POLYPEPTIDE CHAINS OF CHICK EMBRYO HEMOGLOBINS

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Three hemoglobins have been found in the circulating red blood cells of the chick embryo during its development (Fraser, 1964a). These change in concentration such that the dominant one during the early phase of development gradually declines until the time of hatching, at which time it constitutes about 20% of the hemoglobin content (Fraser, 1961, 1964a). A third form, appearing around 7 days of incubation, remains rather uniform in concentration. Apparently all species of hemoglobin reside in each cell, as judged from breaks in the denaturation curves of the hemoglobins within single cells (Matioli and Thorell, 1963). Moreover, we have found (Fraser, 1964b) that both lines of erythrocytes in the circulating blood of 7-day embryos contain the same hemoglobins in the same concentrations.

From the point of view of the chemical embryologist the observation that the hemoglobins progressively change during development is of particular interest. We may imagine a maximum of 6 polypeptide chains used in the formation of the 3 hemoglobins. However, if 1 or more of the chains is used in at least 2 of the hemoproteins, the total number of polypeptide chains would be less. We report here that there are 3 chains in all in the chick embryo, and that the concentration and sequential appearance of these is directly related to the hemoglobin content of the cells during the chick's growth.

Materials and Methods

Blood of White Leghorn chicks of various embryonic ages was taken from either yolk sac or chorio-allantoic vessels as described before (Fraser, 1961). The cells were washed 3 times with cold Ringer's solution (Spratt, 1947; Howard, 1953) buffered at pH 7.6 with 0.067 M phosphate. Following the final centrifugation the cells were lysed with 3 volumes of cold water, and separation of the hemoglobins was performed by electrophoresis on cellulose acetate. The bands were cut from the strips following electrophoresis, and the Hbs were washed individually into 5 ml of cold water. The isolated Hbs pooled from 8 strips were dialyzed for 24 hours against a continuous flow of cold distilled water, and were then treated with acid acetone (Anson and Mirsky, 1930) to cleave the heme. The globin of each fraction was lyophilized, then dissolved in about 0.1 ml of glycine-HCl buffer, pH 2.6, I 0.05 (Long, 1961) containing 6M urea and a trace (0.01 ml/100 ml buffer) of 2-mercapto-ethanol. To this was added the same volume of 60% sucrose, and the solution was mixed.

The chains were separated by acrylamide gel electrophoresis in glass tubes (75 mm x 5 mm I.D.). The preparation of the gels was based on the formula given by McAllister and coworkers (1963). Certain important modifications were incorporated, and only these will be described here. Sufficient gel for 5 tubes was made by mixing 1) 4 ml of Temed catalyst, 2) 2 ml of the glycine-HCl buffer containing 6M urea and a trace of 2-mercaptoethanol, 3) 2 ml of an acrylamide monomer solution, and 4) 2 drops of 2.8% ammonium persulfate. The Temed catalyst consisted of 0.46 ml of Temed (N,N,N',N'-tetramethylethylenediamine) in 40 ml of the glycine-HCl buffer whose formula is given above. After mixing, the fluid was immediately poured into the 5 tubes (which were plugged at the lower end) to a height of 1 cm from the top. A few drops of the glycine-HCl buffer used in the electrode wells (see below) were carefully placed over each gel solution. Gelation occurred within 5 minutes. With an overlayer of fluid the gels form a flat upper

surface showing no meniscus effect.

Electrophoresis was carried out essentially as described by McAllister et al. (1963). We modified the placement of the sample on the top of the gels as follows. One drop of the solution containing the denatured chains was layered carefully on the top of the gel in each tube, after the buffer, previously used during gelation, had been removed. Glycine-HCl buffer (pH 2.6, I 0.05, without urea and mercaptoethanol) was layered very carefully over the samples. This was added until each tube was filled to excess. The same buffer was added to the wells. Care was taken when the buffer was poured into the top well to prevent undue mixing in the buffer overlaying the samples. Air bubbles that may have been trapped at the bottom of the tubes were flushed away with buffer from a bent Pasteur pipet. Each tube was supplied with a current flow of 10 mamps for 2 1/2 hrs. at 4° C.

