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The Plasma of Developing Chick and Pig Embryos.

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(Introduced by E. T. Engle.)

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Several extensive studies[1-4] have been made on the serum proteins from a number of animal species after birth, but no work has so far been reported on the changes occurring in the blood proteins during embryonic development. In order to learn more about the origin and physiological development of the plasma proteins we have obtained electrophoresis, ultracentrifuge, and some chemical data on sera and plasmas from developing chick and pig embryos. By all methods of analyses the embryonic and fetal plasma differs widely from that of the adult, and electrophoretic analyses show rapid changes during development.

Chick Plasma. White leghorn eggs were incubated from 8 to 21 days at 37°. Blood from a large number of embryos of the same age was taken directly from the larger arteries of the living embryos with a tuberculin syringe and then pooled.

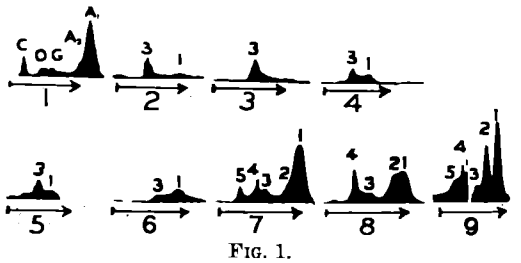

FIG. 1.
Electrophoresis patterns of (1) fresh egg white, (2-8) chick embryo, and (9) adult chicken plasma. Pattern numbers correspond to numbers in first column of Table I. In pattern (1) A_1, A_2 = albumin, G = globulins, O = ovomucoid, C = conalbumin.

All electrophoresis, ultracentrifuge, and diffusion measurements were made after dialysis in viscose casings (average pore diameter $2 - 3 \times 10^{-7}$ cm) at 0° to 4° against a buffer containing 0.02M sodium phosphate and 0.15M NaCl. The electrophoretic data obtained on the chick embryo plasma are given in Table I and the corresponding electrophoretic patterns are presented in Fig. 1. For comparison, similar data on egg white, 3-day-old chick and adult chicken plasmas are also given. The components are classified roughly according to their mobilities and are numbered from 1 to 5 in order of decreasing mobility. Component 3, which has a mobility approximating that of β-globulin in the serum of the adults of most species, is predominant in the earlier embryos, but gradually recedes throughout the development. Component 1 does not become appreciable until about the eleventh day and appears to reach maximum at about the time of hatching (data and patterns not shown). Components 2 and 4, which appear after 16 to 18 days of incubation, varied considerably in blood from fetuses of the same age as well as in newly hatched and adult chickens, but were large in all cases after hatching.

Several samples of whole plasma from the developing chick embryos and a few electrophoretically separated fractions have been studied in the analytical ultracentrifuge (Table II). In the unfractionated chick plasma only a single component was observed as in Fig. 2A, B and C. This component has a sedimentation constant different from either albumin (4.5 Svedberg units) or the bulk of the globulin (7.7 Svedberg units) found in the adult of the same species. The sedimentation constant seemed to decrease slightly with in-

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1 Utheim, K., *Am. J. Dis. Children*, 1920, **20**, 366.

2 Howe, P. E., *J. Biol. Chem.*, 1922, **53**, 479.

3 Jameson, E., Alvarez-Tostado, C., and Sorter, H. H., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 163.

4 Pedersen, K. O., *The Svedberg Anniversary Volume*, p. 490, Upsala, 1944.

TABLE I.
Electrophoretic Fractionation of Chick Embryo Plasma.*

No.		Dilution†	Mobility × 10⁵ cm²/volt sec A_1	A_2	G	O	C	Composition % A_1	A_2	G	O	C
1	Egg white	1:10	4.7	4.2	2.6	1.9	1.0	60	10	7	8	15
	Embryo age days		Mobility × 10⁵ cm²/volt sec 1	2	3	4	5	Composition % 1	2	3	4	5
2	8	1:2	5.8		3.4			10		90		
3	9	1:3	5.4		3.4			15		85		
4	10	2:3	5.8		3.8	2.2		25		70	5	
5	11	1:2.2	5.5		3.5	1.7		30		60	10	
6	13	1:3	5.5		3.9	2.3		60		35	5	
7	17	1:1	5.5	4.7	2.8	2.4	1.6	55	10	8	13	14
8	+3	1:2.4	5.1	4.7	2.9	1.9	1.5	35	25	10	25	5
9	Adult	1:4	5.3	4.4	3.5	2.5		40	25	5	20	10

* Buffer: 0.02M sodium phosphate, 0.15M NaCl, pH 7.4.
† Ratio of plasma volume to volume of solution; thus 1:1 means undiluted.
+3 = 3 days after hatching.

TABLE II.
Sedimentation Constants of Chick Embryo Plasma.

