

From the Department of Dermatology, University of Lund, Sweden.

Head: Professor G. Hagerman, M.D.

## NORMAL VARIATION IN THE COUNT OF CIRCULATING BASOPHIL LEUCOCYTES IN MAN

By

HANS RORSMAN

Very little attention has been paid to basophils in allergic reactions, in contrast to other blood cells (*Chase, Dameshek, Haberman, Samter & Squier* 1955; *Waksman* 1959). This may partly be due to earlier lack of suitable methods for quantitative determinations. After the introduction of a chamber method for the counting of basophils (*Moore & James* 1953), studies on these cells have become much less time-consuming than before, when basophil counts were based on a total leucocyte count and a differentiation of a great number of cells in blood smears. This has stimulated investigations on the numerical variation of basophils in allergic conditions also.

It has recently been demonstrated that urticaria is often combined with a decrease of basophil leucocytes (*Rorsman* 1957; *Rorsman & Rosengren* 1958; *Bersani* 1959; *Meneghini & Levi* 1958 and 1959). Unlike *Rorsman* (1958) and *Schultz, Code & Brunsting* (1959), *Bersani* (1959) found a slight decrease of basophils also in atopic dermatitis, but basopenia has not been demonstrated in other cutaneous or allergic diseases studied (*Rorsman* 1958; *Bersani* 1959; *Tedeschi, Cavazzuti & Angeli* 1959; *Schultz, Code & Brunsting* 1959; *Braunsteiner, Poutuzhek & Thumb* 1960).

The finding of a basophil decrease in urticaria is of special interest as basophils are known to contain considerable amounts of histamine (*Graham, Wheelwright, Parish, Marks & Lowry* 1952; *Ehrich* 1953; *Graham, Lowry, Wheelwright, Lenz &*

Parish 1955; Valentine, Lawrence, Pearce & Bech 1955, Code & Mitchell 1957). Nilzén (1947) stressed the fall of blood histamine in urticaria and Rorsman & Rosengren (1958) showed that the decrease of basophils might be the morphological basis for the lowered blood histamine level.

Little is known about the normal variation of basophils. Further studies on the rôle of these cells in urticaria and related conditions made necessary a study on errors of determination and on physiologic variations of the basophils.

In this work the following sources of variability are studied:

- 1) The error of the counting method.
- 2) The random biologic variation.
- 3) The rhythmic variation.
- 4) The effect of food intake.
- 5) The effect of moderate exercise.
- 6) The influence of sex.
- 7) The influence of age.

Further, the chamber method used is compared with methods for basophil counts based on total leucocyte counts and differential counts in smears.

#### MATERIAL

The *examined normal subjects* were nurses, doctors and students at the Department of Dermatology, workmen at the hospital, pensioners at the Pensioner's homes at Lund and members of the scout corps at Lund. All were in good general health and had no history of allergic or dermatologic diseases. Hemoglobin, erythrocyte count, total leucocyte count, absolute eosinophil count, and differential count were controlled by routine methods (Rorsman & Rosengren 1958). In the persons studied the obtained values were: hemoglobin 12.0 to 15.8 grams per 100 ml, erythrocytes 4.0 to 5.1 millions per  $\text{mm}^3$ , leucocytes 4,000 to 9,900 per  $\text{mm}^3$ , eosinophils 63 to 375 per  $\text{mm}^3$ . Neutrophils constituted 51 to 74 per cent, lymphocytes 18 to 40 per cent, and monocytes 0 to 14 per cent in the dif-

ferential count. The highest sedimentation rate was 9 mm per hour.

*Examined non-healthy subjects* were patients with urticaria treated at the department.

#### METHODS FOR BASOPHIL COUNTS

*Blood samples* for determination of the number of basophil leucocytes were obtained by incision of the finger pulp after cleaning with ethyl ether. The first drop of blood was not used.

When nothing else is stated the *direct counting method* was used for the basophil determinations. The technique used (Rorsman 1957) was a slight modification of that described by Moore & James (1953).

