2.35±1.56 for norepinephrine and 2.5±1.76 for epinephrine. These values are significantly different from the normal with a $p < 0.0005$. Serum iron in this group of patients demonstrated a positive correlation co-efficient of −2.50 with the plasma catecholamines level. There is no correlation between the TIBC and plasma catecholamines. Patients with iron deficiency associated with other diseases, also showed a significant increase in catecholamine levels, 3.8±2.2 for norepinephrine and 2.80±1.9 for epinephrine, with a p value < 0.0005. However, there is no significant correlation co-efficient for the serum iron and catecholamine levels in this group of patients. Catecholamine levels for a heterogeneous group of anemias, non-iron deficient, were not significantly different from control values.

In 6 uncomplicated iron deficient patients following 600 mg of oral ferrosulfate, catecholamine levels were measured and described in table 2. Both levels of norepinephrine and epinephrine fell significantly and approached normal values in all patients studied within 3 days. In 1 patient a response was seen following 3 h after oral iron administration.

The present study demonstrated a significant increase in plasma epinephrine and norepinephrine levels in adult iron deficient patients whether or not their iron deficiency was complicated by other diseases. Furthermore, following 600 mg of oral iron, catecholamine levels promptly fell as early as 3 h following ingestion and returned to normal by 24 h. In contrast, normal catecholamine levels were determined in patients with other types of anemias. These findings are consistent with those reported by Voorhess et al.[3] who found increased urinary excretion of norepinephrine in iron deficient children. However, the present data does not support the hypothesis that elevated catecholamine levels in iron deficiency is due to a decrease in monoamine oxidase levels (MAO). Youdim[5] determined that iron was necessary for the operation of a functional monoamine oxidase and regenerated after 6 days of iron therapy[6]. Thus, it does not appear that the MAO level alone could explain the decrease in catecholamines seen in patients with iron deficiency anemia.

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2 Reprint requests: L.M. Snyder, Department of Hematology, St.Vincent Hospital, 25 Winthrop St., Worcester (MA. 01604, USA).

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Attempts to use carbodiimide (EDCI) to cross-link hemoglobin for transfusions

P. Labrude, B. Teisseire and C. Vigneron

Faculté des Sciences Pharmaceutiques et Biologiques, F-54000 Nancy, Centre Régional de Transfusion Sanguine et d'Hématologie, C.H.U., F-54000 Vandœuvre-les-Nancy, and Service d'Explorations fonctionnelles et INSERM U 138, C.H.U. Henri Mondor, F-94010 Creteil (France), 26 July 1978

Summary. The polymerization of hemoglobin for use as a blood substitute and an oxygen carrier would be of interest because high-mol.wt macromolecules would have a longer vascular retention time than the monomer. We found that the molecules resulting from the treatment of hemoglobin with ethyldimethylaminopropylcarbodiimide did not have a higher mol.wt than free hemoglobin and also had a dissociation curve resembling that of monomers, but seemed more stable.

The use of hemoglobin solutions as a blood substitute has been suggested frequently since the work of Rabiner[1]. Although it is known that free hemoglobin can transport and release oxygen, the low mol.wt and the poor viscosity of the solutions gives them a very short half-life. We have emphasized the care necessary for the storage of preparations free of methemoglobin[2] and demonstrated the changes the pigment undergoes in the transfusion apparatus, especially at interfaces[3]. We have also reported the value of providing a protein environment for hemoglobin and suggested that it could be associated or coupled with other macromolecules to improve its stability and vascular retention.

Attempts to polymerize hemoglobin for use in transfusions have so far been few and limited, since the only reagents tested so far have been diimidates[4,5], while the heme-oxygen relations were mentioned only in 1977 by Horowitz[5]. Therefore, we have tried to 'polymerize' hemoglobin using a carbodiimide and to study the properties of the product obtained: mol.wt as determined by gel-chromatography and analytical ultracentrifugation, viscosity, electrophoretic mobility, isoelectric point, stability, dissociation curve, p 50 and oxygen-binding capacity.

Materials and methods. 1. The hemoglobin solution was prepared according to a method derived from that of Rabiner[2], dialyzed, and kept at −20 °C. The 'polymerization' was performed with magnetic stirring for 3 h at 4 °C and at the pH of the solution (about 6.5), by the addition of powdered ethyldimethylaminopropylcarbodiimide (Fluka) in a 150- to 250-fold excess over the amount theoretically necessary to establish a single peptide bond. The reacted solution was then dialyzed against distilled water for 30 min at 4 °C.

2. The effect of the reagent on the hemoglobin was tested by gel-chromatography (7 runs on an ACA 44 LKB in a 90×2.6-cm column with 0.05 M Tris-HCl buffer, pH 8, flowing at 0.23 ml/min and at 4 °C; the OD was read at 280 nm) and analytical ultracentrifugation (at 20 °C in an

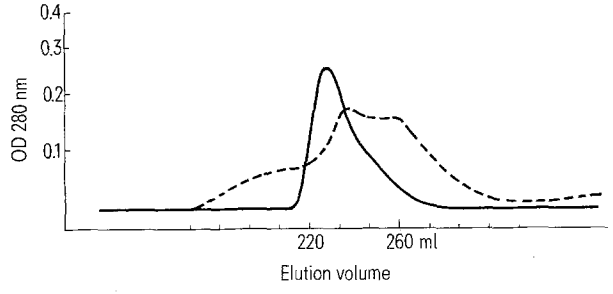

Fig. 1. Gel-chromatography on ACA 44 of 'free' hemoglobin (—) and after treatment with EDCI (– – –). OD at 280 nm is a function of elution volume of Hb in ml.

