Table 1. Amounts of RNA-phosphorus and protein in the cytoplasmic fractions of sliced sweetpotato tissue. Values are expressed in micrograms per gram of fresh tissue.

Measurement	Mw	Pw	S_2	Total
RNA-P of sound tissue from infected slices (A)	1.84	3.54	13.0	18.38
Protein of sound tissue from infected slices (A)	88.0	74.5	390.0	552.5
RNA-P of sound tissue from uninoculated slices (B)	1.74	2.01	14.5	18.25
Protein of sound tissue from uninoculated slices (B)	66.7	47.3	309.0	423.0

tained from the control sample. Both kinds of tissues were then treated in the following fashion.

The tissues were homogenized with 0.25*M* sucrose solution and, after a low-speed centrifugation to remove sediment such as nuclei, starch granules, and the debris of cell walls, the supernatant was fractionated according to Schneider's method (*4*) into three parts: mitochondria (Mw), microsomes (Pw), and supernatant (S_2). Protein nitrogen and ribonucleic acid (RNA) were determined by micro-Kjeldahl digestion and by the modified method of Ogur (*5*), respectively. As is shown in Table 1, protein nitrogen in every fraction of the tissue was higher in the sound parts of infected slices (tissue A) than in corresponding tissue from untreated control slices (tissue B), but the total amount of RNA was the same. However, it can be seen that in tissue A the amount of RNA in the smaller particles (designated as the microsomal fraction) had increased at the expense of the supernatant. Recent studies have led to the conclusion that the microsomes, rich in RNA, are the site of all of the protein synthesis in the cells (*6*). On the basis of these investigations, it may be suggested that the growth of the pathogen in the sweetpotato leads in some manner to a stimulation of synthetic activity (as shown by the large increases in protein in each cell fraction) in adjacent parts of the sweetpotato tissue that are not infected.

The possibility that the observed increases in protein might be due in part to the synthesis of enzyme protein was investigated by comparing the oxidative activity of mitochondrial fractions prepared from tissues A and B.

It is clear (Table 2) that tissue A was superior to tissue B by each of the criteria utilized. The rate of oxygen uptake in mitochondria from the sound part of infected slices was about twice that in those from uninfected slices, and phosphorylative activity was also greater. There was a greater amount of protein nitrogen in the preparation from tissue A, but despite this, the activity of the mitochondria of tissue A (expressed as oxygen uptake per milligram of nitrogen, per hour), was higher than in those from tissue B. The greater intrinsic activity of the mitochondria may be one factor contributing to the higher respiratory rate that has been observed in the intact tissue from which the mitochondria were derived. The P/O value for oxidative phosphorylation was higher in tissue A than in tissue B, but it was lower in both cases than it was in the mitochondria prepared from the tissue of fresh sweetpotato (that is, one which had remained unsliced and untreated). This might be attributed to difficulties experienced in the preparation of the mitochondria which results from the oxidation of polyphenols by a polyphenol oxidase that increases in the tissues when the sweetpotato is sliced or infected (*8*).

It would thus appear that increases in the amount of mitochondrial enzymes (functional protein) do occur in parts of slices adjacent to infected areas and contribute to the protein recovered in this fraction. In addition, however, the intrinsic activity of the mitochondria is apparently increased.

Table 2. Respiratory oxidation and oxidative phosphorylation by mitochondria from sweetpotato. The reaction mixture contained 20 μ*M* phosphate buffer at *p*H 7.2; 200 μmoles of sucrose; 20 μmoles of α-ketoglutaric acid; 100 μmoles of glucose; 3 μmoles of ATP, 10 μmoles of $MgCl_2$; 10 μmoles of Versene; 20 μmoles of NaF; and 1 ml of enzyme in a total volume of 2.3 ml. The mixture was incubated in Warburg vessels for 60 minutes at 30°C. The mitochondria were prepared according to a previously described method (*7*), using 45 g of each tissue, and finally they were suspended in 3 ml of the 0.5*M* sucrose solution containing 0.02*M* phosphate buffer and 0.2*M* NaF as a phosphatase inhibitor.

