Intermediary Metabolism of the Liver

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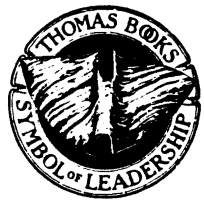

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Chapter 4

METABOLIC STUDIES FOLLOWING ORTHOTOPIC LIVER TRANSPLANTATION

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INTRODUCTION

It is not the intention of this chapter to discuss the general field of liver transplantation, but rather a small and fascinating aspect of this topic. Specifically, it is about the study of several normal or abnormal substances that are manufactured and released by the liver and which can therefore be used to ask important questions about the nature of the liver homograft and its function and about liver disease.

LIVER PROTEIN PHENOTYPES

The first series of observations concerns the tracing of certain proteins. *The conclusion is that liver homografts retain their metabolic specificity after transfer to a new host.* This was first shown by studies of serum haptoglobin[1-4] and the group-specific component of the alpha globulin fraction[3] before and after orthotopic liver transplantation in humans.

The recognition of discrete protein fractions in orthotopic liver recipients was made possible by the earlier studies of Smithies[5] on haptoglobin and of Hirschfeld[6] on group-specific component. These authors had demonstrated that three kinds of haptoglobin *and* group-specific component were identifiable in the human population, that the type present in any indi-

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vidual was subject to genetic control, and that the phenotypic expression could be detected with electrophoretic techniques.

In several of our cases of orthotopic transplantation, the donors have had different haptoglobin and/or group-specific component types than those found in the recipients. Within a few hours to a few days after operation, only the donor protein fractions were present; the changes were complete and permanent.

In Figure 4–1 is an example of a change of haptoglobin. Preoperatively, the patient had Smithies haptoglobin type 2–1, identifiable by the characteristic appearance with starch gel electrophoresis. The donor was haptoglobin type 2–2. After transplantation only the donor type was detectable in the recipient's sera. The child lived for two and one-half years.

By immunoelectrophoresis, analogous observations were made with group-specific component (Fig. 4–2). Before operation

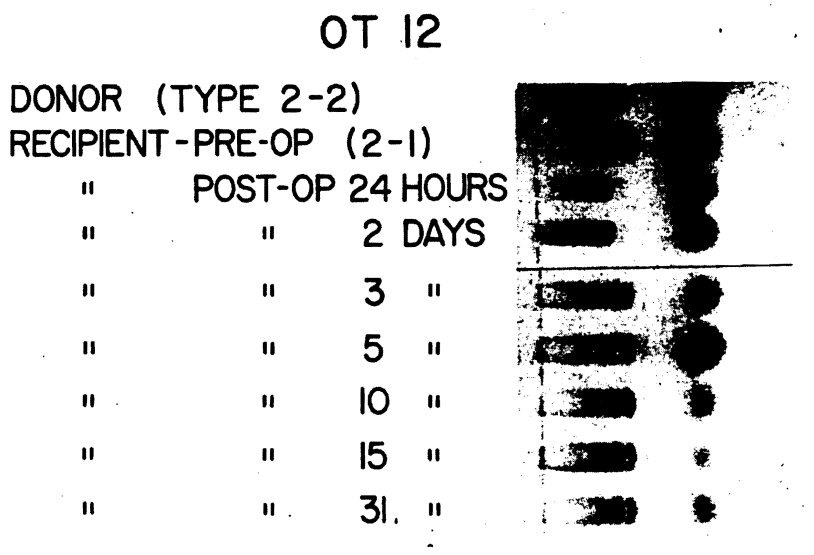

Figure 4–1. Effect of orthotopic liver transplantation on serum haptoglobin (Hp). The studies were in a patient who preoperatively had Smithies Hp type 2–1; her donor was Hp type 2–2. After transplantation, only the donor type was detectable in the recipient sera. The determinations were with starch gel electrophoresis. (By permission of W.B. Saunders Co., *Experience in Hepatic Transplantation*, 1969.)

