data, that the relative TMV concentration for TMV in nonrusted bean, rusted bean, and systemically infected tobacco was about 1 : 10,000 : 2,000,000, respectively. The value of 10,000 for the relative virus concentration in rusted in comparison with nonrusted tissue is not considered as finite or adequately determined. It has been shown to vary with age of leaf, age of virus infection, inoculum level of rust and virus, and bean variety.

If the virus concentration in the nonrusted tissue at about 15 days after inoculation is considered as 1, the virus concentration in other rusted tissues was as follows: 7 for tobacco mosaic in sunflower rust, 59 for alfalfa mosaic in bean rust, 440 for tobacco necrosis in bean rust, and 500 for tobacco ring spot in bean rust. With cucumber mosaic on bean, as assayed by local lesions on sugar beet, no such ratios can be indicated, for except for what is believed to be contamination in one test, none of this virus has been recovered from nonrusted tissue. Therefore the ratio of virus concentration in rusted tissue to that in nonrusted tissue was infinity. The bean variety Bountiful, of which nonrusted young and old leaves have been resistant to infection in the writer's tests, has been infected with TMV only when already infected with rust.

Although normal bean tissues become more resistant with age to TMV, tobacco necrosis, tobacco ring spot, and alfalfa mosaic, rusted tissues of similar age usually remain susceptible. Therefore it is likely that infinite differences in virus concentration between rusted and nonrusted tissues would result if beans of appropriate age were used.

With rust inoculation on sunflower plants already systemically infected with tobacco ring spot virus, no virus increase in association with the rust pustules was detected.

To measure the effect of rusted tissue on virus *in vitro,* fine suspensions of virus-free rusted and nonrusted bean tissues at different concentrations were added to TMV and tobacco necrosis virus at 0.01% tissue concentration. About 20 min after mixing, these suspensions were used as inocula on half-leaves of local lesion hosts (*N. glutinosa* for tobacco mosaic and *N. tabacum* for tobacco necrosis), and the virus suspension without supplement was used as inoculum on the opposite halves of the same leaves. At 0.003% concentration of rusted bean tissue, the number of TMV local lesions was 177% greater than for the control without rust extract, and this corresponds (from the straight line relating local lesions and virus concentration) to about an elevenfold increase in infective virus concentration. By the same token, a 0.01% concentration of bean rust tissue caused a five fold increase in infective tobacco necrosis virus. When these suspensions of virus and bean leaf tissues were allowed to stand overnight and then used as inocula, less stimulation of virus infectivity by rust tissues was detected.

Apparently significant smaller increases in infectivity were produced by nonrusted tissues at slightly higher tissue concentration. However, at 1% concentration, tissues from nonrusted and rusted leaves caused great reduction in virus infectivity, and the rusted tissue caused greater reduction than the healthy.

Although the above results must be considered preliminary, it is concluded that rust-infected plant tissues may increase the invasiveness and infectivity of certain plant viruses. No certain exceptions are known, and the several cases of negative results could be explained on the basis of inadequate trials, inoculation methods, or assay methods for the viruses used.

The cause of these associations has not been determined. That the greater susceptibility of rusted than nonrusted tissues to virus infection is not due to the mechanical punctures made in the cell walls by the rust haustoria is indicated by the finding that infection of bean with *Erysiphe polygoni* or *Colletotrichum lindemuthianum,* which also puncture the cell walls, has not been found to favor virus infection. The finding (unpublished) of Louis Jacobson, of the Division of Plant Nutrition, that rust infection increases the number and amount of free amino acids in bean leaves may have an important bearing on the results reported in this paper.

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Spectrophotometric Determinations of Esterases

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Salicylic acid strongly absorbs ultraviolet light of a wavelength of 290 mμ–300 mμ, whereas acetylsalicylic acid (aspirin) does not absorb at all in this region. This was found to hold true for other fatty acid esters of salicylic acid as well (Fig. 1). This principle provides a convenient and sensitive method for the determinations of esterases in general.

Because of the free carboxy group, these compounds, including the longer chain fatty acid esters, are soluble in solutions of low acidity and can be used as substrates in continuous spectrophotometric measurements. The hydrolysis of as little as 0.01 μM of such an ester can be detected.

The measurements were carried out in the Beckman spectrophotometer. The reference cell contained buffer, substrate, and water to a final volume of 3 ml. The control cell contained buffer, substrate, water, and enzyme to a final volume of 3 ml. The other cells contained, in addition to the elements of the control cell, substances the influence of which upon the en-

zyme reaction were to be observed. The concentration of the substrate was identical in all cells, thus automatically cancelling the influence of spontaneous hydrolysis on the observed increase of absorption. Spontaneous hydrolysis was considerable in many cases.

Simultaneous comparison of the reaction rates in the different cells was completed by reading the extinction at 300 mμ every few minutes during a half-hour period or longer.

