tube, so that all the material is not deposited at the tip. The BEEM Company has modified the design of the tube, however, to eliminate this problem. They have produced experimental lots of tubes which have no facets, and which "neck-down" to either a pointed or a flat tip. We have found that the pointed tip is more desirable for pelleting small amounts of cell-free virus, whereas the flat tip is more desirable for cells. All the particulate material appears to be deposited at the tips of these tubes.

Summary. A method is described which permits cell-free virus or virus-infected cells to be pelleted into the tips of polyethylene tubes in such a way that the pellets can be fixed, plastic-embedded and sectioned *in* place. This permits successful sectioning of pellets so small that they can not be visualized with the unaided eye. Thus thin-section electron microscopy is possible on specimens containing small concentrations of virus or virus-infected cells.

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Platelet Alkaline Phosphatase: Normal Values and Variations in Myeloproliferative Syndromes* (33732)

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Leukocyte alkaline phosphatase (LAP) activity is well established as an aid in the diagnosis of the various myeloproliferative disorders. Measurements of leukocyte alkaline phosphatase have shown elevations in polycythemia vera and infectious leukocytosis, low values in chronic granulocytic leukemia, and variable value in agnogenic myeloid metaplasia and essential thrombocythemia (1, 2). Alkaline phosphatase activity has been measured not only in leukocytes but also in platelets (3, 4). This fact, coupled with the observations of both qualitative and quantitative platelet abnormalities in the various diseases making up the "Myeloproliferative Syndrome," led to our research. The purpose of the present work was to determine whether or not other myeloid cells responded to the same control mechanism which determines the alkaline phosphatase activity of leukocytes. This was tested by measuring alkaline phosphatase activity of leukocytes and platelets simultaneously in conditions in which abnormalities of leukocyte alkaline phosphatase are known to occur. If a single mechanism affected the whole myeloid series one would expect to see similar changes in the activity of alkaline phosphatase in platelets as in white blood cells.

Methods. Whole blood (50–100 ml) was collected from 15 normal controls and from 11 diseased patients using 0.1 vol of 2% disodium ethylenediaminetetraacetate as an anticoagulant. All procedures were carried out at 4°. The plasma was separated by centrifugation at 105*g* for 15 min. The plasma was removed and cell counts were performed. Of the remaining white blood cells, 90–95% were lymphocytes, and a few were eosinophiles. If, however, the white blood cell count was above 5000/mm^3, or more than 10% granulocytes were found, the plasma was respun for an additional 10 min. Platelet counts were done by a phase method (5). The number of platelets per sample varied from 155,000 to 1,795,000 (mean 498,000)/mm^3.

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TABLE I. Leukocyte Alkaline Phosphatase (LAP) and Platelet Alkaline Phosphatase (PAP) Activity in Normal and Diseased Subjects.

Patient	Diagnosis	LAP (score) (normal 10–90)	PAP (μmoles of P_i/hr/10^9 platelets)
1	Normal control	13	0.048
2		19	0.049
3		31	0.041
4		20	0.048
5		56	0.051
6		23	0.022
7		48	0.039
8		56	0.053
9		—	0.047
10		17	0.055
11		12	0.021
12		58	0.034
13		85	0.041
14		12	0.041
15		15	0.035
			(Mean 0.042 ± SD 0.010)
1	Pneumococcal pneumonia	224	0.039
2	Pneumococcal pneumonia	180	0.044
3	Pneumococcal meningitis	190	0.050
1	Myeloproliferative syndrome (myelofibrosis)	96	0.022
2	Myeloproliferative syndrome (myelofibrosis)	113	0.017
3	Myeloproliferative syndrome (not classified)	0	0.046
4	Myeloproliferative syndrome (not classified)	20	0.009
5	Chronic myelogenous leukemia	6	0.113
6	Chronic myelogenous leukemia	3	0.027
7	Polycythemia vera on Myleran	190	0.631
8	Polycythemia vera after P^{32}	259	0.016

The plasma was then centrifuged at 3200*g* for 20 min. The resulting cell button was resuspended in normal saline. This suspension had a concentration of 1.3–14.78 (mean 4.56) $\times$ 10^9 platelets/0.3 mm^3. The suspension was homogenized in a VirTis "45" homogenizer at setting 55 for 15 sec. The homogenate was treated by the method described by Valentine and Beck (6).. Three-tenths ml of homogenate was incubated for 1 hr at 37° with 9.0 ml of sodium β-glycerophosphate (0.026 *M*) buffered to pH 9.9, 0.5 ml of saponin solution (2%), and 0.2 ml of $MgCl_2$ solution (0.050 *M*). Two ml of trichloroacetic acid (30%) was added to end the reaction. The precipitate was removed by centrifugation and the inorganic phosphorus of an aliquot of the supernatant was measured by the method of Gomori(7). Color determinations were made on a Beckman DU spectrophotometer. Control experiments showed the reaction rate to vary in a linear manner with varying number of platelets in the range of platelet concentration employed.

The LAP scores were performed on fresh peripheral blood smears using Kaplow's azo dye coupling technique (8, 9).

Results. The platelet alkaline phosphatase (PAP) measurements in the 15 normal controls (Table I) ranged from 0.021–0.055 μmole of P_i hr 10^9 platelets with a mean value of 0.042 ± SD 0.010 μmole of P_i/hr/10^9 platelets. The LAP values of the controls were all within the normal (10–90) range. The results of the determinations made on the patients with disease are also listed in Table I. Three patients were tested who had severe infections. Their LAP values were quite high. Values for PAP were normal, however.

Eight patients with a variety of myeloproliferative syndromes were tested. In three it was difficult to specifically categorize the syndrome but all had evidence of panmyelopathy. The LAP values were abnormal in all but one, varying from very high to very low. The PAP values were likewise quite abnormal with 7 of the 8 values being more than 2 standard deviations from the mean. They, too, varied either from very low to very high.

Discussion. The results presented demonstrate that alkaline phosphatase can be readily measured in platelets and that in normal patients the results vary relatively little. Values for PAP in infectious states are normal despite large increases in LAP. Values for PAP in patients with myeloproliferative syndromes vary from very low to very high. Values for LAP in these patients also vary from very low to very high, but LAP and PAP vary independently in individuals.

This independent variation is of particular interest in terms of enzyme control mechanisms in the myeloid cells. Although the megakarocyte and the myeloblast may arise from a common myeloid stem cell, these data show that the mechanisms of control of alkaline phosphatase differ in at least these two of the myeloid cells types. The changes in enzyme activity could be due to changes in structural or regulator genes, or control of enzyme activity could be altered at the cytoplasmic level. Changes in LAP isozymes have been shown in some myeloproliferative disorders(10,11.) It would be interesting to know whether electrophoretic heterogeneity of platelet alkaline phosphatase occurs and whether changes in their relative proportions account for some of the alterations reported here.

Summary. Platelets were collected from healthy and diseased subjects and platelet alkaline phosphatase (PAP) was measured and compared to the patient's leukocyte alkaline phosphatase (LAP). Seven out of eight patients with a myeloproliferative disease had PAP levels varying from normal by more than 2 standard deviations. Seven out of the eight patients also had an abnormal LAP, however, the LAP and PAP varied independently. Three out of the three patients with an infectious leukocytosis had a normal PAP while the LAP was elevated. This strongly suggests that there is no single mechanism for control of alkaline phosphatase in myeloid cells.

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