

Distribution of Histamine among Leukocytes and Platelets

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MOST OF THE HISTAMINE of human blood is found in the buffy coat.^{2,3} Earlier attempts to identify the histamine-carrying constituent of this leukocyte-platelet layer have been unsuccessful.⁴ Because human platelets, lymphocytes in lymph nodes, and monocytes from exudates have all been found to contain little histamine, and because blood histamine remains normal in pathological conditions in which these elements are abnormally abundant, histamine has been assigned, by exclusion, to the granulocytes. It has seemed unlikely that neutrophils are rich in histamine because in leukocytosis blood histamine is not greater than normal.⁵ Code stated in 1937 that "the eosinophil is in most cases a source in blood of the histamine-like activity," while in 1952 he wrote that "the histamine content of the blood may or may not be increased when eosinophils are present. Certainly eosinophils often do not contain histamine. Sometimes they may." The concept of eosinophils as facultative histamine carriers is also found in the somewhat later publication of Code and Mitchell.⁶

Because of the high blood level of histamine in chronic myelocytic leukemia, immature forms of granulocytes would be thought to contain large amounts of histamine if it were not for the low blood histamine accompanying even greater abundance of these forms in acute leukemia, for instance. Until very recently,^{1,7} the unique feature of the blood picture in chronic myelocytic leukemia, marked basophilia,⁸ has apparently been overlooked as a possible explanation of the equally unique histaminemia. Shimkin, Sapirstein, Goetzl, Wheeler and Berlin⁹ include basophils among the many leukocyte types whose count is not correlated with histamine level but note a rough parallelism between the total leukocyte count and blood histamine in chronic myelocytic leukemia. Valentine and Lawrence¹⁰ found no close correlation between the level of blood histamine and either total or differential leukocyte count, although examination of their figures shows that histamine content tends to increase with the number of basophils per cubic millimeter.

The present report records measurements of the histamine content of platelets and the various types of leukocytes in human blood, as well as estimates of the contribution of each white blood element to the whole blood histamine in normal individuals. The normal basophilic granulocyte was found to contain histamine at so much higher concentration than any other blood element that, in spite of its scarcity, it accounts for half of the histamine in normal blood. Specific gravity differences between types of leukocytes are also recorded.

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METHODS

1. *Segregation* of individual leukocyte types was attempted by centrifugation of plasma containing white cells at 3000 r.p.m., mean radius 20 cm., for an hour, in the hope of sedimenting the white cells in an orderly array determined by their specific gravity. This centrifugation was carried out at 12 C. in special siliconed tubes consisting of a wide upper portion of 18–25 mm. diameter (10–25 ml. capacity) and a lower, narrow thick-walled segment of 2 or 3 mm. bore and long enough to hold all the formed elements sedimented out of the suspension. After centrifugation the formed elements were removed by constriction pipets in as many distinct portions as desired, and the individual portions were weighed and analyzed for histamine. The buffy coat was isolated in good yield, but although successive portions of the centrifugate varied widely in cellular composition, platelets were the only white blood element obtained sufficiently pure by this technic to provide a satisfactory estimate of their histamine content.

More successful segregation was obtained in specific gravity gradient columns consisting of mixtures of concentrated albumen with plasma containing leukocytes. Prolonged centrifugation then distributed the leukocytes through the column according to their specific gravities.

The columns were prepared in the following way. Blood was diluted with one-tenth volume of isotonic potassium oxalate and the erythrocytes were allowed to settle at 12 C., leaving most of the leukocytes suspended in the plasma. To favor erythrocyte sedimentation, isotonic fibrinogen solution* was added.¹¹ All subsequent procedures until the leukocyte samples were ready for analysis were carried out at 12 C.

From concentrated salt-poor normal serum albumen (human),† a more concentrated, isotonic solution was prepared by evaporation at room temperature followed by dialysis. Water, sodium chloride or N/10 sodium hydroxide was added as needed to give a final solution of pH 7.0, specific gravity 1.085–1.09, and freezing point lowering 0.62–0.64 C. To portions of this isotonic albumen solution various volumes of the leukocyte suspension in plasma with extra fibrinogen were added to make four or five 2 ml. mixtures of specific gravity decreasing from 1.078 to 1.050. These were then cautiously introduced in order of decreasing specific gravity into a 12 to 15 ml. siliconed tube (18 cm. long with 1 cm. bore) which already contained 2 ml. of the albumen solution of specific gravity 1.09. Any excess suspension was added as a top layer and visible separation between all layers was obliterated by a few strokes of a spiral wire stirrer.¹² To secure a smooth, steep specific gravity gradient, excess stirring, especially of the upper, lighter layers was avoided.

The columns were centrifuged for 2 or 3 hours at 3000 r.p.m. at a mean radius of 20 cm. The centrifugation was terminated without braking, 0.5 ml. portions were removed, and each was sampled for (1) histamine analysis, (2) measurement of specific gravity,¹² and total and differential cell count. To obtain a compact mass of cells for smearing on a slide, a small sample from the gradient column was diluted with 0.9 per cent sodium chloride and centrifuged.

Although leukocytes are not in general completely segregated into individual types by this technic, the separation is sufficient to provide estimates of the histamine content and measurement of the specific gravity of the various cell types.

2. *Statistical analysis by multiple regression* offers a means of segregating the contributions of different elements in mixtures of widely differing composition.‡ This method was applied to two series of whole blood samples, one from normal adults, one from patients with chronic myelocytic leukemia. From the histamine content and total and differential

* Prepared from dried Lederle Fraction I (Lot No. 392C), kindly supplied by Dr. James McComb, Director of Biologic Laboratories, Dept. of Public Health, 375 South Street, Jamaica Plains Station, Boston 30, Massachusetts.

