Suspensions for coating the plates are prepared by blending 65 g of mannitol in 100 ml of acetone for 1 minute in a blender. One milliliter of aqueous cornstarch (5 g in 10 ml) is added to the slurry, which is blended for an additional minute. The suspension is then transferred to a capped bottle in which it may be stored for several days. The inclusion of cornstarch in the mannitol suspensions gives better adhesion and does not appear to influence the separation of the pigments.

Application and spreading of the layer must be carried out very quickly because of the rapid evaporation of the solvent. The plates are ready for use within 20 to 30 minutes, and no oven drying is necessary. The plates may be stored under normal laboratory conditions or in a desiccator.

Sucrose plates can be prepared similarly with a suspension of powdered confectioners' sugar (75 g) in acetone (100 ml) with 1 ml of aqueous sucrose (50 percent wt/vol) being added during blending.

In a comparsion of Colman and Vishniac's method (powdered sugar) and plates coated with mannitol, the mannitol plates gave better separation with less streaking or tailing.

Acetone–water (80:20 by volume) extracts of tobacco leaves were partitioned against petroleum ether. Water was removed from the petroleum ether extracts with anhydrous Na_2SO_4 and portions of these extracts were applied to the test plates. The following solvent systems were used to develop the plates: (i) 0.5 percent (by volume) *n*-propanol in petroleum ether (b.p. 30° to 60°C); (ii) 2 percent (by volume) methanol in petroleum ether (b.p. 30° to 60°C); (iii) 5 percent (by volume) acetone in petroleum naphtha (b.p. 60° to 70°C). All plates were developed for an approximately equal length of time (30 to 40 minutes).

One-dimensional development gave pigment R_F values as shown in Table 1, whereas two-dimensional development on mannitol plates gave a separation as shown in Fig. 1.

These results show that mannitol gave a better separation of the individual bands and separation of several additional yellow pigments in the xanthophyll range below chlorophyll *b*. The number of yellow pigments in this region varied with the solvent but was always greater on the mannitol plates than on the powdered sugar plates. The rate of travel of the solvent front was always greater on the mannitol plates.

Table 1. R_F values of tobacco leaf pigments on thin-layer plates developed in three solvent systems. PE, petroleum ether (b.p. 30° to 60°C); PN, petroleum naphtha (b.p. 60° to 70°C); PS, powdered sugar; M, mannitol. The numbers in parentheses are distances in millimeters traveled by the solvent front.

Pigment	2% Methanol in PE		0.5% *n*-propanol in PE		5% Acetone in PN	
	PS(110)	M(156)	PS(114)	M(157)	PS(115)	M(152)
β-Carotene	0.98	0.99	0.97	0.99	0.97	0.99
Chlorophyll *a*	.90	.82	.82	.73	.35–.52*	.28
Lutein	.80	.57	.61	.55	.63	.57
Chlorophyll *b*	.64	.50	.44	.40	.26	.17
		Xanthophyll pigments				
	0.30–0.54*	0.46	0.20–0.40*	0.30	0.50–0.60*	0.44
		.40		.13		.37
		.31		.07		.10
		.24		.04		
		.20				
		.15				

* Pigments did not separate but streaked over a range of R_F values.

Mannitol plates can be used to separate the isomerization and breakdown products of the chlorophyll pigments, such as the pheophytins, chlorophyll a^1, and chlorophyll b^1 from the undegraded pigments. When leaf extracts stand for several hours at room temperature, the gray pheophytin band appears just behind that of β-carotene, and chlorophyll *a* and *b* separate into two distinct bands.

The method has been used successfully to separate the products of chlorophyll degradation produced by γ-irradiation of chloroplasts (*3*).

The thin-layer plates offer advantages to column methods because they are simple to prepare and use. Mannitol plates require only about 20 minutes for 12-cm development with a mixture of propanol and petroleum ether; the high resolution obtained facilitates removal of the pigment bands, without cross contamination, for quantitative work.

