in a medium containing 0.25*M* sucrose, 0.01*M* ethylenediaminetetraacetic acid (EDTA), and 0.1*M* phosphate buffer at *p*H 7.0, no cytochrome oxidase activity was found (Fig. 1) (*1*). The homogenate rapidly turned brown during blending (30 seconds at top speed and 1 minute at a slow speed at approximately 30 v), and after centrifugation of the homogenate at 1000*g* the pellet isolated at 16,000*g* was also brown. The *p*H of the homogenate obtained by such a procedure was 6.0, and the *p*H of the particles suspended in 8 ml of 0.25*M* sucrose and 0.001*M* adenosine triphosphate (ATP) was 5.2 (*2*). The nitrogen content of 0.5 ml of the particulate suspension (derived from 200 g of tissue and 300 ml of homogenizing medium) was about 260 μ. All the phenolase activity from such a preparation was associated with the particulate fraction (Fig. 1), which showed considerable activity.

Quite a different picture emerged when the apples were homogenized in a solution containing 0.15*M* sucrose, 0.01*M*

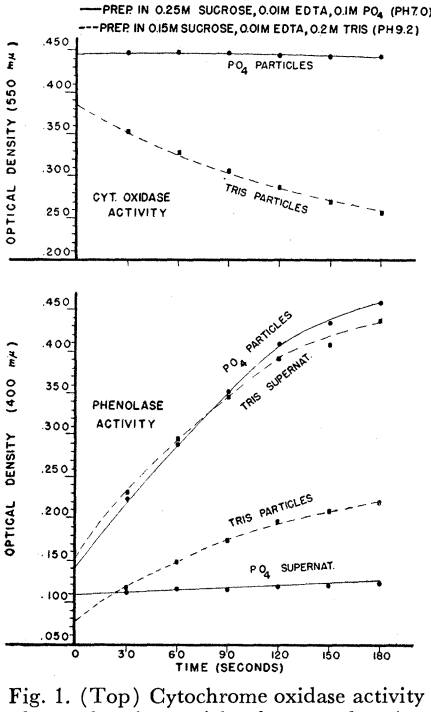

Fig. 1. (Top) Cytochrome oxidase activity of cytoplasmic particles from apples. Activity was determined by the method of Cooperstein and Lazarow (*1*). The crude enzyme used in the assay consisted of 0.03 ml of the particulate suspension. (Bottom) Phenolase activity of cytoplasmic particles and supernatant from apples. Activity was determined by the method of Ponting and Joslyn (*3*) with modifications as follows: 2.99 or 2.90 ml of 0.05*M* catechol in acetate buffer at *p*H 5.0 was the substrate, and 0.01 ml of the particulate suspension or 0.1 ml of the supernatant was added at zero time to a 3-ml cuvette. All assays were made with a Beckman DU spectrophotometer. The values shown are averages of three experiments.

EDTA, and 0.2*M* tris-hydroxymethyl aminomethane (Tris) at *p*H 9.2. The homogenate in the Tris medium had a *p*H of 9.0 and remained green during blending. The isolated particles were green, and the particulate suspension had a *p*H of 6.2. The nitrogen content of 0.5 ml of this particulate suspension (obtained in exactly the same manner as particles isolated in sucrose, EDTA, and phosphate) was about 140 μg. Cytoplasmic particles from apples isolated in this way exhibited good cytochrome oxidase activity (Fig. 1) and showed a different distribution pattern for phenolase activity (*3*). Much of the phenolase activity in this preparation was associated with the supernatant from the high-speed centrifugation, but the cytoplasmic particles also showed significant phenolase activity (Fig. 1).

Three possible explanations for the altered pattern of phenolase activity and for the considerable difference in the nitrogen content of the particulates isolated at the two *p*H levels may be proposed. If it is assumed that phenolase is localized on the cytoplasmic particles *in vivo*, then the enzyme may be removed from the particles by solubilization at *p*H 9.2 but not at *p*H 7.0. A second possibility is that phenolase, present in both soluble and particulate fractions, is agglutinated when apple cells are ruptured in a homogenizing medium at a *p*H of 7.0, but not at the higher *p*H. The third possibility is that phenolase is preferentially adsorbed on the particles in an extracting medium at *p*H 7.0, but that there is no adsorption at *p*H 9.2. Ponting and Joslyn (*3*) found that apple phenolase tightly adsorbed on particles could be solubilized by using a 2 percent sodium carbonate solution of *p*H 11.3. Hagen and Jones (*4*) observed a similar *p*H effect on catalase activity in chloroplast particles and in supernatant fractions from wheat leaves.

Hackney (*5*) reported no cytochrome *c* in apples and concluded that cytochrome oxidase is absent from them. Webster (*6*) could not demonstrate the occurrence of cytochrome oxidase in apple tissue and failed to show a reversal of carbon monoxide inhibition. This report demonstrates cytochrome oxidase in apple particulates by spectrophotometric methods. There are two additional reports (*7*) in which cytochrome oxidase activity was demonstrated by manometric techniques in tissue slices and in cell-free extracts from apples. In our work with apples it appeared that use of homogenizing medium of high *p*H (9.2) is necessary for isolating particulates in which cytochrome oxidase activity can be demonstrated. The cells of the apple fruit have a sap *p*H of 3.5 (*8*) and contain a very active phenolase system (*9*). It seems necessary to counteract the acidity and remove most of the phenolase from the particulates before good cytochrome oxidase activity appears. Since the *p*H of the cell sap of many fruit tissues is very low, and since these tissues usually show high phenolase activity, it may be necessary to use homogenizing media of high *p*H values to isolate cytoplasmic particles that show cytochrome oxidase activity (*10*).

