(Auer procedure): The development of an "Auer colitis" in sensitized rabbits was accompanied by a temporary elevation of the beta globulin in 12 of 16 Auer test rabbits; this trend was not observed in sensitized or unsensitized rabbits.

*Discussion.* The responsiveness of the colon to immunologic reactions has been well documented; and immunofluorescent techniques have provided evidence for an antigen-antibody reaction as the mechanism of the "Auer colitis." The present data indicate that an "Auer colitis" also may be induced by direct intrarectal instillation of specific antigen. An advantage of the rectal over the intravenous method is that it may be repeated in the same animal for long periods, with improved reproducibility and with less likelihood of generalized anaphylaxis.

The colon reaction in the sensitized rabbits receiving formalin enemas, though not as severe as in the "Auer colitis," requires explanation. In earlier control experiments, formalin enemas alone, repeated daily for as long as 6 months in unsensitized rabbits, caused only slight to moderate hyperemia and granularity of the distal colon. Also, in these studies, scattered punctate hemorrhages were noted in 6 of 48 sensitized animals after administration of formalin enemas. The possibility exists that the reaction may have resulted from an antigen-(egg albumin) antibody reaction, facilitated by the increased capillary permeability of the distal colon in sensitized rabbits, induced by exposure to the mild formalin solution.

*Conclusion.* The intrarectal instillation of specific antigen (crystalline egg albumin) in sensitized rabbits may induce a "colitis" in the distal colon, previously exposed to a mild formalin solution, to increase capillary permeability locally. The "Auer colitis" develops presumably on the basis of an antigen-antibody reaction.

1. Auer, J., *J. Exp. Med.*, 1920, v32, 427.
2. Kirsner, J. B., Elchlepp, J. G., Goldgraber, M. B., Ablaza, J., Ford, *H., A.M.A. Arch. Path.*, 1959, v68, 392.
3. Kraft, S. C., Fitch, F. W., Kirsner, J. B., *Am. J. Path.*, 1963, v43, 913.
4. Wissler, R. W., Barker, P. A., Flax, M. H., LaVia, M. F., Talmage, D. W., *Cancer Res.*, 1956, v16, 761.
5. Jenks, W. P., Durram, E. L., Jetton, M. R., *Biochem. J.*, 1955, v60, 205.

Received April 6, 1964. P.S.E.B.M., 1964, v116.

## Change in Serum Haptoglobin Type Following Human Liver Transplantation.* (29363)

D. A. MERRILL, C. H. KIRKPATRICK, W. E. C. WILSON AND C. M. RILEY
(Introduced by D. W. Talmage)
*Departments of Preventive Medicine and Medicine, University of Colorado Medical Center, Denver*

The haptoglobins constitute a population of serum proteins characterized by their ability to form a stable bond with hemoglobin. The molecular heterogeneity of these hemoglobin-binding proteins, demonstrable by starch gel electrophoresis, is under genetic control(1). The 3 major haptoglobin types have been designated 1-1, 2-1 and 2-2.

The opportunity to study a patient before and after liver transplantation provided a unique circumstance in which to evaluate the role of the liver in haptoglobin metabolism. The patient and the donor had different haptoglobin types; and we report here the temporary appearance of haptoglobin of the donor type in the serum of the recipient after liver transplantation.

*Materials and methods.* The subject of this investigation was a 29-year-old Caucasian female with a primary carcinoma of the liver in whom a liver transplantation was performed. A preliminary operation was done on Oct. 2, 1963, and hepatectomy and trans-

* Supported by grants from USPHS.

