Histochemical similarities between newborn and leukaemic myeloid cells and their possible significance

PAUL L. WOLF,¹ BORIS SILBERBERG, SAMUEL ALBERT,
JEROME HORWITZ, AND ELISABETH VON DER MUEHLL

*From the Department of Pathology, Stanford University Medical Center,
Wayne State University School of Medicine, Detroit General Hospital, Hutzel Hospital,
and Detroit Institute of Cancer Research, USA*

SYNOPSIS The neutrophilic alkaline phosphatase activity of 40 newborns was determined and found to be frequently raised, similarly to a leukaemoid reaction. The newborn neutrophil has strong alkaline phosphatase activity and is unlike the myeloid leukaemia cell which has no alkaline phosphatase activity.

We have recently been interested in investigating various enzyme activities in leukaemic cells. It has been established that neutrophils in chronic myeloid leukaemia have decreased or absent alkaline phosphatase activity (Kaplow, 1955; Wiltshaw and Maloney, 1955). Furthermore, studies have demonstrated that in neutrophils the alkaline phosphatase activity is raised in leukaemoid states due to various aetiologies, polycythaemia rubra vera, and in extra- and intrauterine pregnancy.

It is possible that the adult leukaemic myeloid cell may represent an immature cell such as is found in newborn infants. In this study, the problem was to ascertain if the newborn neutrophil possessed alkaline phosphatase activity.

METHODS AND MATERIALS

We studied 40 newborns (first week of life). The infants were equally divided into male and female and Caucasian and Negro groups. The Kaplow (1955) modification of the Gomori alkaline phosphatase method was utilized. The staining method was:

- 1 Fix air-dried peripheral blood smears in 95% alcohol for one minute.
- 2 Incubate for two hours at 37°C in barbital buffered medium containing beta-glycerophosphate, CaCl_2 and MgSO_4 at pH 9.2.
- 3 Wash in dilute CaCl_2 solution.
- 4 Immerse in 1.5% cobalt acetate for five minutes.
- 5 Wash in tap water.
- 6 Place in dilute ammonium sulphide for five minutes (1.5% NH_4S).

¹Present address: Clinical Laboratory, Stanford University Medical Center, Stanford, California 94305.

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- 7 Wash in tap water. Allow to dry.

- 8 Counterstain with Leishman's or Wright's stain.

For the scoring technique we counted 100 consecutive neutrophilic granulocytes, rating them from 0 to 4+ on the basis of the intensity and appearance of the precipitated dye in the cytoplasm as follows: 0 = colourless; 1+ = diffuse pale brown, no granules; 2+ = brown with or without occasional clumps of brown-black precipitate; 3+ = brownish-black, unevenly distributed granular precipitate; 4+ = uniform deep black granular precipitate.

The sum of the ratings of 100 cells is considered the 'score' for a particular smear. For purposes of comparison in scoring, select areas of similar cell thickness, preferably where the erythrocytes just touch.

Normal infants have given scores ranging from 0 to 76 with an average of 22. Infections give much higher values. In granulocytic leukaemia, the granulocytes are negative.

RESULTS

The results indicated that all newborns had neutrophilic alkaline phosphatase activity (Tables I and II). Female and Negro newborns had higher activity. Many had markedly raised activity similar to leukaemoid condition. Furthermore, two populations of neutrophils were seen in many patients. One group of cells showed moderate activity and the other groups none (Table III).

Those infants who were jaundiced, secondary to physiological jaundice or erythroblastosis foetalis, had scores in the lower range of normal.

DISCUSSION

This investigation has demonstrated that the newborn neutrophil has alkaline phosphatase activity.

TABLE I
FEMALE NEWBORNS

Negro	Caucasian
156	208
119	119
14	182
27	108
126	19
170	105
119	175
170	100
98	103
69	101

TABLE II
MALE NEWBORNS

Negro	Caucasian
24	134
92	30
98	90
60	89
86	11
125	64
170	29
38	159
131	83
146	50

TABLE III
PATIENTS' SCORES SUGGESTIVE OF TWO POPULATIONS OF NEUTROPHILS

Patient	Score					Total
	0	+	++	+++	++++	
F/W	34	36	32	0	0	100
F/W	31	35	34	0	0	103
F/N	19	8	74	0	0	156
F/N	8	14	78	0	0	170
F/N	41	22	38	0	0	98

In fact, a large number of newborns had alkaline phosphatase scores which are leukaemoid in type. However, in several newborns who had leukaemoid scores, two populations of neutrophils were delineated with this enzyme histochemical technique. One population had alkaline phosphatase activity and another had no activity as is present in chronic myelogenous leukaemia. The significance and cause for the existence of this population without alkaline phosphatase activity is not known. One might specu-

late that a proliferation of this type of cell is involved in chronic myelogenous leukaemia. Further studies are now in progress to assess further this finding utilizing a computer method which we recently employed to assess cellular populations in cases of megaloblastic anaemia and chronic lymphatic leukaemia (Albert, Powsner, Berman, and Wolf, 1966; Albert, Moore, Potter, Arnold, and Wolf, 1967).

The reason for the presence of the leukaemoid-type scores in a large proportion of the infants is not wholly apparent. It may be related to physiological leucocytosis or stress of birth.

The higher scores were found in infants who had traumatic births, *eg*, prolonged labour or umbilical cords around the neck at birth; circumcision led to leukaemoid scores. Jaundice caused a reduction in the leucocyte alkaline phosphatase. The presence of a raised plasma haemoglobin level, as in intravascular haemolysis, inhibits alkaline phosphatase of granulocytes. We are now evaluating a new indoxyl-alkaline phosphatase substrate (Wolf, Horwitz, Vazquez, and von der Muehll, 1969). This substrate was recently utilized for enzyme histochemistry of alkaline and acid phosphatase in tissues and sera (Wolf, Horwitz, Vazquez, Chua, Pak, and von der Muehll, 1967; Epstein, Wolf, Horwitz, and Zak, 1967).

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