THE NUCLEOTIDE NITROGEN CONTENT OF PATHOLOGIC HUMAN WHOLE BLOOD ^{1, 2}

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Recent investigations suggest that the following properties may be attributed to the purine nucleotides: (1) the apparent ability to stimulate leukocytopoiesis, (2) the ability to produce interference in conduction in the heart, (3) the ability to produce increased tone in isolated smooth muscle, and (4) participation in the co-ferment complex where the catabolism of carbohydrate is involved. Only those nucleotides which contain the component, adenine, display the latter three properties.

Since the nucleotide of the blood contains adenine as its purine component, it would seem to be of value to study the possible quantitative variations in this nucleotide under various pathological conditions. Only two other investigations of this nature have been attempted, that of Rothmann (1), completed before adequate quantitative methods were available, and that of Buell (2), which has been published during the preparation of this manuscript.

Buell (3) has recently revised the Buell and Perkins' (4) method for the estimation of the nucleotide content of the blood. Her paper contains data which were obtained from the analyses of 107 blood samples. The average nucleotide nitrogen content per 100 cc. of whole blood for normal males was found to be 6.2 mgm.; for normal females, 5.2 mgm.

It is generally accepted that, apart from the phospholipins, the "ester phosphorus" compounds of the blood, which include the nucleotides, are, under normal conditions, practically confined to the red blood corpuscles. Buell (3) presents evidence to show that "the apparent difference between the sexes in the nucleotide content of whole blood is associated with the fact that males have more red corpuscles and a correspondingly greater volume of red cells per unit volume of whole blood than do females." This is undoubtedly true under normal conditions where the number and volume of the red blood corpuscles in comparison

TABLE I

Nucleotide nitrogen content of the whole blood of patients with various pathological conditions

Case num- ber‡	Nucleotide nitrogen per 100 oc. whole blood		acleotide itrogen r 100 cc. whole blood		Diagnosis			
	mgm.	per cent	millions					
				Male				
6a* 6b* 6c 66 26a 26b 26c 26d 26c 26d 26c 655 108	2.6 2.7 3.8 4.6 3.7 4.9 2.4 2.5 3.7 4.1 4.6 2.5 3.3 2.9	37 33 47 81 66 90 31 42 68 70 75 43 64 45	$1.38 \\ 1.50 \\ 1.85 \\ 3.90 \\ 3.95 \\ 4.30 \\ 1.26 \\ 2.05 \\ 2.73 \\ 4.28 \\ 4.38 \\ 1.64 \\ 2.55 \\ 1.51 \\ $	3,400 2,800 4,280 5,760 5,760 5,420 7,450 7,450 7,550 5,700 6,700 9,000 7,750	Pernicious anemia Pernicious anemia			
				Female				
17a* 17b* 17c* 17d* 30 38* 75	2.5 2.4 2.2 5.2 3.3 1.6 2.8	44 38 44 55 60 24 28	1.78 1.50 2.15 2.63 3.02 0.82 0.97	3,500 2,800 2,350 4,200 8,600 4,800 6,650	Pernicious anemia Pernicious anemia Pernicious anemia Pernicious anemia Pernicious anemia Pernicious anemia Pernicious anemia			
				Male				
2 9 14 23 14b 43 66 69 81 84 106 109	3.5 4.9 5.0 6.1 2.6 6.2 5.1 4.6 7.8 3.0 4.1	82 85 60 86 60 90 85 61 93 45 90	3.97 4.36 4.88 3.34 4.75 2.80 5.00 5.10 3.31 6.00 2.30 3.70	900 4,150 2,800 1,000 2,550 1,520 4,200 2,400 2,900 4,400 1,250 1,960	Lymphosarcomatosis with multi- ple skin nodules. Leukopenia Bronchopneumonia Leukopenia, after x-ray Leukopenia, after x-ray Lymphoblastoma Eosinophilia Asthma. Lobar pneumonia Hemolytic jaundice Artericselerotic gangrene Lymphatic leukemia Phenol poisoning. Lobar pneu- monia			
				Female				
89	3.8	80	4.48	4,250	Cholecystitis			

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² Aided by a grant given in memory of Laurence A. Myers, Jr., and Thomas J. Maxwell.