Twenty-five mm<sup>3</sup> of blood taken in a control weighed Ellerman pipette (maximum tolerance  $\pm 1\%$ ) was discharged in 215 mm<sup>3</sup> of staining fluid contained in Ellerman tubes, 55  $\times$  10 mm with round bottoms. The staining fluid was measured with a controlled pipette. The tube was shaken by hand for about two minutes to ensure complete mixing. The staining fluid had the following composition (Schellenberg, Wright & James):

|                                           |        |
|-------------------------------------------|--------|
| Disodium hydrogen phosphate, M/15 .....   | 9.3 ml |
| Potassium dihydrogen phosphate, M/15 ...  | 0.7 ml |
| Sodium chloride 0.85 per cent .....       | 5.0 ml |
| Toluidine blue (Merck) 0.1 per cent ..... | 5.0 ml |
| Ethyl alcohol .....                       | 5.0 ml |

After mixing of blood and staining fluid, hemolysis was achieved by addition of 10 mm<sup>3</sup> of a freshly prepared and filtered solution of 10 per cent saponin (Riedel-deHaën) in 20 per cent ethyl alcohol. After the addition of saponin the tube was shaken slightly for about two minutes. The fluid was never permitted to touch the rubber stoppers of the tubes. After hemolysis part of the content in the tube was transferred to two Jessen chambers with a pasteur-pipette. The chambers were allowed to stand in a damp container for 15 minutes before counting so that the leucocytes could settle. The cham-

bers used were from KIFA, Stockholm. They were control-measured and found to have a depth of  $0.400 \pm 0.000 \text{ mm}^3$ . Counting was performed in a Zeiss-Winkel microscope with an achromatic objective  $\times 25$ , num. ap. 0.45 and  $\times 10$  oculars. Blue light was used. A basophil determination was based on examination of two Jessen chambers, i.e.  $20 \text{ mm}^3$ , corresponding to  $2 \text{ mm}^3$  of blood, and the results were given without decimals. The granules of the basophils were stained metachromatically red, which made it possible to distinguish them from neutrophils and mononuclear cells in which only the nucleus, stained orthochromatically, was visible. In eosinophils the greenish-grey, strongly refracting granules could be seen.

*Indirect counting method* for basophils involves total leucocyte count and differentiation of basophils in smears. For the total leucocyte count  $25 \text{ mm}^3$  of blood was transferred in control weighed pipettes to  $475 \text{ mm}^3$  of a fluid with the following composition, contained in Ellerman tubes:

|                                         |        |
|-----------------------------------------|--------|
| Methyl violet solution 1 per cent ..... | 4.0 ml |
| Acetic acid 0.33 per cent .....         | 200 ml |

$0.2 \text{ mm}^3$  of the dilution was counted in a Bürker chamber.

Differential counts were in all cases performed on May-Grünwald-Giemsa-stained smears and on smears stained according to *Undritz* (1950).

May-Grünwald-Giemsa staining:

|                                                                      |           |
|----------------------------------------------------------------------|-----------|
| Methyl alcohol .....                                                 | 4 minutes |
| Undiluted May-Grünwald solution .....                                | 4 minutes |
| Giemsa solution, 5 per cent in 1/15 M phosphate buffer, pH 7.6 ..... | 4 minutes |

Undritz staining:

|                                                   |           |
|---------------------------------------------------|-----------|
| Toluidine blue 1 per cent in methyl alcohol ..... | 5 minutes |
| Short washing in distilled water.                 |           |

At the indirect determination for each staining method the number of basophils per 2,000 leucocytes was determined in

<sup>1</sup> We thank Mr. M. Aust, KIFA, Stockholm, for the checking of the chambers.

the smears and the absolute basophil count was then calculated from the percentage of basophils and the total leucocyte count.

#### EXPERIMENTS

As the planned experiments necessitated a great number of sample takings in the same person, incisions had to be repeated at sites near earlier wounds. It was therefore important to determine the *effect of a previous incision* on the result of a basophil determination performed on blood obtained at a repeated incision. The following experiment was made on ten persons, aged between 23 and 42, six males and four females: Incisions were made into four finger pulps. After 30 minutes, 1 hour, 2 hours and after 4 hours blood for basophil count was obtained from a fresh incision and from reincision into one of the lesions made initially.

The *effect of food intake and of exercise* was studied in ten persons, nine females and one male, aged between 24 and 59. The experiments were performed during three successive days. Each day the experimental persons got up at about 6.30 a.m. They did not take any food and their physical activity was limited as far as possible. From 7.40 a.m. they rested in a recumbent position. Blood samples for basophil counts were taken during the different days at 9.15, 9.30, 9.45, 10.00, 10.30, 11.30 a.m. and at 1.30 p.m.

