granules seems to occur without evident disruption of the ciliary apparatus.

Electron micrographs of the central canal area adjacent to the neurosecretory cells in the caudal system of normal *Albula vulpes*, a more "primitive" teleost, also reveal elementary granules free in the cerebrospinal fluid, paralleling the picture seen in regenerating *Tilapia*. Occasionally elementary granules have been found in the central canal of normal *Tilapia*. The finding of elementary neurosecretory granules in extracellular locations in the insect corpus cardiacum has been reported recently by Normann (*9*); here granule extrusion occurs from the axonal processes. A somewhat different mode of discharge of electron-dense material from caudal neurosecretory axon terminals has also been encountered (*10*).

The abundance of release of neurosecretion into the central canal in the regenerating system may represent a physiologically important pathway for neurohormone release, correlated with the reduced extent of the neurohemal area, which in the regenerated system never reaches the same size as in normal fish. It is also possible that secretion into the central canal is a consequence of the failure of some axonal processes (normally projecting to the urophysis) to establish operative physiological contact with capillaries, resulting in a kind of temporary "pile-up" of secretion at the cellular level.

The significance of this alternative pathway in normal fish is difficult to estimate; however, absorption of neurohormone from the cerebrospinal fluid by the choroid plexuses is conceivable, with eventual vascular transport to the target organ(s) at the periphery. Similarly, neurohormonal effects upon other nervous centers can also be visualized.

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17 January 1966

Predominance of Hemoglobin Gower 1 in Early Human Embryonic Development

Abstract. *Hemoglobin Gower 1, the structure of which is thought to be* ϵ_4, *is the predominant hemoglobin in early human embryonic life. This finding suggests that the production of* ϵ*-chains initially exceeds that of other known* (α, β, γ, *and* δ) *chains.*

Starch-gel electrophoresis of hemoglobins (Hb) from small human embryos has shown two embryonic hemoglobins: Hb Gower 1 and Hb Gower 2 (*1–4*).

Hemoglobin Gower 2 consists of four polypeptide chains, two of which on hybridization and fingerprinting are consistent with α-chains. The other two chains of Hb Gower 2 differ from the β-, γ-, and δ-chains by more than one amino acid substitution in their tryptic-digest pattern and have been termed ϵ-chains (*3, 4*). Hybridization experiments with Hb Gower 1 suggest that it may consist entirely of ϵ-chains (*3, 4*).

In previous studies (*1, 2, 4*) Hb Gower 1 and Hb Gower 2 were detected in all embryos less than 85 mm in crown-rump (CR) length (presumably less than 3 months in gestation). The proportions of the embryonic hemoglobins were greater, the smaller, and presumably the younger, the embryo (Table 1). In all the embryos, fetal hemoglobin (Hb F) was the predominant hemoglobin. The smallest embryo examined was 25 mm in CR length.

Table 1. Proportions of various hemoglobins in human embryos.

Crown-rump of embryo (mm)	Hemoglobin			
	Gower 1 (%)	Gower 2 (%)	F (%)	A (%)
*Previously studied embryos**				
63	1.0	0.5	PH†	
50	3.4	1.3	PH	
46	5	1.7	PH	
34	17	16	PH	
25	24	13	PH	
Our embryos‡				
21.3	24	27	36	14
18.2	42	17	25	16
16.3	42	24	21	13

* Ref. (*2*). † Predominant hemoglobin present. ‡ Proportions of various hemoglobins were estimated by transmission densitometry of photographic negatives of the stained starch gels by use of a Spinco model RB Analytrol.

We here report hemoglobin studies in several embryos with CR lengths of 21.3, 18.2, and 16.3 mm. The 21.3- and 18.2-mm specimens were the products of spontaneous abortions. Their respective gestational ages were estimated as 41 days and 39 days (*5*), and their development was judged by the criteria of Streeter (*5*) to be at horizons XX and XIX. Both specimens were in excellent condition and appeared externally normal. Chromosomal studies of fibroblasts could only be done on the 21.3-mm specimen. These studies disclosed a normal female chromosomal complement. The 16.3-mm specimen was obtained from a tubal pregnancy. Its estimated gestational age was 37 ± 1 days (*5*), and developmentally it was at horizon XVIII. This embryo was likewise in excellent condition and appeared externally normal. Serial sections were made of this embryo (but not of the other two embryos) and appeared normal.