MSE Centriscan 75 analytical ultracentrifuge at 55,000 rpm).
3. Viscosities were measured at 37 °C in a Chaix-Meca coaxial-cylinder viscosimeter (CNRS-Anvar license).
4. The electrophoretic migration was measured on cellulose-acetate (Cellogel) at pH 9.2 and at 150 V, and the electropherograms were stained with Ponceau red and scanned on a Chromoscan (Joyce Loebl). Electrofocusing was conducted for 1.5 h at 1200 V on a 3.5–9.5 pH gradient established with Ampholine (LKB), and the gels were stained with amido-black.
5. The susceptibility to oxidation was determined by measuring the methemoglobin level after polymerization (Evelyn and Malloy's method[6]).
6. The dissociation curve was established spectrophotometrically by the method of Labie and Byckova[7] at pH 7.1 with a DBGT Beckman spectrophotometer. The binding capacity and p 50 were measured as described by Teisseire[8].

Results and discussion. Both the chromatographic behavior (figure 1) and the calculated sedimentation rate show that the mol.wt has not been multiplied and that no polymerization took place. However, carbodiimide reacted with the polypeptide chains, as the shape of the dissociation curve suggests.

The viscosity was not higher than that of unreacted hemoglobin at the same concentration: 1.06 CP versus 1.00 CP (10 determinations). This finding also favors the conclusion that the molecules obtained had an unchanged molecular weight.

When electrophoresis was done at pH 9.2 on Cellogel, the 'polyhemoglobin' migrated less far towards the anode than did hemoglobin, which means that its negative charge is lower. This result was confirmed upon isoelectric focusing, where the band extended from pH 7 to pH 9. This finding may be due to a variable total charge arising from the many opportunities that hemoglobin polypeptide chains offer for the creation of peptide bonds. The methemoglobin levels remained below 4%.

The dissociation curve for oxyhemoglobin reveals an increase in the affinity: The p 50 was therefore very low: 2 to 4 mm Hg in standard conditions instead of 26.6 for normal human blood and 17.5 for 2–3 DPG depleted stored human blood or hemolysates (pH 7.4, pCO_2 40 mm Hg and 37 °C). Also, the oxygen-binding capacity decreased from 1.34 to 0.9 at 1 ml O_2/g hemoglobin. This show a partial denaturation of hemoglobin.

In conclusion, the carbodiimide that we used, like other reagents recently tried (divinylsulfone, 1-4 butanediol diglycidyl ether), did not really polymerize hemoglobin (unpublished results). The unsuccessful attempt reported here shows once more that hemoglobin, because of its unusual structure and properties, resists attempts to modify its polypeptide chains. The change in shape of the hemoglobin dissociation curve resembles that observed when the pigment is coupled with albumin or other macromolecules. Interestingly, all these compounds are more stable than in the free state. Other agents likely to induce the formation of high-mol.wt polyhemoglobins with a less distorted dissociation curve should be tried.

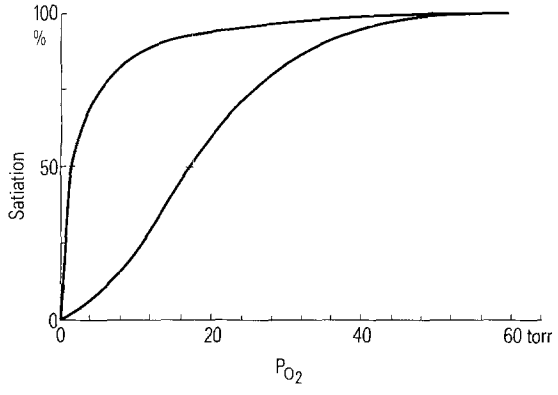

Fig. 2. Barcroft's curve obtained with hemoglobin before treatment with EDCI (right) and after treatment (left). p 50 are respectively 17,4 and 1,7 Torr. Curves are obtained in standard conditions with the Dissociation Curve Analyzer described by Duvelleroy (in Teisseire et al.[8]).

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Immunological recovery of thymectomized and sham-thymectomized lethally irradiated mice reconstituted with syngeneic bone marrow cells

M. Marušić[1]

University of Tennessee-Oak Ridge Graduate School of Biomedical Sciences and Biology Division, Oak Ridge National Laboratory[2], Oak Ridge (Tennessee 37830, USA), and Department of Physiology, University of Zagreb Faculty of Medicine, Zagreb (Yugoslavia), 20 July 1978

Summary. Immunological functions of lethally irradiated mice reconstituted with syngeneic bone marrow cells recover after 5–6 weeks. In mice that had been thymectomized before irradiation and reconstitution, T-cell function is deficient but the B-cell function is preserved.

The bone marrow cells given to lethally irradiated mice require the presence of the thymus in order to reconstitute the host immune potential[3]. Animals that had been thymectomized before irradiation and reconstitution have substantially impaired T-cell-dependent immune reactivity[4]. Accordingly, thymectomized, irradiated, bone marrow reconstituted (TIR) mice were used by numerous investigators as T-cell deficient animals for various purposes, e.g. to grow xenogeneic tumors[5], to study chemical carcinogenesis[6], T-B cell cooperation[7], etc. The purpose of the present study was