The experimental subjects had the following regimen:

First day: Rest without food throughout the experimental period.

Second day: Rest as on the first day, but at 9.30 after the taking of a blood sample a meal was given consisting of bread, butter, cheese, milk and cream, coffee and sugar, which was consumed in ten minutes.

Third day: No food as on the first day, but from 9.30 to 9.40 rapid walking up and down 43 steps for 10 minutes with resulting rise of pulse rate (28–60 per minute).

*The random error of the counting method, the random biological variation, the alteration of the basal level on different*

*days and the possible rhythmic variation of basophils at normal basophil values* was studied in 20 persons, twelve females and eight males, aged between 23 and 59. The study was performed during three successive days in each person and each subject's day was strictly regulated. They all got up at about 6.30, had their breakfast consisting of coffee, sugar, milk, cream, bread and butter at 8.15, lunch consisting of fish or meat, potatoes, vegetables and coffee at 11.30, and dinner of a similar composition as the lunch at 5.30 p.m. Bedtime was between 9 and 11 p.m. The degree of activity during the day was judged to remain approximately the same from day to day. Basophil counts were made each day at 8 a.m., 10 a.m., 12 noon, 2 p.m., 4 p.m. and 6 p.m. At each time two different incisions were made and basophils were determined from each of these incisions. The reason for the double incisions was that we wanted a simultaneous estimate of the whole error of the basophil determination, i.e. the error of the sample taking and the counting.

The *error of the counting method at low basophil levels* was assessed from 40 duplicate determinations in 22 patients with urticaria, 12 females and 10 males, aged between 19 and 61, all with less than 20 basophils per mm<sup>3</sup>.

The *variation of basophils in the course of longer time periods* was studied in 20 persons, aged between 24 and 52, eleven males and nine females. Double determinations of basophils were performed monthly, from January to August, except in April, thus on seven different occasions. Samples for counts were taken between 10 a.m. and 3 p.m. independently of previous meals.

The *importance of sex and age* of the individual for the basophil count was investigated in 166 persons, aged between 11 and 93, 81 males and 85 females. Single determinations were performed and the samples were taken between 7.30 a.m. and 12 noon.

*Comparison of the chamber method for basophil counts with the indirect method* was performed on blood from 15 persons, aged between 29 and 59, nine females and six males. Blood for

direct basophil count, for total leucocyte count and for preparation of smears was taken from the same incision.

## RESULTS

The mean values obtained at basophil determinations in *repeated incisions* performed 30 minutes, 1, 2, and 4 hours after the first incision are given in Table 1. Variance analysis showed that there was no difference between the basophil counts in fresh and repeated incisions performed 30 minutes, 1, 2, or 4 hours after the first incision.

The mean values of the basophil determinations performed *at rest* without food intake are given in Table 2. This table also shows basophil mean values at *food intake* and at *exercise*. Variance analysis showed that in subjects at rest, in sub-

TABLE 1  
*Mean values of basophils per mm<sup>3</sup> blood from fresh and repeated incisions.  
Determinations were made in 10 persons.*

| Time after first incision |               |        |          |         |          |         |          |
|---------------------------|---------------|--------|----------|---------|----------|---------|----------|
| 30 min.                   |               | 1 hour |          | 2 hours |          | 4 hours |          |
| Fresh                     | Re-<br>peated | Fresh  | Repeated | Fresh   | Repeated | Fresh   | Repeated |
| 42.9                      | 41.4          | 43.1   | 40.3     | 40.3    | 41.3     | 41.5    | 41.7     |

TABLE 2  
*Mean values of basophils per mm<sup>3</sup> blood during rest, in connection with food  
intake or exercise. Determinations were made in 10 persons.*

| Condition]                            | 9 <sup>15</sup> a. m. | 9 <sup>30</sup> a. m. | 9 <sup>45</sup> a. m. | 10 <sup>00</sup> a. m. | 10 <sup>30</sup> a. m. | 11 <sup>30</sup> a. m. | 1 <sup>30</sup> p. m. |
|---------------------------------------|-----------------------|-----------------------|-----------------------|------------------------|------------------------|------------------------|-----------------------|
| Rest .....                            | 34.1                  | 34.8                  | 32.9                  | 33.7                   | 34.8                   | 34.2                   | 30.2                  |
| Food .....                            | 33.3                  | 32.1                  | 34.8                  | 33.2                   | 33.8                   | 32.4                   | 34.1                  |
| 9 <sup>00</sup> -9 <sup>00</sup> a.m. |                       |                       |                       |                        |                        |                        |                       |
| Exercise ....                         | 30.5                  | 29.8                  | 30.0                  | 31.7                   | 28.2                   | 28.5                   | 31.9                  |
| 9 <sup>00</sup> -9 <sup>00</sup> a.m. |                       |                       |                       |                        |                        |                        |                       |

jects influenced by food intake or by moderate exercise no systematic change of the basophil counts took place.

