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Reticulocytes of Normal Albino Rats.*

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The literature on nutritional anemia reveals numerous discrepancies in the observations of different investigators. Some workers report that iron alone exerts no hematopoietic effect while others, including ourselves,¹ have obtained partial remission of anemia with such supplements. Although the milk anemic rats used by Drabkin and Miller² failed to regenerate when iron only was administered, prompt recovery from anemia ensued if glutamic acid supplemented the iron. Elvehjem, Steenbock and Hart³ were unable to confirm this observation. Our studies¹ have shown that glutamic acid was ineffective in the case of anemic rats reared from normal mothers, although it exerted a positive effect on hemoglobin regeneration in the case of anemic rats bred from mothers which had been fed a protein-low diet (cystine-deficient). The iron and glutamic acid preparations used in our studies were apparently free from copper and the dry klim, which served as the basal ration, showed no greater copper content than the milk employed by the Wisconsin workers. Furthermore, the same batch of klim and the same preparations of iron and glutamic acid were employed in our 2 series of experiments. Therefore, the dissimilarity in the response of our 2 groups of rats cannot be ascribed to variations in the ration or supplements but rather to differences in the anemic animals or their progenitors.

It is quite possible that some of the discrepancies in the results on milk anemia reported from various laboratories may be due to differences in the copper reserves of the animals. Nevertheless, it was thought desirable to look for other criteria which could demonstrate differences in stock rats used for breeding anemic animals.

The percentage of reticulocytes in the blood of the stock rats employed by Dr. D. L. Drabkin† for the production of his anemic

* In cooperation with the Research Department of the Calco Chemical Co., Bound Brook, N. J.

¹ Stucky, C. J., *J. Biol. Chem.*, 1932, **97**, xiii.

² Drabkin, D. L., and Miller, H. K., *J. Biol. Chem.*, 1931, **90**, 531; 1931, **93**, 39.

³ Elvehjem, C. A., Steenbock, H., and Hart, E. B., *J. Biol. Chem.*, 1931, **93**, 197.

† Personal communication and discussion of data presented in a demonstration at the meeting of the Federation of American Societies for Experimental Biology, Philadelphia, April, 1932. These data will be published shortly.

animals was quite different from that obtained by us on some of the rats from our stock colony. Accordingly, systematic reticulocyte determinations on our normal stock animals were made and these data correlated with age, growth, blood hemoglobin level and the red blood cell count.

Our colony was of the Wistar strain and had been inbred for several generations. The animals were fed a stock ration which was found to be adequate for the nutritional requirements of the rat. The composition of the diet was as follows: Rolled oats 30, whole milk powder 15, dried yeast‡ 10, wheat embryo 10, meat scraps (Swift's 65%) 20, casein 10, cod liver oil 3, sodium chloride 0.5, sodium bicarbonate 0.75 and calcium carbonate 0.75%. In addition, fresh green lettuce was fed at frequent intervals.

Estimations of the hemoglobin, red blood cells and reticulocytes were made at approximately weekly intervals on groups of rats from birth until the animals were 3 months old. Blood was obtained by clipping the tails. Hemoglobin was determined colorimetrically using a calibrated Newcomer disc. "Total hemoglobin" was calculated from blood volume⁴ (total Hb = blood volume × Hb/100

TABLE I.
Changes occurring with age in the reticulocytes and other blood constituents.

Age	No. rats	No. litters	Sex		Wt.	Hb	Blood Estimations			Reticulocytes
			♂	♀			Tot. Hb	R. B. C.	%	
days					gm.	gm. per 100 cc.	gm.	millions per c.mm.	%	millions per c.mm.
0	3	1	2	1	6	10.3	.05	2.7	61	1.7
7	3	1	1	2	16	8.6	.10	3.2	42	1.3
14	3	1	2	1	31	7.0	.15	4.4	31	1.4
21	12	4	5	7	47	8.0	.26	4.8	25	1.2
21	22	4	14	8	43	—	—	—	24	—
21	31	7	11	20	47	7.5	.24	—	—	—
28	12	4	5	7	68	10.7	.47	5.7	18	1.0
28	12	3	3	9	81	11.2	.58	—	—	—
28	4	1	1	3	75	—	—	—	20	—
37	15	6	6	9	100	12.0	.76	7.0	11	.8
43	9	4	5	4	119	13.6*	.99*	7.1*	6	.4
49	12	5	4	8	148	15.0	1.34	8.5	4	.3
56	12	5	4	8	152	15.0	1.37	9.8	3	.3
67†	12	5	5	7	196	15.9‡	1.83‡	9.1‡	3	.3
82†	8	4	2	6	213	17.0	2.09	9.3	2	.2
96†	7	3	4	3	277	17.1	2.67	9.7§	2	.2

* Average of 5 rats. † Mean age. ‡ Average of 10 rats. § Average of 6 rats.

