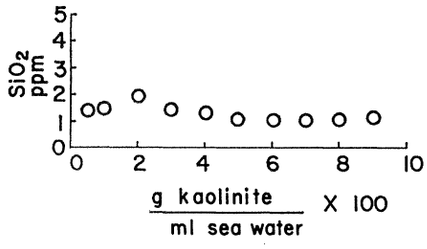

Fig. 2. Silica concentration in sea water supernatants of various weight-percent suspensions of < 62-μ kaolinite at room temperature after 196 hours. Concentrations are corrected for silica content of control. Suspensions were stirred vigorously twice daily to increase the rate of silica release.

the original sea water and the final reading on the sea-water control.

Any detailed interpretation of our results is not warranted at this time, but some speculation and tentative conclusions may be useful. The similarity of the silica values obtained from kaolinite, illite, muscovite, and chlorite indicate incongruent solution, with the formation of an aluminous residue, perhaps an aluminum oxide hydrate. Carroll and Starkey (*3*) showed that silica is removed preferentially to alumina from silicates reacted with sea water, and aluminum oxide hydrates are virtually insoluble at the *p*H of our experiments. Waters draining a mixture of bauxitic and kaolinitic minerals in the Lower Congo (*4*) contain 2 to 4 ppm SiO_2, and Kittrick (*5*) determined the equilibrium silica concentration for coexistence of well-crystallized kaolinite and gibbsite at room temperature as 1 to 2 ppm. The independence of sample size and the silica values obtained by reaction of kaolinite (Fig. 2) also are suggestive of attainment of an equilibrium. On the other hand, the much higher silica values obtained from the montmorillonites suggest that their incongruent solution produces some substance other than an aluminum oxide hydrate—perhaps some ill-defined aluminosilicate. Whitehouse and McCarter (*6*) observed that montmorillonitic material was transformed to illitic and chloritic clay minerals after reacting with a limited volume of sea water for periods of up to 5 years. Perhaps the results we have obtained represent the first stages of a similar transformation, although our work is complicated by the presence of other phases in the montmorillonites used. We have no explanation for the consistent *p*H values at the end of 6 months; x-ray examination showed that no new crystalline phases were formed during immersion of the silicates in sea water.

The major conclusion we wish to draw is that oceanic chemistry is strongly influenced by the reactions of the great tonnages of detrital materials, primarily silicates, that are carried into the marine environment by streams. These same silicates rapidly release silica to sea water low in silica, and the concentrations reached are of the same order of magnitude as the average silica concentration of the oceans. Thus by releasing and, presumably, combining with silica, silicates exert a major control on the silica concentration in the oceans, and probably on other chemical species (*7*).

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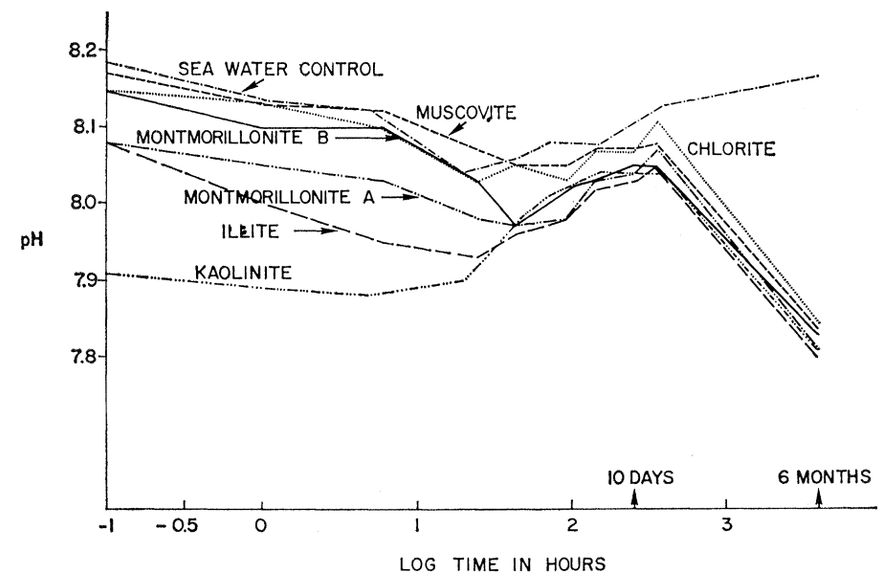

Fig. 3. Changes in *p*H as a function of time for suspensions of silicate minerals and for sea water control.

