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Frank Worthington Allen, … , Salvatore P. Lucia, John J. Eiler

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Research Article

THE NUCLEOTIDE NITROGEN CONTENT OF PATHOLOGIC HUMAN WHOLE BLOOD [1, 2]

By FRANK WORTHINGTON ALLEN, SALVATORE P. LUCIA AND JOHN J. EILER

(From the Divisions of Biochemistry (Berkeley) and Medicine (San Francisco), University of California Medical School)

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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 number	Nucleotide nitrogen per 100 cc. whole blood	Hemoglobin	Red blood corpuscles	White blood cells	Diagnosis
	mgm.	*per cent*	*millions*		
			Male		
6a*	2.6	37	1.38	3,400	Pernicious anemia
6b*	2.7	33	1.50	2,800	Pernicious anemia
6c*	3.8	47	1.85	4,280	Pernicious anemia
6d	4.6	81	3.90	5,760	Pernicious anemia
6e	3.7	66	3.95	9,000	Pernicious anemia
6f	4.9	90	4.30	5,420	Pernicious anemia
26a*	2.4	31	1.26	4,500	Pernicious anemia
26b	2.5	42	2.05	7,450	Pernicious anemia
26c	3.7	68	2.73	7,550	Pernicious anemia
26d	4.1	70	4.28	5,700	Pernicious anemia
26e	4.6	75	4.38	6,700	Pernicious anemia
65a	2.5	43	1.64	7,700	Pernicious anemia
65b	3.3	64	2.55	9,000	Pernicious anemia
108	2.9	45	1.51	7,750	Pernicious anemia
			Female		
17a*	2.5	44	1.78	3,500	Pernicious anemia
17b*	2.4	38	1.50	2,800	Pernicious anemia
17c*	2.2	44	2.15	2,350	Pernicious anemia
17d*	5.2	55	2.63	4,200	Pernicious anemia
30	3.3	60	3.02	8,600	Pernicious anemia
38*	1.6	24	0.82	4,800	Pernicious anemia
75	2.8	28	0.97	6,650	Pernicious anemia
			Male		
2	3.5	82	3.97	900	Lymphosarcomatosis with multiple skin nodules. Leukopenia
9	4.9	85	4.36	4,150	Bronchopneumonia
14	5.0	85	4.88	2,800	Leukopenia, after x-ray
23	5.0	60	3.34	1,000	Leukopenia
14b	6.1	86	4.75	2,550	Leukopenia, after x-ray
43	2.6	60	2.80	1,520	Lymphoblastoma
66	6.2	90	5.00	4,200	Eosinophilia
69	5.1	85	5.10	2,400	Asthma. Lobar pneumonia
81	4.6	61	3.31	2,900	Hemolytic jaundice
84	7.8	93	6.00	4,400	Arteriosclerotic gangrene
106	3.0	45	2.30	1,250	Lymphatic leukemia
109	4.1	90	3.70	1,960	Phenol poisoning. Lobar pneumonia
			Female		
89	3.8	80	4.48	4,250	Cholecystitis

[1] Aided by a grant from the Research Board of the University.

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

TABLE I—*Continued*

Case number‡	Nucleotide nitrogen per 100 cc. whole blood	Hemoglobin	Red blood corpuscles	White blood cells	Diagnosis
	mgm.	*per cent*	*millions*		
			Male		
1	3.7	36	1.92	110,000	Myeloid leukemia
18	7.1	27	2.00	250,000	Myeloid leukemia
31	7.4	95	5.28	14,650	Lymphatic leukemia
34	4.8	70	3.55	8,450	Aleukemic lymphatic leukemia
62	6.4	55	3.06	175,000	Myeloid leukemia
82	2.9	78	4.19	13,050	Lymphoblastoma. Leukemia cutis
86a	6.7	65	3.60	156,000	Myeloid leukemia
86b	8.7	71	3.68	140,800	Myeloid leukemia
86c	11.3	65	4.64	350,000	Myeloid leukemia
96	10.2	45	2.90	530,000	Myeloid leukemia
106a†	3.0	45	2.30	1,250	Lymphatic leukemia
106b	4.2	67	3.33	38,000	Lymphatic leukemia
			Female		
20a	8.5	42	2.32	370,000	Myeloid leukemia
20b	6.2	40	2.90	232,000	Myeloid leukemia
20c	4.8	40	2.16	290,000	Myeloid leukemia
24	6.2	63	3.35	96,000	Monocytic leukemia
72	3.5	92	5.84	24,600	Myeloid leukemia
93	5.7	55	2.85	9,500	Myeloid leukemia
			Male		
3	5.9	118	6.30	9,500	Chronic nephritis. Cardiac decompensation
4	5.6	93	4.30	29,600	Purulent empyema
5	4.2	72	3.90	9,000	Carcinoma of the stomach with metastases to the liver
7	4.6	75	3.90	8,300	Gonorrheal arthritis. Generalized arteriosclerosis. Interstitial nephritis
8	5.4	85	3.85	8,600	Lobar pneumonia
11	4.8	88	4.65	11,650	Infectious arthritis
12	6.1	85	4.94	32,760	Lobar pneumonia
13	4.7	60	3.95	9,600	Gout
15	3.8	92	4.42	17,950	Gout
16	7.8	113	6.37	9,950	Chronic emphysema. Bronchitis. Asthma. Polycythemia
21	5.5	94	5.20	17,600	Lobar pneumonia
22	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
27c	4.6	43	2.31	12,200	Hodgkin's disease
28	5.1	82	4.25	5,280	Neurofibromatosis (von Recklinghausen)
29	2.3	36	1.86	14,000	Chronic pneumonia. Hemolytic icterus
32	5.7	85	4.07	8,900	Pellagra
36	4.5	80	4.33	5,600	Neurosyphilis. Decompensated mitral valvulitis
37	2.2	16	1.35	10,200	Peptic ulcer, posthemorrhagic
40	5.3	90	4.75	6,200	Psychasthenia