† The albumen was donated by the American National Red Cross from blood of volunteer donors.

‡ The advice of Mrs. Barbara Bartels Hixon, Professor George W. Snedecor, and Professor Harold Hotelling in this part of the investigation is gratefully acknowledged.

leukocyte count of each sample, calculations were made as outlined by Johnson,¹³ starting out with the full number of independent variables (types of leukocytes; 5 in the normal, 7 in the chronic myelocytic leukemia series), and eliminating those with partial regression coefficients found not significantly different from zero. In the calculations, segmented and band neutrophilic polymorphonuclear granulocytes were put into one group, myeloblasts into one group, and all other neutrophilic forms into the group labelled "myelocytes". All eosinophilic forms regardless of degree of maturity were put into the group of eosinophilic granulocytes, all basophilic forms into the group of basophilic granulocytes.

3. *Histamine measurements* were made by the chemical method already described.³ In brief, the method consists of elimination of most interfering substances in tissue extracts (and concentration of the histamine in the extracts when necessary) by adsorption on Decalco columns followed by formation of the basic mono-dinitrofluorobenzene derivative of histamine and extraction of this derivative into methyl-n-hexyl ketone and out again into strong hydrochloric acid; the optical density of this acid solution is then read at $\lambda 360 \text{ m}\mu$. The method reports as histamine interfering substances in red cells equivalent on the average to $24 \text{ }\mu\text{g. histamine per liter of blood}$;³ except when otherwise indicated, the histamine levels in *whole blood* given in this paper include this spurious histamine. The wet weight of the samples of buffy coat taken for analysis was usually 20–50 mg. Smaller samples were occasionally used when there was visual evidence of discontinuity in the packed cell mass. All buffy coat samples were diluted with at least an equal weight of water before precipitation with trichloroacetic acid. Samples from the gradient columns varied in size from 75 to $350 \text{ }\mu\text{l.}$ according to the histamine content expected. Samples of normal blood were about 1 ml., those of chronic myelocytic leukemia blood about 0.1 ml. Considerably more histamine than the $0.02 \text{ }\mu\text{g.}$ regarded as minimal for satisfactory measurement was usually present in the samples analyzed.

RESULTS

1. *Analysis of Segregated White Blood Elements*

a. Platelets. By differential centrifugation of blood from a case of polycythemia vera with over 9,000,000 platelets per cu. mm. (table 1), samples of platelets with various minor degrees of contamination by blood cells were obtained. In the best sample, elements other than platelets were rarely seen; certainly there was less than one leukocyte to 10,000 platelets. The histamine concentration of this sample was only $1280 \text{ }\mu\text{g. per liter}$ ($.009 \text{ }\mu\text{g. per } 10^9 \text{ platelets}$) as compared to $12,300 \text{ }\mu\text{g. per liter}$ in the entire buffy coat. Another sample with one white cell per 200 platelets had twice the histamine concentration of the purer sample.

Almost exactly the same value for platelets, $1330 \text{ }\mu\text{g. per liter}$, was obtained in a similar fashion from a normal blood sample. This may be compared with the value for histamine in the average normal buffy coat, $8000 \text{ }\mu\text{g. per liter.}^*$ Platelet histamine, therefore, accounts for not more than a few per cent of normal blood histamine (table 3).

b. Leukocytes. From a 50 ml. portion of oxalated normal blood centrifuged undiluted, it was usually possible to obtain only one leukocyte sample reasonably free of red cells and platelets. Because of the difficulty in obtaining uniform smears, the differential counts from these packed samples were not satisfactory for counting basophils, which were numerically few, but which were suspected of containing much of the histamine.

Blood from patients with chronic myelocytic leukemia proved somewhat more

* Since it is difficult to collect samples of the buffy coat without contamination with minute fibrin clots, this directly measured value for whole buffy coat is lower than that which might be calculated from the sum of its parts.

TABLE 1.—*Histamine and Number of Leukocytes and Platelets per cu. mm. of Blood*

	Hista- mine 10 ⁻⁶ μg	Leukocytes								Platelets
		Total	Neutro- phils	Myelo- cytes	Myelo- blasts	Baso- phils	Eosino- phils	Lympho- cytes	Mono- cytes	
Normal										
Example 1	49	5,500	3,250	0	0	6	55	1,635	550	—
Example 2	101	7,150	4,127	0	0	43	200	2,278	502	—
Example 3	147	5,000	3,040	0	0	30	85	1,495	350	—
Example 4	222	10,400	6,800	0	0	104	198	2,674	624	—
Mean, 25 samples	118	7,000	4,150	0	0	44	165	2,185	456	—
St. dev.	53	2,100	1,719	0	0	34	116	578	195	—
Leukemia										
Chr. myelocytic										
Example 1	12,180	150,300	66,150	51,100	1,503	22,550	4,510	3,006	1,503	750,000
Example 2*	175	5,600	4,985	112	0	168	56	0	112	556,000
Example 3	6,540	79,000	47,400	18,190	0	5,530	2,370	5,530	0	2,360,000
Mean, 13 samples	5,285	66,450	34,108	19,757	256	2,002	7,870	1,945	844	—
St. dev.	4,192	51,580	27,198	20,094	448	8,312	1,716	1,833	935	—
Chr. lymphocytic	124	166,500	833	0	0	0	0	165,000	833	241,800
Polyeythemia vera										
1	122	8,900	5,300	1,024	0	42	934	1,185	418	—
2	443	22,450	20,900	0	0	0	1,122	0	449	1,500,000
3	457	18,900	17,020	0	0	944	0	756	189	—
4	594	24,500	18,000	0	0	2,025	226	2,500	1,790	9,052,000
5	687	22,550	18,080	1,128	0	1,354	677	1,128	226	1,550,000
Leukemoid reaction	85	12,100	10,280	0	0	0	605	485	726	241,800
Eosinophilia										
Trichinosis	111	26,450	3,965	0	0	0	19,310	2,910	264	—
Lymphosarcoma	164	31,000	11,180	0	0	0	15,200	4,030	620	1,000,000
Anemia										
Acute aplastic	249	13,050	6,910	1,435	0	2,218†	0	1,565	912	—
Macrocytic	54	3,400	136	0	0	238	34	2,241	748	17,000
Macrocytic‡	76	3,350	168	0	0	168	670	1,720	640	—
Refractory	74	8,300	5,520	0	0	42	1,163	954	622	240,000

