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Effect of Liver Extract, Injected Parenterally into Pregnant Mother, on Erythrocytes of Newborn Rats.

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A report by Stasney, Higgins, and Mann[1] indicated that the parenteral administration of the concentrated gastric juice of man and swine into the pregnant albino rat caused a reduction of the maximal size of the erythrocytes of the newborn pups. The results suggested that the reduction in the size of the erythrocytes was due to the presence of an anti-pernicious anemia factor. If that were true, we thought that the effect might serve as a laboratory test for the presence of the anti-pernicious anemia factor in liver extract. Accordingly, an experiment has been performed to test such an hypothesis, in which we have directed our attention to the mean, rather than the maximal, diameter or size of the erythrocytes.

Our stock albino rats were used, which are an inbred strain derived from a cross between a Wistar and Chicago alley rat. The pregnant rats were placed in individual cages and fed a diet of Purina dog biscuits and powdered milk, water being available at all times. Ninety newborn of 12 pregnant rats served as controls. Seventeen other pregnant rats were injected intraperitoneally with 0.2 cc of a liver concentrate, a relatively large dose, from 2 to 14 times on separate days prior to delivery. These rats delivered a total of 110 pups. The liver concentrates used were obtained from Eli Lilly and Company (Reticulogen) and from Chappel Brothers (Liver Extract Concentrated), and was known to be potent by actual tests on patients having pernicious anemia. No ill effects from the injections were observed. The blood of the pups of the treated and untreated mothers was collected and studied usually within 2 hours after birth. The blood was obtained by cutting off the tail. The erythrocytes were enumerated with a standardized pipette. Because of the small amount of blood, hematocrit determinations were made on the combined heparinized blood of 3 pups by the method of Rosahn.[2] Hemoglobin was estimated by the New-

[1] Stasney, J., Higgins, G. M., and Mann, F. C., *Proc. Staff Meetings Mayo Clinic,* 1937, **12**, 699.

[2] Rosahn, P. D., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 491.

TABLE I.

No. of Injections	No. of litters	No. of pups	R.B.C. mil.	Hb. g per 100 cc.	Hematocrit	Mean Corp. Vol.	Mean red-cell diam.	A Max.* Mean Size	Min. Mean Size	B Avg† diam. largest cells	C Avg diam. smallest cells
Control — 0	12	90	2.68	13.44	38.3	146	9.08 ± .06	11.72	8.23	11.22	7.82
Treated — 2	2	13	2.74	14.28	44.8	163	9.14 ± .12	9.64	8.11	11.46	7.54
" — 3	1	8	2.74	14.26	37.0	135	9.15 ± .10	9.61	8.69	11.29	7.84
" — 4	1	8	2.72	12.90	39.3	144	9.26 ± .11	9.72	8.61	11.43	7.49
" — 5	2	11	2.33	11.30	38.8	166	8.70 ± .08	9.15	8.29	10.57	7.50
" —Avg	6	40	2.62	13.16	40.5	154	9.07 ± .10				
" — 6	4	26	2.80	12.98	42.1	150	8.95 ± .05	9.24	8.37	11.16	7.85
" — 7	1	8	2.97	12.84	37.0	124	9.04 ± .05	9.29	8.87	11.35	7.99
" — 8	1	7	2.79	14.46	—		8.95 ± .11	9.35	8.47	10.66	7.95
" — 9	1	3	2.71	14.58	40.0	147	8.81 ± .03	8.85	8.76	11.41	6.71
" —11	1	6	2.71	14.04	48.0	177	9.13 ± .45	9.55	8.71	11.41	8.10
" —12	1	4	2.38	11.48	—		9.12 ± .09	9.36	8.98	11.78	7.88
" —13	1	9	2.94	12.84	—		8.84 ± .10	9.33	8.46	11.36	7.65
" —14	1	7	2.50	12.62	42.4	169	9.02 ± .07	9.32	8.70	11.82	7.34
" —Avg	11	70	2.77	13.13	41.9	151	8.97 ± .12				

*The maximum mean red-cell size observed in any newborn rat.
†The average diameter of the largest red cell measure in all newborn rats.

comer method. The mean red cell diameters (Price-Jones counts) were determined under oil immersion (1212X) by a standardized and calibrated disc on blood smears stained with Wright's stain, 100 consecutive cells were measured. Only very thin smears, or smears in which the red cells did not touch one another, were used.

