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# Changes in Serum Haptoglobin and Group Specific Component after Orthotopic Liver Homotransplantation in Humans* (32988)

NOBORU KASHIWAGI, CARL G. GROTH, AND THOMAS E. STARZL
(Introduced by D. W. Talmage)
*Department of Surgery, University of Colorado School of Medicine; and the Denver Veterans Administration Hospital, Denver, Colorado 80220*

Successful clinical liver transplantation should make it possible to more incisively explore certain aspects of hepatic function in man. In the present study this has been done by examining the haptoglobin (Hp) and group specific component (Gc) of the alpha$_2$ globulin serum fraction of 4 infant recipients of orthotopic liver homografts and their donors. The results establish that both protein moieties are manufactured exclusively by the liver. They also provide evidence that liver homografts retain the metabolic specificity of the donor after transfer to a new host.

The previous reports of Smithies on Hp (1, 2) and of Hirschfeld on Gc (3, 4) provided the basis for the experimental approach. They showed that 3 kinds of both Hp and Gc were identifiable in the human population, that the type present in any individual was subject to genetic control, and that the phenotypic expression could be identified with electrophoretic techniques.

time of transplantation. The first patient had a hepatoma; the other 3 had congenital biliary atresia. The cadaveric donors were 2 males and 2 females, aged 14–20 months. Three of the 4 children are still alive, now 180, 136, and 56 days after operation; the fourth died 61 days posttransplantation. A more general account of these cases is published elsewhere (5).

Serum samples were taken from both the donors and recipients prior to transplantation; in Case 1, the parents' sera were also analyzed in order to confirm the Hp genotype. Postoperatively, recipient samples were obtained at variable intervals. The serum Hp genotypes were determined by electrophoresis in a vertical starch gel[1] (6). The wells were charged with a solution consisting of 15 parts of undiluted serum and one part of 10% human hemoglobin solution. The initial current was 6 mA. A constant voltage of 120 was maintained for 24 hours. The gel was removed from the supporting tray and divided horizontally into slices 2–3 mm thick. The gel slice was stained in 0.1% *o*-tolidine in 1% acetic acid and a few drops of 30% $H_2O_2$ were added.

The determination of serum Gc genotypes was made by Hirschfeld's modification (7) of the microimmunoelectrophoresis[2] technique of Scheidegger (8). Commercial horse anti-Gc antiserum and rabbit anti-Gc antiserum were used[3]. Electrophoresis was at 220 volts for 2.5 hours with a discontinuous buffer system (pH 8.6, $\mu = 0.05$ in agar[4] on plate and pH 8.6, $\mu = 0.1$ in buffer tank). Agar plates charged

*Results.* The Hp type of both the donors and recipients was 2–2 in Cases 1 and 4. In Case 1, the same type was present after transplantation in samples obtained on postoperative days 10, 52, and 102. Patient 4 was studied 6, 12, 40, and 58 hours posttransplantation and after 4 and 6 days. During the first 12 hours, there was a 2–1 Hp type mixed with the 2–2, presumably as the result of 1200 ml blood transfusion during operation. The subsequent samples were 2–2 (Fig. 1).

Patients 2 and 3 had preoperative Hp types

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[1] From Otto Hiller, Madison, Wisconsin.

[2] Equipment from LKB-Produkter, Stockholm. Sweden.

[3] From Hoechst Pharmaceutical Co., Kansas City, Missouri.

[4] Special agar-Noble from Difco Laboratories, Detroit, Michigan.

