face-plates. The other face-plate is then placed on top and the two are clamped together. When filled with the solution to be treated the bag bulges out into the recesses of the plates, forming the upper and lower reservoirs, and to a lesser extent in the channel slot, forming the channel. It is convenient to use a double length of tubing folded into a U-shaped bag instead of a single bag of tubing tied at the lower end, since this forms two upper reservoirs, two parallel channels, and a U-shaped lower reservoir. The speed of separation is doubled because of the two channels.

The cell frame shown in Fig. 1 may be used with bags of dimensions from 1 inch by 9 inches (measured when the bag is flattened) up to 3 inches by 12 inches, thus giving a range of capacity from 20 ml to approximately 250 ml.

Fig. 2 is a cross-section of the assembled apparatus. The container is a lucite cylinder 6 inches O.D. by 12 inches high provided with inlet and outlet (not shown) for circulation of the buffer. At the upper end of the U-shaped cell is a sliding gate, consisting of a flat plate of lucite with bevelled edges, which can be slid down between the two upper reservoirs into a V-shaped groove to pinch off the tubing at that point. This prevents mixing of the reservoir contents with the channel contents while the former are being removed.

Either graphite or platinum electrodes can be used. With graphite electrodes a cellophane bag must be placed around the anode to prevent contamination of the buffer by carbon particles.

The apparatus is especially useful in fractionating mixtures where maintenance of sterility is important. The procedure consists of autoclaving a length of dialysis tubing closed at each end, assembling the sterilized bag and frame, opening and filling the cell with the sterile solution using sterile precautions, and reclosing the bag during the run. Bacteria cannot pass through the dialysis membrane even though the external buffer is exposed to bacterial contamination.

Summary. An electrophoresis-convection apparatus for fractionating proteins is described which (1) has variable capacity, (2) is of simple leak-free construction, and (3) can be operated under sterile conditions.

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Improvement of a Hemin Standard by Recrystallization. (19849)

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(Introduced by N. B. Guerrant.)

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The lack of a satisfactory primary standard for the determination of hemoglobin has led to the development of several methods for the preparation of a hemin standard. The present investigation was undertaken to show that the hemin standard proposed by King (5) can be improved by recrystallization. The effect of recrystallization of crude hemin on its purity and stability was studied.

Experimental. Hemin was prepared from both bovine blood and human blood (outdated transfusion blood). Independently prepared samples* of both crude and recrystallized hemin were also used. For the first sample from bovine blood the method of Hans Fischer(3) was used, the increase in purity

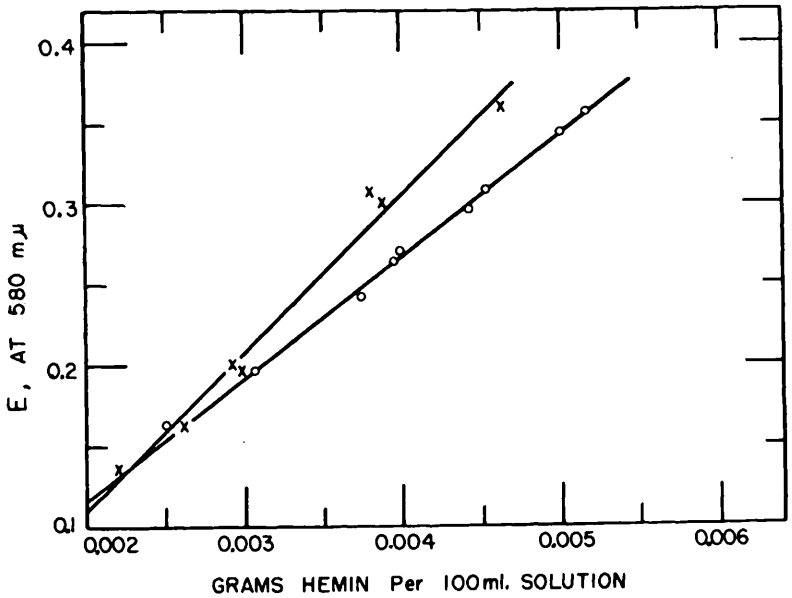

FIG. 1. Extinction vs. concentration. ××—Crude hemin in borate buffer. ○○—Recrystallized hemin in borate buffer.