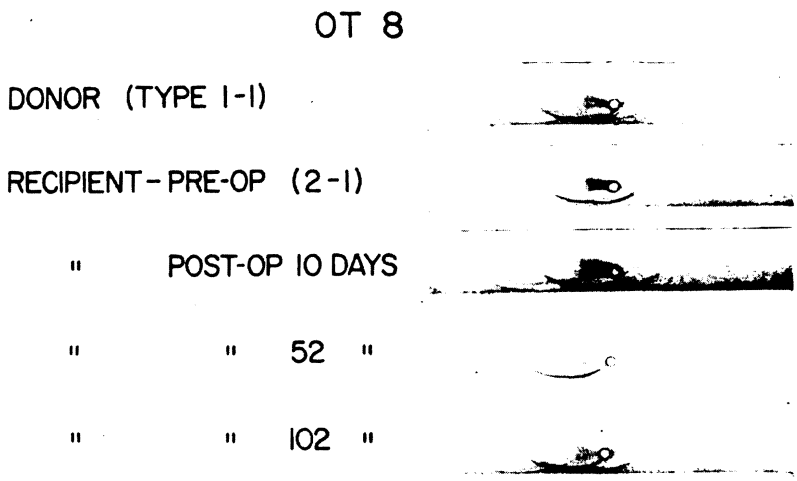

Figure 4–2. The effect of orthotopic liver transplantation on group specific component (Gc). Before operation, the recipient had Gc type 2–1 whereas the donor had type 1–1. After transplantation, only the donor type was detectable. The recipient, whose original disease was hepatoma, died more than 13 months after transplantation, of widespread metastases. The immunoelectrophoretic studies were performed with commercial anti-Gc antisera. (By permission of W.B. Saunders Co., *Experience in Hepatic Transplantation,* 1969.)

this child had group-specific component type 2–1, whereas the donor had type 1–1. After transplantation the recipient's type disappeared and only the donor type was thereafter detectable. Recently, Alper[4] added one of the complement components (C'3) to the proteins mentioned whose polymorphism permits tracer studies to be carried out from a donor to the recipient.

A practical implication of these demonstrations is that liver-based metabolic disorders should be treatable with liver transplantation. The concept has been conclusively tested by Kuster et al. of the Mayo Clinic.[7] Using mongrel canine liver donors, they were able to cure gout naturally present in Dalmatian recipients. Conversely, the transplantation of Dalmatian livers conferred the defect in uric acid metabolism upon mongrel recipients.

The list of liver-based inborn errors of metabolism is impressive; it ranges from phenylketonuria through certain of the

incurable glycogen storage diseases (Type 4 or alpha glucosidase deficiency for example). I will not take time to list other examples, since there are almost 4 dozen of them, of which most are self-evident. Instead, I would like to tell you how the kind of research described has been carried one step farther and used to try to clarify the etiology of Wilson's disease.

Wilson's disease is an inborn error of copper metabolism in which the essential biochemical feature is the saturation of many tissues, including the liver and the brain with copper. Copper deposits in the eye result in the characteristic Kayser-Fleischer rings, by which the clinical diagnosis is often made. A serum protein, ceruloplasm, to which copper is attached may or may not be deficient in quantity in the peripheral blood. Most patients with Wilson's disease can be effectively treated with chelating agents such as penicillamine, providing the diagnosis is made early enough.

For many years, controversy has raged about whether or not Wilson's disease is a liver-based inborn error of metabolism. Observations with one of our patients may be relevant to at least this aspect of the dispute about etiology. An 11-year-old child was admitted to our hospital in an agonal state with terminal liver failure. The diagnosis of Wilson's disease had been considered. Confirmation, however, was missed during the preceding 3 years in hospitals from the East to West coasts, because of misinterpretation of data, or because of failure in one instance to respond to the report of a hepatic biopsy which contained all the features of acute Wilson's disease, including Mallory bodies.[8] When the child was seen, no consideration could be given to penicillamine treatment because he was dying.

Liver replacement was carried out 18 months ago. There was an exceedingly severe early rejection which fortunately was eventually controlled, after the bilirubin had gone to almost 50 mg% (Fig. 4–3). The child now has completely normal function and lives at home.