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Leukocyte Alkaline Phosphatase: Electrophoretic Variants Associated with Chronic Myelogenous Leukemia

Abstract. *Starch-gel electrophoresis of leukocyte alkaline phosphatases, rendered soluble by treating normal leukocytes with butanol, revealed three electrophoretic variants of the enzyme. The phosphatases in similarly prepared extracts of leukemia cells differed from the normal isozymes in electrophoretic mobility. A single variant was detected in one case of untreated leukemia; a similar component and three additional ones were seen in leukemia treated with 6-mercaptopurine.*

Much of the interest in the alkaline phosphatase activity of neutrophil granulocytes centers around the speculation that the locus of the gene which directs the formation of the enzyme is on chromosome 21 (*1*). This view has its primary support in observations made in chronic myelogenous leukemia and in Down's syndrome. Leukocyte alkaline phosphatase activity (*2*) is decreased in chronic myelogenous leukemia. Furthermore,

cultures of the leukemia leukocytes frequently reveal an abnormally small chromosome, the Philadelphia chromosome. This chromosome is probably derived by partial deletion from one of the small acrocentrics of the 21 group. Conversely, an elevated activity of leukocyte alkaline phosphatase and trisomy of chromosome 21 are characteristics of Down's syndrome. These observations have led to the suggestion of a "gene-dose" effect—that is, a relationship between the quantity of chromosome 21 genic material and the quantity of gene product, alkaline phosphatase. Although the cited associations are well established, it should be noted that the activity of the leukocyte alkaline phosphatase can be influenced by a number of factors. Also, the Philadelphia chromosome is not an invariable concomitant of chronic myelogenous leukemia.

Our work was undertaken to determine whether the phosphatases of leukemia cells are also qualitatively different from the normal.

Leukocytes were isolated from 20 ml of blood by a method similar in essentials to that described by Peacock *et al.* (*3*). The cells were washed three times in 0.9 percent NaCl and suspended in 5 ml of an ice-cold dilution (1:20) of the tris–alanine–citric acid buffer, *p*H 9.5, recommended by Boyer (*4*) as an "activating" buffer for phosphatases. The suspension was homogenized in a 10-ml tissue grinder (*5*) operating at approximately 900 rev/min for 60 seconds (20 complete up-and-down movements of the vessel). Two milliliters of *n*-butanol at 0°C were then added drop by drop, the homogenization being continued during the addition. The mixture was heated at 37°C with constant stirring for 5 minutes, cooled by stirring in an ice bath for 5 minutes, and centrifuged at 20,000*g* for 20 minutes at 4°C. Centrifugation separated the mixture into an upper butanol layer, an intermediate layer of denatured proteins, and a lower aqueous layer containing the phosphatases. The lower layer was aspirated with a syringe and needle, dialyzed overnight in 2 liters of the diluted (1:20) activating buffer, centrifuged to remove a small amount of insoluble material, and finally concentrated to approximately 0.3 ml by ultrafiltration (*6*).

Horizontal starch-gel electrophoresis (*7*) was used to separate the phosphatase components. A potential gradient of 8 volt/cm was applied until the brown "borate line" had migrated 8 cm beyond the insertion sites. The sliced gels were then stained for phosphatases (*8*).

