valine N-terminale. Quand l'hydrolyse est plus poussée, il apparaît d'un seul coup de faibles quantités de valine, de proline et d'isoleucine. Ces résultats sont compatibles avec la séquence $CySO_3H$.Gly.Val– proposée par MEEDOM[7]. Ils suggèrent en outre, étant donné les difficultés qu'éprouve l'enzyme à détacher la valine, que la proline occupe la 4° position dans la chaîne A.

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Chromatographic behaviour of hemoglobins

The successful application of adsorption chromatography has been complicated by the instability of protein molecules on supporting media and their tendency to be irreversibly adsorbed or to give strongly curved adsorption isotherms. However, a number of inorganic adsorbents and ion exchangers have been reported as suitable for protein chromatography. Recently, two rather complete reviews were published by MOORE AND STEIN[1] and by SOBER AND PETERSON[2].

The main object of the present work was to study the chromatographic behaviour of hemoglobins on adsorbents like calcium phosphate, hydroxylapatite and the cellulosic cation exchanger, carboxymethyl cellulose (CM-cellulose) and to find out the conditions for the separation of human adult hemoglobin (Hb A) and human fetal hemoglobin (Hb F). The chromatographic behaviour of some avian hemoglobins on Amberlite IRC-50 is also reported.

The blood samples were collected from normal adults by venipuncture and from the umbilical cord. The avian blood was obtained by cutting the jugular vein. The red cells were washed with isotonic saline and hemolysed as described earlier[3,4]. The clear hemoglobin solution thus obtained was stored at —4° in small test tubes and thawed prior to their application on the column.

Hydroxylapatite was prepared as described by TISELIUS *et al.*[5]. CM-cellulose of 0.46 mequiv/g dry material was prepared and regenerated by treating the used material with 0.5 *M* NaOH–0.5 *M* NaCl as described by PETERSON AND SOBER[6]. The columns were washed with the starting buffer till the pH of the effluent reached that of the buffer. Hydroxylapatite columns were packed under 0.8–1.0 kg/cm^2 pressure and slight pressure (0.4–0.5 kg/cm^2) was found to be sufficient for the CM-cellulose columns. Initial treatment of Amberlite resin IRC-50 XE 64 was performed according to

BOARDMAN AND PARTRIDGE[7] and was equilibrated with the starting buffer till the pH of the effluent attained the same pH value.

Columns (15–16 × 0.8–1.0 cm) were used with a flow rate of 1.0–2.0 ml/h. 0.2–0.5 ml fractions were collected with the help of an automatic time-operated fraction collector. The fractions were diluted to 2.0 ml with the developing buffer and the amount of hemoglobin contained in each eluate fraction determined by measuring the absorbance at 540 mμ in a Beckman Spectrophotometer model B using a 0.5-ml cell. The buffer solutions were chilled prior to use and chromatographic operations were conducted in a cold room at 2–3°.

Generally, 0.5 ml hemoglobin solution (approx. 50 mg Hb A) was applied to the hydroxylapatite column (15 × 1.0 cm). The hemoglobin solution was dialysed against the starting buffer (0.005 *M* sodium phosphate buffer, pH 6.8) and then centrifuged at 10,000 × *g* for 30 min at 2° to remove any precipitate formed during dialysis. Stepwise elution with phosphate, pH 6.8, in increasing concentrations, *viz.*, 0.005, 0.04, 0.08, 0.1 and 0.2 *M* were employed. Three peaks emerged respectively at 0.08, 0.1 and 0.2 *M* buffer. These fractions—24, 46 and 30% respectively—were concentrated by pervaporation and were then analysed by paper electrophoresis with barbiturate buffer, pH 8.6, *I*, 0.05, at 220 V applied for 16 h. The first fraction was rather slower moving than the other two. No difference could be observed in the mobilities of the other two fractions. It may, however, be pointed out that no visible sign of denaturation or irreversible adsorption on the columns could be noticed. The absorption spectra—visible and ultraviolet—did not clearly indicate any difference between these components. It may be mentioned that a similar phenomenon has been observed by TISELIUS *et al.*[5] during the elution procedure of phycoerythrin adsorbed on a hydroxylapatite column. Attempts were then made to carry out the experiment at lower pH, *e.g.* pH 5.8, other conditions remaining constant. In this case, two fractions (49.2 and 50.8%) were obtained with 0.3 and 0.4 *M* buffer respectively and paper-electrophoretic analyses did not reveal any difference in the mobilities. Spectrophotometric measurements, however, showed the presence of methemoglobin in the second fraction. Hb A was then applied to the column as HbCO and the developing buffer was saturated with CO immediately before its application to the column. Hb A emerged out of the column as a single peak with 0.1 *M* phosphate, pH 6.5, and without any tailing or irreversible adsorption. Although further work was not undertaken to study the cause of multiple zoning or to characterise the different fractions thus obtained, it seems rather interesting to note that ALLEN *et al.*[9] developed normal human adult hemoglobin on Amberlite IRC-50 with phosphate buffer, pH 7.22–6.91 and obtained three minor components which differed in their isoleucine content.

