A COMPARATIVE STUDY OF THE CIRCULATING EOSINOPHIL LEVEL IN BABIES

Part I : PREMATURE INFANTS

BY

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Studies of circulating eosinophils have been made in many small groups and it has been repeatedly observed that under controlled conditions the circulating eosinophil count may be regarded as a practical clinical test of adreno-cortical function and reserve. The present investigation, the first of a series, was undertaken to find what was the level of circulating eosinophils in premature infants and to study the behaviour of eosinophils in infancy and to find in what circumstances changes in the circulating eosinophil level were effected.

From a preliminary series of daily counts done on premature babies in the Aberdeen Maternity Hospital it quickly became apparent that the circulating eosinophil level in premature infants of the ages of 3 weeks and over was in excess of that quoted as normal for adults by Wintrobe (1946), Whitby and Britton (1950), Forkner (1929) and Washburn (1934 and 1935), but while the final stable level of eosinophils was much higher, this level was only reached after a varying period. In practically all cases counts done on the first or second day of life were extremely low and the rise in eosinophils took place in the ensuing weeks.

To compare this finding with normal infants it was then decided to undertake a series of daily eosinophil counts on full-term infants born in the same hospital. Since these infants were born after the normal gestation period of 40 weeks, the birth weights with few exceptions fell in the 6-8½ lb. weight group and these infants were usually discharged from hospital on the seventh or eighth day. Thus the serial counts for infants in this group are taken over short periods, and the possibility of following these infants for a long period of daily eosinophil counts could not be considered at the time and a prolonged study has not yet been completed. The majority of premature infants had daily counts throughout their stay in the special nursery. The main factor determining the discharge of these infants is the

weight, and as a general rule few infants in this nursery are regarded as ready for home before the weight has reached 5½ lb. In this way it has been possible to make long serial counts on 75 infants and watch the steady eosinophil rise in the early weeks of life. It was found that many of these infants before being discharged home reached a stable level of circulating eosinophils, i.e. that daily counts after a time showed little or no fluctuation although each infant had its own individual level.

Technique

The method described by Randolph (1949) for the differentiation and enumeration of eosinophils in a counting chamber with a glycol stain has been used throughout. For each morning's counts equal parts of the phloxine and methylene blue solutions were freshly mixed and allowed to stand for 15 minutes before being used to dilute the blood sample. White cell counting pipettes were used. After thorough cleansing of the skin surface with an ether-soaked swab, peripheral blood was taken from the heel in all infants. The first drop of blood was wiped away and the second drop taken up. The pulp of the heel was not squeezed. No difficulty was experienced at any time in securing an abundant flow of blood from the infants owing to the higher room temperature of both premature and normal nursery accommodation. A simple spring-release lancet was used in preference to a Hagedorn needle.

White cell pipettes were filled to the 0.5 mark with blood and to the mark 11 with diluting fluid, giving a 1 : 20 dilution. For each patient the number of cells seen in four double counting chambers (eight ruled areas giving a total of 7.2 c.mm.) were counted and the average taken. Variations from one ruled area to another occurred but were never exceptionally wide, and there was close approximation between the eight counts in most cases. The counts were made within two hours of the pipette being filled but control counts from the same pipettes left to stand for at least 16 hours overnight gave results practically identical with the first counts. It would seem, therefore, that if counting is

unavoidably delayed fragmentation of cells and clumping of leucocytes with enmeshing of eosinophils is not troublesome with the use of Randolph's fluid. This disadvantage has been reported when Pilot's fluid was used. Immediately before counting the pipettes were shaken for two minutes, then six drops expelled before filling the counting chamber. Three minutes were allowed for the cells to settle. Evaporation is slow when Randolph's fluid is used, and under low power the eosinophil, stained bright red, is easily distinguishable from the remaining white cells which are azure. Eosinophils were counted daily for each infant and as far as possible all blood samples were taken at approximately the same time every day.

The white cell count was made for each infant on the first or second day of life and thereafter at weekly intervals until discharge. The final count made on the day of, or the day before, discharge was the figure on which the percentage of eosinophils was calculated for each child. The same sample of blood was used for the white count as for the eosinophil count and counting chambers with Neubauer ruling used throughout. An average of two ruled areas was taken for each count, i.e. 1.8 c.mm. were counted.

Material

The 75 premature infants, all of whom were born in the Aberdeen Maternity Hospital, conform to the international classification whereby the birth weight of 2,500 g. ($5\frac{1}{2}$ lb.) or less is the criterion of prematurity. The group has been subdivided into smaller groups depending on the weight, and the cases fell into these weight groups as follows:

2-3 $\frac{1}{2}$ lb.	14 infants
3 $\frac{1}{2}$ -4 $\frac{1}{2}$ lb.	28 infants
4 $\frac{1}{2}$ -5 $\frac{1}{2}$ lb.	33 infants

Results

Surviving Children. One thousand four hundred and seventy-seven counts were made on 75 infants whose birth weights varied from 2 lb. 2 oz. to 5 lb. 8 oz., an average of 19.7 counts for each child. All infants in the two lower weight groups had a complete absence of eosinophils on the first day but the infants in the 4 $\frac{1}{2}$ -5 $\frac{1}{2}$ lb. group had an average of 1.3% eosinophils on the first day. The 1.3% eosinophils calculated on the white cell count represents an absolute count of 122 eosinophils per c.mm. From a study of these counts it is evident that the circulating eosinophil level at birth for many premature infants is zero or very close to it, i.e. there is an eosinophilopenia but over the ensuing weeks the eosinophil level rises sharply to reach figures of the order of 1,500 per c.mm. or even higher so that an eosinophilia up to 15% was a common finding (Table 1).