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Case num- ber‡	Nucleotide nitrogen per 100 cc. whole blood	Hemo- glo- bin	Red blood corpus- cles	White blood cells	Diagnosis	Case num- bert	Nucleotide nitrogen per 100 cc. whole blood	
	mgm.	per cent	millions				mgm.	
		·	·	Male	······			
1	3.7	36	1.92	110,000	Myeloid leukemia	42	5.7	
31 34	7.1 7.4 4.8	95 70	5.28 3.55	250,000 14,650 8,450	Lymphatic leukemia Aleukemic lymphatic leukemia	44 45	5.7 6.4	
62 82 86a	6.4 2.9 6.7	55 78 65	3.06 4.19 3.60	175,000 13,050 156,000	Myeloid leukemia Lymphoblastoma. Leukemia cutis Myeloid leukemia	46 51	4.6 4.7	
86b	8.7	71	3.68	140,800	Myeloid leukemia	52	6.1	l
86c	11.3	65	4.64	350,000	Myeloid leukemia	53	7.6	ł
90	10.2	40	2.90	1 250	Myelold leukemia	56	6.0	l
100a)	4.2	67	3 33	38,000	Lymphatic leukemia	58	4.0	l
			0.00	00,000		61 64a	4.4 5.2	
				Female		64b 70	5.8 6.0	
20.	85	42	2 32	370 000	Myeloid leukemis	71	50	I
20b	6.2	40	2.90	232.000	Myeloid leukemia	73	2.7	l
20c	4.8	40	2.16	290,000	Myeloid leukemia	74	3.4	l
24	6.2	63	3.35	96,000	Monocytic leukemia			l
72	3.5	92	5.84	24,600	Myeloid leukemia	76	6.0	I
93	5.7	55	2.85	9,500	Myeloid leukemia	77	4.5	l
	·	L	I		1	79 90	0.1 6 2	l
				Mala		00	0.5	l
				11010	r	85	4.5	
3	5.9	118	6.30	9,500	Chronic nephritis. Cardiac de-	87	5.4	
					compensation	88	5.7	ł
4	5.6	93	4.30	29,600	Purulent empyema	90	5.1	
5	4.2	72	3.90	9,000	Carcinoma of the stomach with	02	4.0 71	
7	4.6	75	3.90	8,300	Gonorrheal arthritis. Generalized	94	53	
					arterioscierosis. Intersutial ne-	95	4.5	
8	5.4	85	3.85	8.600	Lobar pneumonia	101	6.1	ŀ
11	4.8	88	4.65	11,650	Infectious arthritis	102	4.5	Ĺ
12	6.1	85	4.94	32,760	Lobar pneumonia	103	5.3	
13	4.7	60	3.95	9,600	Gout	104	4.0	
15 16	3.8 7.8	92 113	4.42 6.37	17,950 9,950	Cout Chronic emphysema. Bronchitis.	105 107	9.5 3.7	
21	5.5	94	5.20	17,600	Lobar pneumonia			
$\bar{2}\bar{2}$	5.0	76	3.63	17,050	Lobar pneumonia			
25	6.2	84	5.49	10,000	Malingerer			
27a	3.7	45	2.65	9,900	Hodgkin's disease			_
27b	3.0	50	2.59	6,800	Hodgkin's disease	10	47	
270	4.0	43	2.31	12,200	Hougkin's disease		T.1	
40	9.1	04	4.40	0,280	hausen)	19	14.3	
29	2.3	36	1.86	14.000	Chronic pneumonia. Hemolytic	33	4.5	
				11,000	icterus	35	4.3	
32	5.7	85	4.07	8,900	Pellagra	39	4.9	
36	4.5	80	4.33	5,600	Neurosyphilis. Decompensated	41	5.7	
27	99	1.0	1.22	10 000	mitral valvulitis	59	4.9	
40	5.3	90	4.75	6 200	Pavehasthenia		[
	0.0		1.10	0,200	- DJ CALERDUITOTING	60	3.9	
		i				63	4.7	
		1				07	3.8	