The averaged results are shown in Table I. The normal mature rats in our colony have a mean erythrocyte count of approximately 9.0 million, 14.5 g of hemoglobin per 100 cc of blood, and a mean red cell diameter of 6.29 micra. When these figures are compared with those for the newborn rats, it is evident that the newborn rat has an erythopenia which is compensated for by a macrocytosis, since the hemoglobin values of the newborn are only slightly less than that of the adult. However, the blood picture of the newborn rats was not significantly altered by antenatal injection of the mother with liver extract of known potency. The mean red cell diameter of the pups of the untreated controls was 9.08 ± 0.06 micra (range 11.72 to 8.23); in the pups of the mothers receiving up to 6 injections it was 9.07 micra; in the pups of the mothers receiving 6 to 14 injections it was 8.97 ± 0.12 micra. It is to be noted that the wide range observed in the diameters of the erythrocytes (or the anisocytosis) of the untreated newborn was reduced by the treatment, although the mean diameter, mean corpuscular volume, and other values were not significantly changed. However, there were but 3 normal pups examined whose mean red cell diameters exceeded 9.74 micra (these were 11.72, 10.44, and 10.78 micra).

The mean red cell diameters observed by us for newborn rats (9.07 micra) is at variance with that reported by Stasney, *et al.*,[3] Wills,[4] and Smith.[5] Stasney, *et al.*, obtained a value of 9.54 micra, Wills a value of 8.62 micra on only 4 newborn rats, and Smith a value of 8.65 micra on only 5 newborn rats. Our mean determinations of the number of erythrocytes agree with those of Jolly (2.31),[6] Chisholm (2.69),[7] Jolly (2.76),[8] Sure, *et al.*, (2.8),[9] and Kindred and Corey (2.48).[10] However, the hemoglobin values vary to a greater extent. The differences observed may be due to a dif-

[3] Stasney, J., and Higgins, G. M., *Proc. Staff Meetings Mayo Clinic*, 1937, **12**, 490.

[4] Wills, L., and Mehta, M. M., *Ind. J. Med. Res.*, 1930, **18**, 307.

[5] Smith, C., *J. Path. and Bact.*, 1932, **352**, 717.

[6] Jolly, J., *Comp. Rend. Soc. Biol.*, 1909, **2**, 136.

[7] Chisholm, R. A., *J. Exp. Physiol.*, 1911, **4**, 207.

[8] Jolly, J., *Comp. Rend. Soc. Biol.*, 1919, **82**, 800.

[9] Sure, B., Kik, M. C., and Walker, D. J., *J. Nutrition*, 1929, **1**, 299.

[10] Kindred, J. C., and Corey, E. L., *Anat. Rec.*, 1930, **47**, 213.

ference in diet, breed, or technic. Regardless of the differences in the mean red cell diameters of our newborn rats and those of Stasney, *et al.*, we doubt that there is a trend toward a decreased mean red cell size in the newborn rats of the mothers receiving injections of liver extracts known to contain the anti-pernicious anemia substance.

These results are compatible with the findings of Wigodsky, Richter, and Ivy,[11] that fetal livers of calves are abundantly filled with the anti-pernicious anemia factor. Hence, it can be hypothesized that the addition of more anti-pernicious anemia factor to hemopoietic systems already well supplied should incite no differences in the erythrocyte picture of the animals. These data bear out this hypothesis. Our results should not be interpreted as implying that gastric juice concentrates are ineffective or that our results and those of Stasney, *et al.*,[3] are contradictory.

Wintrobe, *et al.*,[12] have reported similar results in rabbits. They could observe no change in the red blood cell size of the newborn rabbits whose mothers had been injected intramuscularly with a potent liver extract. In one case they succeeded in injecting the extract into one uterine horn; the red blood cells of the fetuses of the injected horn did not differ significantly from those of the fetuses of the uninjected horn at term.

Summary. Data concerning the erythrocyte picture of newborn rats whose mothers had been treated antenatally with liver extracts known to contain the anti-pernicious anemia factor has been presented and shown to be not statistically different from that of untreated controls; or the antenatal injection of liver extract into a pregnant rat has no significant effect on the mean red cell diameter of the newborn rats.

11 Wigodsky, H. S., Richter, O., and Ivy, A. C., *Proc. Am. Phys. Soc.*, 1938, 214.

12 Wintrobe, M. M., Kinsey, R. E., Blount, R. E., and Trager, W., *Am. J. Med. Sc.*, 1937, **193**, 449.