reaching only 19% in the presence of glucose alone after two years at 4 °C in darkness (11); these latter results are close to those of De Venuto et al. (2). It should be emphasized that the stability depended greatly on the lyoprotector used and that with Tris, for example, the proportions of methemoglobin increased rapidly, even at 4 °C (4), to 12.5% in one month and 45% in six months.

Our final point, which is less relevant to these concerns, is the mechanism of action of sucrose and the other products. It is in fact unknown, or almost so—as De Venuto et al. (2) and Bonderman et al. (1) have said. Our experience in this area over several years leads us to reject the hypothesis of an interaction with the iron or the heme (12), but a more precise explanation supported by experimentation cannot yet be put forward.

In conclusion, it appears to us from the consideration of the work of Bonderman and colleagues alongside our own results that the actual production of functional hemoglobin lyophilysates no longer raises difficulties. However, the goal is for them to keep their capability with time. This result has not been attained, and with certain lyoprotectors undoubtedly never will be. But this result must, in our view, be the object of current and future studies, especially if it is hoped eventually to prepare control lyophilysates for a specific and functional use (e.g., blood-gas measurement).

References


Pierre Labrude
Claude Vigneron
Centre régional de Transfusion sanguine et d’Hématologie
54500 Vandoeuvre les Nancy
France

This Letter was referred to the authors of the paper in question. A response follows.

To the Editor:

After careful consideration of the comments of Labrude and Vigneron, and perusal of the references cited, it appears to us that these comments fail to take into account the purpose for which the control was intended. As the title implies, the product was designed as a control for chemical methods for determining hemoglobin. We have shown that the material works admirably for this purpose. Admittedly, the effect shown in our paper could more properly be called lyoprotective since lyophilization is basic to the method of preparing hemoglobin quality controls. However, sucrose exhibits at least some cryoprotective properties, because freezing and thawing solutions without such protection causes destruction of the hemoglobin.

As to the concentration of sucrose used, we have found that the amount necessary for adequate protection is related to the hemoglobin concentration in the solution to be lyophilized. In the references cited (5, 6) above, hemoglobin concentrations of 50 g/L were used, and a lower concentration of protective agent may have provided adequate stabilization. In our system, which contained 100 g of hemoglobin per liter, lower concentrations of protective agents were shown to work initially but failed more rapidly when aging was accelerated by heating at 37 °C for two weeks.

Despite Labrude’s observation concerning mannitol, for the purposes of a precise control for the cyanmethemoglobin procedure this compound, in effective amounts (100 g/L), is useful. Further, we are not unaware of the effect of Ficoll in preparing lyophilized hemoglobin preparations, because we used Ficoll 400 several years ago to prepare hemoglobin electrophoresis controls and found it to be only partly effective. Ficoll has a further disadvantage—namely, high viscosity—which causes any lyophilized solution of hemoglobin fortified with effective amounts of it to constitute very slowly.

As to the working conditions that Labrude et al. do not understand, we regret that they have not stated where our article is less than explicit. We believe that what we reported was described precisely and that our results can be easily duplicated.

With respect to the criticism concerning the high methemoglobin in our, preparations, our hemolysates were prepared from outdated blood-bank units. Outdated units will contain more methemoglobin than does freshly drawn blood. The increase in methemoglobin we report is similar to that which Labrude has reported. We maintain, therefore, that for the stated purpose, the material and method we describe is fully effective, particularly since the analytical procedure to be controlled by the preparations requires that all forms of hemoglobin present be converted to methemoglobin and, finally, into cyanmethemoglobin, which is measured spectrophotometrically.

We agree that the product we describe is not adequate as a whole-blood substitute or as a blood-gas control. This was not our purpose.

Dean P. Bonderman
Dept of Pathol.
Univ Hosp., N440
Indiana Univ.
Indianapolis, IN 46223

The Unidentified Fluorescent Substance in Serum of Patients with Chronic Renal Disease is Also Found in Hemofiltrates, Dialysis Fluids, and Urine

To the Editor:

Serum from patients with chronic renal failure shows increased concentrations of a strongly fluorescent, but as yet unidentified, material (1–5). Fluorescent ligands bound to albumin are responsible for most of the fluorescence in serum (5). The fluorescence emission characteristics of the fluorescent substance in serum have recently been described and a method developed for its extraction from serum (5).

Here, we describe our investigations of the compound in hemofiltrates, dialysis fluids, and urine, as well as in serum.

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