The Absolute Basophil Count and Blood Histamine Levels

Arthur Sohler, Ph.D.[1], and Carl Pfeiffer, Ph.D., M.D.[1]

Abstract

An accurate basophil count can be used as an estimate of blood histamine levels. Basophils deteriorate fairly rapidly in drawn blood. A modification involving the addition of counting fluid immediately on drawing the blood is described which stabilizes the basophil count for a period of four days. Using this procedure for basophil counts we obtained a correlation with histamine levels of 0.864. **Introduction**

Pfeiffer (1972), has suggested that the absolute basophil count may be a useful means of evaluating blood histamine levels. He found a correlation coefficient between histamine and the absolute basophil count of 0.75 for males and 0.87 for females. Therefore, the absolute basophil count might be useful in estimating blood histamine levels in situations where the facilities for measuring histamine directly are lacking.

The absolute basophil count is not without certain inherent difficulties which may make the count unreliable and inaccurate. These difficulties are primarily due to the sparsity of the cell population and to the instability of these cells. We have discovered that often the poor correlation found between the absolute basophil count and histamine levels is due to inaccurate basophil counts as a result of deterioration of the cells on standing. The present study shows that stable basophil preparations can be obtained if a preservative solution is added immediately to the blood. Basophils are then stable for at least four days for counting and the counts of such preserved cells and counts on fresh blood correlate well with blood histamine levels.

Methods

Basophils were counted using the procedure of Gilbert and Ornstein (1975). A Fuchs-Rosenthal chamber was used for the counts. The Gilbert and Ornstein procedure was modified in that the Alcian Blue dye solution was added to the blood immediately after it was drawn. This blood preparation resulted in stable basophil counts over a four day period. Comparative absolute basophil counts were carried out by a commercial laboratory (Met Path), which used the Technician Hemalog D flow through system for counts.

Blood histamine was determined by the method of Iliev, Nichols, and Pfeiffer (1967).

1. Princeton Brain Bio Center
862 Route 518 Skillman, New Jersey 08558.

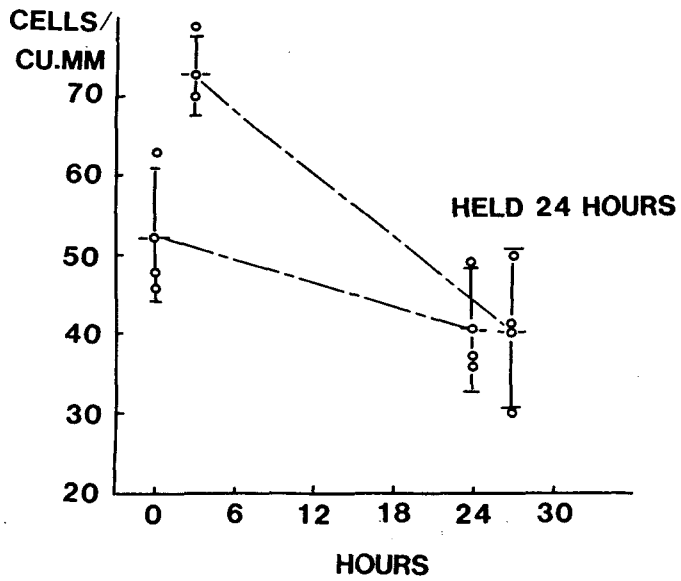

Figure 1. The decay and poor replication of the basophil count of a blood sample sent to a commercial laboratory is illustrated. The amount of time the blood sample sits before being processed markedly affects the basophil count.

Results

Figure 1 presents basophil count results on two identical blood samples from the same individual. One sample was drawn at 9:30 am while the second was drawn at 12:30 pm. The blood samples were divided into aliquots and triplicate samples were sent to the commercial laboratory for absolute basophil counting. The results obtained were not satisfactory in that replication was poor and a marked decrease in counts was noted in samples that had been stored prior to counting. On the basis of this result it was felt the poor correlation between blood histamine levels and the absolute basophil count done by the commercial laboratory might be due to cell deterioration. From subsequent results it became obvious that the storage time between sampling and counting was critical and some measures were needed to stabilize the basophils. The Alcian Blue dye solution contains N-cetylpyridinium chloride which preserves basophil granules and hemolyzes the erythrocytes. Addition of this solution to freshly drawn blood results in a stable basophil preparation. Table 1 lists the basophil count obtained on a sample prepared in this manner compared to a blood sample stored in an EDTA vacutainer tube where the dye solution was added just prior to counting. Storage at room temperature and at 4°C was compared. Storage temperature had little effect on stability. The immediate addition of dye solution to the blood, however, resulted in a preparation which gave essentially reproducible counts over a period of four days.