after each of 4 recrystallizations being studied by determining the percentage of iron in each batch of crystals. A modification of the Delory method(2) was used to prepare hemin from human blood. All hemin samples were analyzed for iron by a micro-modification of the titanous chloride method of Delory(2), and of Wooton and King(6). A modification of the micrometer burette developed by Dean (1), and by Wooton and King(6) was used, the uniformity of the bore being checked by calibration with mercury. Stock solutions of crude and of recrystallized hemin, each containing approximately 3 mg per 10 ml were prepared according to the method of Horecker (4) by dissolving accurately weighed amounts in N/10 borate buffer at pH 9.4. After allowing these solutions to age overnight at about 6°C, they were diluted to give concentrations of hemin ranging from 10 to 50 mg per liter, as recommended by Horecker(4). The conformity to Beer's Law of these solutions was determined by measuring their extinctions at 580 mμ on a DU-Beckman spectrophotometer. The effect of recrystallization of crude hemin on its stability with age was studied by measuring the absorption spectra of these dilute solutions over the range 475-690 mμ at 3 time intervals: immediately after dilution, 16 days after dilution and one month after dilution.

Results and discussion. That recrystallization of hemin causes solutions of it to conform more closely to Beer's Law is shown by the curves in Fig. 1, in which the optical densities at 580 mμ of different concentrations of crude and of recrystallized hemin are shown. Further evidence that the purity of hemin is improved by recrystallization is presented in Table I, the data showing that the iron content is increased by recrystallization.

The variations with respect to wavelength of the extinction coefficients $\left(E_{1\ cm}^{1\ per\ cent}\right)$ of 4 different preparations of crude hemin are

TABLE I.
Iron Content of Crude and Recrystallized Hemin.

Sample	% Fe
Crude crystalline bovine hemin (authors)	7.83
1st recrystallization " " "	8.23
2nd	8.33
3rd	8.47
4th	8.45
Crude amorphous bovine hemin (Armour lab.)	6.91
Recrystallized " " "	8.53
Crude crystalline human " (authors)	8.25
Recrystallized " " "	8.44

* These samples were contributed by the Armour Laboratories and by the Department of Biochemistry at the University of Buffalo.

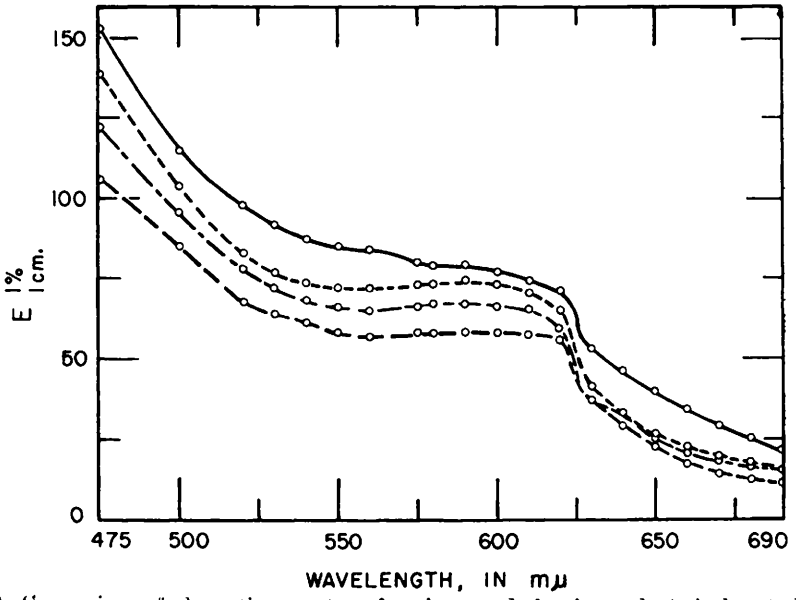

FIG. 2. Comparison of absorption spectra of various crude hemin products in borate buffer.

