We have made the following preliminary observations on the cerebral circulation:

The growth of the cerebral vascular bed from the 9 mm. crown-rump length to the 25 mm. crown-rump length is striking. In the smaller embryo the difference in caliber between main arterial trunk and capillary is relatively slight. After few divisions the artery leads into a simple capillary network which again reunites to form a large vein without apparent anastamosis with other vessels.

Much more complicated is the cerebral vascular bed in embryos of 25 mm. crown-rump length. Here the large arteries and veins are broader and the capillaries appear to be narrower than in the smaller embryos, while the complex capillary bed is separated from the main vessels by a larger number of branches of intermediate size.

In the smaller embryos under magnification of 20 diameters we have obtained beautiful stereoscopic views of the entire cerebral circulation. The deeply placed pulsating arterial flow is readily distinguished from the more superficial and steadily streaming venous flow.

With higher magnifications (100 to 335 diameters) the smallest capillaries appear as minute channels in the cerebral substance through which the red blood cells can be seen to pass but one at a time. Even the endothelial nuclei of the capillary wall may be seen in suitable fields.

This method makes it possible to observe the development and physiology of the cerebral circulation in the *unanesthetized* mammalian embryo with *cranium intact*. Further studies will be carried out in this laboratory.

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Certain Physico-Chemical Characteristics of Muscle Globulin.

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The significance of the protein constituents of muscle for the contractile process has long been recognized. The instability of these bodies, however, has rendered difficult their characterization. Danilewsky[1] described the extraction of muscle protein with sal ammoniac in 1881. The older method of expression, despite rigid

precautions, often led to "denaturation" or "clotting of muscle plasma." The properties of muscle globulin, as of other globulins, are altered both by too low salinity and by too high acidity.[2] Howe[3] has employed phosphate buffers as solvents to overcome these difficulties. In the present investigation an ammonium chloride solution, rendered alkaline by excess of ammonia, was used as solvent and yielded solutions of muscle protein which, for several months, retained solubility in neutral salt solutions.

A globulin-like fraction of ox muscle protein was studied, which presumably corresponded to the "myosin" of von Fürth[4] and the "paramyosinogen" of Halliburton.[5] The method of preparation depended upon: (a) its low solubility in water or dilute salt solution; (b) its greater solubility in the presence of higher concentrations of neutral salt; (c) its precipitation from solutions less than half-saturated with respect to ammonium sulfate. Fresh muscle was finely ground and stirred into a two-molal ammonium chloride solution, to which ammonium hydroxide was slowly added to a concentration one-third normal. This ammonia buffered the acid products and thus prevented "denaturation." The dissolved protein, after centrifugation and filtration, was alternately precipitated by ammonium sulfate, dissolved in more dilute salt solution, and precipitated by further dilution. The reaction was always maintained between pH 8.0 and 8.5 by ammonia. Precipitated by ammonium sulfate, the muscle globulin was freed from albumins[6] and hemoglobin; precipitated by dilution from the serum globulins which dissolve in more dilute salt solutions.

Removal of the excess alkali or of the solvent salt renders the protein less soluble. In the absence of salt, flocculation of the protein occurs over a wide range, extending from pH 4.5 to 8.5, and reaches a maximum, as Collip has reported,[7] in the neighborhood of pH 6.5. The wide flocculation zone of this protein thus coincides with that of the vegetable globulin edestin, and with the zone over which such monoamino monocarboxylic acids as glycine, alanine, and leucine have no buffer effect.

Freed from electrolytes, this fraction dissolved in distilled water, at 25° C., less than 0.025 gram per liter. Water saturated with the protein in this manner had a reaction ranging from pH 6.3 to 6.6.

The addition of alkali to a suspension of isoelectric protein in water first renders it extremely viscous, but finally completely dissolves the protein which combines at saturation with 13×10^{-4} mols of NaOH per gram. Like many globulins,[8] this fraction apparently forms insoluble acid compounds, dissolving only in rather acid solu-

tions and combining at saturation with 15×10^{-4} mols of HCl. The maximal acid- and base-combining capacities of different preparations, calculated from electromotive force measurements,[9] appear in Tables I and II and are in good agreement.