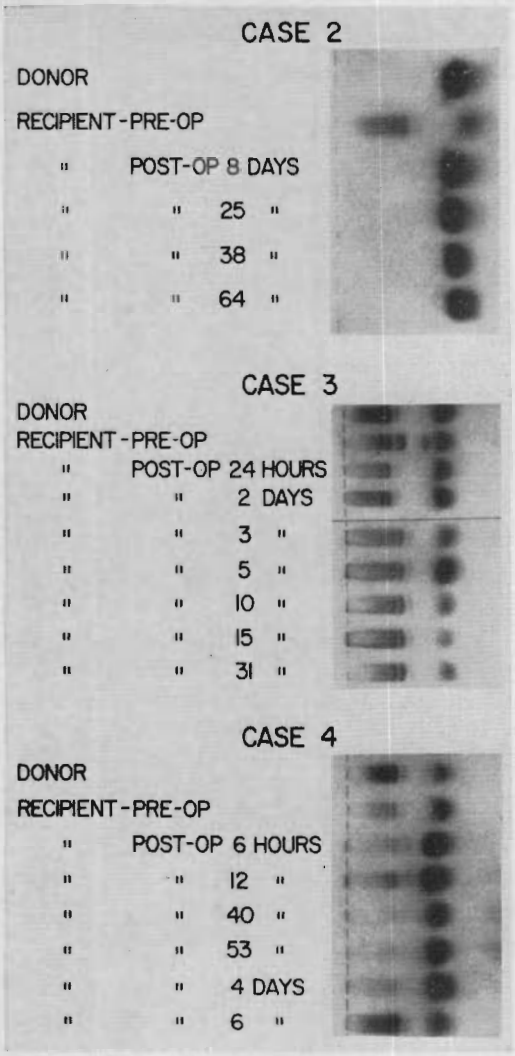


FIG. 1. Effect of liver transplantation upon serum haptoglobin (Hp). Patients 2 (top) and 3 (center) had preoperative Hp types 2–2, and 2–1, whereas their donors had 1–1 and 2–2 , respectively. After transplantation, only the donor type was detectable in the recipient sera. In Case 4 (bottom), both the donor and recipient were 2–2. For the first 12 hours after transplantation, the recipient serum was found to contain Hp 2–1 mixed with 2–2, presumably a consequence of massive blood transfusion during the operation. All subsequent samples were 2–2.

2–2 and 2–1 respectively, whereas their donors were 1–1 and 2–2. After transplantation, both recipients reverted to the Hp types of their donors for the period of their study. In Case 2, the sera tested on days 8, 25, 38, and 64 showed only Hp type 1–1 and no sign of the original Hp 2–2. Patient 3 was studied on days 1, 2, 3, 5, 10, 15, and 31. On all occasions, the only Hp detectable was the donor type 2–2 (Fig. 1).

Patients 3 and 4, who received homografts from donors of the same Gc type, did not have a change in group specific component. In Cases 1 and 2, however, the Gc type of the recipients converted to that of the donors. In patients 1 and 2, the alterations were complete by 8 and 10 days, respectively, when the first analyses were made. These remained unchanged for the subsequent follow-up periods of 4 and 3 months (Fig. 2).

*Discussion.* There have been previous descriptions of changes in these 2 identifiable proteins. In 1964, we reported that an adult recipient of a liver homograft had converted by the second postoperative day to the Hp type of the organ donor (9, 10). However, liver function was poor and after the fourth postoperative day, all detectable haptoglobin had disappeared from the serum. Hirschfeld *et al.* (11) observed that a newborn infant's serum after exchange transfusion contained both his own Gc and that of the blood donor.

In the reports of the earlier liver transplantation (9, 10), it was postulated that the liver was the only source of Hp, a suggestion which was somewhat weakened by the transient nature of the observations. Studies by Prunier *et al.* (12) on various human tissue cultures supported the hypothesis that both Hp and Gc were manufactured solely by the liver. The data obtained in our most recent cases seem to establish this fact beyond doubt.

Nyman (13) has estimated the 50% turnover rate of Hp to be 5.4 days. It is clear that this is accelerated under the special conditions of liver transplantation since all trace of the original recipient Hp was gone at least in one case within 24 hours. The Hp combines with free hemoglobin to form a complex which cannot be filtered by the kidney (14, 15); it has been suggested that this reaction not only prevents excessive losses of endogenous iron, but also shields the tubules from free hemoglobin deposition (16). Thus, hemolysis from massive intraoperative blood transfusion was probably of major importance