Using the modified procedure we compared our basophil counts with our blood histamine levels. We also compared these counts with counts obtained by sending the same blood to the commercial laboratory. Figure 2 is a scatter plot of our histamine and basophil data on thirty individuals. These data are compared to the basophil counts obtained by the commercial laboratory. Our data show less scatter and good correlation between the blood histamine levels and our basophil counts. The correlation coefficient was 0.864 when these data were compared. A comparison of the basophil values of the commercial laboratory with our blood histamine levels gave greater scatter and the correlation coefficient was less, namely 0.553.

The respective coefficients of determination were 0.756 and 0.306 indicating that 75 percent of the variation can be accounted for by the correlation when the proposed method is used in contrast to only 30

Table 1

Stabilization of Basophils by Storage in Alcian Blue Dye Solution

Day	Time	Alcian Blue Dye Solution Added Immediately		Blood Stored EDTA Tube Dye Added Just Prior to Count	
		Room Temp.	4°C	Room Temp.	4°C
DAY 1	10 AM	38*	38	38	38
	12 Noon	35	38	31	34
	2 PM	25	35	31	34
DAY 2	9 AM	35	40	26	26
	2 PM	40	38	30	30
DAY 3	9 AM	38	39	20	30
	2 PM	40	37	25	25
DAY 4	9 AM	36	40	25	25
	2 PM	40	39	30	26
	n = 9				
	$\bar{x}$	36.3	38.2	26.2	27.5
	s	± 4.7	± 1.5	± 9.4	± 9.2

*cells/mm3

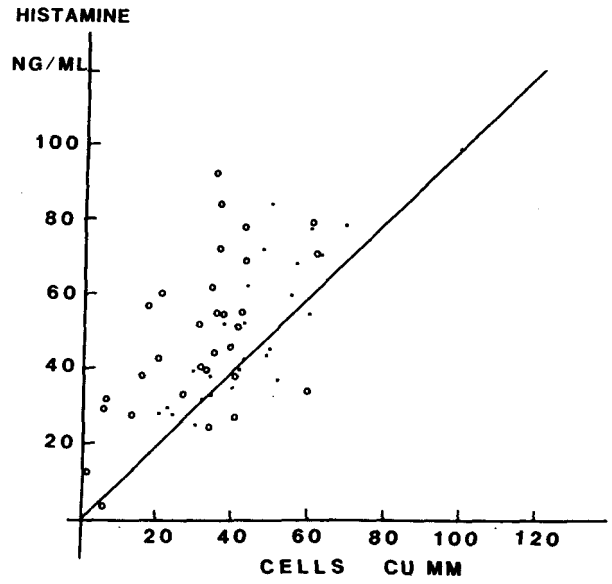

Figure 2. The correlation between the blood histamine level and the absolute basophil count done by our procedure gives an r = 0.864 with a t value of 9.086. For blood histamine compared to the commercial laboratory count the r = 0.553 with a t = 3.509.

percent when commercial laboratory basophil counts are employed.

Discussion

The results presented indicate that it would be advisable to add the freshly drawn blood immediately to the dye solution and acidify as described in the procedure of Gilbert and Ornstein to yield a stable preparation for subsequent counting. This should lend itself to both manual and automated Alcian Blue procedures for basophils. We believe that the modified procedure would be of value for preparing samples for commercial laboratories where a significant time interval occurs between drawing the blood sample and subsequent sample preparation and actual counting. We believe that the manual count with the proposed modification would be of value in the determination of the histadelic and histapenic patients on the basis of an accurate count under circumstances where it is not feasible to measure blood histamine levels such as in a small hospital or a physician's office.

Acknowledgement

We wish to thank Betzi Corkins and Scott LaMola for their excellent technical assistance.

References

GILBERT, H.S. and ORNSTEIN, L: Basophil Counting with a New Staining Method Using Alcian Blue. Blood 46, 279-286, 1975.

ILIEV, V., NICHOLS, R.E., and PFEIFFER, C.C.: A. Combined Chromatographic and Fluorometric Method for Sequential Determination of Histamine, Spermidine, and Spermine. The Pharmacologist 9, 247, 1967.

PFEIFFER, C.C.: Blood Histamine, Basophil Counts and Trace Elements in the Schizophrenias. Rev. Can. Biol. 31, 73-76, 1972.