EVIDENCE FOR A DISTINCT FRACTION OF HEMOGLOBIN, SPECIFICALLY ASSOCIATED WITH THE YOUNG ERYTHROCYTES.°

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Labelling hemoglobin in vivo with ^{59}Fe or ^{14}C and fractionating on alumina (Schapira et al., 1950 and 1955) have given evidence for a metabolic heterogeneity of rabbit hemoglobin.

By electrophoresis on starch block, heterogeneity was also observed and has been explained by the "aging" of a fraction of the hemoglobin. The minor anodic fraction was shown to consist of hemoglobin molecules whose electrical charge was modified during the life span of the erythrocytes (Kunkel and Bearn, 1957 ; Rosa et al., 1960).

In the present work, we study the metabolic significance of heterogeneity by the use of ion exchanger Amberlite IRC 50, according to the technic of Allen, Schroeder and Balog (1958). We have been able to characterize, next to the "old" fraction, a "young" hemoglobin.

EXPERIMENTAL

Rabbits weighing 3 Kg were injected with 60 µc of ^{59}Fe. Several samples of blood were taken at various times after the injection, from the 16th hour to the 38th day.

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Hemoglobin was prepared according to the method of Roche, Derrien and Moutte (1941) by salting out in solutions of phosphate 3,5 M. Before starting the chromatogram, the hemoglobin solution was dialyzed overnight against one hundred times the volume of the developer, and then centrifuged 20 minutes at 20 000 g.

Amberlite IRC 50 was purified according to the method of Hirs, Moore and Stein (1953). The fraction that passed a 250 mesh sieve and was retained by a 325 mesh sieve was used for pouring all columns.

The columns were poured according to the method of Allen, Schroeder and Balog (1958). The developer No. 1 Allen et al. was used. The procedure of operation was that described by Clegg and Schroeder (1959). The tail of the major component was eluted by warming the column to room temperature.

The optical density was determined with a Beckman DU spectrophotometer at 553 mμ without dilution of the fractions (This wavenlength was demonstrated to be the isobestic point of oxyhemoglobin and cyanhemiglobin).

Radioactivity was counted in a scintillation counter (Tracerlab), 2 ml taken from each fraction being counted for 10 minutes.

Specific activity of each sample was computed as

$$\frac{\text{Radioactivity in 2 ml (in counts/min.)}}{\text{Optical density at 553 mμ}}$$

RESULTS

A) Chromatographic behaviour of rabbit hemoglobin

Normal rabbit hemoglobin on Amberlite IRC 50 behaves in a way similar to human hemoglobin as described by Clegg and Schroeder (1959) : one or two fast minor components, and one

major component whose elution is very slow but can be accelerated by heating the column.

By matching the optical density at 553 and 280 mμ, it appears that a non haem protein was eluted with the ascending part of the first minor fraction, but all the other fractions appeared to be pure hemoglobin, according to the spectrophotometrical data.

B) Specific activity (S.A.) of the eluted fractions

Figure 1 shows that the different fractions display differences of labelling ; in addition the S.A. of the different fractions are changing with time.

a) On the first day and up to the third day the most striking fact is the existence of an increase of the specific activity at the tail of the major fraction, which therefore shows a much higher S.A. than the major fraction. On the contrary the fast fraction displays a lower S.A.

b) With time the distribution of S.A. becomes different : the S.A. of the major fraction remains stable, corresponding to the classical "plateau". The fast minor fraction increased progressively in radioactivity : its curve of S.A. crosses that of the main fraction and becomes finally higher. By contrast a striking evolution is seen in the fraction located at the tail of the major component : it goes from a higher S.A. than any fraction to the lowest S.A. of all fractions after the 35th day. These results are summarized in figure 2.

c) In one rabbit reticulocytosis was induced by bleeding. Hemoglobin was prepared on a sample taken 20 h. after injection of ^{59}Fe (fig. 1d).

The heating of the column results in the elution of a discrete peak with a high S.A. (the spectrum of this component is identical to that of pure hemoglobin).

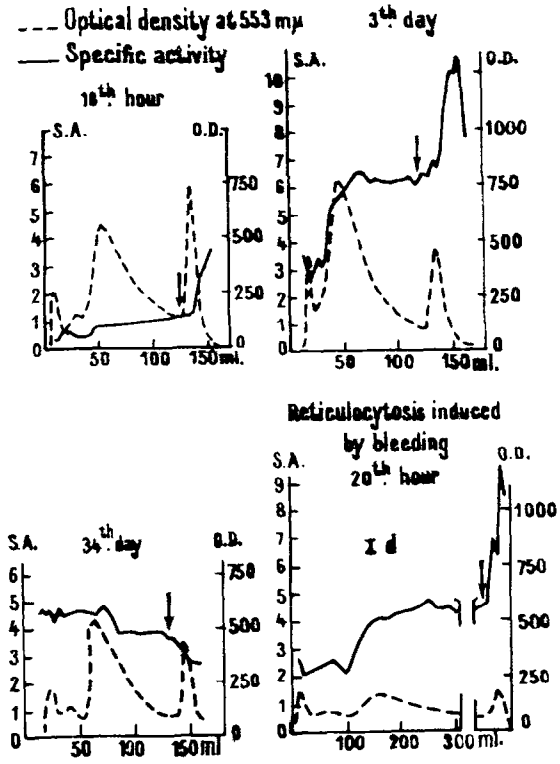

Fig. 1

Chromatographic elution and specific activity of rabbit hemoglobin at various times after the incorporation of ^{59}Fe (Arrow indicates the time of warming of the column).

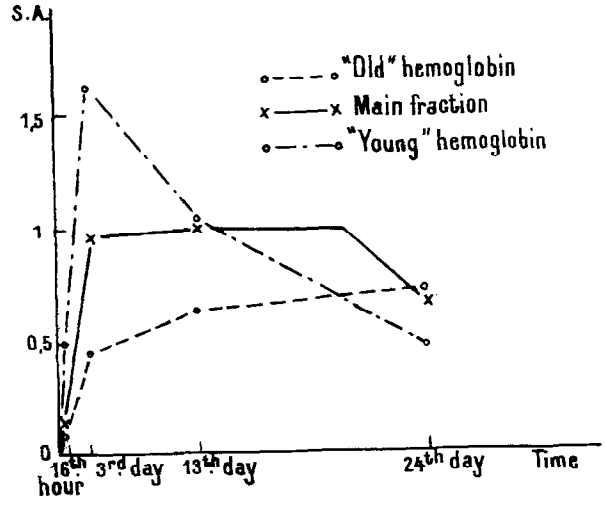

Fig. 2

Variations of the specific activity of the three fractions with time.

INTERPRETATION OF THE DATA

1. The fast fraction probably corresponds to the fast component observed on starch block electrophoresis and discussed previously, and is probably made of "old" hemoglobin.
It shows low S.A. on the first days : the rise in S.A. is due to the accumulation in this fraction of labelled molecules, whose electrical charge is altered in the course of aging.

2. The tail of the main fraction, because of its high radioactivity, must be considered as being made mainly of hemoglobin from young cells. The behaviour of this "young fraction" may be interpreted in two ways :

a) The existence of red cells endowed with a very short life span : if such is the case, it would have to be concluded that these cells possess a special type of hemoglobin more slowly eluted from the columns than that of the other red cells.

b) Alternatively it could be assumed that newly formed red cells contain a special type of "young", conceivably unfinished, hemoglobin, which would, after a few days, acquire the properties of the major fraction.

c) In both cases the observations summarized above reveal the existence of a distinct fraction of hemoglobin, specifically associated with the population of young erythrocytes.

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