Attempts were then made to separate the fetal and adult hemoglobins present in the hemolysates which were obtained from the blood samples collected from the umbilical cord. Both the columns of hydroxylapatite and CM-cellulose were equilibrated overnight with 0.005 *M* phosphate, pH 6.5, and a 0.2 ml sample as HbCO was added to each column. Phosphate of the following concentrations was used after being saturated with CO for the elution procedure, *viz.*, 0.005, 0.05, 0.08, 0.1 and 0.2 *M*. To facilitate a better comparison, columns of identical dimension were used with Amberlite IRC-50 XE 64, with the experimental conditions maintained according to HUISMAN AND PRINS[8]. Fig. 1 shows the chromatographic behaviour of Hb A and

Hb F on different adsorbents. As expected, CM-cellulose was found to be more efficient than the hydroxylapatite column. It is of interest to point out that a good separation can be achieved on CM-cellulose though it contains only one-tenth of the ionizing groups present in IRC-50. The fractions thus obtained from hydroxylapatite and CM-cellulose columns were concentrated by pervaporation, rechromatographed and were found emerging out of the column as a single peak with the same concentration of buffer as used in the original experiment. Good separation could not be achieved with citrate buffer which had to be used in connection with the experiments on Amberlite IRC-50 columns. The chromatographic behaviour of globulins is largely influenced by the cation concentration[6,10]. Graded amounts of NaCl were added to 0.005 *M* phosphate, pH 6.5, to raise the Na^+ concentration. Two peaks emerged

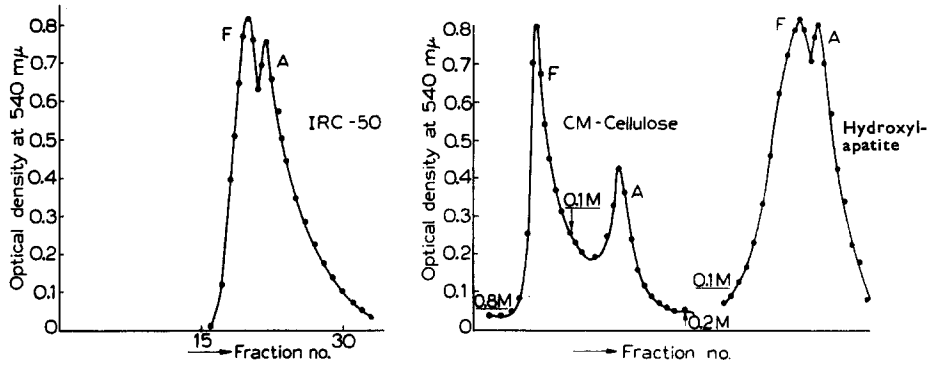

Fig. 1. Chromatographic behaviour of hemoglobins on different adsorbents. Fig. 1A represents the behaviour on IRC-50 XE 64, the experiment being conducted with CO-saturated citrate buffer, pH 6.5, as described in[8]. Figs. 1B and 1C show the stepwise elution of hemoglobins present in the mixture, with phosphate buffer, pH 6.5, saturated with CO. The same amount of hemoglobin has been added in each column and the amount of resolved Hb F emerged out of IRC-50, CM-cellulose and hydroxylapatite columns was found to be 56.6, 61.4 and 54.9 % and Hb A was 43.4, 38.6 and 45.1 % respectively.

one at a time with Na^+ concentration 5.85 and 7.5 g/l respectively. Chromatographic studies with sulphomethyl cellulose[11] showed that it is possible to fractionate the hemoglobin components A and F, though the separation achieved in this case was not as efficient as that with CM-cellulose. Good resolution between the two components present in chick (*Gallus domesticus*) and common Moyena (*Acridotheres tristis* Linn.) has been achieved with both IRC-50 and CM-cellulose and the results were substantiated by paper electrophoresis. Correlation between the mobilities of the different components on electrophoregrams and adsorbent columns was observed to be maintained during the experiments.

Frontal analyses of Little Cormorant (*Phalacrocorax niger* Vieillof) hemoglobin were carried out on CM-cellulose of 0.6 mequiv/g dry material. A short column (3.0 × 0.6 cm) was employed. Hemoglobin was dialysed overnight and then diluted with 0.05 *M* phosphate, pH 6.5, to a 0.1 % solution. Hemoglobin solution thus prepared was passed through the CM-cellulose column. Photometric measurement of the eluate fractions revealed three breaks in the density curve and confirmed the presence of three hemoglobin components revealed by filter-paper electrophoresis.

Starting-agent development technique as employed by HUISMAN *et al.*[8] with

citrate buffer, pH 6.0, containing 0.15 *M* Na^+ has been utilised for the chromatographic study. Hemoglobin solutions of chick, pigeon (*Columba livia*), duck (*Anas anas*) were resolved into two zones and of Little Cormorant into three zones. It seems rather interesting to note that, although the hemoglobins from white-breasted water hen (*Amaurornis phoenicurus* Pennant), crow (*Corvus splendens splendens*) and black-headed oriole (*Oriolus xanthornus* Linn.) could be resolved into three components by paper electrophoresis, under the present experimental conditions only two zones could be observed.

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Nephelometric determination of cell count in synchronously dividing cultures of bacteria

Synchronization of cellular division of bacteria induced by chilling has been described in several recent papers[1-3]. Although this method is simple, the frequent estimation of the number of bacterial cells at short intervals by plate counts is tedious and time-consuming. Determination of direct cell count in a bacterial counting chamber suffers from the same disadvantage. Standard turbidity measurements in the spectrophotometer do not discriminate between relatively small changes in highly diluted, synchronously dividing populations. The same difficulties arise when other methods of synchronization are used.

In our experiments a Pulfrich photometer-nephelometer (C. Zeiss, Jena) has been chosen for these determinations. This highly sensitive instrument measures the turbidity of a bacterial suspension by means of the light scattered at a given angle and gives reliable readings of the increase in cell number of relatively small concentrations of bacteria, while the experiment is in progress.

An actively growing streptomycin-resistant strain of *Escherichia coli* B (3-h culture at 37°) was inoculated in 150 ml tryptose medium (0.5 % Bacto-Tryptose and