A Comparison of the Hemoglobins Occurring in Fetal and Adult Pigs[1] (35808)

STANLEY C. GLAUSER AND ELINOR M. GLAUSER

Department of Pharmacology, Temple University School of Medicine, Philadelphia, Pennsylvania 19140

There are a marked number of similarities between porcine physiology, pharmacology, and biochemistry and that of human beings (1, 2). With wide use of this laboratory animal, the question arose as to whether fetal pigs, like fetal humans, have a hemoglobin differing from that hemoglobin found in the adult of the species. We, therefore, undertook a comparison of hemoglobins from adult pigs and hemoglobins from pigs obtained from the sow uterus before birth, during the second month of gestation. Applying the most commonly used criteria for differentiating adult and fetal hemoglobins, we determined the resistance to alkali denaturation, electrophoretic mobility, and isoleucine content. In addition to these methods, the fingerprint method of Ingram (3), was used to compare fetal and adult hemoglobins.

Materials and Methods. Adult pig blood was obtained immediately after the animal was stunned. Fetal pig blood was obtained from pigs delivered by cesarean section during the second month of gestation. The blood was drawn by cardiac puncture and collected in heparinized containers. The hemoglobin was prepared by the method described by Ingram (4). Alkali denaturation was carried out using the method of Jonxis and Visser (5).

The hemoglobin was digested in 6 *N* HCl for 24 hr in a sealed tube, under nitrogen, at 105° in preparation for amino acid analysis. The amino acid analysis was carried out by using the Spackman *et al.* (6), technique of column chromatography to analyze for all of the amino acids except tryptophan. This gave the isoleucine content and enabled a calculation of the number of amino acids per alpha–beta subunit.

The "fingerprint" technique (3) was carried out on globin prepared from both adult and fetal hemoglobin separately.

Results and Discussion. Hemoglobins from 4 adult pigs and 6 fetal pigs were independently analyzed by the techniques described above.

Electrophoresis of the adult pig hemoglobin showed that it consisted of one major component. Electrophoresis of fetal pig hemoglobin demonstrated that this, also, consisted of one major component (7). The fetal hemoglobin has a lower mobility when electrophoresed at pH 8.6 in barbital buffer than did the adult hemoglobin. This difference in mobility was best demonstrated by taking a mixture of fetal and adult pig hemoglobin and electrophoresing this mixture. This electrophoresis resulted in two well-resolved components (Fig. 1).

Amino acid analysis of the digest of adult pig hemoglobin showed 1 isoleucine residue for every 282 amino acid residues. With the digest of the fetal pig hemoglobin, 1 isoleucine residue/276 amino acid residues was found. This is in contrast to human fetal hemoglobin, where there are 4 isoleucine residues per alpha–gamma dimer of 287 residues, while human adult hemoglobin has no isoleucine residues in the alpha–beta dimer of 287 residues.

On alkali denaturation, smooth curves were obtained when the logarithm of the percentage hemoglobin was plotted against time, for both the fetal and adult pig hemoglobin. However, it was found that the adult pig hemoglobin denatured more slowly and to a lesser extent with 32.1 ± 6.1% of the hemo-

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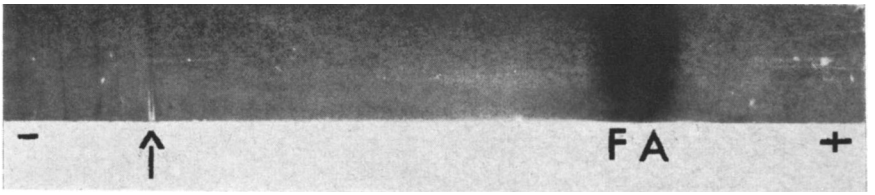

FIG. 1. Electrophoresis of a mixture of purified fetal and adult pig hemoglobin in the standard barbital buffers at pH 8.6 on sepraphore III supporting media for 2 hr and then stained with Ponceau S (0.5%) in 5% trichloroacetic acid for at least 5 min, followed by 3- or 4-min washings in 5% acetic acid, to remove all background color. The strip was then placed on a clean, nonabsorbent surface until almost dry. Finally, the strip was pressed with filter paper to prevent curling of the electrophoresis strips. The slower component (F) corresponds to the mobility of the fetal pig hemoglobin and the more rapidly moving component (A) corresponds to the mobility of the adult pig hemoglobin. The arrow indicates the origin.

globin still undenatured at the end of 15 min. The fetal pig hemoglobin denatured more rapidly and more completely with 16.8 ± 4.4% of the hemoglobin still undenatured at the end of 15 min. This, also, is in contrast to human hemoglobins, where the human fetal hemoglobin is more resistant to alkaline denaturation than is human adult hemoglobin.