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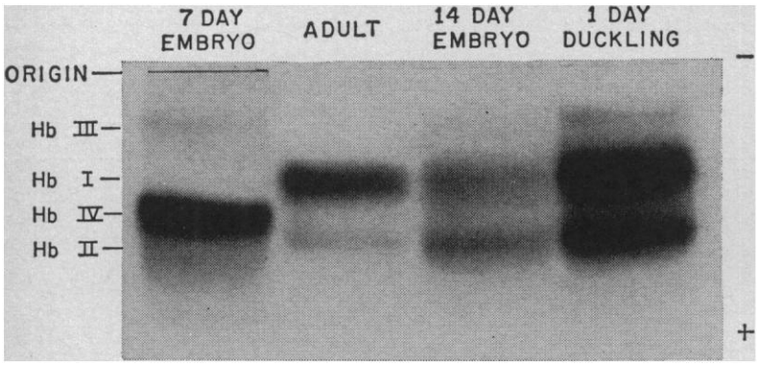
Hemoglobin Heterogeneity: Embryonic Hemoglobin in the Duckling and Its Disappearance in the Adult

Abstract. *The 7-day white Peking duck embryo contains two hemoglobins (Hb's III and IV) which are electrophoretically distinct from the two adult hemoglobins (Hb's I and II). Hemoglobin IV had disappeared in the 14-day embryo, at which time Hb's I and II are evident. The synthesis of Hb III is not restricted to embryonic life but continues in the duckling and ceases before the 10th week of development after hatching.*

In 1955 Cabannes and Serain demonstrated the presence of two hemoglobins in the duck (*1*). Other investigators have confirmed this observation using alkali denaturation (*2*), agar electrophoresis (*3*), and ion-exchange chromatography (*4*). We now report a third hemoglobin in the duck. This hemoglobin originates in the embryo; its synthesis continues after hatching but ceases in the adult.

Ducklings were obtained within 24 hours after hatching; they were bled by cardiac puncture. Adult ducks were bled from the wing vein, and embryo blood was obtained by severing the omphalomesenteric vessels. The eggs containing 7-day-old embryos were opened close to the air space (blunt end of egg), and the yolk was allowed to drain. Adhering material was removed with scissors and forceps. We endeavored to keep the embryo and as much of its circulatory system as possible fixed to the inner shell which served as a retainer when the blood



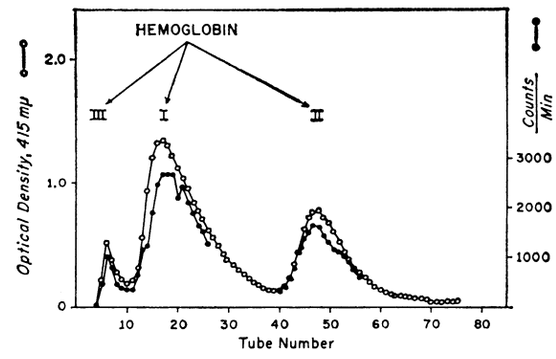


Fig. 1 (left). Acrylamide-gel electrophoresis (*9*) of embryo, duckling, and adult duck hemoglobins. Blood was obtained as described, and the cells were separated by centrifugation at 2400*g* for 10 minutes. After three washings with an isotonic salt solution, the cells were frozen and thawed once and diluted with five volumes of a hypotonic buffer, *p*H 7.4 (*10*). Centrifugation at 105,000*g* removed stroma, nuclear debris, and ribonucleoprotein. The supernatant hemoglobin was converted to cyanmethemoglobin and dialyzed for 18 hours against a tris-EDTA-borate buffer (*11*). The electrophoresis was conducted at 300 volts for 5 hours at 4°C. The hemoglobins were stained with benzidine. Fig. 2 (right). Elution pattern from diethylaminoethyl cellulose columns of hemoglobin labeled with Fe^{59} in vivo. Approximately 60 μc of Fe^{59}, as ferrous citrate, was administered by intracardiac injection to a 13-day-old duckling. Blood was drawn 5 days later by heart puncture, and hemoglobin was prepared as described in the legend of Fig. 1 except that dialysis was performed against 0.005*M* phosphate buffer, at *p*H 8.6, to which 100 mg of KCN per liter was added. Adsorption and elution of the hemoglobin were carried out by a modification of Huisman's procedure (*12*). Fractions containing 5 ml were collected, and optical density measurements were made at 415 mμ. Portions (2 ml) were assayed for activity in a well-type scintillation counter.

vessels were cut. In this manner several drops of blood were collected from each embryo with a Pasteur pipet and transferred to erlenmeyer flasks containing 10 to 20 units of heparin.

Blood was collected from older embryos by severing the blood vessels after placing the entire specimen in a petri dish. Approximately 1.0 to 1.5 ml of cells was collected from 120 7-day or 24 14-day embryos. Twelve 21-day embryos easily provided 2.0 ml of cells.