TABLE I—Continued

TABLE I-Continued

	Case	nitrogen	Hemo-	blood	White				
	num-	per 100 cc.	gio-	corpus-	blood	Diagnosis			
	ber‡	blood	bin	cles	cells				
		mgm.	per cent	millions					
			·		Male				
	42	5.7	75	3.87	9,950	Pulmonary tuberculosis. Arterio-			
	44 45	5.7 6.4	90 80	5.12 4.40	5,100 8,160	Arteriosclerosis. Mitral valvulitis Generalized arteriosclerosis. Em-			
	46 51	4.6 4.7	82 90	3.70 4.93	6,100 9,450	Rheumatic heart disease Jacksonian epilepsy. Hyperten-			
	52	61	00	5 50	8 900	810n Pentia ulaar Neurosthenia			
	53	7.6	95	5.90	8 250	Preumonia (convalescing)			
	56	6.0	85	4.86	8,200	Graves' disease			
	58	4.0	60	3.40	13,700	Malnutrition			
	61	4.4	85	4.45	9,800	Lymphosarcoma			
	64a 64b	5.2	90	6.80	9,800	Rheumatic heart disease. Car- diac failure			
	70	6.0	72	3.60	7,960	Luetic-hepato-splenomegaly. Mor-			
	71	5.9	170	4.84	9,600	Hodgkin's disease			
	74	3.4	73	4.23	8,800	Visceral syphilis. Carcinoma of the stomach			
	76	6.0	80	4.78	7,050	Bronchiectasis. Neurasthenia			
	77	4.5	70	4.35	10,900	Hodgkin's disease			
	80 80	6.3	70	4.04 4.25	6,400 7,240	Congenital heart disease. Duo- denal ulcer			
	85 87	4.5 5.4	85	4.53	6,040	Arteriosclerosis. Syphilis. Chole- cystitis. Cholelithiasis Bruchesthesis. Brutis glass			
	88	57	82	5 25	0,000	Cardiae decompensation Symbilia			
	90	5.1	90	4.90	7,100	Luetic aortitis			
	91	4.8	95	6.30	13,700	Infectious arthritis			
	92 04	7.1	48	2.57	9,400	Chronic nephritis. Plumbism Generalized arteriosclerosis			
	94	5.3	90	4.70	7,400	Mediastinal tumor			
	101	4.0 6 1	80	4.80	9,200	Arterioscierotic heart disease			
	102	4.5	08	5 35	6 320	Synhilie			
	103	5.3	75	3.96	7 520	Luetic aneurysm			
	104	4.6	83	4.83	10,200	Angina pectoris. Coronary scle- rosis. Bronchial asthma			
	105	9.5 3.7	130 90	6.18 4.43	16,450 9,050	Congenital heart disease Psychasthenia			
-					Female	*			
-	10	4.7	84	4.47	9,480	Neurasthenia. Hyperventilation			
	19	14.3	180	10.10	9,200	Polycythemia			
	33	4.5	68	3.93	8,840	Cyst of the liver			
	35	4.3	97	4.61	5,500	Purpura hemorrhagica			
	39 41	4.9 5.7	81 100	4.30 5.10	8,200 6,400	Questionable spinal cord tumor Carcinoma of the ovary with as-			
	59	4.9	88	5.01	7,810	Mitral heart disease. Paroxysmal tachycardia			
	63	3.9 47	85	4.00	7,120	Exophinalmic goiter			
	67	3.8	45	4.20	13 400	Rheumatic heart disease			
	6 8	5.6	85	5.08	9,200	Ovarian tumor. Blindness			
	78	5.3	75	4.95	6,100	Carcinoma of the head of the pan- creas			
	07	4.3	90	4.90	8,800	Sinusitis			
	98	5.2	76	4.49	6 640	Atrophic erthritie			
	99	5.4	88	4.50	7,000	Chronic nephritis with edema			
	100	6.1	84	4.30	8,850	Chronic arthritis			
	1								

with the white blood cells maintain the usual level.

This investigation was undertaken in order to determine the nucleotide nitrogen content of samples of whole blood from pathological cases, and to study the relationships between this factor and the hemoglobin, the red blood corpuscle, and the white blood cell content of the blood.

Over one hundred pathological cases were selected in order to determine the range of the nucleotide nitrogen content in the blood under some * Classified both with the cases of anemia and leukopenia for statistical analysis.

