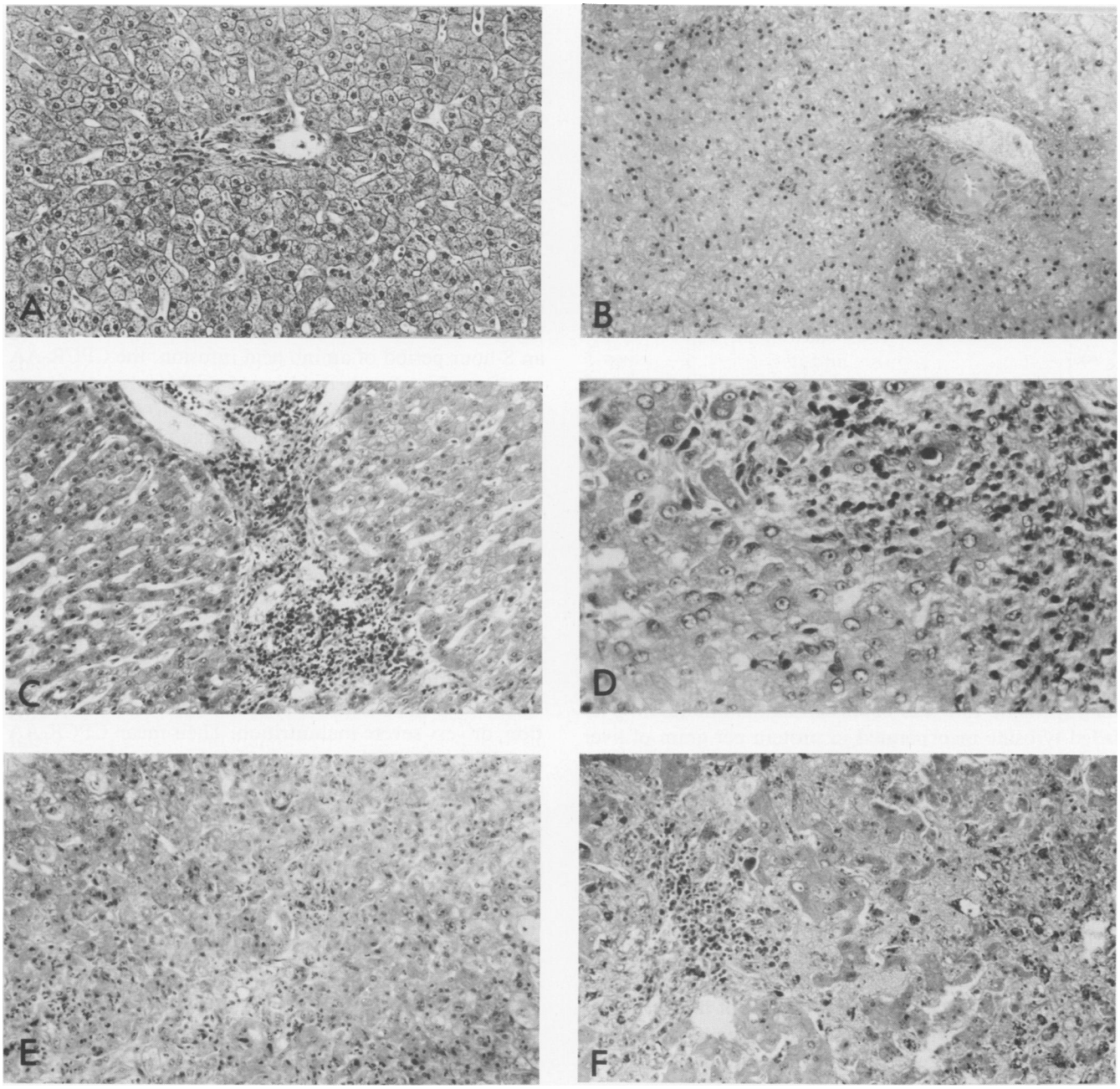
Preoperative measurements of amino acid plasma concentration, peripheral production, and CPCR-AA were made in the resting state following an overnight fast. In order to stress the hepatic amino acid metabolic function, the procedure was repeated in five patients following intravenous infusion of 500–800 ml of 4.5% Freamine II® solution during an 8-hour period. After operation, heparinized blood samples were obtained from an artery and a femoral vein on day 2 and at variable intervals thereafter.

Plasma AA concentrations. Five ml blood samples from both the femoral artery and femoral vein were drawn into heparinized tubes. The plasma concentrations of amino acids shown in Figure 2 were measured employing a Beckman Model 119CL Amino Acid Analyzer (Beckman Instruments, Inc., Fullerton, CA).

