measurement it was shown that the extract hydrolyzed fats such as olive oil and tributyrin very rapidly. Monobutyrin was also attacked, although at a lower rate.

Using the spectrophotometric method described above, it was found that steapsin acting upon acetylsalicylic acid liberated only traces of salicylic acid. When steapsin was allowed to act upon longer chain fatty acid esters of salicylic acid the enzymic hydrolysis proceeded at significant rates.

The data in Table 2 were obtained with butyrylsalicylic acid as the substrate in a concentration of 1.66×10^{-2} *M*. It can be seen that in contrast to the blood serum enzyme, no inactivation by eserine is in evidence, and the enzyme seems to act in the absence of Ca^{++}.

We have the following evidence that the observed hydrolysis of the salicylic acid esters is effected by the same enzyme that hydrolyzes the glycerides.

1. When the steapsin extract acts on insoluble substrates (e.g., olive oil) bile salts are needed for activation. In such cases some additional activation can be observed by Ca^{++}, but even $10^{-1}M$ NaFl does not completely inactivate the enzyme. When a soluble ester such as monobutyrin is used as the substrate, no bile salt is needed, and simultaneously the activity is not influenced by the presence of Ca^{++}. There is no inactivation by $10^{-5}M$ eserine. The enzyme behaves the same when butyrylsalicylic acid is used as the substrate.

2. The ratios of the rates of hydrolysis of butyrylsalicylic acid by different steapsin preparations, including those that were partially inactivated by heat, were the same as values obtained when using monobutyrin as the substrate. Comparison with the activity toward olive oil could not be made, since in this case even in the presence of 2% Na taurocholate no proportionality between enzyme concentration and observed activity could be obtained.

3. The esters of the longer chain fatty acids are attacked preferentially. For instance, at the same concentration caproyl(C6)salicylic acid is hydrolyzed about twice as fast as the butyryl ester. Ethyl acetate is not attacked by the steapsin extract. This is similar to the observation of Nachlas and Seligman (*3*) who found that long chain fatty acid esters of β-naphthol are attacked by "lipase," whereas "unspecific esterase" preferentially attacks short chain esters.

4. The hydrolysis of butyrylsalicylic acid is inhibited competitively by mono- and by tributyrin, the latter being more effective than monobutyrin.

It should be mentioned that the rate of hydrolysis of caproylsalicylic acid, the highest in the homologous series of esters we have prepared thus far, is only of the order of 1/100 of the rate at which olive oil is attacked.

Results with longer chain esters will be reported when these compounds have been obtained in a pure form.

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Resistance of a Protein-Montmorillonite Complex to Decomposition by Soil Microorganisms

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Soils high in clay are known to be much more retentive of soil organic matter than are soils low in clay mineral content. This fact has led to the generally accepted belief that there must be some sort of an interaction between the organic and inorganic colloids in soil, such as physical adsorption, complex formation, or chemical combination.

Considerable work has been done in recent years on the interaction of pure organic compounds with clay minerals, especially montmorillonite (*1–4*), the latter being most reactive because of its high base exchange and swelling capacities. The reaction involves the entry of the organic molecules between the platy sheets of clay, causing an expansion of the crystal lattice structure. Observations have been recorded showing the presence of mono-, di-, and trimolecular layers of organic molecules in the expanded crystal lattice (*2*). Gieseking (*3*) found that large organic nitrogenous compounds behave as do ammonium cations in being strongly adsorbed by montmorillonite, and could be exchanged by other cations of the same size but not by hydrogen. Hendricks (*4*) observed that large organic cations are held to the flat network surface by van der Waals forces between the neutral portions, as well as by electrostatic interaction of the charged parts. Ensminger and Gieseking (*5*) found that proteins, when complexed with montmorillonite, were in large measure resistant to hydrolysis by proteolytic enzymes. Allison *et al.* (*6*), in a study of inorganic soil colloid as a factor in retention of organic matter, reported that the addition of 10% bentonite to sand gave, in several instances, an approximately twofold increase in plant carbon held.

Evidence presented in the present preliminary report shows that a protein-montmorillonite complex is highly resistant to decomposition by soil microorganisms.

A diluted slurry of electrodialyzed Wyoming bentonite having a pH of 2.15 and an equivalent diameter of <0.2 μ was mixed with an aqueous solution of gelatin and shaken for several hours. After the pH was raised to 6.7 by the addition of solid calcium hydroxide, shaking was continued for a few more hours. The complex was removed by filtration, air-dried, and finally dried in an evacuated desiccator over phosphoric anhydride. The carbon content of the complex was found to be 1.54, which corresponds to 3.56% of gelatin.