* Sample from same patient as preceding sample, 6 weeks later, following treatment with X-ray and P³².

† The granules in these cells were doubtfully basophilic.

‡ Sample from same patient as preceding sample, 6 months later.

satisfactory. As many as twenty samples of leukocytes were obtained from 50 ml. of this abnormal blood, and these differed widely in histamine content and cellular composition. Scatter diagrams of the individual series of samples from leukemia blood (a total of 81 leukocyte samples from 7 patients with chronic myelocytic leukemia) plus eight samples from seven normal bloods showed that the concentration of histamine bears no relation to the frequency of monocytes, decreases with increasing frequency of lymphocytes, and increases with increasing frequency of neutrophilic and basophilic granulocytes. There was questionable positive correlation with eosinophils. The results of these experiments are not presented in detail because more convincing evidence of the relationship of histamine concentration to leukocyte type was secured by the use of centrifuged specific gravity gradient columns and by statistical analysis.

The centrifuged specific gravity gradient columns contained one or more

cloudy zones in which the leukocytes were concentrated and to some extent segregated into types. Lymphocytes from a case of chronic lymphocytic leukemia (table 1) were the only type separated completely from other types of leukocytes (one or two granulocytes or monocytes to 10,000 lymphocytes). These purest lymphocytes were rather densely localized (200,000 per cu. mm.) in the zone of the column with specific gravity 1.062 to 1.064. Histamine was found there at the low level of 0.6 $\mu\text{g.}$ per 10^9 lymphocytes as compared to 17 $\mu\text{g.}$ per 10^9 leukocytes of all types in the average buffy coat (table 2). Samples of lymphocytes contaminated with a few other leukocytes (less than 5 per cent) were obtained from other zones of this column and their histamine contents were 2–4 $\mu\text{g.}$ per 10^9 cells.

Lymphocyte samples of this degree of purity obtained from normal blood were likewise found to contain 2–4 $\mu\text{g.}$ histamine per 10^9 cells. Normal lymphocytes may therefore be as low in histamine as leukemia lymphocytes. The histamine content of lymphocytes is then about twice, and their contribution to normal blood histamine about half, that of platelets (table 3).

When samples of normal blood were centrifuged in gradient columns, too few basophils and eosinophils were observed in smears prepared from the layers of the columns to permit estimation of the relative histamine concentration in the three types of granulocytes. In gradient columns prepared from chronic myelocytic leukemia blood, the histamine appeared to be largely determined by the number of basophils (fig. 1). The peak frequency of the basophils occurred at specific gravity 1.070, coincident with the lighter of the two portions containing peak histamine concentration. The histamine found outside the specific gravity

TABLE 2.—*Histamine Content of Leukocytes in Normal and Chronic Myelocytic Leukemia Blood*