† Classified both with cases of leukopenia and leukemia for statistical analysis.

[‡] The number of the case refers to a patient, the letter to successive samples from the same patient.

abnormal physiological conditions (Table I). The amount of hemoglobin, the erythrocyte, and the leukocyte counts presented variations beyond those usually found in normal individuals. Especial attention was directed to patients suffering from anemia, leukopenia, and leukemia. It should be pointed out that there are several instances in which serial specimens were taken from the same patients. These were chosen because of the nature of the pathological changes, the effects of treatment, and the variations in the percentage of hemoglobin and blood counts. (Table I, Cases 6, 17, 20, and 26.) which did not fall into the foregoing classifications were grouped and studied together. Because of the reduced number of cases, this naturally decreased the reliability of any statistical procedure which might be used. By the use of selective grouping, however, it was possible to determine the effect of various concealed factors on the analysis of the total number of cases (Table II).

There was some overlapping among the cases of anemia and leukopenia. Of the eight cases of anemia, four had leukocyte counts so low that they were also classified with the leukopenia

	TABLE II		
Means and standard	deviations of date	ı given in	Table I

	Num- ber	Num- ber of	Nucleonitro	otide gen	Her glol	no- bin	Red corpu	blood 1scles	White ce	blood lls
	cases	mens	Mean	σ*	Mean	σ	Mean	σ	Mean	σ
					per cent	per cent	millions	millions	thousands	thousands
I. Total number of analyses	102	124	5.0	1.8	73.0	22.9	4.01	1.45	30.4	77.0
Male	76	93	5.0	1.7	73.8	20.1	4.06	1.34	26.8	77.2
Female	26	31	4.9	2.2	70.5	29.3	3.86	1.73	41.4	87.0
(b) Classified according to disease	8	21	22	1 0	51 7	178	2 30	1 1 3	57	22
Leukopenia (10 male, 3 female)	13	22	4.0	1.5	61.6	22.4	3.15	1.44	3.0	1.2
Leukemia (12 male, 4 female)	16	18	6.2	2.4	58.5	18.1	3.33	1.07	156.1	147.1
Others (not included above) (52 male, 17 female)	69	72	5.3	1.6	82.4	19.5	4.64	1.20	11.2	12.8

* The standard deviation (σ) is the square root of the arithmetic mean of the squares of all deviations; deviations being measured from the arithmetic mean of the observations $\sigma = \sqrt{\frac{Ex^2}{n}}$. (Yule, G. U., An Introducton to the Theory of Statistics. Charles Griffin & Co., London, 1927, 8th ed., p. 134).

In order to study the nucleotide nitrogen, the quantitative method of Kerr and Blish (5) was used. It was found to be satisfactory. The hemoglobin values were determined by using a Sahli hemoglobinometer (100 per cent equals 14 grams hemoglobin per 100 cc.), and the usual procedures were followed in making blood counts.

The data for each test were correlated with the amount of nucleotide nitrogen in each 100 cc. of whole blood. The Pearson coefficient of correlation was used.

In order to treat the material statistically, the data were classified according to sex. They were then grouped and studied according to certain diagnoses; i.e., pernicious anemia, leukopenia (cases in which the leukocyte count was less than 5000 per c. mm.), and leukemia. Those cases cases. This double classification occurred in nine instances. In one instance, that of a case of leukemia under treatment (Case 106), the first leukocyte count was so low that the blood was grouped with the leukopenia as well as with the leukemia cases.

When the total number of cases is considered, there are significant correlations (Table III) between the amount of nucleotide nitrogen and the percentage of hemoglobin, red blood corpuscle count, and white cell count. This does not indicate a cause and effect relationship. As one value increases; i.e., the percentage of hemoglobin, the red blood corpuscle count, or the white blood cell count, the amount of nucleotide nitrogen also increases.