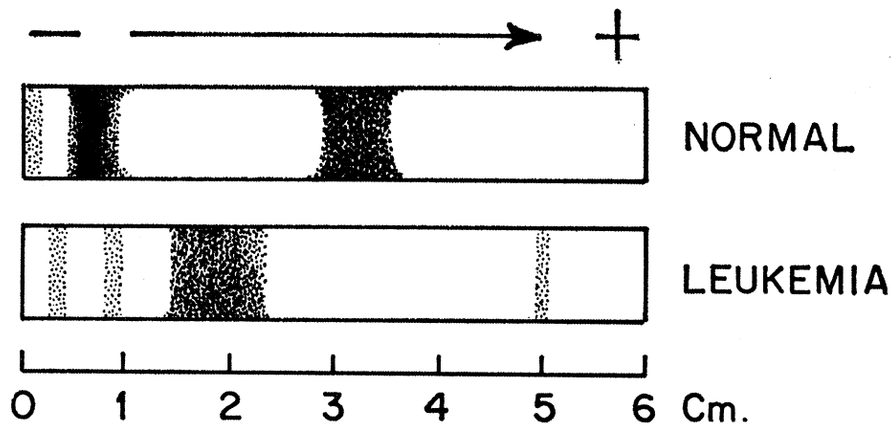

Fig. 1. Drawings of alkaline phosphatase zymogram patterns of extracts of normal and leukemia leukocytes. Starch gels were stained by incubation in a buffered solution containing β-naphthyl phosphate and fast blue RR. Three bands are discernible in the extract of normal cells. Only one band, at 2 cm, was present in the extract of leukocytes from a case of untreated leukemia; in patients treated with 6-mercaptopurine there were three weak bands in addition.

The isozyme patterns of the phosphatases extracted from leukocytes were obtained in seven normal individuals (three males and four females) (Fig. 1). The preparation of phosphatases from two of the individuals was repeated after 3 weeks, and identical patterns were obtained. The band at the origin was variable in intensity, being barely visible in some specimens.

A leukemia pattern, with a single band near 2 cm, was obtained from the leukocytes of a 43-year-old female 1 month after the onset of symptoms. At the time the phosphatase extract was prepared, her leukocyte count was 130,000 per cubic millimeter, the leukocyte alkaline phosphatase activity was 16 (normal: 25 to 173), the Philadelphia chromosome was not detected, and she had received no therapy. One month after treatment with 6-mercaptopurine was begun, the isozyme pattern revealed three additional bands centering at aproximately 0.4, 1.0, and 5.0 cm (Fig. 1). Patterns which were indistinguishable from this, except for the relative intensity of the bands, were obtained from four other cases of myelogenous leukemia. Three of the patients were being treated with 6-mercaptopurine; the fourth was receiving cytosine arabinoside, but had been treated with 6-mercaptopurine 1 month previously. The ages of these four patients, one female and three males, varied from 18 to 43 years. Duration of illness was from 3 months to 3 years. Leukocyte cultures revealed the Philadelphia chromosomes in only two of the cases.

All of the bands of the leukemia pattern, with the exception of the one at 2 cm, were usually faint. The variability encountered in the first case may have been related to initial low activity of these enzymes, to faulty extraction, or to therapy received after the first isozyme pattern was obtained.

Extracts of acute myelogenous leukemia cells (four cases) were essentially the same as normal, there being only a slight retardation of the mobility of the 3-cm band.

Because of the increased number of circulating leukocytes in the patients with leukemia, the isolation procedure yielded a correspondingly larger-than-normal mass of leukocytes. Consequently, greater significance can be attached to the loss of phosphatase components than to the presence of additional ones in the extracts of leukemia cells. That is to say, components corresponding to those on the leukemia zymograms may be present in normal extracts, but in amounts below the sensitivity of the method. Since the number of enzyme units (*9*) in the phosphatase preparations from control leukocytes (7 to 86 units) was in the same range as that from leukemia leukocytes (13 to 67 units), the observed

differences in isozyme patterns are not related to the total phosphatase activity of the preparations, but rather to qualitatively different phosphatase components.