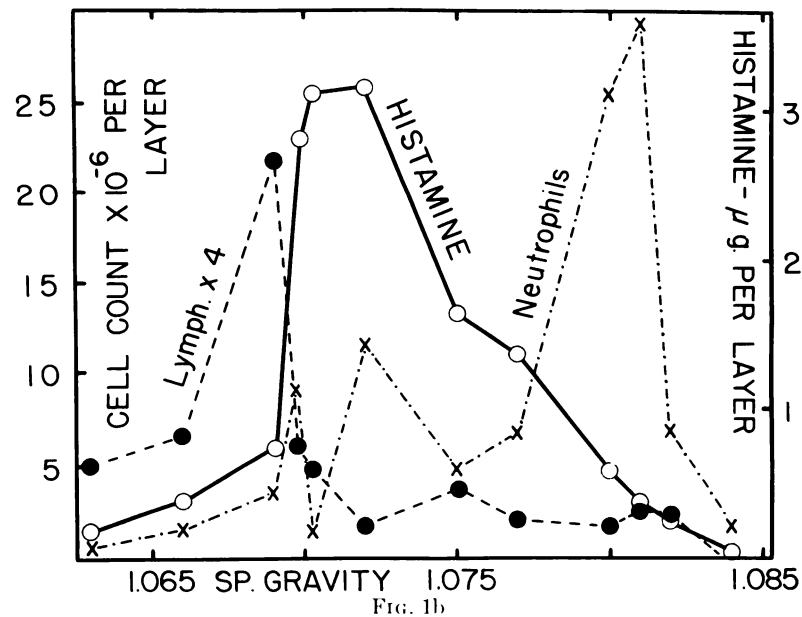
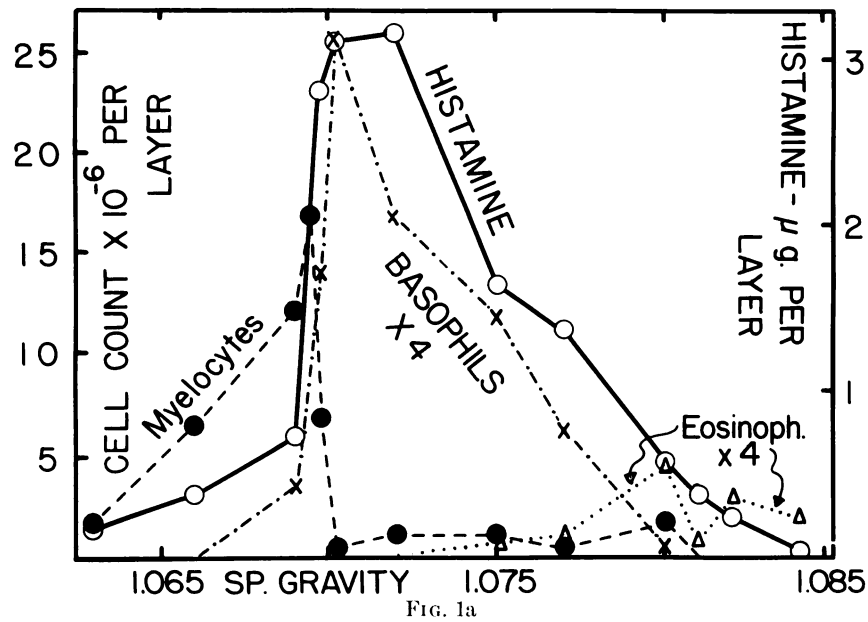
Histamine, $\mu\text{g.}$ per 10^9 cells, calculated by multiple regression from (A) 25 samples of blood from 21 normal adults, and (B) 13 samples of blood from 7 patients with chronic myelocytic leukemia.

	A. Normal		B. Chronic myelocytic leukemia	
	5 independent variables	Variables with significant partial regression coefficients	7 independent variables	Variables with significant partial regression coefficients
Leukocytes, total.....	17*	—	78*	—
Granulocytes				
Neutrophilic.....	7.3(5.3)†	3.1(4.8)‡	143(100)	69(26)
Basophilic.....	990(248)	1026(236)	367(240)	305(84)
Eosinophilic.....	96.0(81)	153(66)	271(966)	—
Myelocytes (neutral).....	—	—	—99(120)	—
Myeloblasts.....	—	—	763(4276)	—
Lymphocytes.....	18.7(16.3)	—	164(814)	—
Monocytes.....	—58.5(44.8)	—	—1527(1813)	—
Histamine not allocated, $\mu\text{g./L.}$	14.7(36)	34(20)	—296(1403)	535(1097)

* $10^9 \times$ mean histamine level divided by mean total leukocyte count.

† The figures in parentheses are standard errors.

‡ See text for explanation of retention of this coefficient here.



FIGS. 1a and 1b.—Amounts of histamine and numbers of leukocytes in successive 0.5 ml. layers of a specific gravity gradient column. Column prepared from a leukocyte suspension derived from 2.46 ml. chronic myelocytic leukemia blood which contained 26.2 μg . histamine and 230×10^6 leukocytes classified as follows: neutrophilic polymorphonuclear granulocytes 130, neutrophilic myelocytes 46, myeloblasts 1, basophils 31, eosinophils 9, lymphocytes 7, monocytes 6, all $\times 10^6$. Histamine recovered from column including 3.7 μg . in clotted cell mass at bottom of column, 18.2 μg . (69 per cent); leukocytes recovered, 189×10^6 (82 per cent). Monocytes and myeloblasts are not plotted because of their rare appearance in the gradient column. Note that numbers of lymphocytes, basophils and eosinophils have been multiplied by four for plotting.

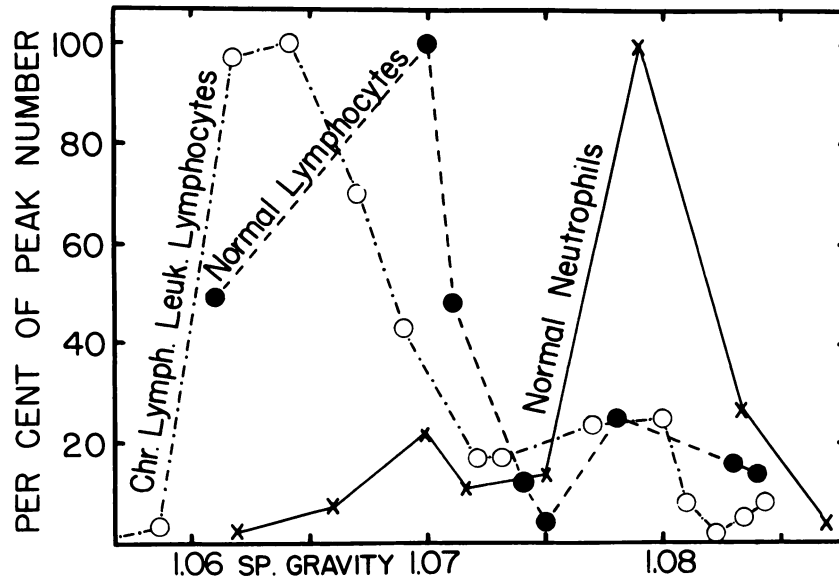


FIG. 2.—Lymphocytes and neutrophilic granulocytes in successive 0.5 ml. layers of specific gravity gradient columns, plotted as per cent of the maximum number found. Normal lymphocytes and neutrophils were obtained from 10 ml. normal blood which contained 100×10^6 leukocytes, of which 72 per cent were neutrophils and 19 per cent were lymphocytes. Leukemia lymphocytes were obtained from 2.9 ml. chronic lymphocytic leukemia blood which contained 480×10^6 leukocytes, of which 99 per cent were lymphocytes.

range of basophils may be ascribed to eosinophils and neutrophils in the heavier layers; the histamine in the lighter layers seems too great to be explained by the neutrophils present unless these lighter neutrophils were especially rich in histamine. The results of multiple regression analysis (table 2) suggest that the myelocytes cannot account for the histamine present in these layers.