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4 October 1957

Alteration of Clay Minerals by Digestive Processes of Marine Organisms

The possible importance of the highly acid chemical environment in animal stomachs upon clay minerals suspended in marine water was brought to our attention by two papers (*1*) which were concerned primarily with the effects of inorganic particles upon organisms. These papers demonstrated that many plankton-feeding and ooze-feeding fishes, clams, plankton animals, and other organisms are apparently unable to separate organic food particles from inorganic clay particles of the same size and cannot avoid passing suspended clay material through their digestive organs. An analysis of the stomachs of mullet (*Mugil cephalus*) from the surf at Port Aransas, Texas, showed that each fish contained an average of 1.6 g of clay within its stomach and intestine. Similar clay-filled guts were observed in menhaden, shrimp, and anchovies. That changes in the clay mineral composition of sediments being brought to the bays

and estuaries of the Texas Gulf Coast occur has been shown by Grim and Johns (*2*). This investigation (*3*) is concerned with the part played by marine organisms in bringing about these changes.

The ultimate purpose of this study is (i) to determine the amount and type of change in various clay minerals caused by representative organisms and (ii) to compare these changes in the digestive tracts with changes brought about by exposure of clays to sea water alone.

Representatives of Mollusca, Echinodermata, the filter feeding fish, and several planktonic organisms have been used for this work, to date. All organisms were placed in aquaria of filtered sea water and starved, to rid them of any previously contained clay matter. Then the organisms were transferred to fresh tanks of filtered water into which food—either algae (*Platymonas*) or yeast—and the clay mineral to be studied were introduced. Controls were set up in which these same minerals were exposed to filtered sea water of the same salinity, without the animals. Only clay material of less than 2 μ in size was used, to insure similarity between clay particle size and food particle size and to avoid contamination from other minerals which generally occur in larger grain size.

Fecal matter was carefully collected, as being representative of material which had actually passed through the animal. The fecal material was washed free of salt and treated with 30 percent hydrogen peroxide to rid it of organic matter. X-ray diffraction, differential thermal analysis, and electron microscopy were used to characterize the minerals before treatment, after passage through organisms, and in controls. All samples used for diffraction work were conditioned at 50 percent relative humidity at 20°C, except during special treatments. Data were obtained both from x-ray diffraction photographs and from spectrometer recordings.

The major part of the work has been done with a soil containing randomly interlayered clays, a bentonite, and kaolinite. Kaolinite does not appear to be affected by either sea water or organisms. However, the interlayered minerals and the bentonite are readily subject to modification and exhibit appreciable changes both within the control samples and in animal tanks after periods of only 24 hours.

Representative data are presented in Table 1. These experiments tend to indicate a build-up of magnesium within the exchangeable cation positions upon exposure to sea water alone which, for the bentonitic montmorillonite, produces no change in 001 peak position during the 24-hour period. A similar build-up of magnesium within the soil clay, however, tended to prop the structure open to 15.5 A, thus blanketing out the effect of the poorly developed brucite interlayer already present. Heat treatment of this organization then collapsed the mica layers containing magnesium but only gradually affected the portions containing interlayered islands of brucite.

Table 1. Effect of organisms on x-ray characteristics of ingested clays; (001) *d* values from orientated aggregates of clays before treatment, in controls, and after passage through organisms. All work was done with Cu*Kx* radiation.

Treatment	(001) *d* values (A)								
	24-hour exposure					5-day exposure			
	Original	Control	Oyster	Clam	Mullet	Control	Oyster	Clam	Mullet
				Soil montmorillonite					
50% relative humidity	14.7	15.5	12.6	12.1	14.7	14.9	12.9	12.3	14.3
Glycol	17.0	17.0	17.0	17.0	17.0	17.0	17.0	17.0	17.0
300°C	12.6	14.5	12.6	11.8	12.5	14.5	12.9	12.1	12.6
				Bentonitic montmorillonite					
50% relative humidity	15.5	15.2	14.7	14.3	14.3	14.7	12.9	12.9	12.6
Glycol	17.0	17.0	17.0	17.0	17.0	17.0	17.0	17.0	17.0
300°C	10.0	10.0	10.0	10.0	10.0	12.8	10.0	10.0	10.0
				Kaolinite					
50% relative humidity	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3

The same bentonitic montmorillonite, when exposed to sea water for a period of 5 days, appears to begin the development of a brucite configuration around the magnesium ions in the manner described by Grim and Johns (*2*); this results in a shifting of the 001 peak position from 15.5 A to 14.7 A. This new structure expanded completely upon glycolation but only gradually collapsed through 500°C. Exposure of the soil clay to the control water for the same length of time resulted only in an enhancement and slight shift in position of the original 14.7 A peak.

Exposure of these same clays to the organisms for like periods of time always resulted in a large shift in the 001 peak position toward 10.0 A. Although glycol apparently produces complete expansion, we suggest that potassium in the form of an exchange cation is being taken up by all samples upon exposure to the animals. It also seems likely that, within the soil clay, the brucite islands are being at least partially destroyed by the stomach acidity of the organisms. A relative decrease in influence from the 14.0 A component was noted in the soil clay, and no development of such a component could be observed within the bentonitic montmorillonite.

Thus, the two processes of change may be working independently of one another. According to this interpretation, clays of the 2:1 layer type, upon exposure to sea water, begin the uptake of magnesium within the exchangeable cation positions almost immediately and gradually begin the development of a brucite configuration between mica layers. Similar clays which pass through organisms, however, suffer a loss of such brucite and build up potassium as an interlayered cation. Whether or not this potassium begins to exert any more influence upon the structure with time and constant reexposure may be determined in future work.

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30 September 1957