Basophil determinations in the experiment concerning the *random error of the counting method, the random biological variation, the alteration of the basal level during different days* and the possible *rhythmic variation* gave a mean value of 35.3 cells/mm<sup>3</sup>.

The following statistical model was adopted for every person in the analysis:

$$x_{ijk} = \mu_i + \beta_j + e_{ij} + e_{ijk}$$

$i$  denotes the day ( $i = 1, 2, 3$ ).

$j$  denotes the hour ( $j = 1, 2, 3, 4, 5, 6$ ).

$k$  denotes the replicate ( $k = 1, 2$ ).

$x_{ijk}$  denotes the variate value corresponding to the  $k$  th replicate on the  $i$  th day at the  $j$  th hour.

$\mu_i$  denotes the individual value.

$\beta_j$  denotes the rhythmic variation.

$e_{ij}$  denotes the random biological variation.

$e_{ijk}$  denotes the error of the method.

For every person sums of squares were calculated as follows:

A. The 36 squares of individual values were added up.

B. The 18 squares of the sums of duplicate values were added up and divided by 2.

C. The 6 squares of the sums of values at the same hour were added up and divided by 6.

D. The 3 squares of the sums of values from each day were added up and divided by 12.

E. The square of all added 36 values was divided by 36.

Mean values and the calculated sums of squares are given in Table 3.

The following differences were calculated:

A-E, which is the sum of squares of deviations from mean.

This sum may be further analyzed:

A-B, which depends on the value of  $e_{ijk}$ , here called  $\sigma_1$

$$A-B = 18 \sigma_1^2$$

B-C-D + E, which depends on the value of  $e_{ij}$ , here called  $\sigma_2$ , and of  $e_{ijk}$

$$B-C-D + E = 20 \sigma_2^2 + 10 \sigma_1^2$$

C-E, which depends on the value of  $\beta_j$

$$C-E = 6 \Sigma \beta_j^2 + 10 \sigma_2^2 + 5 \sigma_1^2$$

D-E, which depends on the variation between days

$$D-E = 12 \Sigma (\mu_i - \mu)^2 + 4 \sigma_2^2 + 2 \sigma_1^2$$

TABLE 3

*Mean values of basophils per mm<sup>3</sup> blood and certain sums of squares in 20 persons. For explanation cf. the text.*

| Person   | Mean value<br>basophils/<br>mm <sup>3</sup> | Sums of squares |         |         |         |         |
|----------|---------------------------------------------|-----------------|---------|---------|---------|---------|
|          |                                             | A               | B       | C       | D       | E       |
| 1        | 15.0                                        | 8693            | 8581    | 8391    | 8183    | 8130    |
| 2        | 21.0                                        | 16496           | 16370   | 15983   | 15916   | 15876   |
| 3        | 22.8                                        | 19119           | 19040   | 18923   | 18792   | 18783   |
| 4        | 23.4                                        | 20426           | 20029   | 19774   | 19772   | 19693   |
| 5        | 25.3                                        | 24079           | 23696   | 23037   | 23503   | 22952   |
| 6        | 25.5                                        | 24543           | 24266   | 23699   | 23490   | 23460   |
| 7        | 28.2                                        | 29495           | 29157   | 28685   | 28687   | 28617   |
| 8        | 28.2                                        | 29413           | 29081   | 28802   | 28737   | 28617   |
| 9        | 29.4                                        | 32411           | 32199   | 31623   | 31171   | 31152   |
| 10*      | 30.8                                        | 23546           | 23356   | 22874   | 22817   | 22816   |
| 11       | 31.7                                        | 37000           | 36481   | 36148   | 36170   | 36100   |
| 12       | 34.0                                        | 42883           | 42521   | 41975   | 41983   | 41684   |
| 13       | 34.5                                        | 44531           | 44070   | 43111   | 43442   | 42918   |
| 14       | 37.4                                        | 51367           | 51047   | 50368   | 50286   | 50251   |
| 15       | 38.3                                        | 53424           | 53271   | 52962   | 52833   | 52747   |
| 16*      | 46.1                                        | 52398           | 52237   | 51582   | 51320   | 50968   |
| 17       | 51.0                                        | 95989           | 95697   | 94792   | 93755   | 93738   |
| 18       | 51.5                                        | 98843           | 98440   | 95900   | 96949   | 95378   |
| 19       | 61.2                                        | 141723          | 140803  | 136068  | 138699  | 134811  |
| 20       | 71.4                                        | 185273          | 184879  | 184016  | 184053  | 183612  |
| $\Sigma$ |                                             | 1031652         | 1025221 | 1008713 | 1010558 | 1002303 |