Peripheral production of amino acids. The rates of release of each amino acid and the sum of all amino acids from one leg were determined by the product of arterial-venous plasma difference (ΔA-FV) multiplied by the estimated leg plasma flow. In ten preoperative patients, the cardiac index (CI) was measured by thermodilution. In the remainder, CI was assumed to be 3.5 $L/M^2/min$ for these patients in the resting state, a value previously observed in patients with liver failure.[7] After operation, measurements of CI by Swan Ganz catheter and thermodilution were employed in all cases. Leg blood flow is approximately 5.15 ± 0.45% of CI in such patients.[7] Thus, leg plasma flow, adjusted for hematocrit, is (1 − hematocrit) × 0.0515 × 3.5 ($L/M^2/min$). Since the musculature of the leg, from which the great majority of amino acids are mobilized,[12] is approximately one fifth of the skeletal muscle in the entire body,[13] the total peripheral production of amino acids (PP-AA) equals ΔA-FV-AA × (1 − hematocrit) × 0.18 $\mu M/M^2/min$. If an infusion of amino acids is in progress, the rate of amino acid infusion must be added to PP-AA to obtain the total entry rate of amino acids into the plasma pool.

Central plasma clearance rate of amino acids (CPCR-AA). Because of the impracticality of measuring arteriovenous differences and blood flow in the liver, bone marrow, spleen, lymph nodes, and other actively metabolizing organs that take up amino acids, the concept of CPCR-AA was developed to measure the rate of clearance by these organs as a unit.[12] Assuming a state of equilibrium, the net rate of amino acid uptake by these organs at a given arterial plasma concentration must equal their rate of entry into the plasma from muscle and by infusion when there is no enteric food intake. The release or uptake of amino acids by gut is an integral part of the net amino acid uptake by the liver and other central tissues. It is convenient to consider CPCR-AA as the quantity of plasma from which all amino acids would be extracted by central tissues at a given arterial concentration in a given time.[7] Therefore:

$$\text{CPCR-AA} = \frac{\text{entry rate of amino acids}}{\text{arterial plasma amino acid concentration}} = ml/M^2/min$$



FIGS. 1A–F. *A.* Grade I = normal transplanted liver. *B.* Grade IIa = ischemic injury with hepatocyte swelling and polymorphonuclear infiltrates. *C.* Grade IIb = moderate rejection with lymphocyte infiltration in portal triads. *D.* Grade III = viral hepatitis with inclusion bodies (cytomegalovirus). *E.* Grade IVa = severe ischemic injury with hepatocyte necrosis diffuse polymorphonuclear infiltrates. *F.* Grade IVb = severe rejection with extensive lymphocyte infiltration and confluent hepatocellular necrosis.

Hepatic protein synthesis measured in vitro *in the resected liver.* The hepatectomy specimen was generally removed from the abdomen within 15–20 minutes of severing the hepatic artery and portal venous inflow. A wedge biopsy, weighing approximately 10 g, dampened with Ringer's lactate solution, was placed in a container suspended in an ice bath for prompt transport to the laboratory. There the liver tissue was cut into slices less than 0.5 mm in thickness. Each slice was weighed and incubated for 3 hours in Krebs–Ringer's bicarbonate buffer containing 2.0% bovine albumin and essential amino acids at physiologic concentrations. The medium was equilibrated with a gas mixture (95% O_2, 5% CO_2), and 14C-uniformly labeled tyrosine (0.5 μCi/flask) was added. Following incubation, the total hepatic protein synthetic rate was determined by the method of Stakeberg et al.[13] Ra-