In figure 1, the peak concentration of myelocytes falls close to that of basophils, near specific gravity 1.07, that of eosinophils is close to that of neutrophils, 1.08 or higher. The neutrophils in the normal blood used for figure 2 reach their peak concentration at essentially the same specific gravity (1.079). The specific gravity distribution of lymphocytes is also closely similar in normal and chronic myelocytic leukemia blood (peak at 1.07), but it appears from figure 2 that in chronic lymphocytic leukemia the lymphocytes are lighter (peak at specific gravity 1.063).

2. Relationship between Amounts of Histamine and Numbers of Leukocytes in Whole Blood

The above observations suggest that the basophilic granulocytes are the leukocytes richest in histamine, at least in chronic myelocytic leukemia. A scatter diagram of whole blood histamine vs. basophil count in normal and chronic myelocytic leukemia blood (fig. 3) indicates moreover that in normal blood the basophils are actually richer in histamine than are the basophils in chronic myelocytic leukemia blood. Lines drawn by eye through the two sets of points in the figure suggest a ratio of 3 or 4 in favor of the normal basophil.

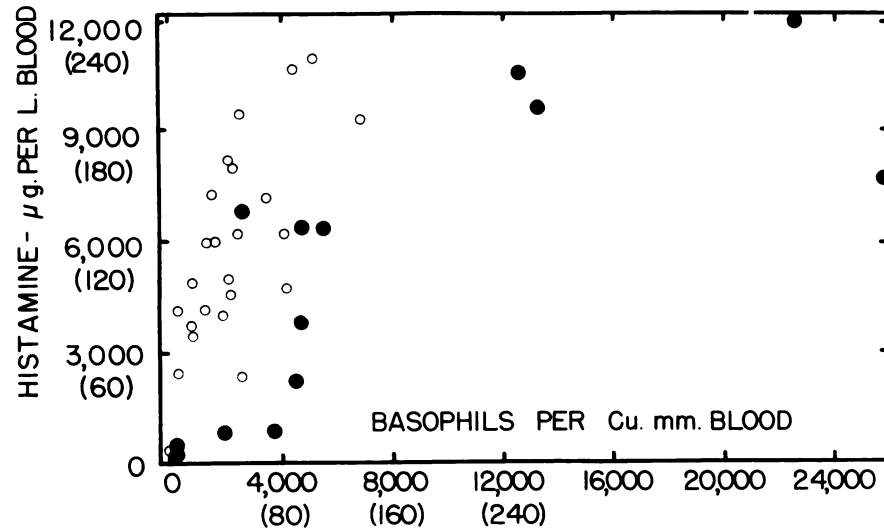


FIG. 3.—Basophil count and histamine content of whole blood. Figures in parentheses on both axes apply to normal blood samples (○); other figures (1/50 of scale for normals) apply to blood samples from patients with chronic myelocytic leukemia (●).

In order to obtain more quantitative information regarding the amount of histamine in basophils as well as in other types of granulocytes, analysis by multiple regression was applied to the series of samples of normal blood and of blood from chronic myelocytic leukemia patients summarized in table 1. A preliminary complete analysis by multiple regression gave partial regression coefficients (measures of the amount of histamine per cell) for lymphocytes and monocytes with a 20 to 85 per cent probability of not differing significantly from zero in both series (table 2). The coefficients for myelocytes and myeloblasts in the leukemia series were equally unreliable. Since there is independent evidence that none of these four types of cells carries much histamine, they were eliminated from the regressions by conventional statistical procedures.

The partial regression coefficients for neutrophils is not significant in the normal series but has been retained in the table because it suffices to show that the histamine content of these granulocytes is very much less in normal than in chronic myelocytic leukemia blood.

In normal blood, eosinophils rank next below basophils in histamine content; the absence of correlation between the eosinophil and basophil counts strengthens the conclusion that both cell types are rich in histamine. The coefficient for eosinophils is not significant in the chronic myelocytic leukemia series and furnishes no helpful clue as to the relative histamine content of eosinophils in the two kinds of blood. For basophils, the values of the coefficient are significant in both series and differ by a factor of three, but even so a significant difference between the two values cannot be demonstrated statistically.

Values for the histamine content of the three types of granulocytes differing considerable from those in table 2 were obtained in preliminary statistical analysis of the normal blood sample data.^{1, 3} These earlier values were calculated from differential counts on 200 leukocytes; in order to obtain better estimates of the

frequency of the important but normally relatively scarce basophilic and eosinophilic granulocytes, their counts among 1000 leukocytes were used for the calculations on normal blood in the present report. In the earlier calculations as in those leading to table 2 here, basophils were found to be the leukocyte type much the richest in histamine. The coefficient calculated for eosinophils from the differential count of 200 leukocytes was not significant and neutrophils ranked next to basophils in histamine content. The extent of the change brought about by the more extensive enumeration emphasizes the difficulty of obtaining accurate measures of the frequency of basophils and eosinophils from the examination of smears.