‡ Obtained through the courtesy of Mr. Leo Wallerstein of the Wallerstein Laboratories, New York, N. Y.

⁴ Donaldson, H. H., *The Rat*, Philadelphia, 1924, 287.

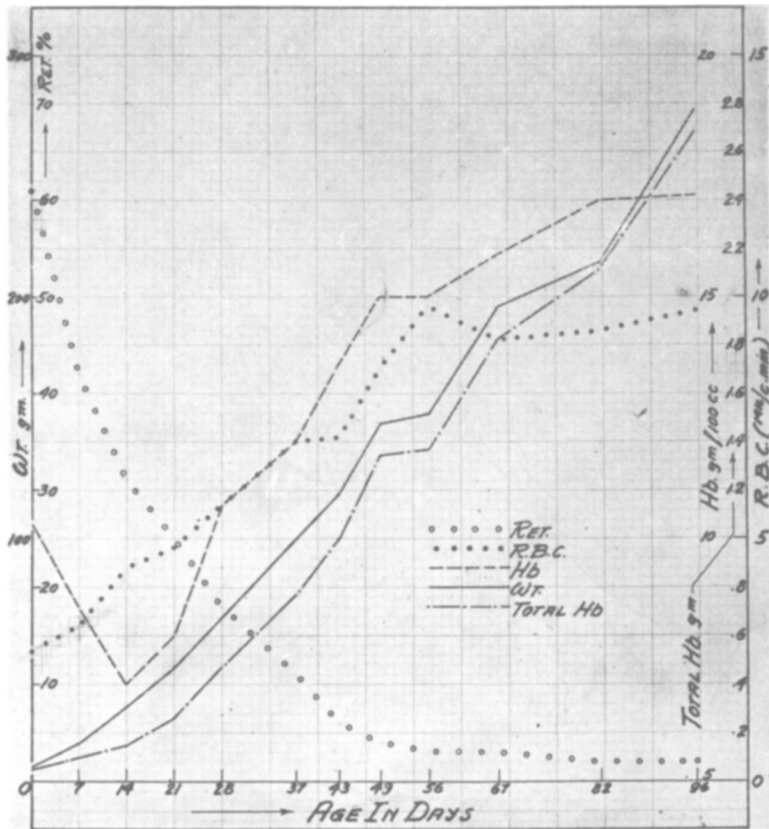


FIG. 1.

gm.). For the preparation of smears for the reticulocyte counts, a slight modification of the usual technique was introduced. In the customary method, the blood is mixed directly with the dried stain on the slide. In our procedure one drop of a 0.2% solution of sodium citrate is dried on the slide in a spot free from the brilliant cresyl blue. Then a drop of blood is mixed first with the citrate and then with the dye. The remainder of the procedure is carried out as usual. In this way uniform blood smears may be obtained easily. §

The data are presented in the table and chart. There is a rapid decrease in the number of reticulocytes from birth (approx. 60%) until the animals are 2 months old. Thereafter their number remains fairly constant (2 to 3%). It is interesting to note that the well-known drop in the hemoglobin level during the nursing period

§ The complete details will be described elsewhere by Miss E. M. Kapp.

is associated with a progressive decrease in the percentage of reticulocytes and an increase in the number of red blood cells. Only a few rats were used for the reticulocyte estimations during the first 3 weeks of life but it is believed that the figures are fairly representative. When the rat pups are weaned at 21 days of age, the reticulocytes have dropped to a 25% level. The values for the other blood constituents are in accord with those in the literature, the maximum hemoglobin being 17 gm. % (approx.) and the maximum red blood cell count being 9-10 millions for our young stock adults.

Since young adult rats, 2 to 3 months old, show a very low and constant reticulocyte count (2 to 3%), it would appear that such a criterion might be used as another index of the physiological condition of the stock colony. Thus, values above this level might indicate a condition of potential anemia. Hence, it might serve as an additional basis of comparison for the stock rats of different laboratories. It is conceivable that such information might help in the interpretation of some of the conflicting data on nutritional anemia reported by different investigators.

Summary. The percentage of reticulocytes in the blood of young rats bred from an original Wistar strain varying in age from birth to 3 months was determined. These data have been correlated with growth, hemoglobin level, total hemoglobin and red blood cell count. The drop in the hemoglobin level during the nursing period is associated with a progressive decrease in the number of reticulocytes. Rats from our colony 2 to 3 months old show 2 to 3% (.2 to .3 millions per c.mm.) of reticulocytes. The significance of the number of reticulocytes in young adult stock animals as a possible criterion of potential anemia has been indicated.

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Picrotoxin-Stramonium Antagonism.

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The actions of picrotoxin and stramonium on the upper central nervous system suggest the possibility of their possessing some degree of mutual antagonism. The site of the principal action of picrotoxin upon the central nervous system has been localized by