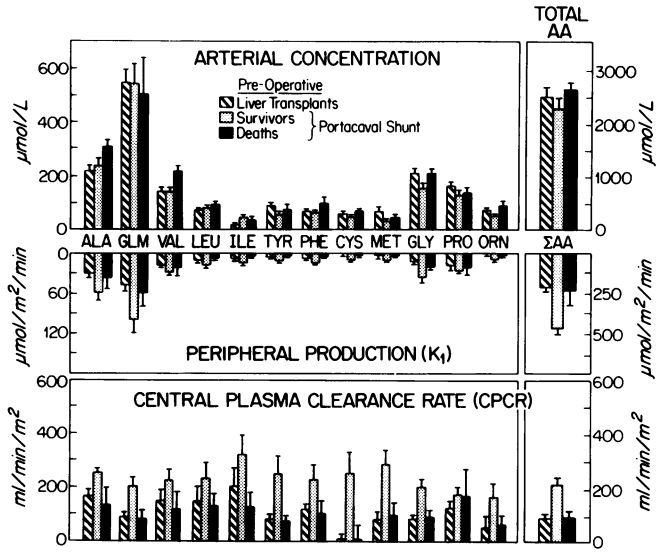


FIG. 2. Arterial plasma concentrations, peripheral release rates, and CPCR-AA comparing preoperative liver transplant candidates with preoperative cirrhotics (survivors *vs.* deaths). Note that CPCR-AA in the transplant candidates is the same as that in the cirrhotics who die after operation.

dioactivity of 14C tyrosine in all precipitable protein (liver tissue + incubating medium) was compared with that of a blank flask containing medium alone. The *de novo* protein synthetic rate was expressed as nanomoles 14C-labeled tyrosine incorporated in protein per gram of liver tissue (wet weight) per 4 hours.

The values of hepatic protein synthetic rate obtained from this assay were correlated with CPCR-AA determinations made within a few hours before the operation in a total of 11 patients.

Results

Preoperative Selection of Patients

Each of the 41 patients who underwent hepatic transplantation was in a terminal state of liver failure as judged by the usual clinical criteria. The most common diagnosis was primary biliary cirrhosis, with chronic active hepatitis, and submassive hepatic necrosis being next in frequency (Table 1). Some degree of culture documented infection was present in 37% of the population. Ages ranged from 17 to 60 years, with an average of 43.6 years. Serum bilirubin and alkaline phosphatase levels were elevated in all (mean: 19 mg/dl and 154 units, respectively), with the most abnormal results being found in the patients with sclerosing cholangitis (mean: 27 mg/dl and 220 units, respectively). Encephalopathy was present before operation in 46% of the patients and in 100% of those with submassive hepatic necrosis.

Serum proteins. Serum albumin was moderately depressed in each patient. The average of 2.7 g/dl compares with a total protein of 5.9 g/dl. The mean prothrombin time (PT) was 17 seconds compared with a normal laboratory control value of 11–13 seconds, indicating a deficit of coagulation factor production. This finding was particularly evident in the group of six patients with submassive hepatic necrosis in whom the PT value averaged 28.5 seconds.

Preoperative Amino Acid Metabolism

The total CPCR-AA was 91 ± 9 ml/M^2/min in the 28 patients from whom a preoperative value could be obtained. In five who were tested a second time following an 8-hour period of amino acid infusion, the CPCR-AA rose from 53 ± 8 only to 118 ± 15 ml/M^2/min. As shown in Figure 2, the value for both fasted CPCR-AA and amino acid concentration did not differ significantly from cirrhotic patients in a previous study who failed to survive portacaval shunt or other surgical procedures.[7]

In addition to the patients who underwent hepatic transplantation, 19 others considered as candidates for the procedure were not accepted for various reasons. One man was found to have a fasted CPCR-AA of 204 ml/M^2/min and is alive. Four patients with a mean CPCR-AA of 79 ± 21 underwent portasystemic shunt procedures with a mortality of 75%. Fourteen were not accepted because of age greater than 60 years, uncontrollable infection, or very severe malnutrition. Their mean CPCR-AA was 38.6 ± 23. Of these people, four have survived to date and 10 have died (mortality 71%).

In vitro *hepatic protein synthesis.* The *in vitro* total incorporation of 14C tyrosine into protein by incubated slices of resected recipient liver was 73 ± 16 nM/g/4 h. In eleven patients, CPCR-AA was measured immediately before the operation for comparison with the rate of total protein synthesis in the resected liver, as shown in Figure 3. There was a significant positive correlation between the two measurements ($r = 0.72$, $p < 0.02$).

Mortality

Sixty-three per cent of the 41 patients were discharged from the hospital and are alive at periods ranging from 1 month to 3 years after the operation. As presented in Table 2, six patients died during the operation or shortly thereafter of uncontrollable hemorrhage related to insurmountable technical problems (4 patients) or primary graft nonfunction (2 patients). Four died of infection within 8 weeks, three being retransplanted because of severe rejection (2 patients) or preservation injury (1 patient) to the liver. At the time of death, one patient had a biopsy indicating Grade III damage and two patients had Grade IV hepatic damage. Of the remaining five deaths, all died of overwhelming infection at times up to 15 months after

operation (Table 2), with viral cultures being positive in three.