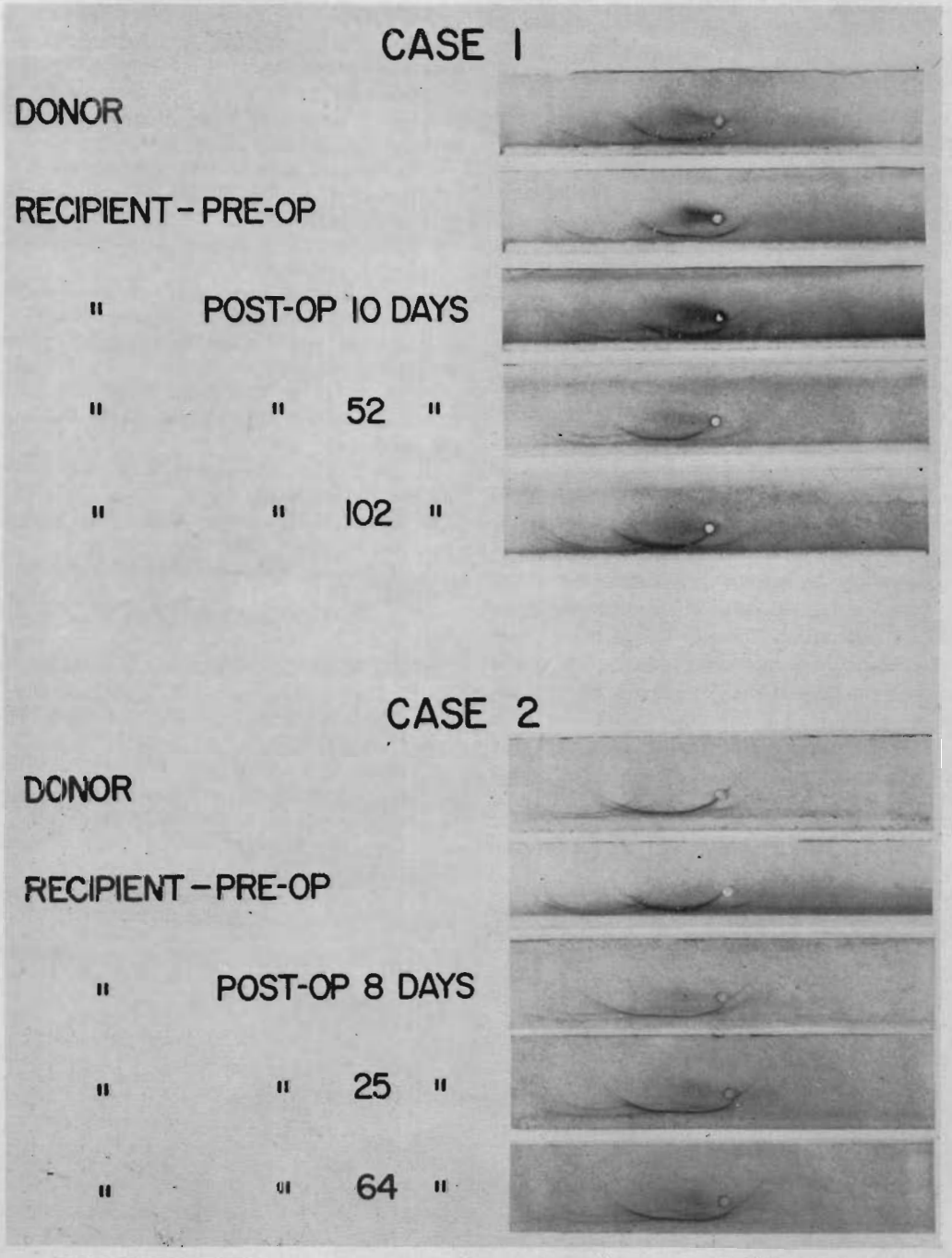


FIG. 2. In Cases 1 (top) and 2 (bottom), the group specific component (Gc) types of the recipients which were 2–1 and 1–1, were converted after transplantation to the 1–1 and 2–1 types of their donors.

in increasing Hp utilization. An additional factor could be an increased rate of red cell destruction which is known to be caused by the presence of liver homografts (17). Trauma and steroid therapy also influence Hp metabolism (16, 18). It is probable that the conversion of the Gc phenotype was also rapid. There is no information on the biologic function of Gc protein.

A practical implication of the demonstration that the liver retains its metabolic specificity after transplantation is that certain liver-based metabolic diseases might be treatable by such a procedure. This concept has been conclusively tested by Kuster *et al.* (19). Using mongrel canine donors, they were able to cure the gout present in Dalmatian recipients. Conversely, the transplantation of Dalmatians' livers conferred the defect in uric acid metabolism upon mongrel recipients.

*Summary.* In human recipients of orthotopic liver homografts with different haptoglobin (Hp) and group specific component (Gc) types than their respective donors, the donor phenotype permanently replaced that previously present. The findings prove that the liver is the sole source of Hp and Gc, and that it retains its metabolic specificity after transplantation to a new host.

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