The Morphology of Buffy Coat in Normal Human Adults

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EXAMINING peripheral blood for L.E. cells, we encountered cells which are not usually found in blood smears. We decided therefore to study the morphology of leukocyte concentrates of healthy adults.

R. Klima of Vienna has since 1949[3,4] used the above method to investigate peripheral blood morphology in various diseases. Although his material was vast, we could find little that concerned healthy people. We were also interested in semiquantitative evaluation of the unusual cells.

We examined buffy coats from peripheral blood of 55 normal adults, and surprisingly enough, in all we found atypical mononuclear cells and nuclear fragments of megakaryocytes and in the majority myelocytes and/or metamyelocytes (fig. 9).

Material and Methods

We examined leukocyte concentrates (buffy coat from peripheral blood) of 55 healthy adults, among them physicians, nurses and laboratory workers. There were 29 men and 26 women, their age varying from 17 to 45 years, the average being 28 years.

The leukocyte concentrates were prepared as follows (fig. 1): 10 ml. of venous blood were mixed with 0.1 ml. of heparin (5000 U. per milliliter). At the same time, a few drops of venous blood without heparin were used for the estimation of hemoglobin, leukocyte and thrombocyte counts, and standard blood smears. The heparinized blood was centrifuged for 15 minutes at 800 rpm, and then the plasma was discarded. The buffy coat was removed with a small amount of erythrocytes and transferred to Wintrobe's hematocrit tubes: the amount was sufficient for 2 to 3 tubes, depending on the number of leukocytes. The hematocrit tubes were centrifuged for 7 minutes at 1800 rpm; subsequently the plasma was removed with the help of a syringe with a long needle, and the remaining leukocyte concentrate was transferred to a watch glass in a moistened Petri dish. The leukocytes were well but gently mixed.

The leukocytes were counted in a pipet for erythrocytes after dilution of 1:200. Four hundred small squares were counted, and the results were multipled by 2000. Every count was repeated twice.

From 10 cu.mm. of leukocyte concentrate 5 smears were made and stained with May-Grünwald Giemsa.

We looked for nuclei of megakaryocytes with the magnification of 80 in all the five slides. Whole nuclei and nuclear fragments were counted, and the resulting number represented the amount in 10 cu.mm. of the concentrate. Three thousand granulocytes were counted with the 800 magnification, and the number of myelocytes and metamyelocytes was recorded. Two thousand mononuclear cells were counted and the number of atypical mononuclear cells registered.

The number of nuclei of megakaryocytes or their fragments was calculated in 1 ml. of blood according to their number in 10 cu.mm. of leukocyte concentrate. There was a fairly constant ratio in the leukocyte concentrates between the number of nuclear fragments of megakaryocytes and leukocytes. By extrapolation the number of megakaryocytes

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Submitted Sept. 8, 1959; accepted for publication Dec. 15, 1959.

in 1 ml. of blood was calculated. The ratio was examined between the megakaryocyte fragments and leukocytes in the blood which remained after removal of the buffy coat and was found to be identical with that in the buffy coat.

Results

In the smears made from buffy coats the distribution of cells was not always identical with that in the peripheral blood. We observed that similar cells tended to aggregate. It is therefore desirable to obtain uniform distribution of leukocytes and to avoid crowding of cells (fig. 2).

The results are summarized in tables 1 and 2.

Morphologic Observations

A number of blasts was among the cells observed by us and included in table 1. The majority of these were of the atypical mononuclear cell type; a few others could not be definitely classified; these were of medium size, with basophilic cytoplasm and a round nucleus with loose chromatin structure; in the nucleus 2 to 3 pale nucleoli were distinguished.

The so-called atypical mononuclear cells were similar to those found in infectious mononucleosis. Their protoplasm was strongly basophilic with a pale area near the nucleus. The nucleus was made of coarse chromatin aggregates, often the chromatin structure was loose and one pale nucleolus could be seen. Structurally those cells resembled lymphocytes or plasma cells (fig. 3).[11]

Mitoses were observed on three occasions, and only in atypical mononuclear cells; in two cases we saw leaf-shaped nuclei in those cells (fig. 4).

Lymphocytes in concentrates were similar to those seen in peripheral blood. In two cases we noticed numerous azurophilic, coarse granules in the cystoplasm; in one case a large inclusion body having the color of chromatin was found near the nucleus. In all our material only one tissue mast cell was seen. In five cases polyploidy of nuclei was seen: in three lymphocytes and one atypical mononuclear cell twin nuclei were present (fig. 5); and one neutrophilic granulocyte was present in the form of a macropolycyte (fig. 10).