After the elimination of all non-significant partial regression coefficients from the two regression equations of table 2, the equations become: (1) for normal blood,

$$\begin{aligned} \mu\text{g. histamine per liter blood} = & 1076 \times 10^{-9} \times \text{no. basophils per cu. mm.} \times 10^6 \\ & + (162 \times 10^{-9} \times \text{no. eosinophils per cu mm.} \times 10^6) + 43, \end{aligned}$$

and (2) for chronic myelocytic leukemia blood,

$$\begin{aligned} \mu\text{g. histamine per liter blood} = & 305 \times 10^{-9} \times \text{no. basophils per cu. mm.} \times 10^6 \\ & + (69 \times 10^{-9} \times \text{no. neutrophils per cu. mm.} \times 10^6) + 535. \end{aligned}$$

The standard error of estimate of the equation for normal blood is 42; therefore, for normal blood containing, for example, 50 basophils and 150 eosinophils per cu. mm., the expected histamine, $1076 \times 10^{-9} \times 50 \times 10^6 + (162 \times 10^{-9} \times 150 \times 10^6) + 43 = 121 \mu\text{g.}$ per liter would be within 42 $\mu\text{g.}$ of the actual amount in two-thirds of the cases. Similarly, in blood from a chronic myelocytic leukemia patient with 60,000 neutrophils and 10,000 basophils per cu. mm., the expected histamine, $69 \times 10^{-9} \times 60,000 \times 10^6 + (305 \times 10^{-9} \times 10,000 \times 10^6) + 535 = 7725 \mu\text{g.}$ per liter, in two-thirds of the cases would be within the standard error for this equation, 2276 $\mu\text{g.}$ per liter, of the actual value.

The constants in the equation, 43 and 535, represent histamine from other sources than the specified cell types. Although the constant for the chronic myelocytic leukemia series is larger than that for the normal series, it is smaller relative to the amount of histamine present, and it is not statistically significant. There is probably no increase in non-leukocyte histamine in chronic myelocytic leukemia. With careful handling, plasma from patients with this disease contains little more histamine than does normal plasma.¹⁴ Determinations in this laboratory confirm this and indicate that in this disease erythrocyte "histamine" is also not much greater than normal.

3. Histamine in various abnormal bloods

Observations made on diseases with abnormal blood pictures other than leukemia are included in table 1.

a. *Polycythemia.* Valentine, Pearce and Lawrence⁵ report an average blood histamine level in polycythemia about 3 times normal. An increase in blood histamine is confirmed in the present study, and accounted for by the high numbers of basophils and eosinophils found. It appears, however, that in this condi-

tion the basophils and possibly the eosinophils contain somewhat less than the normal amounts of histamine.

b. Leukemoid reaction. The normal blood histamine reported by Valentine et al. for subjects with leukemoid reaction has been confirmed by one case in the present series. No basophils were found in this blood.

c. Eosinophilia. In two blood samples showing eosinophilia, the blood histamine levels were average and high normal values. The high normal accompanied a large number of neutrophilic granulocytes. No basophils were observed in either blood. The eosinophils in these cases could not have contained more than $\frac{1}{10}$ to $\frac{1}{20}$ the normal amount of histamine.

d. Anemia. In the four blood samples from patients with anemia, the histamine blood level is lower than would be predicted from the numbers of granulocytes present. Certainly in the case of aplastic anemia and probably in the others, the "basophils" were relatively poor in histamine. The eosinophils may also have contained subnormal amounts of histamine.

The findings in these bloods bear out the statement that blood histamine is high when the basophil count is high.

DISCUSSION

On the basis of the observations presented, it is concluded that histamine is distributed among normal blood elements as shown in table 3. The estimates of the histamine in neutrophils and monocytes is little more than a guess. It was obtained in the following way. From the mean value (43 μg . per liter of blood) of the constant in the normal regression equation (histamine from other sources than basophils and eosinophils), have been subtracted the amounts of histamine contributed to a liter of blood by plasma (1.4 μg .³), by platelets (2.5 μg ., table

TABLE 3.—*Distribution of Histamine among Normal Blood Elements*

The figures in parentheses are calculated on the assumption that the concentration of histamine in monocytes is the same as that in lymphocytes (see text). The spurious histamine of erythrocytes is omitted from this table.*

	Histamine Concentration in Normal Blood Elements			Distribution of Normal Blood Histamine		
	Histamine per 10 ⁹ cells or platelets μg .	Cell or platelet volume μ^3	Histamine per liter μg .	Cells or platelets per cu. mm. blood number	Histamine per liter blood μg .	Fraction of whole blood histamine %
Granulocytes						
Neutrophilic.....	(3.3)	450†	(7,000)	4,200	(13.5)	14
Basophilic.....	1080	450†	2,400,000	44	47.0	51
Eosinophilic.....	160	450†	360,000	165	26.7	29
Lymphocytes.....	0.6	230†	2,600	2,200	1.3	1
Monocytes.....	(1.2)	470†	(2,600)	450	(0.5)	0.5
Platelets.....	.009	8‡	1,280	250,000§	2.5	3
Plasma.....	—	—	2.6*	—	1.4	1.5

* Lowry et al.³

† Tivey, Li and Osgood's¹⁵ median volume of neutrophils assigned here to basophils and eosinophils also; median volume of lymphocytes; mean volume of monocytes.