Calcium bentonite was prepared by neutralizing the electrodialyzed bentonite with calcium hydroxide, the pH of the product being 6.5.

The decomposition studies were conducted in 2-qt Mason jars. The additions were as follows: (*a*) 16.23 g protein-montmorillonite complex, containing 0.25 g carbon, plus 84 g quartz sand; (*b*) 0.58 g gelatin (0.25 g carbon), plus 15.65 g calcium bentonite and 84 g quartz sand; and (*c*) 0.58 g gelatin plus 100 g quartz sand. To each of these mixtures were added 10 ml nutrient medium, containing phosphorus, potash, and minor nutrients, and 2 ml soil infusion to supply an active soil population. Each of the first two treatments, where montmorillonite was present, received 8 ml water. Determinations of the evolved CO_2 were made daily during the first 4 days and on every third day thereafter. The data, presented graphically in Fig. 1, are typical of those being obtained.

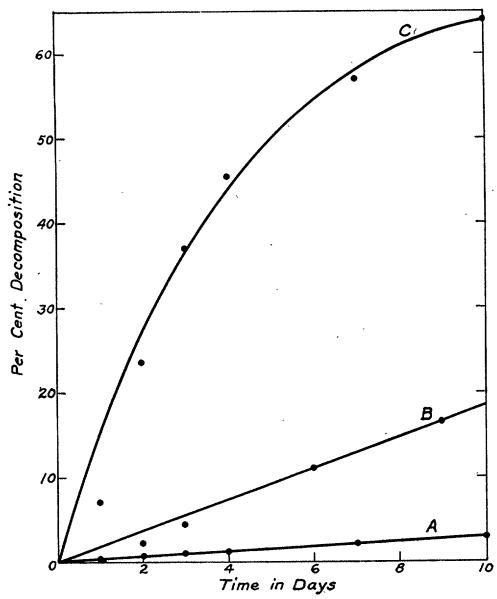

FIG. 1. Decomposition of gelatin by soil microorganisms: *A*, gelatin-bentonite complex; *B*, gelatin-bentonite mixture; *C*, gelatin alone.

The rate of decomposition of the gelatin in the gelatin-montmorillonite complex was considerably less than in the mixture of the two substances, but for a given material the rate was fairly constant during the period of the experiment. On the other hand, the gelatin in mixture with sand decomposed very rapidly at first, the rate decreasing markedly later. At the end of 10 days' incubation only 3.0% of the protein in the complex had decomposed, compared to 18.5% in mixture with bentonite, and 63.8% in the sand. These marked differences suggest that a considerable portion of the gelatin did enter into the crystal lattice where neither microorganisms nor their excreted enzymes could get to it. According to Ensminger and Gieseking (*7*), the (001) spacing of the complex containing only 3.57% protein would be < 16 A. Presumably the small amount of decomposition that takes place in the gelatin-complex preparation is limited to the protein attached to the external surfaces and edges of the montmorillonite. The low rate of decomposition in the gelatin-bentonite-sand mixture also suggests that considerable interaction between the protein and clay mineral occurred even under these conditions. The significance of these findings in connection with soil organic matter maintenance is obvious.

These researches are being extended to include other organic compounds, as well as a study of factors pertinent to complex formation and decomposition.

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Screening Effect of Vitamin C on the Inactivation of Leaf Phosphatase by Ultraviolet Light

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It is known that enzymes are inactivated by exposure to ultraviolet light. In an earlier publication (*1*) from this laboratory it was reported that vitamin C protects the enzymes—phosphatase, amylase, and pepsin—against inactivation by ultraviolet irradiation. But the mechanism of the reaction involved in the protection of the enzymes against inactivation by the vitamin has not been elucidated. The object of the present paper is to present results which throw light on the role of vitamin C in the reaction.

Ten ml of the phosphatase solution prepared from French-bean leaves purified by fractional precipitation with alcohol (*2*) was put into two quartz tubes. The solution was adjusted to pH 7.0. Ten ml of water and 10 ml of 10 mg of vitamin C solution in another set of two quartz tubes were used as screening materials and introduced between the light source and the

TABLE 1

Time (min)	Phosphatase activity in mg phosphorus after exposure to ultraviolet light filtered through water	Inactivation (%)	Phosphatase activity in mg phosphorus after exposure to ultraviolet light filtered through vitamin C solution	Inactivation (%)	Vitamin C (mg)
0	2.58	—	2.58	—	10
30	2.42	6.2	2.54	1.5	—
60	2.40	7.0	2.54	1.5	9.1
90	2.33	9.69	2.49	3.2	—
120	2.06	20.00	2.35	8.9	—
180	1.67	35.2	2.30	10.8	6.5