The nuclei of megakaryocytes were of different size, usually round, occasionally with 2 to 4 lobules, often with small amount of pale, basophilic cytoplasm. In a few instances the nuclei were surrounded by normal platelets; nuclei without cytoplasm and without platelets were also encountered (figs. 6, 7 and 8).

Phagocytosis: in three cases we found "tart" cells, i.e., mononuclear cells which ingested granulocytes. The structure of the phagocytised cells was clearly distinguished. In 11 cases phagocytosis of platelets was seen, usually by monocytes, only in two instances by granulocytes.

Degenerative changes: degeneration of nuclear chromatin was observed in 18 cases; twice in lymphocytes, once in monocyte and nine times in granulocytes. Vacuoles were seen frequently in monocytes, and sometimes platelets were found in large vacuoles, occasionally homogenous acidophilic inclusions. In six cases we noted large vacuoles in neutrophils, twice in eosinophils and once in megakaryocyte.

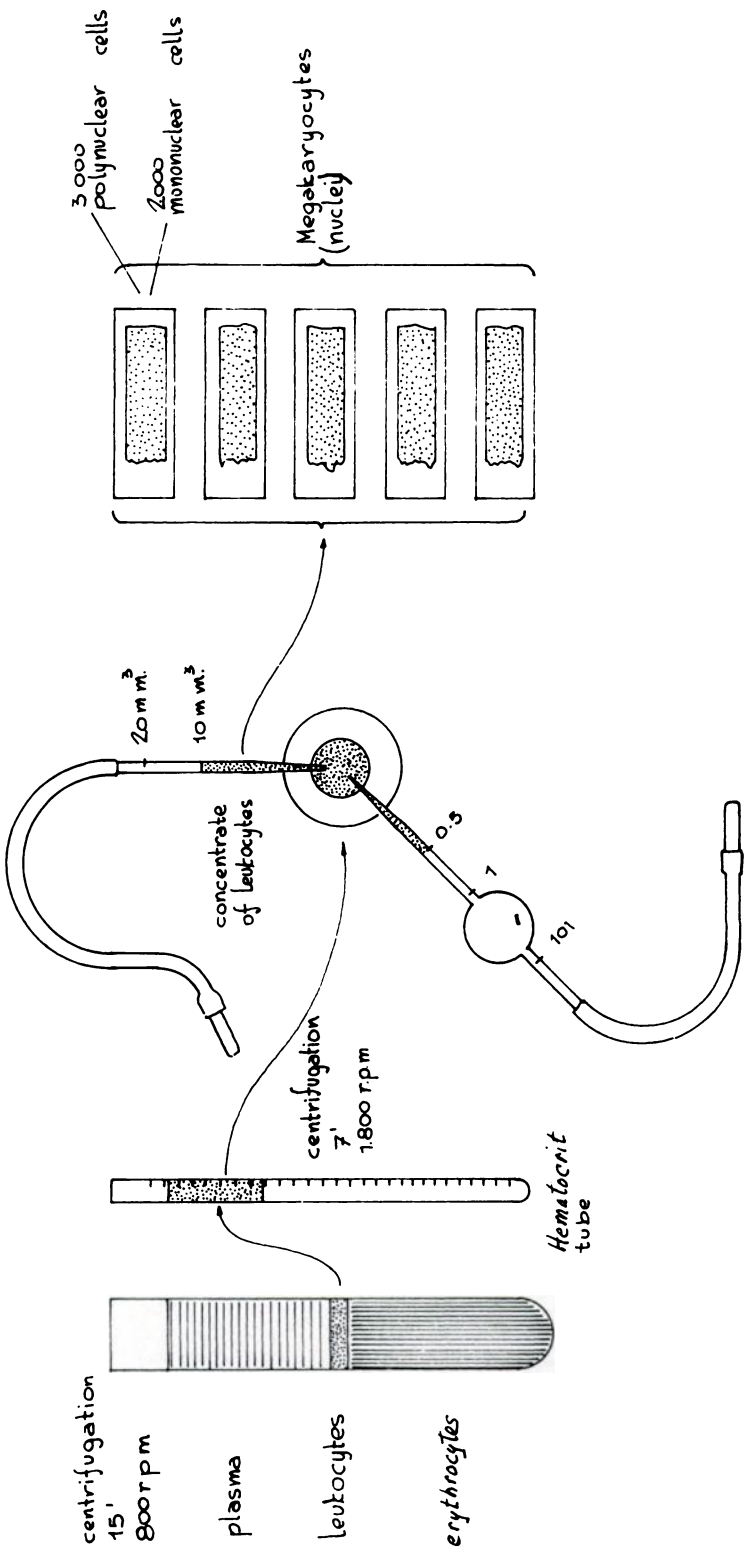
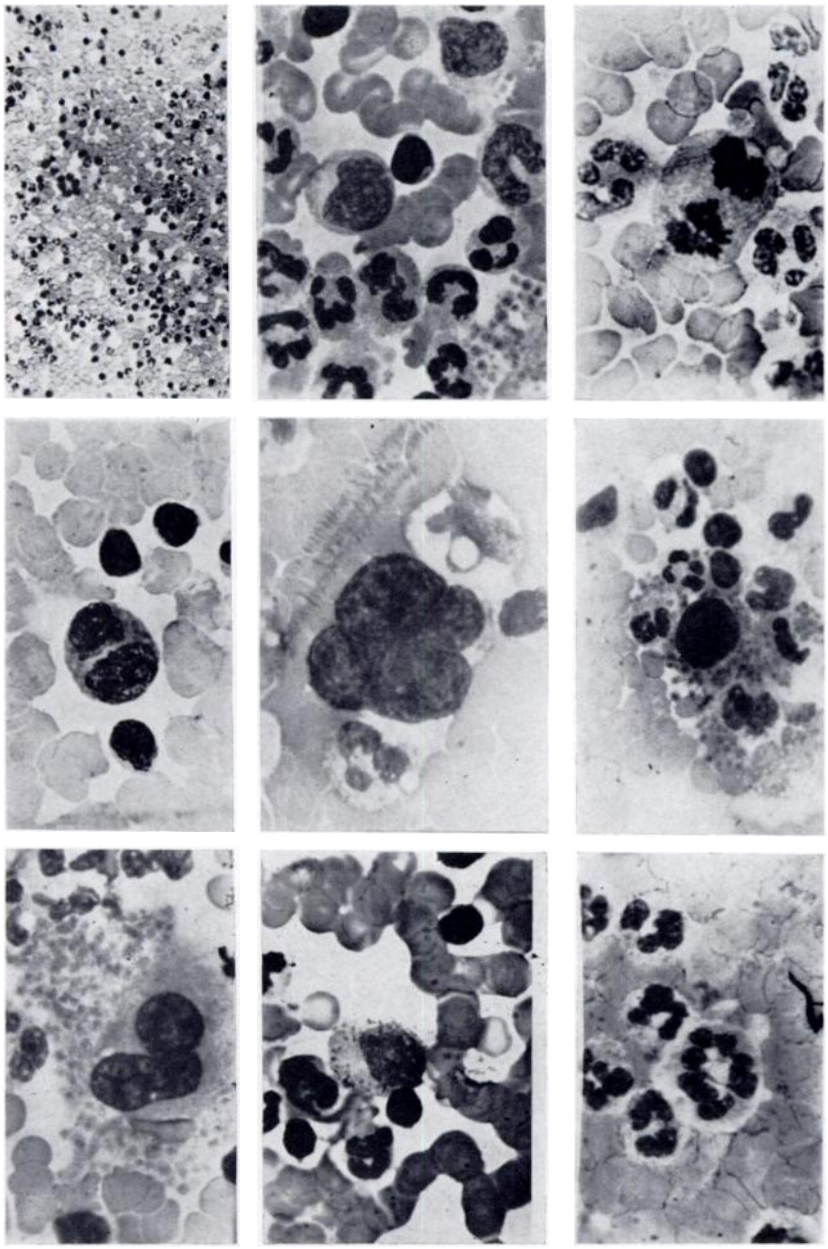


Fig. 1.—Technic of preparation of leukocyte concentrate.



Figures 2–10

FIG. 2 *(top, left)*.—Leukocyte concentrate—general view. (Magnification 128×) FIG. 3 *(top, center)*.—Atypical mononuclear cell, lymphocytes, monocyte and granulocytes. FIG. 4 *(top, right)*.—Mononuclear cell in mitosis. FIG. 5 *(middle, left)*.—Binucleated atypical mononuclear cell. FIG. 6 *(middle, center)*.—Nucleus of megakaryocyte. FIG. 7 *(middle, right)*.—Nuclear fragment of megakaryocyte surrounded by platelets. FIG. 8 *(bottom, left)*.—Megakaryocyte with rest of cytoplasm. FIG. 9 *(bottom, center)*.—Neutrophil myelocyte. FIG. 10 *(bottom, right)*.—Neutrophil "macropolycyte" and mature granulocytes.