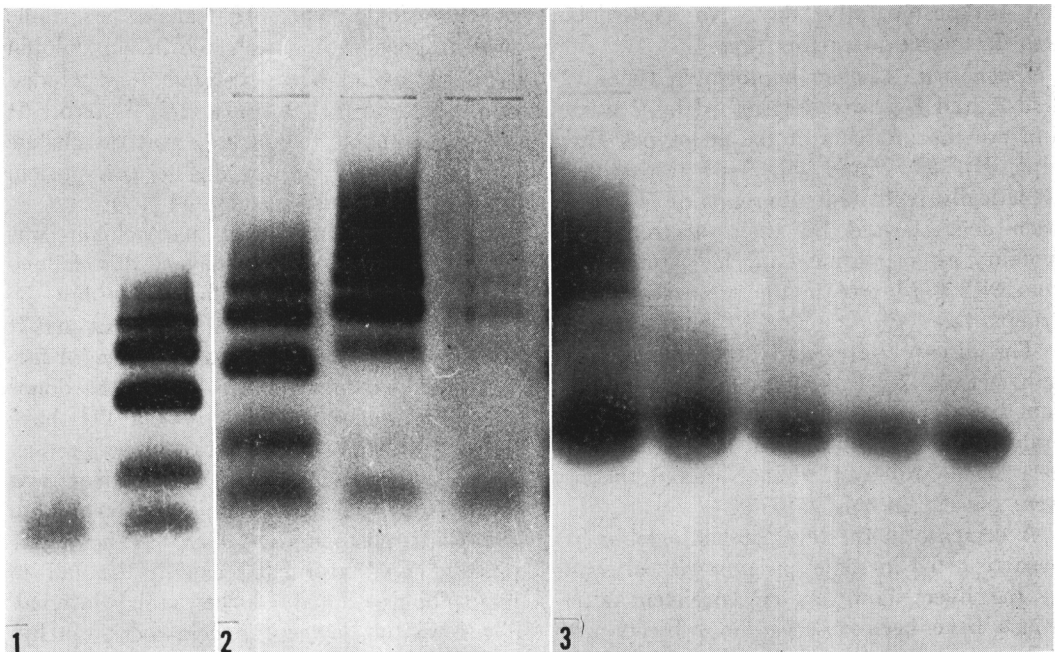


FIG. 1. Starch gel electrophoresis of recipient's serum stained for haptoglobin. The serum obtained after the first-stage operation (left) contained no demonstrable haptoglobins and is compared with the preoperative pattern (right).

FIG. 2. Starch gel electrophoresis of serum stained for haptoglobins from recipient (left) and donor (center) prior to transplantation. The donor pattern was demonstrated in recipient's serum on second day after transplantation (right).

FIG. 3. Starch gel electrophoresis of serum stained for haptoglobin. These sera were obtained from recipient 2, 4, 6, 10 and 19 days after transplantation of the liver.

plantation on Oct. 5, 1963. The clinical course of this patient has been described elsewhere(2). The donor organ was obtained from a 66-year-old Caucasian male who had just died of a gunshot wound of the head.

Serum samples from the patient were obtained prior to both surgical procedures, between the first stage operation and the transplantation, and serially following implantation of the liver. A serum sample was obtained from the donor on the day he died.

The serum haptoglobin genotypes were determined by electrophoresis in a vertical starch gel(3)† The wells were charged with a solution consisting of 15 parts of undiluted serum and 1 part of 10% hemoglobin solution. The initial current was 15 ma and 120 volts, and a constant voltage was maintained for 18 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.

*Results.* Electrophoresis of the recipient's serum prior to the surgical procedures revealed her haptoglobin type to be 2-1. A second serum sample, obtained 3 hours after completion of the 11 hour preparatory operation, contained no demonstrable haptoglobins (Fig. 1).

The first post-transplant serum was obtained 2 days after the liver had been implanted. Electrophoretic analysis of this serum revealed that the haptoglobin was 2-2, the type of the liver donor. The haptoglobin patterns of the donor and the recipient, before and after transplantation, are shown in Fig. 2.

Fig. 3 illustrates the loss of demonstrable haptoglobin during the postoperative period. The 2-2 haptoglobin bands on the gel were faint but probably present on Oct. 9, 1963,

† The hydrolyzed starch was the product of Connaught Medical Research Laboratories, Toronto.

the 4th postoperative day. No haptoglobin was detectable after this time.

*Discussion.* Human haptoglobin types (1-1, 2-2, and 2-1) are determined by 2 alleles and are the products of the genotypes $Hp^1/Hp^1$, $Hp^2/Hp^2$, and $Hp^2/Hp^1$ respectively. Genetically regulated subgroups of $Hp^1$ have been characterized by their electrophoretic mobility after treatment of the parent molecule with 8 M urea in the presence of thiol groups(4).

The disease states and other factors that influence the level of haptoglobins in the serum have been recently reviewed(5,6). Several of these conditions, liver disease, surgery, hemolysis and adrenal steroid therapy were present in this patient.

A decrease in the level of haptoglobin has been reported in some patients with cirrhosis of the liver; however, no consistent alterations have been observed in subjects with primary or secondary liver neoplasms(6). Preoperatively, the patient's 2-1 haptoglobin type was clearly demonstrated.

The serum obtained 3 hours after the first stage operation, during which the patient received 2000 ml of blood, contained no demonstrable haptoglobins. Two factors may be responsible for this change. Free hemoglobin in the transfused blood would be bound by the patient's haptoglobin and the complexes rapidly cleared from the circulation. The level of free hemoglobin in this serum sample was 3 mg/%. A second consideration is that, although surgery is usually associated with an increase in haptoglobin levels, in patients with liver disease low values may be seen(6).

Following hepatectomy and liver transplantation, haptoglobin of the donor's type was found in the serum of the recipient. However, the patient also received 7000 ml of blood during the operation. Although it is not possible to define precisely the role of the transfusions in the appearance of the new protein, much of the transfused haptoglobin would be bound to free hemoglobin and removed from the circulation. Since the 2-2 genotype is present in only 35% of the Caucasian population(5), it is unlikely that the patient would receive 14 units of blood of this single type. If transfusions alone were responsible for a change in haptoglobin type, we would have expected a mixed distribution and not a pure 2-2 pattern. It seems reasonable to conclude that the change in haptoglobin type was due to new protein released from the transplanted liver.