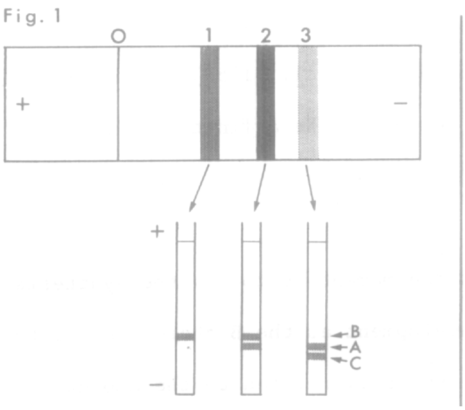
The gels were then removed from the tubes, stained overnight with 0.5% Amido Black in 7% acetic acid, and destained in a photopolymerized 6.0% acrylamide solution containing 0.5 mg % riboflavin, as described by McAllister and coworkers (1963).

Similar analyses were made on the polypeptide chains of unfractionated hemoglobins from chick embryos of various ages.

Results and Discussion

A diagram of the results of the acrylamide gel electrophoresis of the chains from the 3 Hbs of the 14-day embryo is presented as Fig. 1. In all, there are 3 chains present in the 3 Hbs. Hb 3* yields chains A and C, while Hb 2 consists of chains A and B. Hb 1, however, is made up of B chains only. The electrophoretic behavior of the chains is consistent with that of the hemoglobins from which they were derived. Hb 3, which is the most basic of the three, alone contains polypeptide C, which in turn is the most basic of the 3 chains.

*We number the hemoglobins according to their electrophoretic mobility, while the polypeptide chains are given letters making them analogous to their mammalian counterparts, for the sake of the discussion that follows.



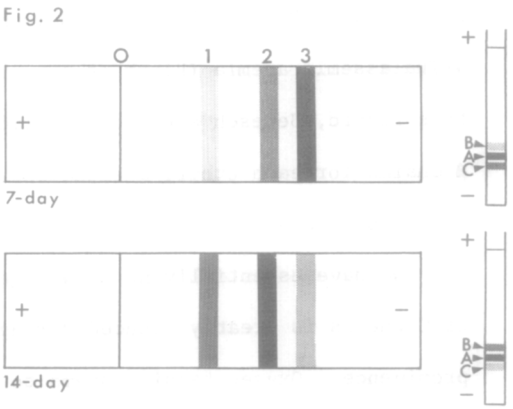


Fig. 1. Polypeptide content of the 3 hemoglobins from the 14-day chick embryo. Hb separation performed by cellulose acetate electrophoresis in tris buffer, pH 8.9, I 0.05; chain separation by acrylamide gel electrophoresis.

Fig. 2. (Left) Band patterns of the hemoglobins of 7-day and 14-day embryos in cellulose acetate electrophoresis. (Right) Polypeptide content of whole, unfractionated hemoglobins from embryos of the same ages.

On the left of Fig. 2 is shown the distribution and relative concentration of the hemoglobins from the 7- and 14-day embryo as determined by cellulose acetate electrophoresis (Fraser, 1964a). To the right of each strip is shown the pattern of the polypeptides in polyacrylamide gels from whole, unfractionated hemoglobins from embryos of corresponding ages. During development there is an obvious shift from the synthesis of C to B chains, similar to the change from γ to β chains in the human.

Indeed, in this respect, Hbs 2 and 3 of the chick embryo behave much like mammalian Hbs A and F respectively. It should be pointed out, however, that the transition in the chick occurs gradually during embryogeny (Fraser, 1961), such that the newly hatched bird has essentially the same hemoglobin content as the adult.

The presence of Hb 1 in the blood of the chick embryo has its counterpart in the human as well. In the bird the overproduction of the B chains is a normal event resulting in a hemoglobin consisting solely of it. In the human the excessive synthesis of the β chain is an abnormal occurrence,

resulting in Hb H, a hemoprotein found in varying amounts in persons with α-Thalassemic anemia (Rigas et al., 1956; Jones et al., 1959). Finally, in this regard, Benesch and Benesch (1964) recognize the affinity of the β chains for each other, a situation that must also exist between the B chains of the avian embryo.

We have essentially no knowledge at the moment as to why the synthesis of C chains is greatly reduced during development as the B chain comes into prominence. Oxygen tension seems to be inversely related to the amount (Hammel and Bessman, 1966), but not to the species of hemoglobin synthesized (Jonxis, 1965). Most credence is given to a hormonal mechanism involved in the regulation of Hb forms (Bromberg et al., 1957; Moss and Ingram, 1965). We have initiated a study of such pertinent factors in the regulation of synthesis of embryonic chick hemoglobins.

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