Vertical-gel electrophoresis of hemoglobins from embryo, duckling, and adult duck is shown in Fig. 1. The 7-day embryo has two hemoglobins (Hb's III and IV) which are electrophoretically different from the adult hemoglobins (Hb's I and II). Hemoglobin IV, with an electrophoretic mobility intermediate between Hb's I and II, is the major embryonic hemoglobin at 7 days but has disappeared in the 14-day embryo. Hemoglobin III, which is present in the embryo and duckling, has disappeared in the adult. Hemoglobins I and II, absent in the 7-day embryo, are evident by 14 days and persist in the adult as the major and minor components, respectively. Purification by ammonium sulfate fractionation did not alter the mobility of duckling or adult hemoglobins. The proportion of the two adult hemoglobins was also unaffected.

Manwell (5) has demonstrated in 4-day-old embryos of three different species of chicken and in the California turkey two embryonic and two adult hemoglobins. The minor embryonic hemoglobin is the slowest anodal component and would correspond to Hb III in the duck. The major embryonic hemoglobin in the chicken and turkey has an electrophoretic mobility intermediate between the two adult hemoglobins, and in this regard is similar to Hb IV in the duck. In contrast to our findings in the duck, Manwell's data on chicken and turkey, in agreement with the results of D'Amelio and Salvo (*6*), indicate that only traces of embryonic hemoglobins exist in the 11-day-old embryo.

Analytical ultracentrifugation of duckling hemoglobin reveals a single homogeneous peak and indicates that Hb III is not a polymeric artifact (see *7*).

The duckling hemoglobins labeled in vivo with Fe^{59} were separated by chromatography (Fig. 2). This method confirms the presence of three hemoglobins during the development period after hatching. Specific activities of Hb's I, II, and III, expressed as count/min per optical-density unit at 415 mμ,

Table 1. Cell hemoglobin during embryonic development and maturation of the duck after hatching. Ducks normally hatch at approximately 28 days. Cell counts (by hemocytometer) and hemoglobin determinations (*13*) were done in triplicate. The gram absorption coefficient for human hemoglobin was assumed for the duck. Blood was prepared for hemoglobin separation as described in the legend for Fig. 2. The individual hemoglobins were separated by starch-block electrophoresis (*14*), eluted, and optical density was measured at 415 mμ. Cell contents of individual hemoglobins in the embryo and 1-day-old duckling were calculated from the total hemoglobin per cell, and from the hemoglobin proportions determined from duplicate electrophoretic separations on three samples of pooled blood. Duplicate separations were also performed on samples of hemoglobin from ducklings older than 1 day and on adult ducks. The figures in parentheses indicate the number of animals used.

Age (days)	Hemoglobin/cell (pg)				
	Total	Hb I	Hb II	Hb III	Hb IV
		Embryo			
6	55.8				
7	72.2	Absent	Absent	18.8	53.4
9	70.2				
14	48.6	16.7	24.0	7.9	Absent
21	42.8	18.4	19.0	5.4	Absent
		After hatching			
1	37.9	21.6	12.5	3.8	Absent
14(4)	36.3	22.0	11.4	2.9	Absent
38(2)	40.4	29.0	10.0	1.4	Absent
84(2)	44.8	36.9	7.9	Absent	Absent
180(1)	45.2	37.1	8.1	Absent	Absent

were 201, 205, and 193, respectively. The nearly equal specific activities indicate that Hb III is synthesized in the duckling and that its presence does not represent simply a carry-over from late embryonic life.

The chronology of cell hemoglobin values is shown in Table 1. The times of the activation and deactivation of hemoglobin synthesis have not yet been determined. Cell hemoglobin content is maximum in the 7- to 9-day embryo. The subsequent decrease at 14 days is conincident with the disappearance of the so-called "primitive" cells (*8*). The reciprocal change in cell content of Hb's I and II stabilizes in the adult, while Hb III decreases and ultimately disappears.

We suggest that the appearance of Hb III in the duck embryo, its presence in the duckling, and its disappearance in the adult are similar to characteristics of fetal hemoglobin in the human. According to current epigenetic concepts, the inactivation of genes for duck embryonic hemoglobins occurs at widely separate times, that for Hb IV early in embryonic life and that for Hb III after hatching.