It is of interest to note that the mean preterminal CPCR-AA value in the patients who died after operation was 48 ± 6 ml/M²/min (Table 2).

Postoperative Values

The histological assessment of the degree of liver damage obtained by needle biopsies is compared in Table 3 with simultaneous CPCR-AA and other laboratory values in relation to the survival rate.

Survival in relation to biopsy grade, calculated to include all deaths which occurred when patients shifted into less favorable groups, reveals that with Grade I "normal transplanted liver," if observed at any time, 89% recovered to survive; with "moderate injury" (Grade II) biopsies, 67% survived; with viral hepatitis (Grade III), 50% survived; but with severe injury (Grade IV) only 17% ultimately survived (Table 3).

CPCR-AA was measured 104 times in conjunction with liver biopsies, permitting comparison with the histological grade. The results are shown in Figure 4. In the presence of normal transplanted liver, the mean CPCR-AA value was 263 ± 22 ml/M²/min. When liver biopsies demonstrated moderate degrees of injury (Grade II) or the presence of viral inclusion bodies (Grade III), the CPCR-AA was depressed, respectively, to 195 and 186 ml/M²/min. By contrast, patients in whom the liver biopsies showed severe damage (Grade IV), the CPCR-AA was only 93 ± 19 ml/M²/min, which does not differ from the mean preoperative value but is significantly different from those patients with postoperative Grade I biopsies ($p < 0.001$).

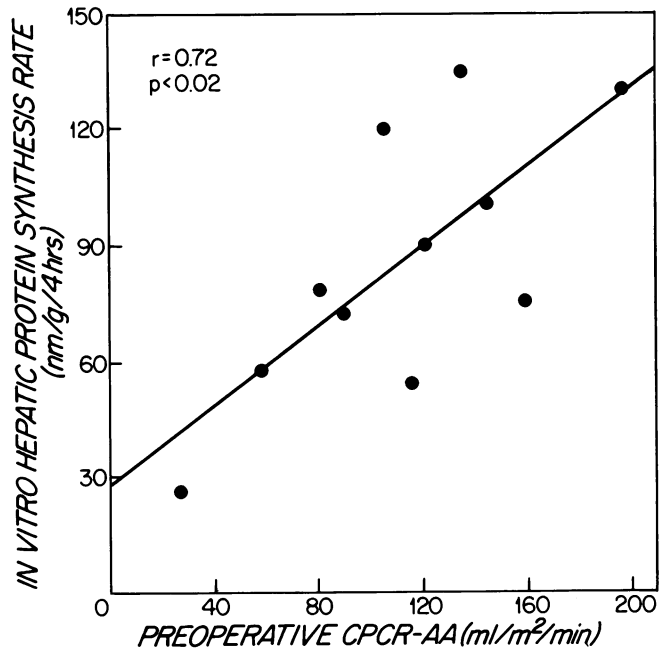


FIG. 3. Total hepatic protein synthetic rates in incubated slices of liver biopsy specimens obtained at operation with CPCR-AA values measured immediately prior to transplantation in the same patients.

TABLE 2. *Cause of Death, Infections, and Premortem CPCR-AA*

Time of Death	Months Survived	Cause of Death	Fungus	Virus	Bacteria*	Liver Biopsy Histological Grade	CPCR-AA
Perioperative (N = 6)	0	Hemorrhage	—	—	—	N/A	—
Early postoperative (N = 4)	1 ± 0.5	Sepsis	Candida (4)	CMV (2)	Pseudomonas (1) *K. pneumonii* (4) *S. faecelis* (1) Enterobacter (1) *S. aureus* (1)	I (0) II (0) III (1) IV (2)	49 ± 18
Late postoperative (N = 5)	10.4 ± 2	Sepsis	Aspergillis (2)	CMV (3) HSV (1)	*S. aureus* (2) *E. coli* (2) Streptococcus (1) *C. freundii* (1) *K. pneumonii* (1) *P. mirabilis* (2) Diptheroids (1) *M. morgagni* (1) *P. carinii* (1)	I (1) II (2) III (0) IV (0)	47 ± 3
Total 15 deaths of 41 patients	4.1 ± 1	Hemorrhage (6) Sepsis (9)	6/9	6/9	9/9	I (1) II (11) III (1) IV (11)	48 ± 6

* Number of patients with each isolate.
Grade I = NL liver = normal transplanted liver.
Grade II = moderate ischemic injury or rejection.
Grade III = viral hepatitis.
Grade IV = severe ischemic injury or rejection.