TABLE I—*Continued*

Case number‡	Nucleotide nitrogen per 100 cc. whole blood	Hemoglobin	Red blood corpuscles	White blood cells	Diagnosis
	mgm.	*per cent*	*millions*		
			Male		
42	5.7	75	3.87	9,950	Pulmonary tuberculosis. Arteriosclerosis
44	5.7	90	5.12	5,100	Arteriosclerosis. Mitral valvulitis
45	6.4	80	4.40	8,160	Generalized arteriosclerosis. Emphysema
46	4.6	82	3.70	6,100	Rheumatic heart disease
51	4.7	90	4.93	9,450	Jacksonian epilepsy. Hypertension
52	6.1	90	5.59	8,800	Peptic ulcer. Neurasthenia
53	7.6	95	5.90	8,250	Pneumonia (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	5.2	90	6.80	9,800	Rheumatic heart disease. Cardiac failure
64b	5.8	106	8.50	11,200	Congenital heart disease
70	6.0	72	3.60	7,960	Luetic-hepato-splenomegaly. Morphinism
71	5.9	70	4.84	9,600	Hodgkin's disease
73	2.7	45	3.75	6,800	Chronic hypochromic anemia
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
79	5.1	76	4.64	6,400	Chronic arthritis
80	6.3	75	4.25	7,240	Congenital heart disease. Duodenal ulcer
85	4.5	85	4.53	6,040	Arteriosclerosis. Syphilis. Cholecystitis. Cholelithiasis
87	5.4	85	4.85	18,080	Psychasthenia. Peptic ulcer
88	5.7	82	5.25	9,800	Cardiac decompensation. Syphilis
90	5.1	90	4.90	7,100	Luetic aortitis
91	4.8	95	6.30	13,700	Infectious arthritis
92	7.1	48	2.57	9,400	Chronic nephritis. Plumbism Generalized arteriosclerosis
94	5.3	90	4.70	7,400	Mediastinal tumor
95	4.5	88	4.85	9,200	Arteriosclerotic heart disease
101	6.1	88	4.69	8,900	Arteriosclerotic heart disease
102	4.5	98	5.35	6,320	Syphilis
103	5.3	75	3.96	7,520	Luetic aneurysm
104	4.6	83	4.83	10,200	Angina pectoris. Coronary sclerosis. Bronchial asthma
105	9.5	130	6.18	16,450	Congenital heart disease
107	3.7	90	4.43	9,050	Psychasthenia
			Female		
10	4.7	84	4.47	9,480	Neurasthenia. Hyperventilation tetany
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	4.9	81	4.30	8,200	Questionable spinal cord tumor
41	5.7	100	5.10	6,400	Carcinoma of the ovary with ascites
59	4.9	88	5.01	7,810	Mitral heart disease. Paroxysmal tachycardia
60	3.9	80	4.53	7,120	Exophthalmic goiter
63	4.7	85	4.32	5,500	Arteriosclerotic heart disease
67	3.8	45	4.20	13,400	Rheumatic heart disease
68	5.6	85	5.08	9,200	Ovarian tumor. Blindness
78	5.3	75	4.95	6,100	Carcinoma of the head of the pancreas
83	4.3	90	4.90	8,800	Sinusitis
97	6.0	76	4.10	12,300	Adenocarcinoma of the kidney
98	5.2	76	4.49	6,640	Atrophic arthritis
99	5.4	88	4.50	7,000	Chronic nephritis with edema
100	6.1	84	4.30	8,850	Chronic arthritis

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 data given in Table I

	Number of cases	Number of specimens	Nucleotide nitrogen		Hemoglobin		Red blood corpuscles		White blood cells	
			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
(*a*) Classified according to sex										
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										
Anemia (4 male, 4 female)	8	21	3.2	1.0	51.7	17.8	2.39	1.13	5.7	2.2
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)

	Number of cases	Number of specimens	Correlations of nucleotide nitrogen with		
			Hemoglobin	Red blood corpuscles	White blood cells
I. Total number of analyses	102	124	*0.534*	*0.572*	*0.431*
(a) Classified according to sex					
Male	76	93	*0.447*	*0.502*	*0.523*
Female	26	31	*0.726*	*0.690*	0.214
(b) Classified according to disease					
Anemia	8	21	*0.858*	*0.796*	0.276
Leukopenia	16	22	*0.798*	*0.856*	0.112
Leukemia	13	18	*−0.757*	0.096	*0.770*
Others	69	72	*0.969*	*0.663*	−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 nucleo-

tide 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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