TABLE 1
EOSINOPHIL LEVEL IN 75 PREMATURE INFANTS

Weight Group (lb.)	Number in Group	Average Stay in Hospital (days)	Range of Eosinophils	Percentage of Eosinophils on First Day	Percentage of Eosinophils on Discharge
2-3 $\frac{1}{2}$	14	41	0-1,496	0	15.4
3 $\frac{1}{2}$ -4 $\frac{1}{2}$	28	35	0-1,650	0	15.7
4 $\frac{1}{2}$ -5 $\frac{1}{2}$	33	17	0-1,496	1.3	11.7

Average white cell count on first day of life: 9,400 per c.mm.
Range of white cell count: 2,800 to 16,500 per c.mm.

On discharge the percentage of eosinophils for the 75 infants was as follows:

1-4%		12 infants
4.1-10%		48 infants
10.1-12%		8 infants
12.1-16%		7 infants

Thus only 12 infants out of 75 had a percentage of eosinophils within the adult range.

The range over which counts occurred in premature infants was from 0 per c.mm. on the first day of life (and even up to the fifth day in infants whose survival was precarious) up to 1,650 per c.mm. for an infant (of 3 lb. 10 $\frac{1}{2}$ oz. birth weight) on the forty-fourth day of life. Many of these babies showed a tendency towards the development of a stable circulating eosinophil level during their later counts and close estimations over several consecutive days seemed to indicate that the infant had reached a stable level. Two infants in particular reached their individual levels in the third week of life, and when discharged in the fifth and sixth weeks respectively still had the same levels (Fig. 1). The rise in the circulating eosinophil

FIG. 1.—Increase in circulating eosinophils to reach a stable level at the fifteenth day. The level was still the same on discharge on the forty-first day. Premature infant (birth weight 4 lb. 8 $\frac{1}{2}$ oz.).

level was slightly more rapid in the infants in the 4 $\frac{1}{2}$ -5 $\frac{1}{2}$ lb. weight group but the levels for the other

Fig. 2.—Fall in eosinophils during an episode of respiratory infection in a premature infant (birth weight 3 lb. 12 oz.).

two groups were almost identical. Since reproduction of individual graphs for all infants is not practicable, several typical graphs are reproduced and an average curve constructed from available data for each weight group. For the average curves, averages were struck at five-day intervals and the graph plotted from these findings (Fig. 3).

Fig. 3.—Graph of average counts for each of three weight groups.

White cell counts in this study of premature babies numbered 422. Of the 75 infants in the series, 68 had a white cell count made on the first day of life with an average count of 9,400 white cells per c.mm. Twenty-two infants had counts on the twenty-ninth day of life and the average was 11,800 white cells per c.mm.

These figures correspond closely to those quoted by Smith (1951) from Lichtenstein's work where 10 premature babies were examined and 7,500 per c.mm. found to be the average white cell count on the first day of life.

Infants who Died. Twenty-nine counts were made on eight infants who did not survive longer than the thirteenth day. The striking feature in these infants was the persistent eosinophilopenia. In all cases on the day of death there was a complete absence of eosinophils.

Eleven white cell counts were made on these eight infants, but the average count was similar to that of the large group of surviving infants. The white cell count for these infants averaged 8,600 per c.mm. (Table 2).

TABLE 2
EOSINOPHIL LEVEL IN PREMATURE INFANTS WHO DIED

Infant	Age at Death (days)	Daily Eosinophil Counts (per c.mm.)	White Cell Count
T.	3	0, 0, 0.	4,500
W.	3	0, 22, 0.	8,900
T.	4	0, 44, 22, 0.	11,600
M.	5	0, 0, 22, 0, 0.	9,800
H.	4	0, 44, 0, 0.	13,400
Mi.	2	0, 0.	8,900
R.	7	0, 66, 44, 0, 22, 0, 0.	14,300
Mcl.	13	0, 0, 0, 22, 0, 44, 66, 22, 0, 0, 0, 0.	12,900
			6,100
			4,200

Discussion

The figures for circulating eosinophils in adults vary from author to author. Few recent surveys have been made in children and fewer in infants. Actual figures quoted in the literature for eosinophil counts on infants are rare and only small series have been followed, the best known being the work done by Forkner (1929) on infants but there is no mention made in his work of premature infants. Among the many conditions in which eosinophilia occurs the state of prematurity is mentioned only by Whitby and Newns (1949). Eosinophils may be expressed as the direct count per c.mm. of blood or as a percentage of the total white count. Thus, for adults the direct count is quoted variously as lying between 60 to 200 eosinophils per c.mm. and the percentage accepted as normal is 1 to 4. The higher absolute counts of infants and children are not quoted, but Klein and Hanson (1950) remark that we have come to accept as normal for older children a higher eosinophil level than is quoted for adults. Smith (1951) however says: 'Eosinophils and basophils may occur in the blood smears of earliest infancy in about the proportions represented by these cells in later life'. He does not specifically mention eosinophils in premature infants.