‡ Wintrobe,¹⁶ p. 233.

§ Wintrobe,¹⁶ p. 241.

3), by lymphocytes (1.3 $\mu\text{g.}$, table 3), and by erythrocytes (24 $\mu\text{g.}$ spurious histamine³). This leaves 14 $\mu\text{g.}$ per liter blood to the neutrophils and monocytes. From the volumes adopted for these two types in table 3 and from their average counts, it is calculated that the neutrophils in a liter of blood occupy about 1.9 ml. and the monocytes about 0.21 ml. The monocyte volume is so small that the concentration of histamine assigned to it makes relatively little difference in the over-all picture; the figures in parenthesis in table 3 are based on the assumption that histamine is at the same concentration in monocytes as in lymphocytes. This leaves 13.5 $\mu\text{g.}$ histamine for the neutrophils which are accordingly entered in the table as containing 3.3 $\mu\text{g.}$ histamine per 10^9 cells at a concentration about three times that in lymphocytes.

In respect to increasing histamine concentration, the order of the buffy coat elements of normal blood is platelets, lymphocytes, neutrophils, eosinophils and basophils. The basophils contain histamine at a concentration 20,000 times that of platelets and a million times that of plasma. Half of the normal blood histamine is located in basophils, about a third in eosinophils, and probably most of the remaining sixth in neutrophils. In chronic myelocytic leukemia, depending on the differential count, from 10 to 80 per cent of the blood histamine may be present in the basophils.

How are the intracellular concentrations of histamine achieved? For the basophils it may be assumed that their granules are somehow associated with the presence of unusually large amounts of histamine. This assumption follows naturally from the concept that the histamine of mast cells^{17, 18} is present in the metachromatic granules of these cells. This concept in turn is based on the hypothesis of combination between the base histamine and the acid heparin, heparin being thought of as fixed in the granules.^{19, 20} That is, heparin is the basophilic stuff and histamine is the base which it "loves." The presence of large amounts of histamine in basophilic granulocytes led to finding even larger amounts in mast cells,^{1, 18} and conversely the presence of heparin in mast cells led to evidence in favor of an anticoagulant of similar chemical structure in basophilic leukocytes.^{21, 22} Although there are a number of differences between these two types of cell,²³ they may well be qualitatively similar in regard to the mechanism by which they retain extraordinary amounts of histamine. It remains to be seen whether the not inconsiderable amounts of histamine in eosinophils (and neutrophils) are held by heparin-like substances or through some other binding mechanism.

The findings presented suggest, but do not prove, that in chronic myelocytic leukemia the basophils are poorer and the eosinophils and neutrophils richer than normal in histamine. This would be reasonable since in this disease (1) the basophils are smaller and contain fewer granules than normal,⁸ (2) basophilic granules are not infrequently reported in eosinophils^{24, 25} and (3) neutrophilic myelocytes containing "granules which are quite basophilic" occur in the blood. Between these and true neutrophilic granulocytes, "all transitions" are to be found. The basophilic-neutrophilic myelocytes are distinguished from basophilic myelocytes chiefly in size.⁸ Although the average neutrophil in chronic myelocytic leukemia contains more histamine than normal, it appears from figure 2, that some of the neutrophils are little richer than normal. (For example, if all the histamine in the

zone of specific gravity 1.081 is assigned to the neutrophils of that zone, there would be 12 $\mu\text{g.}$ per 10^9 cells.)

The observations included in table 1 on blood samples from other diseases than chronic myelocytic leukemia suggest that in these diseases the histamine content not only of basophils but of eosinophils and possibly of neutrophils is lower than normal. It now seems possible that not only eosinophils but all types of granulocytes may contain quite different concentrations of histamine under different conditions.

From the observations of Valentine et al.⁵ it is clear that in leukocytosis the neutrophil may contain less histamine than the 3 $\mu\text{g.}$ per 10^9 cells reported here for normal blood. In severe infections as little as 0.3 $\mu\text{g.}$ per 10^9 myeloid cells was found by the earlier workers. Possibly infection or fever causes release of histamine or calls forth neutrophils containing subnormal amounts of histamine.

The function of histamine in leukocytes also remains a matter for speculation. The location of many mast cells along blood vessels led to their being considered as a source of heparin for the circulation,¹⁹ and more recently as a source of histamine also.²⁶ The store of anticoagulant and of histamine in blood cells is so much smaller than that in mast cells that the leukocyte storehouse seems superfluous when the supplies from mast cells are readily available. However, the distribution of mast cells is exceedingly erratic, possibly governed by the connective tissue function that has been suggested for them.²⁷ It may be that the histamine-bearing leukocytes constitute a mobile source of a limited amount of histamine (and/or anticoagulant material) immediately available wherever needed to tide over any lag period before the arrival of more adequate supplies from a nearby, fixed source.

Little quantitative information on the amount of histamine in specific types of leukocytes has been published. Valentine et al.⁵ reported 25 $\mu\text{g.}$ histamine per 10^9 myeloid cells in the blood of normal, non-fasting adults; this value is based on the assumption that all blood histamine is contained in myeloid cells. The corresponding amount calculated from our normal series is 27 $\mu\text{g.}$ Humphrey and Jaques²⁸ recently estimated the histamine content of the whole buffy coat to be 15 $\mu\text{g.}$ per 10^9 leukocytes, in agreement with the value of 13 $\mu\text{g.}$ per 10^9 mixed leukocytes obtained after correction for erythrocyte "histamine" in the present report. Humphrey and Jaques conclude however that platelets because of their number are the predominant contributors to blood histamine. If our platelet analyses are correct—and they agree with the lowest value of Humphrey and Jaques—this cannot be true for human blood. Higher values for platelet histamine in some of their and our results appear to be due to admixture of a few leukocytes. One average leukocyte contains as much histamine as 1000 platelets.