When the data are classified according to sex,

TABLE III

Correlations between the amount of nucleotide nitrogen per 100 cc. of whole blood and per cent of hemoglobin and total red and white blood counts, the sample having been classified as in Table II

(124 specimens from 102 patients)

	Num-	Num-	Correlations of nucleotide nitrogen with				
	ber of cases	ber of speci- mens	Hemo- globin	Red blood corpus- cles	White blood cells		
I. Total number of analyses	102	124	0.534	0.572	0.431		
(d) Classified according to sex Male Female (b) Classified according to dis-	76 26	93 31	0.447 0.726	0.50 8 0.690	0.5 23 0.214		
ease Anemia Leukopenia Leukemia Others	8 16 13 69	21 22 18 72	0.838 0.798 -0.757 0.969	0.796 0.856 0.096 0.663	0.276 0.112 0.770 0.295		

practically the same correlations as were obtained in the analysis of the total number of cases, hold for the males, but not for the females. The larger proportion of males, of course, affects the total picture.

In considering the correlations when the data are grouped according to diagnosis, it is found that in the case of the patients with anemia, although there are four men and four women represented, and the number of specimens divide as to sex into fourteen from men and seven from women, the correlations follow those for women rather closely. It appears that in these cases of anemia, when the amount of hemoglobin and the number of red blood corpuscles are very low, the nucleotide nitrogen is also low. An increase in hemoglobin and red blood corpuscles is accompanied by a simultaneous increase in the nucleotide nitrogen content. There is no significant correlation between nucleotide nitrogen and the white blood cell count in the group of cases of anemia.

In the specimens showing leukopenia, the same correlations hold as for the cases of anemia. In this instance, it must be remembered that there is some overlapping of specimens; i.e., nine samples of blood common to both pernicious anemia and leukopenia.

When considering the cases of leukemia, there is a significant negative correlation between the nucleotide nitrogen and the hemoglobin content of the blood. There is no relationship at all between the nucleotide nitrogen and the number of red blood corpuscles. On the other hand, there is a significant positive correlation between the nucleotide nitrogen and the number of white blood cells. It will be noted (Table II) that the mean hemoglobin and the red blood corpuscle content do not differ appreciably from either the anemic or the leukopenic groups, but that, as would be expected, the mean number of white blood cells is very high with a large variation (M = 156.000): S. D. = 147.100). The mean amount of nucleotide nitrogen is also high when compared with other values in the table (M = 6.2, S, D, = 2.4). It is evident from the positive correlation of 0.770 that these high values for nucleotide nitrogen must be in some way associated with the increased white blood cell count

In the group of other diagnoses, the nucleotide nitrogen is significantly correlated with both the hemoglobin and the number of red blood corpuscles. The correlation with the white blood cells, although negative, is not statistically significant.

DISCUSSION

The correlations between nucleotide nitrogen and the percentage of hemoglobin and the red blood corpuscle count are high in all the conditions which were studied, except in leukemia. This indicates that in those conditions in which the number and volume of the red blood corpuscles are great in comparison to that of the white blood cells, the amount of nucleotide in whole blood is practically dependent upon the number and volume of the red blood corpuscles (3). In other words, the amount of nucleotide which may be present in the white blood cell would be masked completely by the larger quantity of nucleotide which is confined to the red blood corpuscles.

The correlation coefficient of 0.770 between nucleotide nitrogen and white blood cell count indicates that in leukemia the leukocytes and possibly the plasma may participate in elevating the nucleotide content of whole blood. An examination of the cases of leukemia demonstrates variations from the mean which may be associated with the progressive or regressive phase of the leukemia. This may indicate the presence of nucleotide in the white blood cell, as well as nucleotide in the plasma. The nucleotide of the plasma may be derived from the breakdown of the nucleic acid which is contained within the nucleus of the white blood cell. Certain experiments in progress, where the nucleotide nitrogen of both the whole blood and the plasma was determined, indicate that there is an appreciable amount of nucleotide nitrogen in the plasma of leukemic bloods. The high nucleotide content of leukemic bloods possibly accounts for the high uric acid values so often observed in leukemia.

SUMMARY

1. The nucleotide nitrogen content of 124 samples of pathological blood obtained from 102 patients was studied. The values were correlated with the percentage of hemoglobin, the red blood corpuscle, and white blood cell counts.

2. The correlations between nucleotide nitrogen and the percentage of hemoglobin and the red blood corpuscle count are high in all conditions, except in leukemia. A high correlation was found between nucleotide nitrogen and the white blood cell count in leukemia. An explanation for this is offered.

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