The pattern of donor haptoglobin was readily apparent on the second day following transplantation. By the fourth day the pattern was only questionably present and it could not be detected thereafter. Several factors could account for the loss of the donor protein. Glucocorticoids and ACTH have been described as inhibitors of the production of haptoglobins(7). The patient was given large doses of prednisone starting on the third postoperative day. A hemolytic process could also have caused the fall in haptoglobins noted during this interval. There was no decrease in hematocrit but hyperbilirubinemia prevented reliable measurement of plasma hemoglobin levels.

Immunologic processes must be considered in the pathogenesis of these observations. A humoral antibody mediating the disappearance of donor haptoglobin is unlikely. There is no information concerning the antigenicity of homologous haptoglobin types in man. A recognized immunologic process present in the patient was the allograft rejection reaction which was directed against the transplanted liver. The rising serum bilirubin and the serum enzyme changes indicated marked cellular destruction and impairment of function of the residual tissue. The post mortem examination confirmed the advanced degree of liver cell destruction.

The appearance of a new haptoglobin with donor characteristics in the serum of a hepatectomized recipient of a liver allograft has a number of implications regarding haptoglobin metabolism. Although the site of production of the hemoglobin-binding proteins has not been conclusively demonstrated, the liver has been postulated to be the synthesizing organ. The observations in this patient are evidence in support of this postulate. It should be noted that, at a time when haptoglobin could be demonstrated (the second postoperative day), no recipient-type hapto-

globin was present, presumably because of the hepatectomy. Furthermore, since donor-type haptoglobin was present, it seems clear that the liver is at least a site of storage or activation of haptoglobin which can be released under appropriate conditions.

Haptoglobin-hemoglobin complexes are cleared from the circulation by the liver(7), but the subsequent fate of the haptoglobin molecules is not known. The present investigation suggests that the haptoglobin is degraded after sequestration by the liver, since the haptoglobin which appeared following the transplantation was of donor specificity and not heterogeneous as would be expected if bound recipient haptoglobin and bound haptoglobin from the transfused blood were released by the transplanted liver.

1. Smithies, O., Walker, N. F., *Nature,* 1959, v178, 694.

2. Starzl, T. E., Marchioro, T. L., Rifkind, D., Rowlands, D. T., Jr., Waddell, W. R., *Southern Med. J.,* in press.

3. Smithies, O., in Adv. in *Protein Chem.,* eds., Anfinsen, C. B., Jr., Anson, M. L., Bailey, K., Edsall, J. T., Academic Press, 1959, v14, 65.

4. Connell, G. E., Dixon, G. H., Smithies, O., *Nature,* 1962, v193, 505.

5. Galatius-Jensen, F., *The Haptoglobins, A Genetical Study,* Dansk Videnskabs Forlag, Kobenhavn, 1960.

6. Nyman, M., *Scand. J. Clin. Lab. Invest.,* 1959, Supp. 39.

7. Murray, R. K., Connell, E. G., Pert, J. H., *Blood,* 1961, v17, 45.

Received April 8, 1964. P.S.E.B.M., 1964, v116.

## Effect of Na+ and K+ on the Adenylate Kinase of Human Erythrocytes.* (29364)

AMIR ASKARI AND JOSEPH C. FRATANTONI (Introduced by Jay Roberts)
*Department of Pharmacology, Cornell University Medical College, New York City*

The presence of a Na+, K+-activated adenosinetriphosphatase (ATPase) in erythrocyte membranes has been reported by several investigators(1-3). Evidence suggesting the participation of this enzyme in the process of monovalent cation selectivity of erythrocytes has been reviewed(4). The various enzyme preparations described thus far all release two equivalents of phosphate from each adenosinetriphosphate (ATP) molecule. A possibility which could account for the observed release of 2 phosphates is the presence of an adenylate kinase in the ATPase preparations. This investigation was undertaken to determine: (1) if adenylate kinase is present in the erythrocyte membrane preparations and (2) the effect of Na+ and K+ on whatever enzyme activity is responsible for release of the second phosphate of ATP. A present finding shows that contrary to a previous report(5), kinase activity can be detected in membranes prepared by several different methods and that this enzyme is also activated by monovalent cations. After the completion of this work Post and Sen(6) also reported the presence of adenylate kinase in erythrocyte membrane preparations used for the study of ATPase. They did not, however, study the effect of Na+ and K+ on the kinase activity.

*Materials and methods.* Sodium salts of ATP and adenosinediphosphate (ADP) were obtained from Sigma Chemical Co. (St. Louis), and converted to the tris salts by passage through columns of cation exchange resin Dowex 50 in tris form. Adenosine-5′-monophosphate (AMP) and yeast hexokinase, either crystalline or Type IV, were also purchased from Sigma. All other chemicals used were of reagent grade quality.

Adenylate kinase of various preparations was measured, according to Colowick and Kalckar(7), by incubating aliquots with 5 μmoles of ADP, 100 K.M. units of hexokinase, 5 μmoles of $Mg^{++}$, 100 μmoles of tris-

* Supported by USPHS grants.