After the transplantation, the ceruloplasm in this child has been normal or even elevated (30 to 55 mg%). (Normal range of ceruloplasm is 22–43 mg%.) The serum copper has been 80 to 170 μg%. Postoperatively, there was a major cuprure-

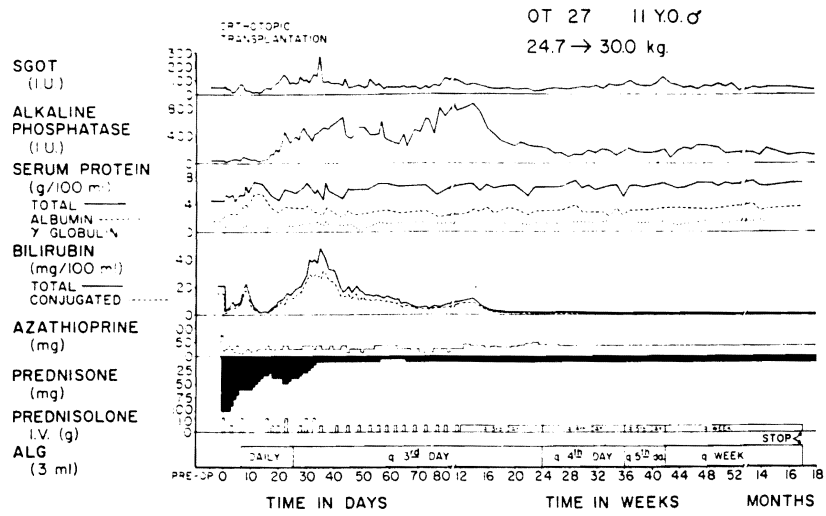

Figure 4–3. Course after liver replacement. ALG—horse antilymphocyte globulin. (By permission of *Lancet, 1*:505, 1971.)

sis which lasted for almost 6 months (Fig. 4–4). Even then, there was an additional outpouring of copper in the urine during a 3-day course of penicillamine.

In Wilson's disease, there is often in fact a high copper urine excretion, but this is associated with high copper levels in the liver. The child had his homograft biopsied 6 and 17 months after transplantation. His native liver contained the extraordinary copper level of 216 μg/g wet liver tissue, which confirmed the diagnosis of Wilson's disease. (Normal range of liver copper is 5μg-20μg/gm wet liver tissue.) At 6 months, following the chronic cupruresis, the liver copper of the homograft was 15μg/g and after 17 months it was 13.6μg (both values normal). Consequently, from these studies we conclude that there has been clearance of body copper stores, without accumulation of copper in biopsies of the transplanted liver. Further follow-up will be necessary before deciding whether the Wilson's disease has been cured by liver replacement and whether this constitutes proof that this disorder is an inborn error of hepatic metabolism. The observations so far are consistent with these conclusions although they do not prove them.[9]