Embryo age days			Sedimentation constant*		
9			4.2		
10			4.2		
11	A†		4.2		
12			4.0		
13			3.9		
15	B		4.1		
16			4.0		
21	C		3.6		
21			3.6		
21 slow	D	1.9	4.0		
Adult fast			4.5		
Adult slow				7.7	19.0‡

* In Svedberg units = 10^{-13} cm/sec per unit field.
† See indicated pattern of Fig. 2.
‡ Trace only.

creasing age of the embryo but did not vary appreciably with concentration (0.6% maximum concentration used). Components 3, 4 and 5 (see Fig. 1) were electrophoretically separated from the plasma of a newly hatched chick and were found to contain the component having $S_{20} = 4.0$ and a trace of material, possibly masked by the predominant component in whole plasma, having a lower sedimentation constant (see Fig. 2D).

Diffusion constants were determined on 2 pooled plasma samples from embryos incubated 12 and 21 days and were calculated to be 1.6 and 2.5 × 10^{-7} cm²/sec respectively, at 20°. Diffusion measurements on serum (no anti-coagulant added to the blood) from 11-day embryos gave $D_{20} = 1.8 \times 10^{-7}$ cm²/sec. No difference in the sedimentation patterns of serum and plasma was detected. These values of S_{20} and D_{20} would indicate a molecular weight of between 200,000 and 300,000, the molecules having a high frictional ratio ($f/f_0 = 3.0$). The partial specific volume is as yet undetermined but preliminary measurements indicate that it is not greatly different from most serum proteins, *i.e., ca.* 0.75.

TABLE III.
Electrophoretic Fractionation of Pig Embryo Plasma.*

No.	Embryo length mm		Dilution†	Mobilities × 10⁵ cm²/volt sec 1	2	3	4	5	6	Composition % 1	2	3	4	5	6
1	30	P‡	1:4	6.2	5.0	3.9		1.9		10	50	15		25	
2	130	P	1:2	5.3	4.5	3.5	2.5			35	35	20	10		
3	160	P	1:2	5.1	4.3	3.8	2.7			45	40	10	5		
4	180	S	1:1	4.7	3.5		2.7	1.6		45	40		10	5	
5	250	S	1:1	4.5	3.3		2.4	1.7		55	35		7	3	
6	300	P	1:2	4.6	3.3		2.5		0.9	50	30		5		15
7	Adult	S	1:4	4.8	3.3		2.4		1.2	50	15		15		20

* Buffer: 0.02M sodium phosphate, 0.15M NaCl, pH 7.4.
† Ratio of volume of plasma or serum to volume of solution, thus 1:1 means undiluted.
‡ P = plasma, S = serum.

TABLE IV.
Sedimentation Constants of Pig Embryo Plasma.

Embryo length mm		Sedimentation constant*	
30	A†	3.9	
95		3.7	
180		3.8	
180 slow		3.7	
250	B	3.5	
250 fast	C	3.4	
250 slow	D	2.9	5.9

* In Svedberg units = 10^{-13} cm/sec per unit field.
† See indicated pattern of Fig. 4.

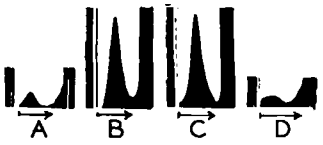

FIG. 2.
Ultracentrifuge patterns of chick embryo plasma. (See Table II.)

After dialysis against 0.9% NaCl a sample of serum from 13-day-old embryos was found to contain 0.66 mg N/ml of orginal serum. The protein was dialyzed against H_2O, lyophilized and dried in vacuum to constant weight at room temperature over P_2O_5. 6.0% N by Kjeldahl and 2.0% ash were found.† Several samples from embryos of various ages were analyzed for non-diffusable carbohydrate by a colorimetric[5] method and found to contain several times as much per unit N as was found in adult chicken serum.‡ These analyses were made after dialysis against either phosphate-NaCl buffer or H_2O. Electrophoretically separated components both contained carbohydrate, but considerably more was associated with the faster fraction. A great part of the sugar is evidently bound with the protein. The nature of this carbohydrate is under investigation by Dr. Dische and will be reported later.

Pig Plasma. Umbilical cord or heart blood was collected from an entire litter of pig embryos obtained from freshly slaughtered sows.§

[5] Dische, A., and Popper, H., *Biochem. Z.*, 1926, **175**, 371.

† We are indebted to Dr. Manfred Mayer for these determinations.

‡ We are indebted to Dr. Zacharias Dische for these measurements.

§ Kindly furnished by Figgie and Hutwelker Co., slaughterers, New York, N. Y.

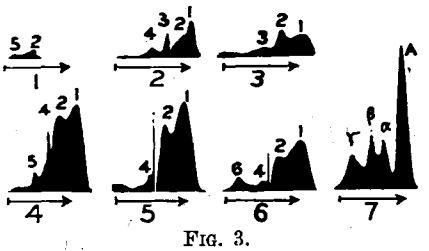

FIG. 3.
Electrophoresis patterns of pig embryo plasma and sera. Pattern numbers correspond to numbers in first column of Table III.