The fingerprint pattern of the tryptic digests of adult and fetal hemoglobins are shown in Fig. 2. There were a number of striking differences in these patterns. An example of this was a change in the relationship of peptides four and five. Peptide four was identified in both patterns as it was positive to the α-nitroso-β-naphthol test for tyrosine in both cases. With the position of the 4 peptide established, it was possible to see a shift in the 5 peptide from a position above and to the left of the 4 peptide, in the adult pig hemoglobin, to a position below and to the left of the 4 peptide, in the fetal pig hemoglobin. The entire pattern in the region of the 12, 13, and 14 peptides, when going from the adult hemoglobin fingerprint to the fetal hemoglobin fingerprint, was distorted. Peptides 37 and 23, which were present in adult pig hemoglobin, were absent in fetal pig hemoglobin. However, a number of peptides did remain the same. The triplet, in the lower left hand corner of the fingerprint was essentially the same. Thus, more than half of the peptides were mapped on the same position in both the fetal and adult pig hemoglobin fingerprints, while somewhat less than half either shifted in position significantly, or disappeared and gave rise to new peptides. The number of these shifts was too large to be sure which new peptides, in the fetal hemoglobin fingerprint, came from which peptides in the adult hemoglobin fingerprint.

Summary. Hemoglobin was prepared from adult pigs and from fetuses in the second month of gestation. Both the adult and fetal pig hemoglobin consisted of one major component on electrophoresis in barbital buffer at pH 8.6. However, the adult pig hemoglobin had a faster mobility under these electrophoretic conditions than did the fetal pig hemoglobin. On amino acid analysis, using the accelerated Moore and Stein column technique, it was found that both the fetal and adult pig hemoglobin had one isoleucine per dimer subunit. On alkaline denaturation, it was found that the adult pig hemoglobin denatured more slowly and to a lesser extent than did fetal pig hemoglobin. The adult pig hemoglobin had 32.1 ± 6.1% of the hemoglobin still undenatured at the end of 15 min, while fetal pig hemoglobin only had 16.8 ± 4.4% still undenatured. The peptide fingerprint pattern showed that less than half of the peptides had been altered when comparing adult and fetal hemoglobin. It is concluded that there are two distinct hemoglobins in the pig; an adult hemoglobin and a fetal hemoglobin.

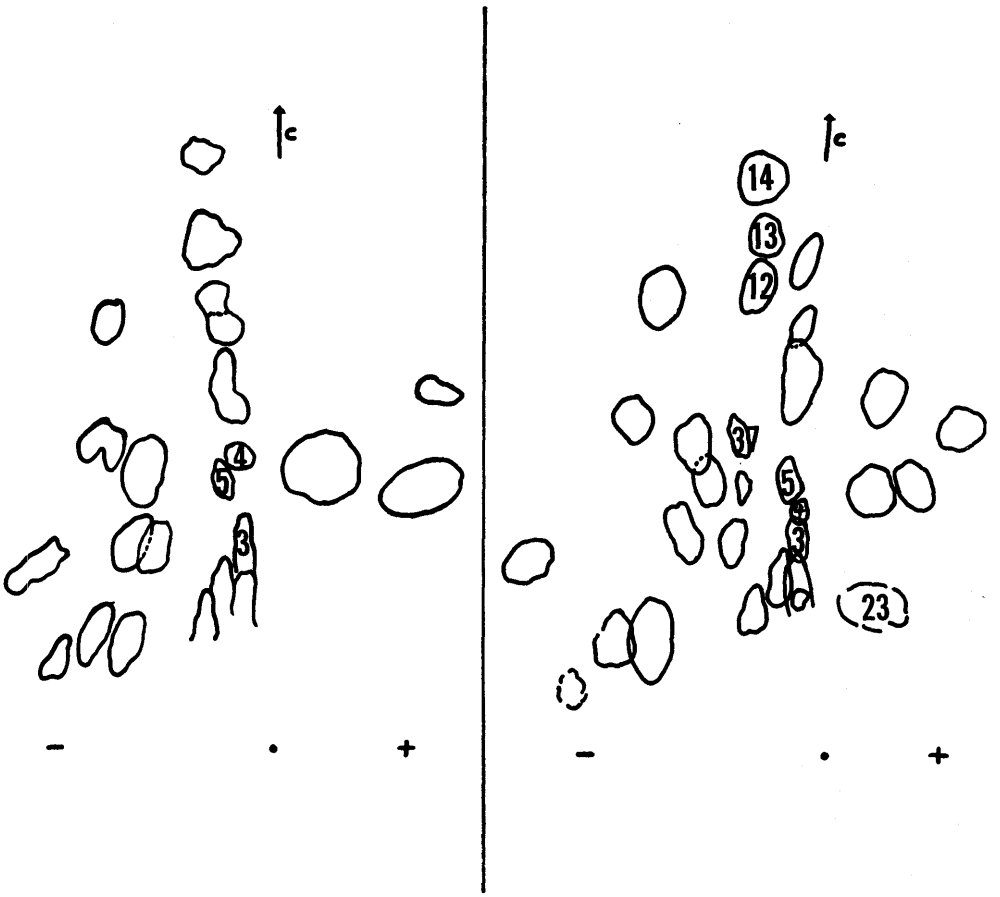

FIG. 2. Fingerprint pattern of pig hemoglobins: The globin was digested with trypsin, then electrophoresed, and then chromatographed in the *n*-butanol:acetic acid–water solvent (3:1:1) described by Ingram (3). The chromatography direction is indicated by the arrow (C), while the direction of electrophorsis is at right angles to that indicated for chromatography; and the polarity of electrophoresis is as indicated; the origin is indicated by a dot. The location of the spots was determined with Ninhydrin reagent. The fingerprint of the fetal pig hemoglobin is on the left while the fingerprint of the adult pig hemoglobin is on the right.

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