TABLE I. Maximum Acid-combining Capacity.

Preparation and Exp. No.	Protein in one liter (p)	Conc. of HCL (a)	pH	p[HCL]* (pH-pγ)	Conc. of free HCL (b)	Conc. of bound HCL (a-b)	HCL bound per gm. protein (a-b)/p
	gm.	N			N	N	mols
VIII A5	7.25	0.018	2.19	2.14	0.007	0.011	0.0015
″ A6	″	.020	2.09	2.04	.009	.011	.0015
″ A9	″	.022	2.01	1.96	.011	.011	.0015
″ A7	″	.024	1.92	1.87	.014	.010	.0014
″ A8	″	.026	1.86	1.81	.016	.010	.0014
″ A11	″	.028	1.78	1.75	.019	.009	.0012
″ A10	″	.030	1.77	1.72	.019	.011	.0015
XIV A12	7.6	0.014	2.67	2.63	0.002	0.012	0.0016
″ A13	7.5	.016	2.41	2.36	.004	.012	.0016
″ A14	6.9	.018	2.16	2.11	.008	.010	.0014
″ A15	6.8	.020	2.10	2.04	.009	.011	.0016
″ A16	7.4	.023	2.07	2.01	.010	.013	.0018
						Average..................	0.0015

*The negative logarithm of the concentration of acid, pHCL, is equal to the difference between the negative logarithm of the hydrogen ion activity, pH, and the negative logarithm of the activity coefficient, pγ. The same relations obtain between pNaOH, pOH and pγ.

TABLE II. Maximum Base-combining Capacity.

Preparation and Exp. No.	Protein in one liter (p)	Conc. of NaOH (a)	p[OH]	p[NaOH] (p[OH]-pγ)	Conc. of free NaOH (b)	Conc. of bound NaOH (a-b)	NaOH bound per gm. protein (a-b)/p
	gm.	N			N	N	mols
VIII B5	7.25	0.016	2.11	2.02	0.009	0.007	0.0010
″ B9	″	.016	2.13	2.11	.008	.008	.0011
″ B13	″	.016	2.16	2.13	.008	.008	.0011
″ B2	″	.020	2.06	2.01	.010	.010	.0014
″ B8	″	.020	2.00	1.95	.011	.009	.0012
″ B12	″	.020	2.07	2.02	.010	.010	.0014
″ B4	″	.024	1.96	1.88	.013	.011	.0015
″ B7	″	.024	1.85	1.80	.016	.008	.0011
″ B1	″	.028	1.80	1.75	.015	.013	.0018
XII 4.5	8.4	0.018	2.20	2.15	0.007	0.011	0.0013
″ 5.0	″	.020	2.08	2.03	.009	.011	.0013
″ 5.5	″	.023	2.03	1.98	.011	.012	.0014
″ 6.0	″	.025	1.94	1.88	.013	.012	.0014
						Average..................	0.0013

Throughout the physiological range the fraction studied is appreciably soluble only in the presence of salt. Although at reactions alkaline to pH 6.0 this globulin dissolves in salt solutions, at more acid reactions the solvent action of neutral salts is greatly diminished. In this respect also, the behavior of this muscle globulin suggests that of edestin.[8]

This muscle globulin fraction appears to be of a different type than hemoglobin or the serum globulins. All of these animal globulins are more soluble in water than the muscle globulin studied. The solvent action of neutral salts on muscle globulin is, however, more pronounced than on hemoglobin.[10] The globulin-like properties of muscle protein therefore acquire additional significance. Whereas the solubility of hemoglobin is increased only six-fold by neutral salts, the solubility of muscle globulin has been increased several hundred-fold in these studies. Inasmuch as the solubilities of the serum globulins are increased to at least this extent, while their solubilities in water are greater, lower concentrations of salt suffice to dissolve larger amounts of serum globulin than of muscle globulin. Such distinctions have a significance not only for separating these proteins, but for understanding the electrically charged condition of their molecules and their physical state.

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