Cytochemical Demonstration of "Acid" Phosphatase in Bone Marrow Smears

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Since the statement of Gomori (*3*) that blood cells gave a negative reaction for the "acid" phosphatase, to our knowledge no other report has been published on this subject.

Using Gomori's technique we have regularly demonstrated the presence of "acid" phosphatase in human and animal bone-marrow smears. These were made on cover slips, dried in air, fixed in chilled acetone for 30 sec, rinsed in distilled water, and incubated at 38° C for

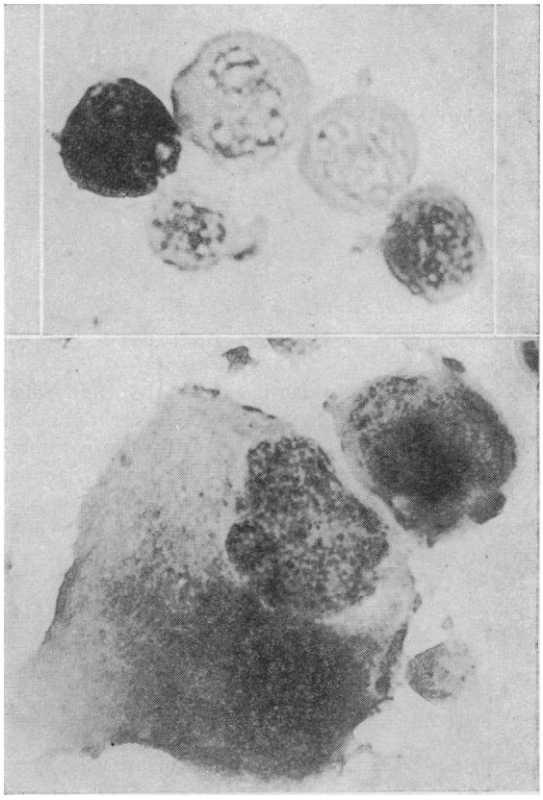

FIG. 1. All cells stained by the method described. About 1,600 diameters. *Above,* from left to right: an eosinophile, an erythroblast, two neutrophile "stabs," and an unidentified cell. *Below,* left, a mature, and right, a young megakaryocyte.

6–10 hrs in the following medium: 5 parts distilled water, 3 parts acetate buffer at pH 4.0, 1 part 2% sodium glycerophosphate (52% alfa, May & Baker), and 1 part 2% lead nitrate. Incubation mixtures at pH 3.5 and 4.5 consistently gave the same results. At pH 4.0, very little cloudiness (probably from the lead hydroxide formed) was noted.

Control cover slips were incubated in the same mixture, to which 2 mg of NaF was added (final concentration about M/300). After incubation the cover slips were washed in running tap water for 2 min, immersed in dilute yellow ammonium sulfide for 2 min, rinsed in water, dried, and mounted in Canada balsam. Upon examination, the sites of phosphatase activity were brown-black and the control cover slips entirely negative. Attempts to obtain controls using other substances as inhibitors were, unfortunately, not successful. Ethyl alcohol (as a fixative or in the substrate solution), formol (as a fixative), sodium molybdate, and sodium cyanide did not interfere with the phosphatase activity. A perusal of the literature revealed that inhibitors *in vitro* and conditions of inhibition of "acid" phosphatase vary greatly with the organ studied. Thus, "acid" phosphatase of the prostate gland is not inhibited by formol, whereas the reverse is true for the enzyme of red blood cells (*1*). Although realizing that chemical results cannot be extended *in toto* to cytochemical techniques, we feel that these facts can explain our failure to obtain inactivation by means of chemical agents other than fluoride. Colchicine, urethane, and pteroylglutamic acid did not affect the reaction in normal and pernicious anemia marrow. Bone-marrow smears obtained by sternal puncture from three subjects without hematological disorders and from one case of Addisonian pernicious anemia, one case of chronic myeloid leucemia, and two cases of lymphoid leucemia were studied. The results were constant, although in the same smear there were regions which were more intensely stained than others.

In the normal marrows the eosinophiles gave a strong reaction on the granules, sometimes masking the nucleus. The nonspecific granules of the myeloblasts stained less intensely. Neutrophile granules were negative.

No basophiles were found in the marrow material, but in rat's mesentery we confirmed the work of Montagna and Noback (*4*). The nuclei of the myeloblasts were nearly negative but appeared to have a distinct filamentous aspect in more mature elements of the white cell series.