Table 1

Case no.	Leucocytes per cu.mm. of blood, (10^3)	Leucocytes per cu.mm. of concentrate (buffy coat) (10^3)	Megakaryocyte nuclear fragments per 10 cu.mm. of concentrate	Megakaryocyte nuclear fragments per 1 ml. of blood	Myelocytes and meta-myelocytes per 3000 granulocytes	Atypical mononuclear cells per 2000 mononuclears
1 m	8.9	528	20	33.9	2	10
2 m	8.05	448	29	51.8	10	6
3 m	4.5	360	11	8.5	4	18
4 m	6.4	410	18	28.1	13	8
5 f	13.5	490	4	11.1	0	11
6 m	4.0	302	20	26.7	1	8
7 m	6.1	460	27	36	8	4
8 m	5.0	286	3	5.3	1	8
9 f	4.1	310	8	10.5	0	10
10 f	5.2	344	4	6.1	1	14
11 m	7.4	326	13	24.1	7	10
12 m	7.45	302	12	29.3	9	10
13 m	6.1	448	25	34.2	2	8
14 f	6.3	593	7	7.4	–	–
15 m	9.6	482	22	44	1	6
16 m	9.9	509	12	23.5	8	12
17 m	9.3	324	20	57.1	8	11
18 m	4.4	260	49	83.1	2	2
19 f	6.7	442	2	3	0	11
20 m	7.5	446	14	23.7	18	14
21 m	9.7	388	10	25	1	12
22 f	4.85	380	11	14.1	7	3
23 f	5.0	301	2	3.3	0	10
24 f	6.1	337	5	9.1	2	10
25 f	7.9	330	14	26.2	3	14
26 m	7.3	230	4	12.5	9	9
27 m	10.0	452	13	28.9	2	36
28 f	9.7	460	8	17	0	11
29 f	5.9	360	3	4.9	1	14
30 f	6.1	356	4	6.9	0	9
31 f	4.1	414	4	4	1	12
32 f	5.5	312	4	7	1	13
33 f	7.2	381	18	34	2	8
34 f	7.4	609	6	7.3	–	–
35 f	4.5	295	9	13.7	1	11
36 m	4.2	362	45	52.3	1	13
37 m	4.85	490	4	3.9	1	9
38 m	6.4	353	23	41.8	6	16
39 m	4.4	353	6	7.5	6	6
40 f	8.0	427	2	3.8	3	12
41 f	5.65	334	15	25.4	0	5
42 f	4.0	408	22	21.6	2	9
43 f	5.0	317	11	17.3	0	13
44 f	5.2	421	8	9.9	7	9
45 f	4.9	370	20	26.5	2	10
46 f	4.4	326	18	24.3	0	10
47 m	12.9	440	15	41	6	6
48 m	4.9	300	9	14.7	0	12
49 f	8.45	370	25	57.1	10	10
50 m	5.35	292	4	7.3	–	–
51 m	5.2	394	12	15.4	3	6
52 m	6.8	350	14	27.2	4	5
53 m	5.5	390	7	9.9	7	4
54 m	4.5	260	11	19	5	15
55 f	5.0	400	7	8.7	2	10

Table 2

	Mean value (men and women)	Mean value (in men)	Mean value (in women)	Difference between men and women	*t* value
Megakaryocytes, nuclei (in 1 ml. of blood)	21.79 ± 17.27	28 ± 17.6	14.6 ± 12.1	13.6 ± 4.04	3.3
Myelocytes and/or metamyelocytes (per 3000 granulocytes)	3.65 ± 3.9	5.2 ± 4.3	1.9 ± 2.6	3.3 ± 0.96	3.4
Atypical mononuclear cells (per 2000 mononuclear cells)	10.2 ± 4.8	10.1 ± 6.3	10.3 ± 2.6	0.2 ± 1.3	0.15

Discussion

Various authors described immature granulocytes and nuclei of megakaryocytes in peripheral blood.[5,6] Their presence was confirmed by Klima[3,4] in his studies of leukocyte concentrates. The investigations carried out to the present have dealt with pathologic conditions, mainly myeloproliferative disorders with extramedullary hemopoiesis. It was generally believed that nuclei of megakaryocytes were normally unable to pass through pulmonary capillaries, and in various pathologic states they were found in large numbers in lungs.[5,9] They were, however, observed in glomerular capillaries in healthy persons.[1]

Examination of leukocyte concentrates has been carried out lately in search for L.E. cells, tumor cells,[6-8] in the diagnosis of various blood disorders, megaloblastic anemias,[2,4,5] extramedullary hemopoiesis and leukemic conditions. It is advisable, therefore, to acquaint oneself with normal morphology of leukocyte concentrates in order to distinguish between the normal and the pathologic elements.