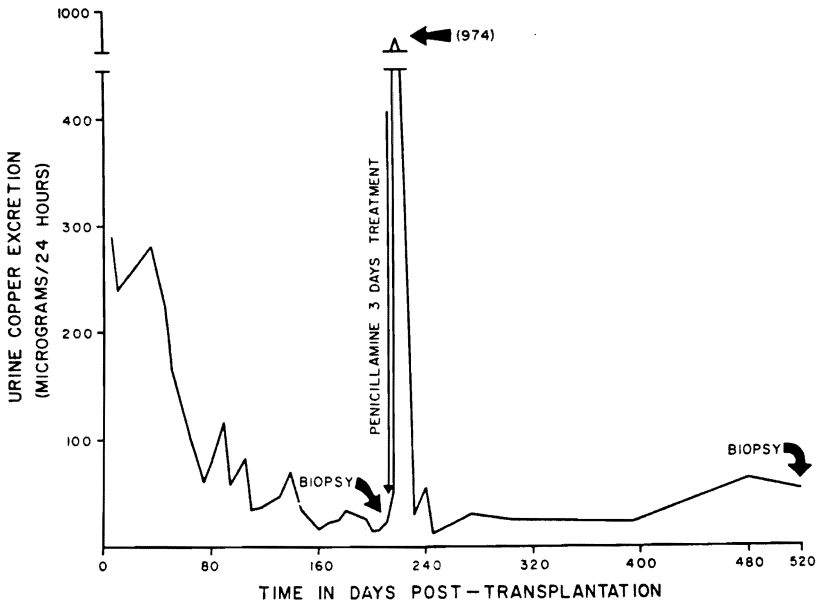

Figure 4–4. Urinary excretion of copper after the orthotopic liver transplantation for Wilson's disease. (By permission of *Lancet, 1*:505, 1971.)

GAMMA GLOBULIN STUDIES

Thus far, I have pointed out that a liver homograft can and does synthesize proteins of new types, thus contributing to the internal environment in which the organ must live. The proteins that have been precisely studied (referring again to haptoglobin, group-specific component, and C'3 complement) have come under such close scrutiny primarily because techniques are available for identifying the phenotypes of these proteins. While interesting, the fact that protein phenotypes changed was not particularly surprising in those instances where there was prior evidence that the protein studied was of hepatic origin.

Now in the second part of this presentation I would like to examine the effect of liver transplantation on immunoglobulins, and especially gamma G globulin, that have long been thought to be exclusively of *extra*hepatic origin. In spite of this, it has been found in human recipients of orthotopic livers that im-

munoglobulin (or Gm) types of donor specificity are regularly conferred upon the recipient.[10,11]

At least 21 gamma G globulin phenotypes have been identified in man. The so-called Gm markers have been used by Mathe[12] to determine the success or failure of bone marrow transplantation. The standard technique for identification of the Gm types is the indirect method of Martensson,[13] in which the patient's serum is added to specific commercial antisera that are directed against known Gm types. If that particular Gm type is present, it consumes the antiserum, which then fails to cause agglutination of a complex antigen (human O-type red cells coated with IgG) which serves as an indicator. With absence of the Gm type being looked for, the antiserum is not consumed, and there is agglutination in the indicator system.

The Gm investigations were carried out by Dr. Noboru Kashiwagi[10,11] on a number of recipients of orthotopic liver homografts. In his studies, 3 of the Gm types (1, 2, and 12) were looked for. Initially, it was found that after operation all Gm types became transiently positive whether or not these had been present previously either in the donor or the recipient. This indiscriminate conversion of Gm types was apparently due to the multiple blood transfusions which all these patients received in the course of their operations.

In subsequent patients, quantitative studies of the Gm types were made. The most interesting cases were those in which Gm types were represented in the donor but not the recipient. After operation, significant quantities of the donor-specific Gm types were conferred upon the recipient. These persisted for as long as the first post-transplantation year. In some cases there was a slow dying away of the new Gm type over a period of many months, but this was never complete. In other instances the new Gm type was fully maintained without loss of strength. Whatever Gm types were previously present in the recipient were maintained throughout the period of study, although the strength of these also sometimes tended to decrease.

The conclusion from these studies was that the preexisting Gm types of the recipients were not altered by transplantation, a finding that supports the long held concept that immuno-

globulin production is not hepatic dependent. However, the results also showed that the liver could introduce and support a new Gm type not previously present in a given patient. Thus, the circulating IgG in the patients was of both donor and recipient origin. There were no easily detectable specific adverse consequences in these cases of having a genetically heterogenous admixture of immunoglobulins.

Special studies, however, were carried out to examine the possibility of an interaction between the immunoglobulins of host and graft origin. Antigamma globulin antibodies, which collectively have been termed rheumatoid factors, were studied quantitatively after 3 transplantations. In two patients rheumatoid factor titers increased enormously, to as high as 1:1000 and 1:512 after 3 and 5 months. These host gamma M antibodies were shown to be specifically directed against the new Gm types that had been introduced into the recipient circulation as a consequence of the transplantation.