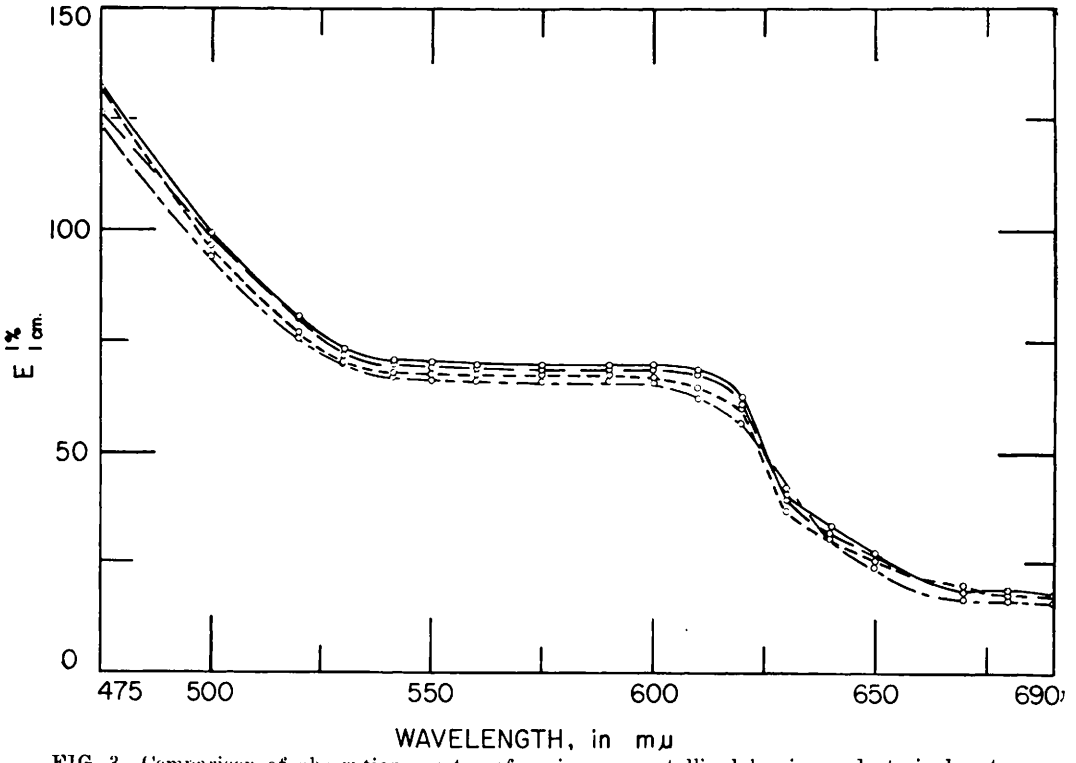

FIG. 3. Comparison of absorption spectra of various recrystallized hemin products in borate buffer.

shown in Fig. 2. and the same relationships for the same preparations after one recrystallization are presented in Fig. 3. Since replicate values for the various dilutions of each product were in excellent agreement, average values are plotted, each curve representing

TABLE II. Ratio of Extinction Coefficients of Dilute Solutions of Crude and of Recrystallized Hemin at 580 mμ over a Period of One Month.

Sample	Ratios of $E^{1\ per\ cent}_{1\ cm}$ at 580 mμ 16 days	1 mo
Crude crystalline bovine hemin (authors)	1.066	1.108
1st recrystallization bovine hemin (authors)	1.015	1.015
2nd	1	1
3rd	1	1
4th	1.014	1.014
Crude amorphous hemin (Armour lab.)	1	1
Recrystallized hemin (Armour lab.)	1.015	1.029
Crude crystalline human hemin (authors)	1.014	1
Recrystallized human hemin (authors)	1.015	1.015

the mean extinction coefficient for 3 concentrations of each preparation. It is evident that recrystallization of hemin makes for significantly greater uniformity and reproducibility among several preparations. Another way to show this effect of recrystallization is by calculating the ratio of the highest extinction coefficient at a particular wavelength to the lowest at the same wavelength. For the crude hemin preparations this ratio at 590 mμ is 1.36; for the recrystallized material it is 1.05, indicating a much smaller variation in the degree of light absorption by the recrystallized hemin.

Ratios of extinction coefficients are also used to present the effect of age of hemin solutions on their stabilities. In Table II are presented the ratios of $E^{1\ per\ cent}_{1\ cm}$ for dilute solutions of the several hemin preparations 16 and 30 days after dilution to the values of $E^{1\ per\ cent}_{1\ cm}$ of the same solutions immediately after being diluted. All ratios are calculated at 580 mμ and represent the maximum deviations of the aged solutions from the fresh dilutions. It is evident that the recrystallized samples were more consistently stable than the crude preparations.

Similar studies on the stock solutions indicated that concentrated solutions of hemin are stable regardless of purity, as the ratios of $E^{1\ per\ cent}_{1\ cm}$ were very close to unity for all samples.

Summary. 1. The purity of crude hemin preparations used, as determined by their iron content, was improved by recrystallization. 2. Crude hemin preparations in borate buffer showed variable extinction coefficients at any wavelength selected along the flat portions of the absorption spectra. Recrystallized hemin preparations from the above crude hemin samples showed very nearly equal extinction coefficients at the same wavelength. 3. Dilute solutions of recrystallized hemin from different sources were more uniformly stable than dilute solutions of crude hemin during a one-month period.

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