TABLE 3. *Correlation of CPCR-AA, Histology, and Liver Function Tests with Survival*

Liver Biopsy	Survival	CPCR-AA (ml/M²/min)	Prothrombin Time (Seconds)	Bilirubin (mg/dl)	SGOT (IU/L)
I = normal liver	89% (16/18)	263 ± 22 (47)	13.2 ± 0.2 (42)	10.4 ± 1.1 (45)	170 ± 42 (44)
II = moderate injury (ischemia or rejection)	67% (10/15)	195 ± 15† (38)	14.8 ± 0.5‡ (26)	13.2 ± 1.3 (32)	542 ± 191 (32)
III = viral hepatitis	50% (2/4)	186 ± 37 (10)	14.7 ± 0.7* (10)	15.9 ± 3.8 (10)	319 ± 130 (10)
IV = severe injury (ischemia or rejection)	17% (1/6)	93 ± 19§ (14)	18.1 ± 1.3‡ (8)	23.7 ± 3.5§ (9)	1104 ± 348* (9)

* = $p < 0.05$
† = $p < 0.02$
‡ = $p < 0.01$
§ = $p < 0.001$

The relationship of the posttransplant clinical course in the hospital to serial postoperative values of CPCR-AA is presented in Figure 5. The 18 patients with an "early response" had a rapid rise of CPCR-AA to 296 ml/M²/min, with a fall of total arterial plasma AA concentration to 2098 ± 110 μM/L. Although all but two of those with an early response had an infection in one or more sites, each responded to therapy. The convalescence of each person in this group was relatively uneventful. The average time in the intensive care unit (ICU) was only 10 ± 4 days and discharge was 1.5 ± 0.4 months after operation. The CPCR-AA of a second group of 12 patients, called "delayed response," remained below 150 ml/M²/min for several days to weeks, during which time the patients all had life-threatening infections. Two patients who received retransplanted livers are in this group and were discharged. Subsequently, the mean CPCR-AA rose to 210 ± 15 ml/M²/min. Because of infections and other serious complications, this group remained in the ICU for an average of 30 days, and discharge from the hospital was delayed to an average of 3.1 ± 0.9 months. A third group of six patients failed to elevate the CPCR-AA above 112 ± 12 ml/M²/min. Three underwent retransplantation, and all died. Each of the six was seriously infected, and only one survived to be discharged from the hospital.

Prothrombin times. Measured at the time of postoperative biopsies (presented in Table 3), prothrombin times are closely related to the morphological grade of liver injury, ranging from 13.2 seconds (Grade I) to 18.1 seconds with severe injury (Grade IV). A similar but inverse correlation of prothrombin time exists with CPCR-AA, presented both in Table 3 and Figure 6 ($r = 0.57$, $p < 0.001$).

Serum bilirubin. Although volume of bile drainage from the common duct T-tube is related to hepatocyte function and is a determinant of serum bilirubin concentration, no exact relationship was found between these variables.

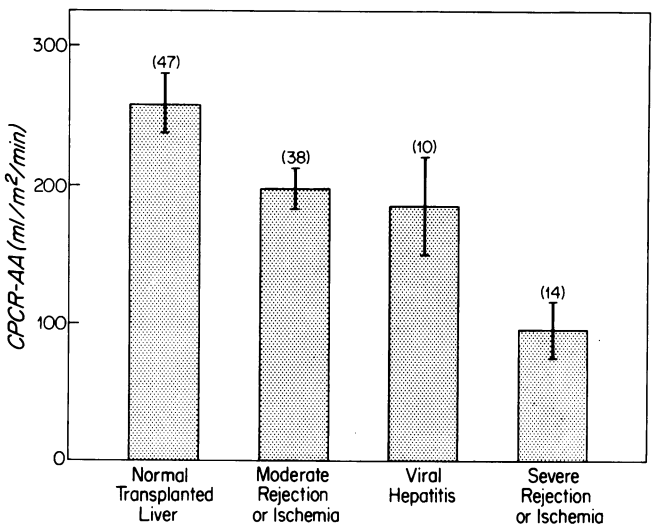


FIG. 4. CPCR-AA compared to simultaneous liver biopsy histological results in the same patients.

