8720 C

Nucleotide Nitrogen Content of Human White Blood Cells.*

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In a previous communication[1] on the nucleotide content of pathologic human whole blood, it was stated that the correlations between nucleotide nitrogen and the percentage of hemoglobin and the red blood corpuscle count are high in all of the conditions which were studied, except in leukemia. In the latter case there is no relationship between the nucleotide nitrogen and the number of red blood corpuscles, but there is a significant positive correlation between the nucleotide nitrogen content of the whole blood and the number of white blood cells. In other words, it would seem that the high values for nucleotide nitrogen observed in leukemia must be associated in some way with the increased white cell count. Under conditions where the number and volume of the red blood corpuscles in comparison with the white blood cells maintain the usual level, the amount of nucleotide which is present in the white blood cell is masked completely by the larger quantity of nucleotide which is confined to the red blood corpuscles.

Insofar as we are aware, there is only one analysis of the nucleotide content of the leucocytes published in the literature. Buell and Perkins[2] reported negative results for the nucleotide content of the lymphocytes taken from the thoracic duct of the dog. It therefore seemed desirable to attempt the separation of the white blood cells from the red blood corpuscles in those cases of leukemia in which small quantities of blood would give a quantity of white cells sufficient for the quantitative analysis of the nucleotide content.

The following procedure was used for the separation of the red blood corpuscles from the white blood cells. Five millimeter glass tubing was cut the size of ordinary hematocrit tubes and sealed at one end. The blood was introduced into the tubes with a syringe which had been fitted with a long needle. The tubes containing the blood were then centrifuged for 25 minutes at approximately 2500 r.p.m. After the centrifugation, the tubes contained 3 separate

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1 Allen, F. W., Lucia, S. P., and Eiler, J. J., *J. Clin. Invest.*, 1936, **25**, 157.

2 Buell, M. V., and Perkins, M. E., *J. Biol. Chem.*, 1928, **76**, 95.

and distinct layers, corresponding to red blood corpuscles, white blood cells with a few enmeshed red corpuscles, and plasma. The length of each layer in the 5 mm. tubes varies from one inch or more, dependent upon the relative volume of the red blood corpuscles and the white blood cells. The tubes were broken, with the aid of a file and gentle pressure, at the juncture between red corpuscles and white cells and also at the juncture between white cells and plasma. The cells or plasma were then aspirated from each section. If carefully done, quite an efficient separation of the three fractions may be effected. After the separation, the total volume of each cell fraction was determined, and Ringer's solution was added until the volume in each case (determined by hematocrit) was the same as that occupied by the fraction as it appeared in the original sample of whole blood.

Thus, with a sample of blood from a case of leukemia it was found possible to obtain a white blood cell suspension with a cell count comparable to that of the original blood, but with the red blood corpuscles present in very small percentages. Table I contains the results of the analyses of 2 samples of blood taken from patients with myeloid leukemia. Sample No. 1 may be taken as an example of a very marked separation of white blood cells from red blood corpuscles (less than 20,000 R.B.C./c.mm.), while sample No. 2, due to the greater diameter of the white cells with their tendency to enmesh the smaller corpuscles, is representative of one of the cases of a less marked separation (690,000 R.B.C./c.mm.). The nucleotide nitro-

TABLE I.
Partition of Nucleotide Nitrogen Between the Formed Elements of the Blood of Patients with Myeloid Leukemia.

	Nucleotide nitrogen per 100 cc., mg.	Red blood corpuscles, millions	White blood cells
Sample No. 1			
(a) Whole Blood R.B.C. Hematocrit 16.5% W.B.C. " 15.5%	5.7	2.26	790,000
(b) White Cell Fraction	3.0	0.02	500,000
(c) Red Corpuscle Fraction	1.8	1.70	8,300
(d) Plasma	1.2	—	—
Sample No. 2			
(a) Whole Blood R.B.C. Hematocrit 20.0% W.B.C. " 44.0%	8.0	3.34	360,000
(b) White Cell Fraction	6.8	0.69	464,000
(c) Red Corpuscle Fraction	3.0	2.34	13,200
(d) Plasma	0.9	—	—

gen was estimated by the quantitative method of Kerr and Blish.[3] Examination of the table shows that in cases of myeloid leukemia in which the white blood cell counts are high there is more nucleotide in the white blood cell fraction than in the red blood corpuscle fraction.

Table II contains an example of a case of myeloid leukemia upon which a serial study of the nucleotide nitrogen content has been made coincident with the regression of the white blood cell count. It will be noted that during the 20-fold decrease in white blood cell count with an approximately constant red blood corpuscle count there is a 2-fold decrease in nucleotide nitrogen content of the whole blood.

TABLE II.
Nucleotide Nitrogen Content of the Whole Blood of a Patient with Myeloid Leukemia.
(Serial samples taken during the regression of the disease.)

Date 1935	Nucleotide nitrogen per 100 cc. whole blood, mg.	Hemoglobin %	Red blood corpuscles, millions	White blood cells
10/29	9.6	50	2.93	460,000
10/31	7.3	54	3.20	390,000
11/16	4.8	67	3.68	24,000

8721 C

A Bile Fistula Procedure in the Rat.

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In this laboratory the rat has been found to be a very useful experimental animal for purposes of studying bile function. Large numbers of animals may be used. Moreover, the rat is not particularly subject to infections, and special aseptic operative procedures do not need to be employed in preparing bile fistulas.

The internal bile fistula technique which has been found useful in dogs cannot be applied to the rat. This animal has no gall blad-

[3] Kerr, S. E., and Blish, M. E., *J. Biol. Chem.*, 1932, **98**, 193.

* E. R. Squibb and Sons Fellow.