The cytoplasm of the red cell series stained poorly, but the nuclear structure appeared distinctly more granulous than filamentous. More mature elements showed a condensation of the nuclear structure. Red blood cells were entirely negative. Megakaryocytic nuclei stained heavily with a coarse, reticulated pattern. The cytoplasm gave a weak reaction, but a heavily stained juxtanuclear zone was frequently observed. Platelets were negative, as were nucleoli. The general pattern of the nuclei is not comparable to that obtained by the Feulgen method, iron hematoxylin stains, or the reaction for alkaline phosphatase. White and red cell series, in the case of chronic myeloid leucemia, gave reactions strictly comparable with those of the normal marrows. Lymphoid leucemia calls gave a very faint reaction in the nucleus and cytoplasm. Megaloblastic series in the pernicious anemia marrow did not present quantitative differences when compared with the normoblastic series. Chicken

and rat bone-marrow smears showed essentially the same reaction even after 16–18 hrs of incubation. The reaction was found exclusively in the nucleus of chicken red blood cells. This is in accordance with Dounce and Seibel's work (*2*).

A more detailed account will be published in the future.

References

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IN THE LABORATORY

A Simple Tangent Meter

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One is in frequent need of an instrument for the determination of the angle which a tangent to a curve at a given point will make with the coordinate axis. Apparently there is no instrument designed for this purpose on the market.

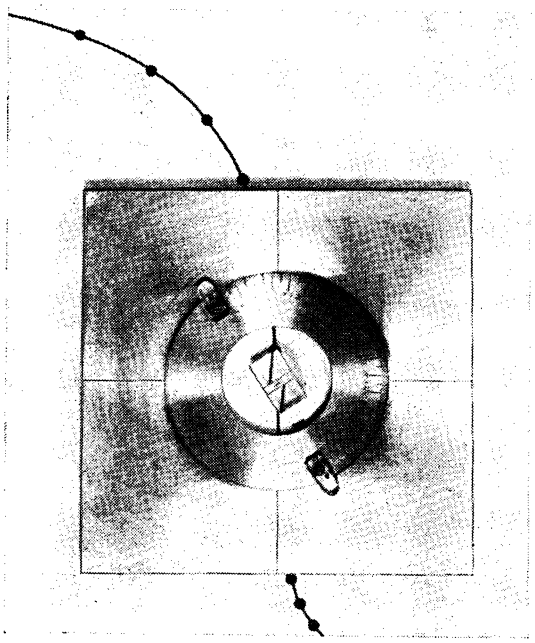

Fig. 1

The simple instrument shown in Fig. 1 has been found useful in determining the slopes of curves at particular points. This tangent meter is not as simple in construction as the one described by Latshaw (*1*), but it does have the advantage of permitting one to read the tangent angle directly. In the measurement of the angle the instrument is placed over the curve with the center of the prism directly over the point of interest, and, with the help of a T-square, it is held firmly in place with the edges of the meter parallel to the coordinate axis. The ring is then rotated by means of the two knobs until the lines in the prism intersect to give a continuous line. The angle is then read from the scale.

The drawing shown in Fig. 2 is to scale. The instrument was built from $\frac{3}{8}$″ aluminum plate, and the window upon which the prism is placed is of Lucite, which was pressed into the ring. The triangular prism is glued to

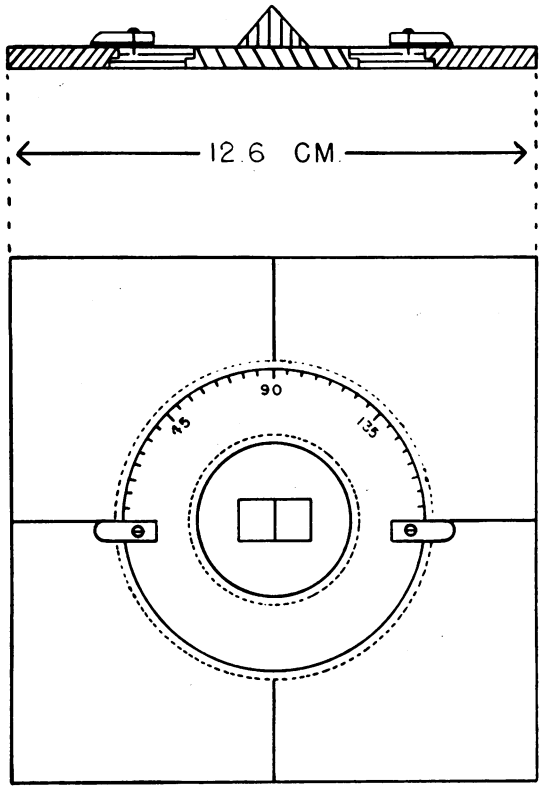

Fig. 2

the Lucite with Canada balsam, and the prism is centered so that the hairlines on the rectangular plate intersect in the center of the prism. When the curve is drawn on coordinate paper, the hairlines are of considerable help in centering the tangent meter.

This simple device may be modified in several particulars. Figures for the angle could be placed on the sta-