Comparison of percentages of eosinophils shows that the adult figure of 1 to 4 is exceeded unquestionably by the majority of infants in this series. The absolute count for adults is 150 to

400 per c.mm., and Discombe (1946) found that only 1% of normal adults had an eosinophil count exceeding 240 per c.mm. Sixty-three infants in this series had more than 4% circulating eosinophils with direct counts from 376 to 1,650 per c.mm.

The results of serial eosinophil counts were expressed graphically for each patient studied, and consideration of these graphs showed that invariably a premature infant had no eosinophils circulating at birth. This may be closely connected with depression of eosinophils in the mother in the first stage of labour and her eosinophilopenia at delivery (Davis and Hulit, 1949). Where the progress of the infant was entirely satisfactory the eosinophil count rose steadily to reach a high level by the third to the fifth week. Counts were made on eight babies who did not survive (Table 2). It was a constant finding that these infants showed no tendency for the eosinophil count to rise and before death the eosinophil count was 0%. This might be regarded as an indication of an infant's chance of survival, the satisfactory eosinophil rise being indicative of a satisfactory prognosis or, conversely, that the lack of eosinophils could be taken to mean that all was not well with the child.

The eosinophilopenia in infants whose survival was precarious has a parallel in the low circulating eosinophil level of ill children and adults and in the very low post-operative values for eosinophils (Roche, Thorn and Hills, 1950). It has long been accepted that eosinophils appear in increasing numbers with the return of health, and eosinophilia has been described by Lams, quoting Chantemesse and Rey (1899), as 'an index of convalescence'.

In this series of premature infants there were too few instances of infection for the response of the circulating eosinophil level to infections to be studied in detail. One infant, however, with a moderately severe upper respiratory infection in the fourth week of life showed a clear-cut fall in eosinophils. The infectious episode lasted four days, but from the onset of infection seven days elapsed before the eosinophil count was as high as before the first symptoms (Fig. 2). Two other infants with similar infections showed the same pattern of response but less clearly defined, perhaps because the illness was of a very mild character.

Among the infants examined there were seven

pairs of twins, of whom two pairs were uniovular. The number of twins studied is too small for any conclusion to be drawn regarding the eosinophil counts but it is of interest that uniovular twins showed serial counts that were almost identical while binovular twins had individual counts showing marked differences. One of the pairs of uniovular twins seen at six months still had similar counts but lower than when the hospital series was concluded.

Summary

Seventy-five premature infants had daily eosinophil counts performed throughout their stay in the Aberdeen Maternity Hospital Special Nursery. Counts were also made on eight premature infants who died.

There is a progressive rise in the eosinophil count from the birth level of zero to a high stable level of circulating eosinophils. This level varied from 900 to 1,650 eosinophils per c.mm. and was reached in three to five weeks.

Infants whose progress was unsatisfactory, and who later died, had a marked eosinophilopenia.

The white cell count of 68 infants on the first day averaged 9,400 per c.mm. and agrees well with figures quoted in the literature. Twenty-nine infants had an average count of 11,200 per c.mm. on the twenty-ninth day.

The percentage of circulating eosinophils in counts done on the day of discharge in this group of premature infants lay between 5 and 12% in 50 of the 75 infants.

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REFERENCES

- Davis, M. E. and Hulit, B. E. (1949). *J. clin. Endocr.*, 9, 714.
- Discombe, G. (1946). *Lancet*, 1, 195.
- Forkner, C. E. (1929). *Bull. Johns Hopk. Hosp.*, 45, 75.
- Klein, R. and Hanson, J. (1950). *Pediatrics*, 6, 192.
- Lams, H. (1907). *C. R. Soc. Biol. Paris*, 62, 489.
- Randolph, T. G. (1949). *J. Lab. clin. Med.*, 34, 1696.
- Roche, M., Thorn, G. W. and Hills, A. G. (1950). *New Engl. J. Med.*, 242, 307.
- Smith, C. A. (1951). *The Physiology of the Newborn Infant*, 2nd ed. Springfield, Illinois.
- Washburn, A. H. (1934). *Amer. J. Dis. Child.*, 47, 993.
- (1935). *Ibid.*, 50, 413.
- Whitby, L. E. H. and Britton, C. J. C. (1950). *Disorders of the Blood*, 6th ed. London.
- and News, G. H. (1949). In Garrod, Batten and Thursfield's *Diseases of Children*, ed. Paterson, D. and Moncrieff, A. Vol. 2, ch. 14, 4th ed. London.
- Wintrobe, M. M. (1946). *Clinical Hematology*, 2nd ed. London.