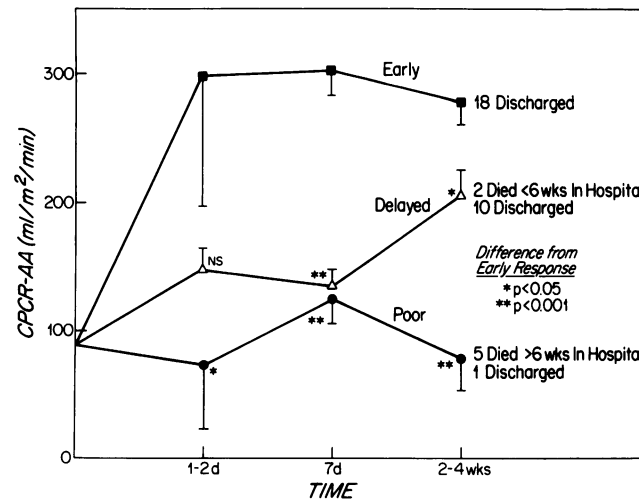


FIG. 5. Serial CPCR-AA determinations in 36 patients during the first 30 days posttransplant, correlating clinical course with the metabolic response as determined by CPCR-AA.

When compared to the histological grade of injury for the pooled data in the postoperative period, the serum bilirubin was found to average 10.4 mg/dl in the presence of normal transplanted livers (Grade I) and to be 23.7 in those with severe rejection of hypoxic injury (Grade IV). No significant differences from Grade I were found in Grade II or III (Table 3). Neither was there a significant correlation between CPCR-AA and the serum bilirubin concentration.

Serum glutamic oxalate transaminase (SGOT). Being representative of other serum enzymes, SGOT was 170 ± 42 units/L in patients with Grade I liver histology and was elevated significantly to 1104 ± 348 IU/L only in those with Grade IV hepatic injury. No correlation between SGOT and CPCR-AA was found.

Discussion

Improvement in surgical techniques and a more satisfactory means for immunosuppression have made liver transplantation a practical method of treating end stage liver disease. In this series of 41 consecutive adult patients, 63% have survived 3 months to 3 years following liver replacement. In consideration of the potential morbidity and mortality, only selected patients in immediate danger of death from hepatic failure for whom no alternative therapy exists are currently considered to be candidates for liver transplantation.

Excluding the six perioperative deaths from insurmountable technical problems or primary graft nonfunction, the corrected mortality remains 25% (9 of 35) (Table 2). Improved methods for preservation and transport of the donor liver are needed to obviate the dangers of initial hypoxic injury, which necessitated three of the five retransplantations in our series. Of the retransplanted patients, only one survived long-term. Significant pharmacologic advances (*i.e.*, OKT_3 monoclonal antibody)[9] in the prevention or treatment of refractory rejection may further reduce the incidence of fatal liver allograft failure, which contributed to the four deaths within the first six postoperative weeks (Table 2). All early deaths were characterized by histologic evidence of liver injury coupled with reduced CPCR-AA. In both early and late death categories, overwhelming infection was the principal cause of death and was associated with compromised hepatic function, as measured by significantly reduced CPCR-AA.

Three principal methods exist for assessing the state of the liver before and after operation: (1) Needle biopsy[14,15] of the liver delineates the degree of histological damage present but gives no direct evidence of the physiologic competence of the hepatocyte mass. (2) CPCR-AA[8] is a means for measuring not only the ability of the liver and other central tissues to clear amino acids, as an important measurement of metabolic function, but serves also as an indicator of the hepatic protein synthetic rate, as shown in Figure 3. (3) Routine liver function tests (SGOT, bilirubin, *etc.*) give indirect evidence of cellular injury or destruction but do not correlate with the actual metabolic competence of the hepatocellular mass except in the most severe injuries (Grade IV biopsies). Prothrombin time has proved to be the only standard laboratory parameter that correlates with CPCR-AA after operation, but only when in the abnormal range (Fig. 6).

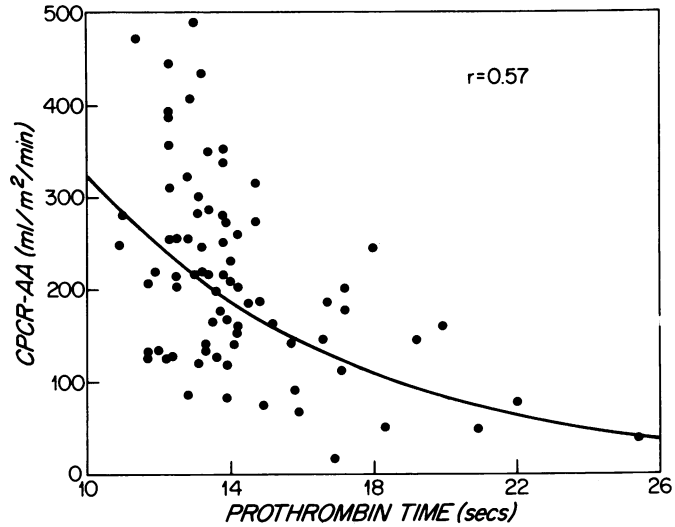


FIG. 6. Multiple postoperative CPCR-AA determinations and prothrombin time results taken simultaneously in the same patients.

