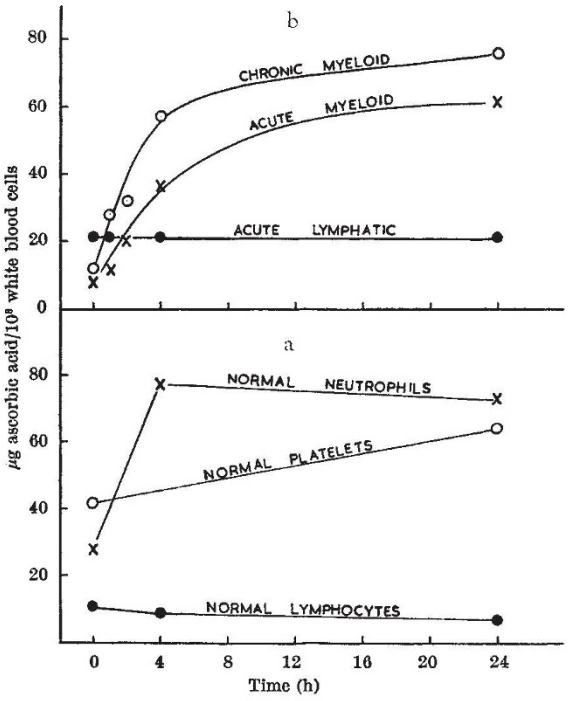

Fig. 2. *a*, Uptake of ascorbic acid as in Fig. 1 by neutrophils, lymphocytes and platelets. The levels for platelets are shown as $\mu g/5 \times 10^9$ platelets. *b*, Uptake of ascorbic acid as in Fig. 1 by leukæmic cells

The fractions were suspended in diluent plus ascorbic acid at a final concentration of 2·5 mg/100 ml. and the uptake at 4° C after 4 and 24 h determined. The results are shown in Fig. 2*a*. The increases in ascorbic acid content of the neutrophils after exposure to diluent plus ascorbic acid are less than would be anticipated in comparison with the normals of Fig. 1. This may be due to cell damage occurring during the process of separation. The lymphocytes, which appeared morphologically intact after the separation, showed no uptake of ascorbic acid.

The white blood cell ascorbic acid levels and uptake of leukæmic cells at 4° C are shown in Fig. 2*b*. In each case, the amount of blood added to diluent was adjusted so that the ratio of cells to diluent was comparable with that in the normals. The results support the findings of Waldo and Zipf[3], who obtained low levels of plasma ascorbic acid in leukæmia and other conditions involving blood-forming organs. Fig. 2*b* also shows that immature granulocytes take up ascorbic acid, but the data are too few for relative assessment. It is conceivable that this is an apparent result, and that a higher uptake is masked by a greater utilization of ascorbic acid by immature cells. Abnormal cells of the lymphocyte series in common with the normal lymphocytes of Fig. 2*a* showed no uptake of ascorbic acid.

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Free Amino-Compounds in Blood Platelets

Platelets contain 0·31–0·39 mg nitrogen per 10^9 platelets. Of this, 83–92 per cent is protein nitrogen[1]. Although platelets are the main source of histamine and serotonin (5-hydroxytryptamine) in blood[2], nothing is known of the free amino-acids therein. It was thus of interest to determine the total free amino-acid content of platelets and to identify individual components.

Total α-amino nitrogen: The concentrations in platelets from normal individuals and certain patients, some with platelet anomalies, are given in Table 1.

Table 1. Total Free Amino-Nitrogen in Platelets

Condition	No. of cases	Platelet count/mm³ (10^3)	Total amino-nitrogen ($\mu g/10^9$ platelets)
Thrombocytopenia			
No operation*	2	90–170	9·1–16·0
Post-splenectomy	6	275–620	4·9–8·2
Allergic purpura	4	350–525	5·1–5·7
Thrombocythæmia	1	1,200	10
Polycythæmia	2	276–672	6·4–9·0
Haemochromatosis	2	210–240	5·4–9·0
Normal individuals	5	220–280	5·8–9·2

* Treated with steroids.

Identification of amino-acids: Using two-dimension paper chromatography, four amino-acids, namely, aspartic and glutamic acids, serine and glycine, were identified as well as a peptide with R_F 0·55–0·60 in phenol and 0·10–0·12 in butanol–acetic acid. After acid hydrolysis, alanine, tyrosine and leucine(s) appeared and the spots produced by glycine and serine showed increased density. This distribution would appear to be specific, being different from that in plasma[3] and in normal and leukæmic white blood cells[4].

The possible identification by paper chromatography of serotonin and histamine in appropriate extracts of platelets was also investigated. Table 2 shows the R_F values of these substances in 3 solvents. To detect these substances, however, aliquots containing at least 10 μg nitrogen were required. Since 10^9 platelets contain less than 0·1 μg histamine and 0·2–0·4 μg serotonin[2] no corresponding spots could be identified in platelet extracts with a total amino-nitrogen of 45 μg.

Table 2. R_F Values of Histamine and Serotonin

Compound	Solvent		
	Phenol	Butanol–acetic acid	77% ethanol
Histamine	1*	0·16	0·7
Serotonin	0·96	0·58	0·8

* Moving with solvent.

Effect of glass contact: All the above investigations were carried out in siliconed glass. Further experiments demonstrated that using untreated glass for washing platelets before extraction resulted in an appreciable loss of amino-acids, the peptide being the only compound detected on a chromatogram.

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