\* Values obtained from two days only were used in the statistical analysis, as some data were missing from the third day, owing to a technical error.

Variance analysis showed that the mean error of a single determination in the whole material was  $\pm 4.3$  cells. The mean error seemed to depend on the level of the basophil count, being  $\pm 3.1$  in the subjects with mean value below 25,  $\pm 4.1$  in subjects with mean value between 25 and 30,  $\pm 4.5$  in subjects with mean value between 30 and 40, and  $\pm 5.1$  in subjects with mean value above 40. In the urticaria group the mean value was 7.8 cells per  $\text{mm}^3$  and the standard error of a single determination was  $\pm 2.5$ . The standard error is thus absolutely lower but relatively higher for low basophil counts and is proportional to the square root of the counted number of cells.

The *random biological variation* in the whole material was  $\pm 3.5$  cells and thus somewhat smaller than the standard error of a single determination. The random biological variation was found to vary with the mean value of the individual. The random biological variation was  $\pm 2.0$  in subjects with mean value below 25,  $\pm 3.0$  in subjects with mean value between 25 and 30,  $\pm 3.3$  in subjects with mean value between 30 and 40, and  $\pm 5.0$  in subjects with mean value above 40.

Generally no alteration of the *basal level* of basophils from day to day was found, but in two of the cases (5 and 19) a probable change occurred.

Variance analysis with regard to *rhythmic variations* of basophils showed that there was no rhythmic variation in common in the studied individuals. In two persons, however, (number 1 and 3) a rhythmic pattern was found with a maximum at 8 o'clock in the morning in both subjects and a minimum at 4 p.m. in subject 1 and at noon in subject 3. Although a rhythmic variation was not present in the group studied, it may thus be found in some individuals.

The average biological variation during a period of three days was  $\pm 5.1$  cells.

The mean values of basophil determinations during 8 months are given in Table 4.

Analysis of the basophil *variations during a longer time period* yielded no significant difference between the different

TABLE 4

*Mean values of basophils per mm<sup>3</sup> blood in 20 healthy persons at determinations during nine months.*

| Jan. | Feb. | March | May  | June | July | Aug. |
|------|------|-------|------|------|------|------|
| 33.9 | 28.0 | 30.4  | 33.1 | 33.8 | 36.7 | 31.4 |

TABLE 5

*Basophil mean values and standard errors in different age groups.*

| Age   | Sex | n  | Mean value<br>basophils/mm <sup>3</sup> | Mean value in both sexes<br>± standard error |
|-------|-----|----|-----------------------------------------|----------------------------------------------|
| 10-19 | M   | 17 | 41.6                                    | 41.1 ± 3.7                                   |
|       | F   | 26 | 40.7                                    |                                              |
| 20-29 | M   | 15 | 38.5                                    | 38.8 ± 3.9                                   |
|       | F   | 22 | 39.0                                    |                                              |
| 30-39 | M   | 20 | 42.1                                    | 43.5 ± 4.7                                   |
|       | F   | 6  | 48.2                                    |                                              |
| 40-49 | M   | 11 | 53.7                                    | 57.6 ± 6.0                                   |
|       | F   | 5  | 66.0                                    |                                              |
| 50-59 | M   | 4  | 49.3                                    | 56.7 ± 8.0                                   |
|       | F   | 5  | 62.6                                    |                                              |
| 60-69 | M   | 2  | 22.0                                    | 36.9 ± 9.1                                   |
|       | F   | 5  | 42.8                                    |                                              |
| 70-79 | M   | 5  | 45.2                                    | 36.3 ± 6.9                                   |
|       | F   | 7  | 30.0                                    |                                              |
| 80-89 | M   | 5  | 30.4                                    | 35.0 ± 6.9                                   |
|       | F   | 7  | 35.7                                    |                                              |
| 90-99 | M   | 2  | 28.0                                    | 24.5 ± 12.0                                  |
|       | F   | 2  | 21.0                                    |                                              |

months. The average biological variation during the studied period was  $\pm 7.7$  cells.