The results of these experiments neither substantiate nor refute the hypothesis of genetic control of leukocyte alkaline phosphatase by chromosome 21. Since, however, the Philadelphia chromosome may persist in acute transformation of chronic granulocytic leukemia while at the same time the leukocyte alkaline phosphatase is elevated, and since the zymogram in the acute disease resembles the normal, the hypothesis of simple genetic control may not be adequate. As Teplitz *et al.* (*10*) have suggested, modifier genes of leukocyte alkaline phosphatase, rather than the structural gene, may be located on chromosome 21.

The results do, nevertheless, help to rationalize the decreased activity of the leukocyte phosphatase in chronic myelogenous leukemia. Apparently the mechanism for controlling the synthesis of the phosphatases is altered to such an extent in the neoplastic cells that decreased amounts, if any, of the usual enzymes are synthesized. Furthermore, those phosphatases which are present in leukemia cells have low activity in the test system. There are, of course, other possible interpretations of the observed difference in the zymogram patterns. For example, the migration rates of the leukemia phosphatases could be the result of alteration of charge on the usual phosphatase components by the action of intracellular degradative enzymes (*11*).

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A Wandering Enteropneust from the Abyssal Pacific, and the Distribution of "Spiral" Tracks on the Sea Floor

Abstract. *Certain coiled tracks appear in photographs from the bottom of most oceans and are abundant in some regions. Enteropneusts are among the forms responsible for such tracks although, despite earlier evidence, they are rarely considered to be either active or abyssal.*

Strange animal burrows and tracks perplex alike the student of deep-sea photographs and the paleontologist; the tracks and burrows may be plentiful enough, but it is often hard to find the animals that made them.

One kind of track, in the form of large coiled or "spiral" patterns (20 to 200 cm in diameter), sometimes appears in photographs of the abyssal sea floor. The patterns begin and end abruptly; we have never discovered traces leading toward their centers or away from their outer ends.

In some places, these tracks are quite plentiful (Fig. 1). On a cruise between Wellington, New Zealand, and Tahiti, the camera recorded them at nearly every station made in depths greater than 4000 m (*Vema*, Cruise 18; *1, 2*). They appeared at 17 of these deep stations but were absent at 69 shallower ones. Later, the U.S.N.S. *Eltanin* photographed them at 60 of more than 200 stations made in the southeast Pacific and the Scotia Sea. Still more "spirals," about 20, have been found in pictures from the North Atlantic, Indian Ocean, and North Pacific. But in our survey they have appeared most often in pictures from the high southern latitudes; the southern South Pacific seems a particularly good place for them (*3*).

At the first of the Pacific stations where the patterns were found (29°40'S, 176°43'W, 4735 m) we photographed very clearly not only the track but the animal responsible (Fig. 2). It is a giant enteropneust, or acorn worm (Hemichordata), about 1 m long, and 5 cm thick just behind the collar. The "spiral" turns out to be the fecal cast, marking the area methodically covered as the animal feeds upon the oozes. If it feeds in the usual way (*4*), particles are trapped in a strand of mucus which is secreted by the proboscis and, by ciliary action, either passed into the mouth or rejected at the collar (our specimen's proboscis is almost hidden at this camera angle). A trace of that mucus, mixed with bits of sediment, can be seen in Fig. 2 to run back from the collar, parallel to the cast.

Hardly anyone mentions enteropneusts as members of the deep-sea fauna. It should be remembered, though, that they are extremely fragile animals and unlikely to survive a trawling, so that their absence from collections (like the absence of the big squids) must be, sometimes at least, the fault of the fishing methods. Our pictures show tracks concentrated in places where comparatively little trawling has been done.

But it would be quite wrong to take the distribution of the tracks as the distribution of abyssal acorn worms. Those few pictures which show animals as well as tracks have, regrettably, been too unclear for sure identification. Possible records come from the North Atlantic, the New Hebrides Trench, the Peru-Chile Trench, and the Bellinghausen Sea, but the North Atlantic record is particularly doubtful. One photograph from the Pacific, however (Fig. 3), does show the same sort of track as the enteropneust's, but it was made by a distinctly different animal. Perhaps it was an echiuroid worm; a number of abyssal echiuroids have been collected in the Pacific by the *Vityaz* (*5*).