Of special interest in our present work is the finding of megakaryocyte nuclei or their fragments in all healthy subjects under investigation.

In 42 of 52 subjects examined we found metamyelocytes and/or myelocytes, their percentage in peripheral blood being 1/1000 granulocytes.

We would like to stress the point that the so-called atypical mononuclear cells were found in all subjects. Their number was small, 5/1000 mononuclear cells including lymphocytes. These cells have been considered characteristic of viral infections; they were also observed in spirillosis and rickettsiosis. Our findings throw some doubt as to their pathologic significance. Moreover, our recent studies revealed those cells in cord blood of newborn infants who had no obvious infection. We believe, therefore, that their status should be reconsidered; they are probably normal although unusual elements of peripheral blood, whose number tends to increase in certain diseases.

The difference in number of megakaryocyte nuclei, myelocytes and metamyelocytes found in men and women was statistically significant. However, the standard deviation was too high in comparison with the mean value and a larger series of samples is necessary before definite conclusions can be drawn.

The one tissue mast cell we found in all the slides does not seem to us of any importance. It might have been aspirated into the needle during the puncture of vein from subcutaneous tissue.

The degenerative changes observed in a few cases were probably artifacts produced during preparations of smears.

Summary and Conclusions

The technic of preparation of smears from buffy coats was described.

Fifty-five samples of buffy coat from healthy adults were examined. Twenty-nine were from men, 26 from women.

In all cases nuclear fragments of megakaryocytes were found, on the average 21.8 nuclear fragments per 1 ml. of blood.

In all cases atypical mononuclear cells, 10.2/2000 mononuclear cells, were found.

In 42 of 52 examined subjects metamyelocytes and/or myelocytes were found, 3.65/3000 granulocytes.

Metamyelocytes, myelocytes and nuclear fragments of megakaryocytes were more commonly found in males than in females.

So-called atypical mononuclear cells were found in small number in all the subjects. Their possible pathologic significance was discussed. It was suggested that these cells were probably normal though rare elements of peripheral blood.

The advantage of this method in various pathologic states was emphasized.

Summario in Interlingua

Es describite le technica del preparation de frottis ab coagulo blanc.

Esseva examinate 55 specimens de coagulo blanc prendite ab adultos in bon sanitate. Vinti-novem veniva ab homines e 26 ab feminas.

In omne casos, fragmentos de megacaryocytos esseva trovate—al media 21,8 fragmentos nuclear per 1 ml de sanguine.

In omne casos, atypic cellulas mononuclear—10,2 per 2000 cellulas mononuclear—esseva trovate.

In 42 del 52 subjectos examinate, metamyelocytos e/o myelocytos—3,65 per 3000 granulocytos—esseva trovate.

Metamyelocytos, myelocytos, e fragmentos nuclear de megacaryocytos esseva trovate plus frequentemente in masculos que in femininas.

Le si-nominate atypic cellulas mononuclear esseva trovate in micre numeros in omne le subjectos. Le possible signification pathologic de iste cellulas es discutite. Il es suggerite que iste cellulas es probabilemente elementos normal, ben que rar, del sanguine peripheric.

Le avantages del application de iste methodo in varie statos pathologic es sublineate.

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A NEW TECHNIQUE FOR THE HISTOCHEMICAL STUDY OF SMEARS. *M. S .Burstone and I. J. Flemming.* From National Institute of Dental Research, Bethesda, Md. J.Histochem.& Cytochem. *7*:203, 1959.

This report deals with a new technic for the study of blood films and other types of smears with an emphasis on the application of enzyme technics. In many instances routine smears on glass slides exhibit poor adherence, and this may necessitate fixation or coating with a collodion film. Such procedures may inactivate enzyme systems or may prevent substrate from penetrating to the smear. The new technic utilizes a polyester (polyethylene terephthalate) plastic film to which cells readily adhere.—*O. P. J.*