From these data it appeared certain that the new livers were responsible in some way for the new immunoglobulin types. At this point, the unanswered question was how it had occurred, since it was not believed that the homograft hepatocytes were responsible for IgG synthesis. One hypothesis might have been that the reticuloendothelial cells of the grafts were responsible by differentiation into lymphoid cells of donor genotype. To investigate this possibility, 9 livers donated by males to female recipients were studied by Dr. K.A. Porter[14] of London (Table 4-I). In the specimens obtained before 100 days, the Kupffer cells remained male. After this time, the Kupffer cells were female with typical nuclear Barr bodies, and therefore of host origin. At the same time the endothelial cells of the hepatic arteries and portal veins had *not* changed sex and did not do so for as long as one postoperative year.

The findings just described disproved the idea that Kupffer cells were responsible for new Gm types, since in order to be a candidate for a new gamma globulin source it would have been necessary for the graft Kupffer cells to retain their donor sex identity. An alternative hypothesis to explain the new Gm types in these patients was that lymphoid tissue of donor origin

TABLE 4–1
ANALYSIS OF SEX OF CELLS

Patient OT Number	*Days Graft Residence*	*Sex Change in Graft (Male to Female)*	
		Kupffer Cells	*Endothelial Cells*
5	23	No	No
8	400	Yes	No
12	105	Yes	No
14	380	Yes	No
17	35	No	No
18	4	No	No
20	1/2	No	No
21	1/2	No	No
24	11	No	No

Analysis of the sex of the Kupffer and vascular endothelial cells in nine patients in whom orthotopic livers were transplanted from male donors to female recipients.

had been accidentally carried with the liver homografts. Consequently, a special search was made by Dr. Porter for lymphoid tissue in 12 human homografts. Lymphoid deposits were found in each case,[11,14] containing prominent lymphoid follicles, prominent germinal centers, and many plasma cells.

In summary of the second portion of this presentation, new Gm types of donor origin were conferred upon recipients of orthotopic liver homografts, without altering the previously present recipient Gm types. The source of the new Gm types was apparently lymphoid tissue transplanted along with the liver homografts. As an incidental finding in this study, it was shown that the Kupffer system of the homografts ultimately changed and became made up of host cells. The same transformation could *not* be shown by sexing techniques to have occurred in the vascular endothelium.

ABNORMAL SUBSTANCES

Two bits of miscellaneous information follow that might be of special interest to those concerned with hepatology.

Fetoglobulin

The first is the measurement of alpha fetoprotein as a means of following the course of patients being treated for hepatoma. The work described was carried out by Dr. Eliott Alpert and

Dr. Kurt Isselbacher of Boston on serial samples collected in Denver from recipients whose indication for liver replacement was hepatoma.

In patients who developed clinically obvious metastases after liver replacement, the fetoprotein was always positive. In these cases, there was little need for the fetoprotein determination except as a matter of interest. However, in two of our patients, the presence of fetoprotein clearly indicated that there was persistent tumor, although this could not be detected by other diagnostic tests. Conversely, we have followed a child whose fetoglobulin serum concentrations fell to zero after orthotopic transplantation. We believe that the complete disappearance of this primitive protein from the peripheral blood is an indication of a cure. The child is now more than a year postoperative with perfect liver function and with no evidence of recurrent malignant disease.

The Australia Antigen Marker

The second piece of miscellaneous information in this section is in the nature of a case report. On August 9, 1970, we removed the liver of a 28-year-old woman who was dying of chronic aggressive hepatitis. She was referred to us by Dr. Alan Rediker of Los Angeles who knew that she had Australia antigenemia for more than one preceding year. She had a history of acute serum hepatitis in 1965.