Hepatic needle biopsies may significantly contribute to more accurate preoperative diagnosis of the liver disease suggested by clinical findings. These are presented in Table 1 and indicated the nature of diseases accepted for transplantation. However, the histologic abnormalities fail to quantitate the extent to which important liver functions have been impaired. On the other hand, in the postoperative period, frequent histological assessment of liver injury[14,15,22] has proven to be a valuable tool for the early differentiation of hypoxic damage, viral infection, or rejection. The method is rapid and may be used for grading the degree of hepatic damage, as shown in Figure 1. The histological grades, presented in Tables 2 and 3, have an important quantitative relationship to the incidence of infection and mortality.

A shift from Grade I or II to a lower level has served usefully to indicate the need for aggressive immunosuppressive therapy. The presence of viral inclusion bodies, as found in Grade III (see Fig. 1), demonstrates the need for acyclovir or other antiviral therapy. Valuable as the postoperative hepatic histology has been, there are two instances when other indicators (CPCR-AA and prothrombin time) have demonstrated failure of adequate protein synthesis in patients with Grade II hepatic biopsies.

CPCR-AA is the ratio of the entry rate of amino acids into the plasma pool divided by the arterial plasma amino acid concentration. The CPCR-AA value derived represents the quantity of plasma cleared of amino acids per unit of time by the liver and other central tissues. Certain shortcomings have been previously reviewed extensively.[8] The most important of these concerns is the production of amino acids from peripheral tissues. This value is dependent on the arterial–femoral vein concentration difference and the leg plasma flow rate. The latter is usually estimated as 5.15 ± 0.45% of cardiac index based on previous observation.[12] However, the overall error in deriving CPCR-AA has been estimated to be no more than ±12%. This is relatively insignificant compared with the magnitude of difference observed in various clinical states (trauma, sepsis, cirrhosis, and transplantation) that differentiate survivors and deaths.[7,8,16,17] Others[18,19] have devised other methods for measuring the clearance of amino acids, but all agree that liver failure for any cause results in significant reductions.

The importance of CPCR-AA determinations is the relationship of CPCR-AA to the synthetic rate of proteins in visceral tissues. CPCR-AA is a measure of the uptake of amino acids by the liver and other central tissues, whether they be furnished by peripheral muscle protein degradation or by intravenous infusion of amino acid containing solutions. Since Cuthbertson's observation[11] of an accelerated urea excretion following trauma, increased utilization of amino acids for gluconeogenesis and energy production has been recognized. More recently, it has become evident that survival from trauma or infection requires a three- to fourfold increase of amino acid uptake by the liver and other central tissues for synthesis of coagulation factors, antibodies, complement, and other components of the immune system.[8,16] The acute phase proteins produced by the liver include not only fibrinogen, C-reactive protein, and amyloid A, but also antiprotease agents such as alpha-2-macroglobulin, which protect normal uninvolved tissues from injury or destruction by the activated immune system.[19] If this metabolic response mediated by proteolysis inducing factor (PIF), probably in conjection with stress hormones[20] fails, overwhelming infection, multisystem failure, and death usually follow.[8]

Somewhat as a surprise, previous observations[7] revealed that cirrhotic patients who were not infected also exhibited an accelerated flux of amino acids from the periphery to the central tissues, similar in most respects to the metabolic response of septic or injured patients with normal liver function. In the presence of severe hepatocyte injury, clearance of amino acids becomes insufficient, resulting in encephalopathy and inability to secrete proteins adequately.[21] Cirrhotic patients who survived surgical procedures had high preoperative CPCR-AA values of 220 ± 26 ml/M^2/min. In those who died after operation, usually of infection, CPCR-AA was 97 ± 16 ml/M^2/min. Furthermore, the *in vitro* hepatic protein synthetic rate in liver biopsies correlated well with the measured CPCR-AA ($r = 0.73$, $p < 0.01$).[7]

Application of CPCR-AA to assessment of preoperative patients selected for liver transplantation revealed the amino acid concentration to be slightly elevated to 2980 ± 250 μM/L, but the mean CPCR-AA was only 91 ± 9 ml/M^2/min. In Figure 2, the values for this group of patients are compared with data previously obtained from cirrhotic surgical patients who underwent other procedures. In every respect, blood amino acid concentrations, peripheral amino acid release rate, and CPCR-AA of these patients do not differ from those of the cirrhotics who died after surgery. By these criteria, any of the patients selected for transplant would have had a high probability of dying. This concept is supported by the high mortality rate of patients who were studied but not accepted for transplant.