The mean values of the different groups in the study on the importance of *sex and age* are given in Table 5. The mean value was the same for males and females, namely, 41.9 cells per mm<sup>3</sup>. The standard deviation in the material was  $\pm 24.0$

cells. The mean values in the age groups 40 to 59 years were slightly higher than the mean values in the other groups ( $0.05 > p > 0.01$ ).

*Comparison between direct and indirect methods for basophil determination* showed that the direct counting method gave about the same mean value (56.9 cells per  $\text{mm}^3$ ) as the two indirect counting methods (May-Grünvald-Giemsa 57.0; Undritz 60.1 cells per  $\text{mm}^3$ ). Diagnosis of basophils was most easily performed in smears stained according to Undritz.

#### DISCUSSION

The study on the possible effect of a previous lesion of the blood vessels on the basophil count did not show any change of the basophil count at determinations 30 minutes, 1, 2, and 4 hours after a single earlier incision. *Graf & Swensson* (1950) and *Harm* (1954) found an increase of leucocytes and particularly of basophils after repeated lesions of a vein in the rabbit. The absence of any demonstrable basophil increase after a mechanical lesion in man may be due to the sampling technique used or to a species difference, but it is also possible that local basophilia is not induced by lesions of smaller vessels.

There was no effect of food intake on the number of basophils in the ten studied non-allergic persons. No earlier studies on the influence of food intake on basophil leucocytes have been performed.

There was no reaction of the basophils after moderate exercise. Absence of significant basophil changes after heavy manual work has been reported by *Karvonen & Kunnas* (1953), but a study of *Dunér & Pernow* (1958) may suggest a basophil increase during physical work.

The factors influencing the result of a single basophil determination are the error of the counting procedure and the biological variation. Both seem to depend on the level of the basophil count. The error of the method decreases absolutely with lower basophil counts but the ratio  $\frac{\text{method error}}{\text{mean value}}$  increases at lower basophil levels. In the material with a mean value of

35.3 cells per  $\text{mm}^3$  the error of the method was  $\pm 4.3$  cells or  $\pm 12\%$  of the mean value, while the material with a mean value of 7.8 cells per  $\text{mm}^3$  had a method error of  $\pm 2.5$  cells or  $\pm 32\%$  of the mean value. Also the biological variation decreased absolutely with lower basophil counts, but here the ratio  $\frac{\text{biological variation}}{\text{mean value}}$  was relatively unchanged. The bio-

logical variation was  $\pm 14\%$  of the mean value during a three-day period and  $\pm 24\%$  of the mean value during an eight-month period. The biological variation of basophils was of a random type in the studied group. Only in two persons did the basal level change from day to day and in two other persons a rhythmic variation was found. The absence of a rhythmic basophil fluctuation between 8 a.m. and 6 p.m. is in agreement with the findings of *Osada* (1956), who found no significant changes during the day but a rise in the basophil count during the night. Our results do not agree with earlier reports on the tendency towards basophil rise during the morning (*Labendzinski* 1956), at noon (*Sundberg & Wasastjerna* 1953), or in the afternoon (*Boseila* 1959).

In four studied persons *Labendzinski* (1956) found no change in the per cent values of basophils at weekly determinations. This seems to be the only earlier study on the biological variation of basophils for a longish time period. Our study shows a greater variation during an eight-month period than during a period of three days.

The present study did not show any difference in the mean basophil count of men and women. The only report on a sex difference is that of *Angeli, Tedeschi & Cavazzuti* (1955), who found a lower mean value in females over 60 years of age than in females below 60 years of age and in males of both age groups. The present material showed a probably higher basophil count in the age groups between 40 and 59 years when both sexes were analysed together. *Boseila* (1959) found a slightly higher mean value in subjects of both sexes, aged between 40 and 49, compared to other adult age groups, but *Braunsteiner & Thumb* (1958) found no age differences.