Blood (less than 0.1 ml) was obtained by cannulation of the umbilical vessel from each embryo. The erythrocytes were washed with isotonic saline and disrupted with distilled water, and the hemolyzate was examined promptly by starch-gel electrophoresis in a tris-ethylenediamine tetraacetate–borate (*6*) or tris-citrate-borate (*7*) buffer system at *p*H 8.4 These buffer systems allowed clear separation of both embryonic hemoglobins and separation of Hb F from Hb A (adult hemoglobin). The starch gels were stained with benzidene and photo-

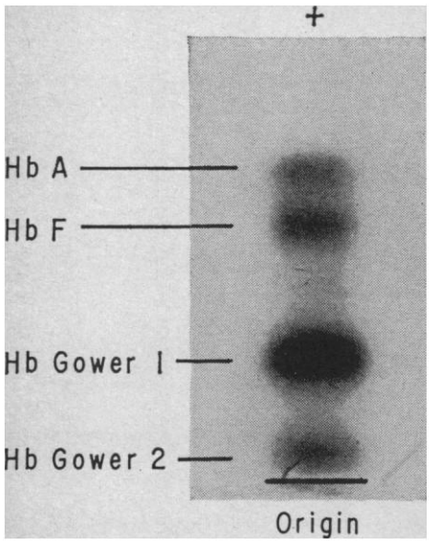

Fig. 1. Starch-gel electrophoresis of hemoglobins from 18.2-mm embryo in tris-citrate-borate buffer system, *p*H 8.6; stained with benzidene.

graphed (Fig. 1). The proportions of the various hemoglobins were estimated by transmission densitometry of the negatives; a Spinco model RB Analytrol was used.

In these three small embryos the combined proportion of the embryonic hemoglobins (Hb Gower 1 and Hb Gower 2) increased with decreasing embryonic size (Table 1). In the two smaller embryos Hb Gower 1 was the predominant fraction (*8*).

In each of the embryos the hemoglobin with the electrophoretic mobility of Hb A appeared to constitute at least 10 percent of the total hemoglobin. This concentration of Hb A is unexpectedly high for humans at this stage of development, in view of the finding that the proportion of Hb A is about 8 percent after a 35-week gestation period and in the range of 20 ± 10 percent at 40 weeks (*9*). Thus it might have been inferred that the concentration of Hb A should be much lower early in gestation.

Our data (Table 1) do not necessarily constitute evidence that the production of embryonic hemoglobins is initiated before that of Hb F and Hb A. The data do, however, indicate that there is either preferential production of Hb Gower 1 or preferential destruction of other hemoglobins in early embryonic life. This would signify either preferential production of polypeptide ϵ-chains, presumably the only ones in Hb Gower 1, or preferential destruction of other known (α, β, γ, δ) chains. We tend to favor the possibility of preferential production of ϵ-chains.

The significance of Hb Gower 1 early in human development may be that early embryos require a different type of hemoglobin for oxygen transport.

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21 December 1965

Hydroxylation of Proline and the Intracellular Accumulation of a Polypeptide Precursor of Collagen

Abstract. *Autoradiographs of embryonic cartilage indicated that labeled protein accumulated intracellularly when the tissue was incubated with tritiated proline, and when the hydroxylation of proline was inhibited by anaerobic conditions or by a chelator for ferrous iron. The labeled protein apparently corresponds to protocollagen, the polypeptide precursor of collagen which serves as a substrate for the enzymatic synthesis of hydroxyproline.*

Although essentially all the hydroxyproline in vertebrate proteins is found in collagen, isotopic studies indicate that free hydroxyproline is not incorporated into collagen (*1*). These observations have recently been explained by evidence (*2*) suggesting that the hydroxyproline in collagen is synthesized by the hydroxylation of proline in a proline-rich polypeptide precursor of collagen. A proline-labeled polypeptide precursor of collagen was prepared by incubating embryonic cartilage with proline-C^{14} under anaerobic conditions (*3*). Because atmospheric oxygen was required for the hydroxylation (*4*), the synthesis of collagen hydroxyproline-C^{14} was inhibited to greater extent than the incorporation of proline-C^{14} into protein was inhibited. When protein fractions from the anaerobically labeled cartilage were subsequently incubated aerobically with a chick embryo homogenate, a net increase in protein-bound hydroxyproline-C^{14} was observed. The substrate for the hydroxylation was a large polypeptide for which the name "protocollagen" has been suggested (*3*). Since the enzymatic hydroxylation was inhibited by sodium (*3*), it occurred to us that the hydroxylation of protocollagen probably occurs intracellularly. We have found that when the hydroxylation is inhibited in embryonic cartilage, labeled protein which apparently corresponds to protocollagen accumulates within chondrocytes.

Tibiae which consisted primarily of cartilage were removed from 10-day-old chick embryos under sterile conditions. The tibiae were incubated with