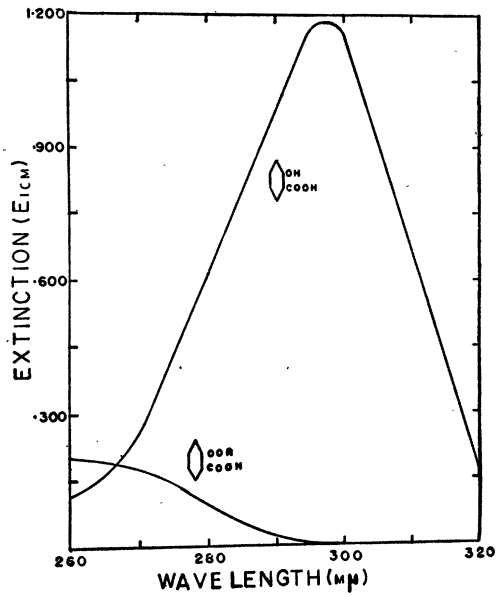

FIG. 1. Absorption spectra of $3.3 \times 10^{-4} M$ solutions of salicylic acid and of salicylic acid esters at pH 8.0.

Acetylsalicylic acid was obtained commercially. The other esters were prepared from the acid anhydrides as starting material. They were recrystallized from alcohol until free from unchanged salicylic acid.

Solutions of the substrates were freshly made up before each experiment by heating the free acid with a few drops of alcohol and then mixing the melt with the buffer (0.075 *M* veronal, pH 8.0) to which an amount of *N* NaOH, equivalent to the substrate, had been added. The following results were obtained with two different types of esterases.

Choline esterase: Table 1 shows the influence of a variety of activators and inhibitors on the esterolytic activity of normal human blood serum (specimen pooled from several individuals) with acetylsalicylic acid as the substrate. The concentration of the ester was 1.66×10^{-2} *M*, such high concentration being necessary to obtain maximum activity.

The observed activities appear to be due entirely to choline esterase (*s*-type) since there is practically complete inhibition by eserine in concentrations less than 10^{-5} *M*, which is supposed to be characteristic for the choline esterases. Moreover, the hydrolysis of acetylsalicylic acid is inhibited by acetylcholine. The inactivation by compounds such as parathione and tri-*o*-cresylphosphate and the activation by Ca^{++}, Mn^{++}, and Mg^{++} also point toward this type of enzyme.

Acetylsalicylic acid has been used as a substrate for "true" choline esterase as well (*1, 2*). It seems to be very probable, therefore, that the activity of this enzyme can be determined with the present method.

TABLE 1

HYDROLYSIS OF ACETYLSALICYLIC ACID ($1.66 \times 10^{-2} M$) BY NORMAL HUMAN BLOOD SERUM AT pH 8.0

Influence of bivalent ions and of eserine on the reaction rate		
Additions to 0.025 ml serum	$\Delta E^{300\ m\mu}_{1\ cm}$ /30 min	μM Salicylic acid liberated/ml serum/hr
None (control)	0.040	2.7
NaFl, $10^{-2} M$	.019	1.5
$CaCl_2$, $10^{-2} M$	.280	18.6
NaCl, $10^{-1} M$	.050	3.3
$CaCl_2$, $10^{-1} M$	.320	21.3
$CaCl_2$, $10^{-1} M$ 0.025 ml serum	.630	21.0
None (control)	.045	3.0
$MnSO_4$, $10^{-2} M$	.372	24.8
$MgCl_2$, $10^{-2} M$	.184	12.3
$CaCl_2$, $10^{-1} M$ (control)	.240	16.0
$CaCl_2$, $10^{-1} M$	0.013 (Total)	0.9
Eserine,* $10^{-5} M$	0.005 (First 6 min × 5)	0.3
$CaCl_2$, $10^{-1} M$	0.049 (Total)	3.3
Eserine* $1.7 \times 10^{-7} M$	0.005 (First 6 min × 5)	0.3
$CaCl_2$, $10^{-1} M$ (control)	0.269	17.9
$CaCl_2$, $10^{-1} M$ Parathione,* † $10^{-6} M$	0.035	2.3
$CaCl_2$, $10^{-1} M$ Tri-*o*-cresylphosphate,* ‡ $10^{-6} M$	0.105	7.0

* Poisons added to enzyme 20–30 min before the substrate.
† Furnished by C. H. Hine, University of California.
‡ Furnished by T. C. Daniels, University of California.

TABLE 2

HYDROLYSIS OF BUTYRYLSALICYLIC ACID ($1.66 \times 10^{-2} M$) BY AN AQUEOUS EXTRACT OF COMMERCIAL BOVINE LIPASE AT pH 8.0

Influence of NaFl and of eserine on the reaction rate		
Additions to 1 ml 1% steapsin	$\Delta E^{300\ m\mu}_{1\ cm}$ /30 min	μM Salicylic acid liberated/ml extract/hr
None (control)	0.412	0.69
NaFl, $10^{-2} M$	.414	.69
Eserine, $10^{-5} M$	0.412	0.69

Pancreatic lipase: As a source of the enzyme a commercial powdered bovine lipase preparation (steapsin) was used. A 1% aqueous extract was filtered over celite, resulting in a clear solution. By titrimetric

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.