The youngest subject in our material was 11 years, but there are earlier reports on basophil counts in children. *James, Wright, Wilkersson & Schellenberg* (1955) found a higher basophil count in newborn than in older children. *Mitchell* (1955) analyzed in detail the variation of basophils in newborn and found an increase of the basophils during the first 24 hours after the birth, but a later decrease with a minimum five days after the birth. Then an increase started, which continued for six weeks. In a material of children between 6 months and 12 years of age the mean value was higher than at six weeks after birth, but the difference was not significant (*Mitchell* 1958; *Mitchell & Cass* 1959). *Labendzinski* (1956) found lower per cent values of basophils in newborn children than *Mitchell*.

The good agreement between the results of the direct basophil method and the two indirect methods for basophil determination in the present study is in conformity with the findings of *Moore & James* (1953). *Graf & Swensson* (1950) found toluidine blue stained smears superior to May-Grünwald-Giemsa stained smears, but *Harm* (1954) did not find differences between the results of the two staining methods. The actual study shows that May-Grünwald-Giemsa staining or toluidine-blue staining may both be used for indirect determinations of basophils. The error of the method in an indirect basophil determination was not determined, but will depend on the error of the total white cell count (*Berg* 1945) and on the error of the differential determination of basophils. The last-mentioned error will be that of a Poisson frequency distribution (*Ricker* 1937).

#### SUMMARY

The influence of method error on basophil counts and the biological variations of basophils were studied with the following results:

1. Mechanical lesion of the small blood vessels at the site of sample taking did not influence the result of later basophil determinations.

2. Food intake did not influence the basophil level.
3. Moderate exercise did not influence the basophil level.
4. There was no general rhythmic variation of basophils between 8 a.m. and 6 p.m.
5. The error of the method decreased absolutely with lower basophil counts but the ratio  $\frac{\text{method error}}{\text{mean value}}$  increased at lower basophil levels.
6. The biological variation was  $\pm 14$  per cent of the mean value during a three-day period and  $\pm 24$  per cent of the mean value during an eight-month period.
7. The mean value of basophils was the same in men and women.
8. The mean value of basophils was probably higher in subjects between 40 and 59 years of age.
9. Direct basophil counting and indirect basophil counting performed on May-Grünwald-Giemsa- or Undritz-stained smears gave the same mean values.

#### ACKNOWLEDGEMENTS

My thanks are due to Mrs. *E. Wiehe* for skilful technical assistance and to Professor *C.-E. Quensel*, who has given me advice and performed the statistical analysis.

#### REFERENCES

- Angeli, G., Tedeschi, G. & Cavazzuti, F.*: Studio sul comportamento dei basofili ematici in soggetti di età avanzata. *Acta gerontol.* 5, 28, 1955.
- Berg, W. N.*: Blood cell counts. Their statistical interpretation. *Am. Rev. Tuberc.* 52, 179, 1945.
- Bersani, R. B.*: The blood basophil in urticaria and other dermatoses. Thesis, Zürich, 1959.
- Boseila, A.-W. A.*: The normal count of basophil leucocytes in human blood. *Acta med. scandinav.* 163, 525, 1959.
- Braunsteiner, H., Poutuzhek, O. & Thumb, N.*: Variations observées dans le nombre des leucocytes basophiles chez l'homme. Leur significations fonctionnelles. *Rev. hemat.* 15, 241, 1960.
- Braunsteiner, H. & Thumb, N.*: Quantitative Veränderungen der Blutbasophilen und ihre klinische Bedeutung. *Acta haemat.* 20, 339, 1958.