Except for platelets (sp. gr. 1.03²⁹) quantitative estimates of the specific gravity of individual white blood elements are also lacking in the literature, although qualitative, comparative information compatible with the data presented here is available.^{30, 31} The absolute specific gravities are of course affected by tonicity and possibly other environmental factors, and the few observations presented do not define the normal and pathological ranges.

SUMMARY

1. Platelets and lymphocytes free of other white elements of blood have been analysed for histamine by a microchemical method. Platelets were found to contain 1280 μg . per liter or .009 μg . per 10^9 platelets, lymphocytes 2600 μg . per liter, or 0.6 μg . per 10^9 cells.

2. The histamine content of neutrophilic, eosinophilic and basophilic granulocytes has been calculated by multiple regression from histamine measurements on one series of blood samples from normal adults and another from subjects with chronic myelocytic leukemia. The normal values are 1080 μg . per 10^9 basophils and 160 μg . per 10^9 eosinophils or 2,400,000 and 360,000 μg . per liter respectively. The corresponding values for neutrophils are 3 μg . per 10^9 cells and 7000 μg . per liter. The values for the histamine concentration per cell are statistically significant for basophils and eosinophils, but not for neutrophils.

In the leukemia series, the values for neutrophils and basophils are respectively 69 and 305 μg . per 10^9 cells, and they are statistically significant. The difference from normal in the value for basophils was not statistically validated. There are, however, in chronic myelocytic leukemia, histological changes from the normal that are consonant with a decreased histamine content in basophils and an increased histamine content in eosinophils and neutrophils.

3. Although definite values for the histamine content of monocytes and the immature forms seen in chronic myelocytic leukemia have not been obtained, there is reason to believe that the histamine of these cell types does not exceed that of neutrophils.

4. By centrifuging white cells in a specific gravity gradient column, partial segregation of cell types was obtained. Peak concentrations were observed at the following specific gravities: lymphocytic leukemia lymphocytes, 1.063; myelocytes, normal lymphocytes and basophils, all near 1.070; neutrophils, 1.080, and eosinophils, 1.080 or higher. Although individual concentration peaks were usually quite sharp, there was considerable overlapping at intermediate specific gravities.

5. About half of the histamine of normal blood is in the basophils, one-third in the eosinophils and the remaining one-sixth in all the other blood elements combined. In blood samples from patients with chronic myelocytic leukemia, the fraction of blood histamine carried by each type of granulocyte varies widely.

SUMMARIO IN INTERLINGUA

1. Plachettas e lymphocytos libere de altere leucoelementos del sanguine esseva analysate microchimicamente in re lor contento histaminic. Esseva constatate 1280 μg histamina per litro plachettas e 2600 μg histamina per litro lymphocytos. Isto es a dicer que 10^9 plachettas contineva 0,009 μg histamina e 10^9 lymphocytos contineva 0,6 μg histamina.

2. Le contento histaminic de granulocytos neutrophile, eosinophile, e basophile esseva calculate per multiple regression super le base de mesurationes del histamina in un serie de specimens de sanguine ab adultos normal e in un altere serie ab individuos con chronic leucemia myelocytic. Le valores in le serie normal es 1080 μg per 10^9 basophilos e 160 μg per 10^9 eosinophilos, i.e.

2.400.000 μg histamina per litro eosinophilos e 360.000 μg histamina per litro basophilos. Le valores correspondente pro neutrophilos es 3 μg per 10^9 cellulas e 7.000 μg per litro. Le valores obtenite pro le concentration de histamina in le cellula individual es statisticamente significative in le caso de basophilos e eosinophilos sed non in le caso de neutrophilos.

In le serie de pacientes de leucemia, le valores pro neutrophilos e basophilos es 69 e 305 μg histamina per 10^9 cellulas. Ambes es statisticamente significative. In le caso del basophilos le deviation ab le norma non esseva statisticamente validate. Nonobstante, in chronic leucemia myelocytic il occorre cambiamentos histologic que esserea de accordo con un decrescentia del contento de histamina in le basophilos e un accrescentia del contento de histamina in le eosinophilos e le neutrophilos.

3. Ben que nos non ha obtenite definitive valores pro le contento histaminic del monocytos e del formas immatur que se observa in chronic leucemia myelocytic, il ha rationes pro concluder que le contento histaminic de omne iste typos cellular non excede le contento histaminic del neutrophilos.

4. Le segregation partial del varie typos cellular esseva obtenite per centrifugar cellulas blanc in un columna a gradiente de gravitates specific. Concentrationes maximal esseva constatate in connexion con le sequente gravitates specific: lymphocytos de leucemia lymphocytic, 1,063; myelocytos, normal lymphocytos e basophilos, omnes vicin a 1,070; neutrophilos, 1,080; e eosinophilos, 1,080 o plus. Ben que le varie maximos de concentration esseva generalmente multo clar, il habeva un considerable interfusion in le areas del gravitates intermediari.

5. Circa un medietate del histamina de sanguine normal es in le basophilos, un tertio es in le eosinophilos, e le remanente sexto es distribuite inter omne le altere elementos sanguinee. In specimens de sanguine ab pacientes de chronic leucemia myelocytic, il ha grande differentias inter le portiones del histamina sanguinee que es portate per le varie typos de granulocyto.

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