To some samples enough citrate was added to prevent coagulation. The electrophoretic data and patterns are given in Table III and Fig. 3. In the plasma from 30 mm (measured from snout to tail root) embryos, components 1 and 3 are only indicated, component 2 being the predominant one. Component 1 increases, however, throughout the embryonic and fetal development, but component 2 remains an appreciable proportion of the total even in the 300 mm fetus. The body length at parturition is probably between 350 and 400 mm.

Ultracentrifugal analyses of unfractionated plasma and sera indicated only a single component (Table IV, Fig. 4) whose sedimentation constant ranged from 3.9 to 3.5 Svedberg units. Components 1 and 2 (pattern 5 of Fig. 3) separated electrophoretically were homogeneous in the ultracentrifuge (Fig. 4C), having $S_{20} = 3.4$, but the slower fractions (components 4 and 5) revealed two components (Fig. 4D) with $S_{20} = 2.9$ and 5.9 (in whole serum these were possibly masked by the main component). The component having a sedimentation constant of 2.9 Svedberg units is probably the one described by Pedersen.[4]

Diffusion constants were determined on unfractionated sera from 180 and 250 mm pig fetuses and found to be 5.9 and 5.3 $\times$ 10^{-7} cm^2/sec, respectively. These figures are very different from those obtained on the plasma

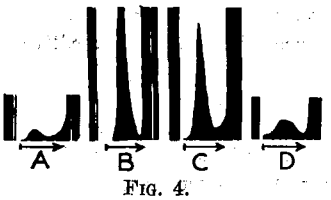

FIG. 4.
Ultracentrifuge patterns of pig embryo plasma. (See Table IV.)

from chick embryos of various ages, and lead to a molecular weight of about 60,000.

Total solids in serum samples from 180 and 250 mm pigs were found to be 17.2 and 17.4 mg/ml respectively after dialysis against H_2O and lyophilization. Nitrogen determinations yielded 12.5% and 13.2% respectively, values which did not change measurably after a portion of the solids were refluxed for 24 hr with alcohol-ether under N, indicating little or no substances soluble in organic solvents. Three small aliquots (1 to 3 mg each) from each sample of lyophilized solid were analyzed for phosphorus but were found to contain only traces.‖

The ratio of carbohydrate to nitrogen in the pig embryo serum, like that in the chick, was high, being 2 to 3 times that in the serum of adult swine. Whether or not all of this is bound to the protein, further investigation will show.

A correlation of these findings with the morphogenesis and organogenesis of the embryos is being made.

Summary. Electrophoresis patterns show rapid changes in the plasma of developing chick and pig embryos. In the ultra-centrifuge only a single component is apparent in the unfractionated plasma. After electrophoretic fractionation of the plasma or sera from older fetuses, traces of other components are revealed.

Diffusion and sedimentation constants of 11-day chick embryo serum would indicate a molecular weight of more than 200,000. The diffusion rate of the plasma from older chick fetuses was greater. Pig fetus plasma had a much larger diffusion constant, indicating a molecular weight of approximately 60,000 for the bulk of the plasma protein from 180 and 250 mm fetuses.

Thirteen-day-old chick serum protein contained 6.0% N and 20 to 30% carbohydrate, whereas 180 mm pig embryo serum protein contained 12.5% N and 15 to 20% carbohydrate.

‖ We are greatly indebted to Miss Helen Fabricant for these analyses.

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Simple Method of Preparing Potent Blood Grouping Sera.*

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The properties A and B are antigenic for human beings whose erythrocytes lack the corresponding agglutinogens, as was demonstrated by observations made following unintentional transfusions of blood of an incompatible group.[1,2] Aubert, Boorman, and Dodd[3] observed a similar increase in isoagglutinin titer in patients transfused with serum prepared from the blood of donors belonging to an incompatible group. Wiener[4] applied this observation as a method of preparing high-titered blood grouping sera, by immunizing professional blood donors with transfusions of dried pooled plasma (Sharp and Dohme) reconstituted with distilled water.† Satisfac-

* The work in this paper was done under a contract recommended by the Committee on Medical Research between the Office of Scientific Research and Development and the Jewish Hospital of Brooklyn. The work was also aided in part by a grant from the United Hospital Fund of New York City.

1 Wiener, A. S., Oremland, B. H., Hyman, M. A., and Samwick, A. A., *Am. J. Clin. Path.*, 1941, **11**, 102.

2 Wiener, A. S., *J. Immunol.*, 1941, **41**, 181.

3 Aubert, E. F., Boorman, K. E., and Dodd, B. E., *J. Path. and Bact.*, 1942, **54**, 89.

4 Wiener, A. S., unpublished observations.

† Based on these findings, individuals of group 0 who have previously received plasma transfusions should not be used as universal donors, unless it is proved by careful titration tests that their sera do not contain dangerously high concentrations of isoagglutinins.