- Chase, M. W., Dameshek, W., Haberman, S., Samter, M. & Squier, T. L.*: The role of the formed elements of the blood in allergy and hypersensitivity. *J. Allergy*. 26, 219, 1955.
- Code, C. F. & Mitchell, R. G.*: Histamine, eosinophils and basophils in the blood. *J. Physiol.* 136, 449, 1957.
- Dunér, H. & Pernow, B.*: Histamine and leucocytes in blood during muscular work in man. *Scandinav. J. Clin. & Lab. Invest.* 10, 394, 1958.
- Ehrich, W. E.*: Histamine in mast cells. *Science*. 118, 603, 1953.
- Graf, W. & Swensson, A.*: Experimental investigations on local changes in the white blood cell picture following perforating injury to blood vessels (veins). *J. Path. & Bact.* 62, 419, 1950.
- Graham, H. T., Lowry, O. H., Wheelwright, F., Lenz, M. A. & Parish, H. H.*: Distribution of histamine among leucocytes and platelets. *Blood*. 10, 467, 1955.
- Graham, H. T., Wheelwright, F., Parish, H. H., Marks, A. R. & Lowry, O. H.*: Distribution of histamine among blood elements. *Fed. Proc.* 11, 350, 1952.
- Harm, H.*: Über Schwankungen der relativen Basophilen - Zahl im peripheren Kaninchenblut, unter verschiedenartigen Entnahme- und Färbebedingungen, sowie nach Colchicinbehandlung. *Folia haematol.* 72, 129, 1954.
- James, G. N., Wright, D. V., Wilkerson, V. & Schellenberg, R.*: Observations on the absolute basophil count in health and disease. *Clin. Research. Proc.* 3, 31, 1955.
- Karvonen, M. J. & Kunnas, M.*: Factor analysis of haematological changes in heavy manual work. *Acta physiol. scandinav.* 20, 220, 1953.
- Labendzinski, F.*: Recherches quantitatives sur les basophiles du sang périphérique au moyen de la goutte épaisse. *Sang.* 27, 432, 1956.
- Meneghini, C. L. & Levi, L.*: Istamina cutanea e mastociti in alcune dermatosi pruriginose. *Minerva Dermat.* 33, 247, 1958.
- Meneghini, C. L. & Levi, L.*: Istaminemia e mastociti circolanti in alcune dermatosi allergiche. *Gior. ital. dermat.* 100, 449, 1959.
- Mitchell, R. G.*: Circulating basophilic leucocyte count in the newborn. *Arch. Dis. Childh.* 30, 130, 1955.
- Mitchell, R. G.*: Basophilic leucocytes in children in health and disease. *Arch. Dis. Childh.* 33, 193, 1958.
- Mitchell, R. G. & Cass, R.*: Histamine and 5-hydroxytryptamine in the blood of infants and children. *J. Clin. Invest.* 38, 595, 1959.
- Moore, J. E. & James, G. W.*: A simple direct method for absolute basophil count. *Proc. Soc. Exper. Biol. & Med.* 82, 601, 1953.
- Nilzén, A.*: Studies in histamine (H-substance) with special reference to the conditions obtaining in urticaria and related skin-changes. *Acta dermat.-venereol.* 27, suppl. 17, 1947.
- Osada, Y.*: Diurnal rhythms of the numbers in circulating basophils and eosinophils in healthy adults. *Bull. Inst. Pub. Health.* 5, 5, 1956.

- Ricker, W. E.*: The concept of confidence or fiducial limits applied to the Poisson frequency distribution. *J. Am. Stat. Assn.* 32, 349, 1937.
- Rorsman, H.*: Basophil leucocytes and urticaria. *Acta dermat.-venereol.* 37, 121, 1957.
- Rorsman, H.*: Basophil leucocytes in asthma, atopic dermatitis and psoriasis. *Acta dermat.-venereol.* 38, 175, 1958.
- Rorsman, H. & Rosengren, E.*: Basophil leucocytes and blood histamine in urticaria. *Acta dermat.-venereol.* 38, 377, 1958.
- Schellenberg, R. F., Wright, D. V. & James, G. W., III*: Personal communication, 1955.
- Schultz, H. J., Code, C. F. & Brunsting, L. A.*: Blood histamine and basophil-eosinophil counts in skin diseases. *A.M.A. Arch. Dermat.* 80, 44, 1959.
- Sundberg, M. & Wasastjerna, C.*: Förekomsten av basofilt granulerade leukocyter i blodet. *Finska läk.-sällsk. handl.* 96, 208, 1953.
- Tedeschi, G., Cavazzuti, F. & Angeli, G.*: Variazioni quantitative dei granulociti basofili del sangue periferico in diversi condizioni patologiche. *Minerva med.* 50, 1535, 1959.
- Undritz, E.*: in *Hämatologische Tafeln* Sandoz, Basel, Sandoz AG. 1950, p. 23.
- Valentine, W. N., Lawrence, J. S., Pearce, M. L. & Beck, W. S.*: The relationship of the basophil to blood histamine in man. *Blood.* 10, 154, 1955.
- Waksman, B. H.*: The toxic effects of the antigen-antibody reaction on the cells of hypersensitive reactors, in Lawrence, H. S.: *Cellular and humoral aspects of the hypersensitive states*, New York, Hoeber, Inc., 1959, p. 123.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.