Since the preoperative CPCR-AA measurements in the transplant group also correlated with the *in vitro* hepatic protein synthetic rates ($r = 0.72$, $p < 0.02$), CPCR-AA as a method for defining deficiencies of hepatocyte function and protein synthesis becomes valuable in the selection of patients for hepatic transplantation.

The results presented in Table 3 and Figure 4 clearly demonstrate a significant postoperative relationship between the degree of histological damage to the transplanted liver and the CPCR-AA. Furthermore, there is an inverse correlation of CPCR-AA and prothrombin time, which represents the availability of those clotting factors, predominantly produced in the liver (Fig. 6). However, determinations of the arterial and femoral vein blood plasma concentrations of amino acids required for calculation of CPCR-AA is time consuming. For this reason, urgent decisions must be based on conventional clinical criteria. The two most important uses for CPCR-AA after operation include the ability to define the state of amino acid metabolism and, by inference, protein synthesis after transplantation as follows:

First, patterns of response can be established by serial CPCR-AA observations, which have an important prognostic value. Early responders, shown in Figure 5, who within 2 days had high CPCR-AA values to almost 300 ml/M^2/min, all survived to be discharged with minimal complications. These are the patients with easily controlled infections, whose intensive care time was brief and whose hospital stay averaged 6 weeks. The metabolic response of this group resembles the behavior of cirrhotics who survived other surgical procedures and who had mean CPCR-AA values of 212 ± 24 ml/M^2/min.[7] In short, they were synthesizing the proteins required for healing and control of infection. By contrast, those who failed to develop CPCR-AA values near 200 ml/M^2/min after trans-

plantation either did so at a later date with a protracted hospital course or died within 6 weeks of overwhelming infection. Of this group, five required retransplantation; there was but one survivor.

A second use is illustrated by one patient who had a successful transplantation with only Grade II histological abnormalities but compromised metabolic function, as demonstrated by a decreased CPCR-AA of only 122 ml/M^2/min. Ultrasound examination of the abdomen revealed thrombosis of the portal vein. Within 1 day after portal flow was re-established by thrombectomy, the CPCR-AA rose to 343 ml/M^2/min. Subsequently, the patient recovered. Another example was a patient with progressively rising bilirubin concentrations who was febrile and exhibited swollen hepatocytes in the liver biopsy. The differential diagnosis lay between an intraperitoneal infection and hepatic failure from rejection. However, CPCR-AA was found to be 502 ml/M^2/min. An infected hematoma was removed with excellent early recovery.

Liver function tests, with the exception of bilirubin and prothrombin time, have proven to be relatively inexact in defining the state of the liver. The enzymes rise following hypoxic injury or during acute rejection, but commonly fall when the liver is "dead." Serum bilirubin is prone to be elevated for 1–2 weeks in all patients despite output of bile from the T-tube. Only in severe injury, Grade IV, shown in Table 3, does the bilirubin rise to very high levels, differentiating the patient with severe liver injury. Prothrombin time reflects hepatic protein synthesis of clotting factors and appears to be a useful marker of transplant damage that correlates inversely with CPCR-AA (Fig. 6).

In summary, survival from hepatic transplantation is dependent on adequate function of the donor liver within the recipient. Biopsies of the liver can reveal histologic abnormalities of varying grades but fail to quantitate the accompanying metabolic abnormalities. Of the numerous important metabolic functions of the liver—secretion of bile, gluconeogenesis, degradation of toxic and other compounds, and utilization of amino acids for protein synthesis—the latter appears to be the easiest to measure quantitatively in the clinical setting. For this purpose, CPCR-AA was devised and correlates with hepatic protein synthesis measured *in vitro*. Other commonly available clinical tests give only indirect evidence of liver destruction or dysfunction by blood concentrations of various substances. With the occasional exception of bilirubin and prothrombin time, none was found to reflect either liver function or the extent of injury. Thus, percutaneous needle biopsies and CPCR-AA in combination have proven value, not only in understanding the nature of injury and functional impairment of the transplanted liver, but also in guiding the selection of patients and deciding the course of postoperative management.

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