During the orthotopic liver transplantation, samples of her peripheral venous blood were obtained every 15 minutes. All of these contained the Au antigen until her native liver was excluded from the circulation and removed. Then, within 15 minutes, the Australia antigen disappeared (Fig. 4–5) as measured by a battery of tests ranging from the insensitive Ouchterlony immunodiffusion (AG), the more sensitive immunoelectro-osmophoresis (IEOP), and the highly sensitive complement fixation (CF), to the anticomplementary activity (ACA) titers as recently described to be an indication of antigen-antibody complexes by Shulman and Barker.[15]

She remained Au negative for about 6 weeks and then had a

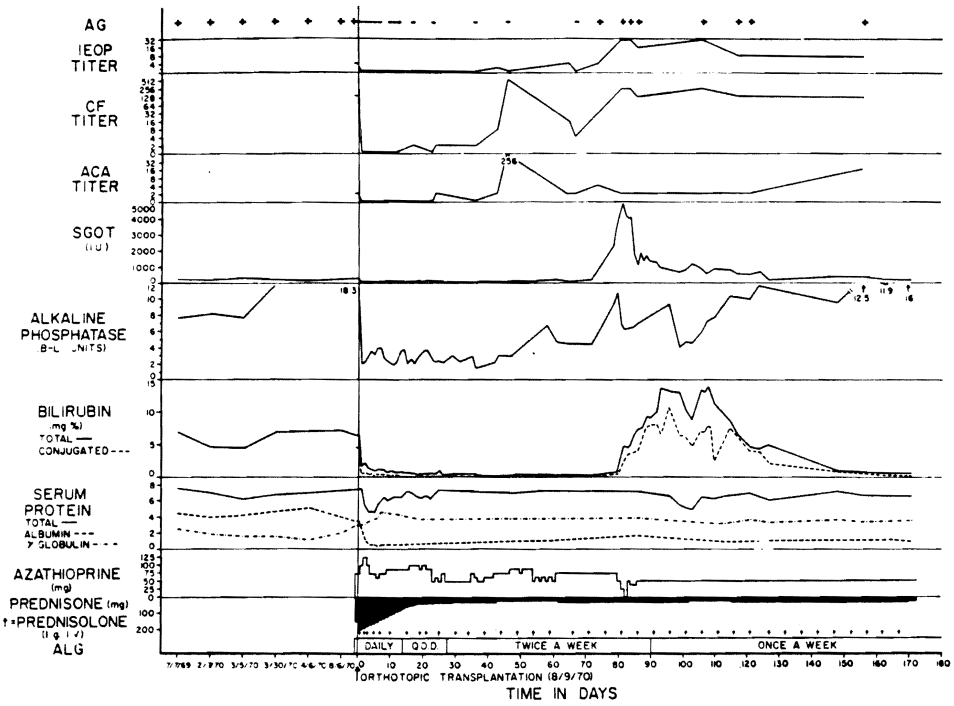

Figure 4–5. The course of a patient who was terminally ill with chronic aggressive hepatitis, Australia (Au) antigen positive. She was treated by liver replacement. Note that all serologic evidence of serum hepatitis disappeared immediately after operation only to return some weeks later. AG—agarose gel micro-Ouchterlony test for Au antigen. IEOP—quantitative immunoelectro-osmophoresis test for Au antigen. CF—complement fixation test for Au antigen. ACA—anticomplementary activity which is thought to reflect the presence of circulating antigen-antibody complexes; the test is not immunologically specific for Au antigen. Normal Bessey-Lowry (B-L) units for alkaline phosphatase are less than 3.

return of the Au marker. About 10 days after the reappearance of the Au antigen, she developed hepatitis of her hepatic homograft. The clinical course of her new disease was somewhat prolonged, about 2 months. Now she has largely recovered, but with chronic Au antigenemia just as she had previously (Fig. 4–5). She is now 6 months post-transplantation.

From the study of this patient, we have reached three conclusions. First, the treatment of people dying of viral hepatitis by liver replacement may be justified on an experimental basis,

since this woman's life has been significantly prolonged. Second, the liver in patients with chronic aggressive hepatitis appears to be the major reservoir from which the Au antigen emanates. Third, the incubation period of serum hepatitis, which is customarily thought of in terms of the host immunologic apparatus and its response, must be viewed in this case as a latent period intrinsic to the target organ, the liver. There will be other important observations from this kind of patient, but these will require further periods of followup.

SUMMARY

A series of observations were presented about different kinds of normal or abnormal substances that come from liver homografts and that appear in the peripheral blood. These markers can be and have been used to study several aspects of hepatic physiology and disease.

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