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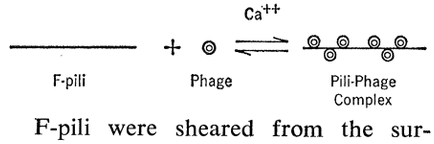
Complexes of F-Pili and RNA Bacteriophage

Abstract. *Cell-free filtrates of F+ strains of* Escherichia coli *"inactivate" RNA phages. The active agents have been identified in the electron microscope as thin appendages called F-pili. Complexes of F-pili and phage may be easily assayed because of their retention by membrane filter pads. Calcium ion is required for formation of the complex.*

The thin filaments growing out from the surface of male (F+ and Hfr) strains of *Escherichia coli* and related organisms are called F-pili (*1, 2*). While several types of morphologically similar pili (also called fimbriae, *2*) are synthesized by Gram-negative bacteria, Crawford and Gesteland (*1*) observed in the electron microscope that a male-specific bacteriophage, R-17, was adsorbed only to pili of an Hfr and F+ strain of *E. coli* but not to pili of an F− strain. Brinton, Gemski, and Carnahan (*2*) obtained similar results with the RNA phage M12, and found that usually only one or two F-pili were present on each male cell along with large numbers of more common pili, Type I pili, which were unrelated to maleness. The F-pili were easily distinguished in electron micrographs since at high concentration the phage almost covered the surface of the F-pili without interacting with other pili on the same bacterium (*2*). The F-pili were genetically controlled by the fertility factor of *E. coli* (*2*). Brinton *et al.* (*2*) have proposed the name F-pili for this kind of appendage and suggested the interesting idea that F-pili may serve as hollow tubes through which nucleic acid may be transferred. An assay in vitro for complexes of bacteriophage and F-pili would have obvious advantages. We have devised a simple and rapid filtration assay which has enabled us to study RNA phage adsorption to F-pili in vitro. Evidence will be presented for the following reaction:



F-pili were sheared from the surface of *E. coli* cells by treatment in the Waring blendor (*3*). In a typical experiment, 20 g of cell paste of an F+ strain of *E. coli* (K 12) grown to late log phase in tryptone–yeast extract broth (*4*) with gentle aeration were suspended in 200 ml of water and blended for 2 minutes. After centrifugation the cell-free supernatant containing the F-pili fragments was dialyzed and stored at 0°C. This "F-pili concentrate," which also contained Type I pili and other impurities, was sufficient for several thousand assays. Radioactive virus used for the F-pili assay were prepared by infecting 20 ml of cell culture containing radioactive phosphorus with coliphage f2 (*5*). Approximately 3×10^{12} phages (plaque-forming units) containing P^{32} (1.6×10^7 count/min) were obtained after purifi-

Table 1. Adsorption of P^{32}-labeled phage to F+ cells (*E. coli*, K 12). A culture containing 4×10^8 cells/ml was infected with about 3×10^7 f2 per ml (1300 count/min; P^{32}-labeled). After 10 minutes (for adsorption), the cells were centrifuged to remove unattached phage, precipitated with 5 percent cold trichloroacetic acid, and the precipitate was collected on glass filter pads for counting. The cell supernatant was assayed for viable phage, and the number of adsorbed phage was calculated by difference. In the blending experiment cells were treated for 2 minutes in the Waring blendor. Incubation was at 37°C.

Conditions	Adsorbed phage	
	Radioactivity (count/min per 10 ml)	No. (plaques/ml)
Complete	156	2.2×10^7
Minus cells	6	0
F− cells	9	1×10^6
Complete (100 μg/ml ribonuclease)	14	
Complete (cells blended before adsorption)	83	
Complete (su-1 defective added instead of f2, 1600 count/min)	5	
Complete (3 min incubation instead of 10)	89	1.6×10^7
Complete (cells blended after 3 min adsorption)	11	4×10^6

Table 2. "Inactivation" of phage by F-pili. In this experiment 5 ml of F-pili were added to a suspension of 7×10^7 phage per milliliter. After incubation (37°C) with F-pili, the remaining viable phage was assayed by plaque count. Blending of the complexes was carried out for 2 minutes.

Conditions	$10^7 \times$ Viable phage remaining (plaques/ml)
Control (0 time sample)	6.5
Complete (10 min incubation)	3.6
Complete (complexes blended)	6.0
Complete (complexes blended; reincubated 40 min)	3.2