10968 P

Influence of Anti-Anemic Principles upon Size of Erythrocytes of Opossums (*Didelphis virginiana*) in Maternal Pouch.

JOSEPH STASNEY AND EDWARD L. BURNS (Introduced by Kenneth L. Burdon.)

From the Department of Pathology and Bacteriology, School of Medicine, Louisiana State University, New Orleans.

The red blood cells of the mammalian fetus are constantly larger than those of adults of the same species, and resemble very closely the erythrocytes found in individuals with pernicious anemia.[1] It has likewise been shown experimentally that the mean maximal diameter of erythrocytes of newly born rats can be reduced at birth by the administration of concentrated normal gastric juice to the mother during pregnancy.[2]

The inaccessibility of common mammalian fetuses to direct injection made it desirable to devise a scheme by which anti-anemic principles could be injected directly into the developing animals themselves. The opossum *(Didelphis virginiana)* is suitable for this purpose because intrauterine life lasts only 10.5 ± 1.7 days, after which the immature young live in the maternal pouch for 90 days.[3]

Materials and Methods. Forty-nine immature opossums, varying in crown-rump lengths from 5.5 to 7.0 cm, were used in these experiments. Twenty were injected over periods varying from 3 to 13 days with 0.8 to 2.2 cc of concentrated normal human gastric juice. Ten untreated pouch mates served as controls. Nine were injected over periods varying from 3 to 14 days with from 0.3 to 2.6 cc of concentrated solution of liver extract. Ten untreated litter

TABLE I.
Comparison of Mean Maximal Diameter and of Mean Corpuscular Volume of Erythrocytes of Immature and Adult Untreated Opossums.

	Untreated immature opossums	Untreated adult opossums
No. of animals	49	10
Mean max. diam. of erythrocytes in μ	8.90	7.30
Variance of means	0.00240	0.00200
No. of animals	39	10
Mean corpuscular vol. of erythrocytes in μ^3	162.71	88.49
Variance of means	2.90406	5.91789

1 Wintrobe, M. M., and Shumacker, H. B., *J. Clin. Invest.*, 1935, **14**, 837.

2 Stasney, J., Higgins, G. M., and Mann, F. C., *Am. J. Med. Sci.*, 1939, **197**, 690.

3 Hartman, C. G., *J. Morph. and Physiol.*, 1928, **46**, 143.

TABLE II.

Effect of Normal Human Gastric Juice and of Concentrated Liver Extract Solution on the Mean Maximal Diameter and Mean Corpuscular Volume of Erythrocytes of Immature Opossums.

	Gastric Juice				Liver Extract			
	Experimental animals		Control animals		Experimental animals		Control animals	
	Beginning of exp.	End of exp.	Beginning of exp.	End of exp.	Beginning of exp.	End of exp.	Beginning of exp.	End of exp.
No. of animals	20	20	10	10	9	9	10	10
Mean max. diam. of erythrocytes in μ	8.87	7.90	9.05	8.74	8.87	7.93	8.86	8.98
Variance of means	0.0031	0.0032	0.0180	0.0069	0.0152	0.0028	0.0036	0.0064
No. of animals	19	19	8	8	5	5	7	7
Mean corpuscular vol. of erythrocytes in μ^3	161.63	114.94	166.00	161.50	158.20	122.60	165.14	164.57
Variance of means	12.5768	9.5761	24.0714	35.0357	12.5400	6.1600	14.9085	2.7466

mates served as controls. In addition, the cardiac blood of 10 untreated adult animals was examined.

Samples of blood were obtained from the tails of the immature animals before and 2 days after each series of injections. In both immature and adult groups of animals the mean maximal diameter of the erythrocytes was determined by direct measurement and the mean corpuscular volume was calculated. The data thus obtained were analyzed statistically.[4]

Results. In the immature, developing opossum the mean maximal diameter of the erythrocytes was greater and the mean corpuscular volume larger than these respective values in adult animals of the same species (Table I). The direct injection of either normal human gastric juice or of a concentrated solution of liver extract into immature opossums significantly reduced the mean maximal diameter and the mean corpuscular volume of the red blood cells as compared with untreated litter mate controls (Table II). These changes were effected through a relative increase in the number of smaller erythrocytes. The mean maximal diameter and the mean corpuscular volume of the erythrocytes of the treated immature opossums approached but did not equal these respective values in the blood of adult opossums (Tables I and II). Normal human gastric juice and a concentrated solution of liver extract, used in the manner described in this experiment, were equally effective in reducing the mean maximal diameter and the mean corpuscular volume of the erythrocytes of opossums developing in the maternal pouch.

[4] Fisher, R. A., *Statistical Methods for Research Workers,* 4th ed., Oliver and Boyd, London, 1932.