Source of mitochondria	Uptake (μatom/flask, per hr)		P/O	Protein N (μg/flask)	O_2 uptake (μatom/mg of N, per hr)
	O_2	P			
Tissue A (sound) from infected slice 48 hr after cutting	6.7	3.6	0.54	577	11.6
Tissue B (from uninoculated control slice) 48 hr after cutting	3.6	0.59	0.16	419	8.6
Tissue A 72 hr after cutting	7.61	3.2	0.42	595	12.8
Tissue B 72 hr after cutting	2.77	0	0	426	6.5
Fresh sweetpotato	2.0	2.15	1.08		

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New Insoluble Active Derivative of an Enzyme as a Model for Study of Cellular Metabolism

It has long been recognized by those interested in cytochemistry and cell physiology that an interface would be the preferred model structure for the study of certain enzymatic reactions (*1*). If the enzyme forms the surface of a stationary phase and the substrate is dissolved in the adjacent liquid or mobile phase, the system simulates conditions inside a living cell (*2*). Other conditions which are imposed on such an ideal working model are that the enzyme is not inactivated in forming the model and that the model responds to environmental changes.

In these studies, catalase was combined with cellulose anion exchanger; this combination was enzymatically active. It is believed that this is the first recorded demonstration of an active derivative of an enzyme resulting from the electrostatic interaction of the enzyme and an insoluble polyelectrolyte. The new derivative is remarkable because of the ease with which the soluble enzyme can be liberated.

The new catalase-cellulose form can be easily demonstrated with crude or crystalline preparations of catalase and

a weak cellulose anion exchanger. The insoluble derivative of catalase retains a high order of enzymatic activity which, according to estimates, is as high as 70 percent of that of its soluble form (*3*). No measurable amount of enzyme is removed by washing with water, but the solubility of the enzyme is affected by a shift in *p*H or an increase in the ionic strength of the surrounding solution. Certain other proteins can displace the enzyme, whereas the substrate, hydrogen peroxide, is acted upon but does not appear to free the enzyme.

Preparation of the catalase derivative was accomplished as follows. A column of 1 g of cellulose anion exchanger (0.2 to 0.5 milliequivalent/g, *4*) as the free base was reacted with 10 Keil units (*5*) of purified liver catalase (*6*) in 10 ml of water. This product was washed with many volumes of distilled water. The catalase-cellulose was exposed to 3 to 6 percent hydrogen peroxide solution at the maximum rate at which the solution could flow through the column. No hydrogen peroxide was detected in the effluent. The catalase continued to function for 4 days at room temperature—tests were made for several hours each day—without any visible decrease in the evolution of oxygen. At the end of this period, the catalase was desorbed by the addition of salt solution. A green band moving down the column corresponded to the active enzyme. This type of experiment was repeated more than 20 different times with slight variations.

Catalase preparations containing salts, acids, or other proteins require larger amounts of exchanger to react with a corresponding amount of the enzyme. The width and intensity of the colored band on a column of exchanger depends on the concentration and purity of the enzyme. Purification can be effected by selective elution. The mild nature of the cellulose exchanger for purification of catalase is demonstrated by the quantitative recoveries of soluble catalase that one can accomplish while working at room temperature over a period of several days.

One can speculate that some insoluble enzymes in the cell function in a state similar to this catalase-cellulose form. Nucleic acids, structural proteins, mucopolysaccharides, or phospholipids may serve as ion-exchange agents in the cell. Enzymes that are not active as the insoluble derivative with these "natural" ion exchangers might be activated by at least two different mechanisms. Some of these insoluble derivatives could be solubilized by the substrate. For example, cathepsin of kidney was liberated from its protein nucleates by a peptide (*7*). The product of the enzyme action may not maintain the enzyme solubility in the presence of the polyelectrolyte; in this case, the insoluble derivative is reconstituted. Removal of the product could have the same end-result.

Other environmental changes, such as local changes in *p*H or ionic strength in the cell, may be even more important in releasing electrostatically bound enzymes. Some support for this model is that carbon dioxide can dissociate many of these insoluble protein-cellulose ion-exchanger derivatives (*8*). Selective permeability of the cellular membranes to substrates and other ions, together with some system of insoluble polyelectrolyte derivatives of enzymes, may be responsible for control of enzymatic processes. This new model, although admittedly crude, might lead other workers to a better understanding of cellular metabolism.

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Protection against Clostridium perfringens Type A Toxin by a Metal-Chelating Compound

The alpha toxin of *Clostridium perfringens* type A is an enzyme, a lecithinase (*1*), and there is evidence that it is the most important lethal factor in infections caused by this organism (*2*). This enzyme is activated by calcium, and it was hypothesized that, if the calcium were removed from the area of an infection, the ability of the organisms to spread and cause death would be decreased. In an attempt to test this hypothesis, a lethal dose of organisms was mixed with sodium oxalate and sodium citrate and injected intracutaneously into mice, but there was no decrease in mortality (*3*).

In an extension of this work, ethylenediamine tetraacetic acid (EDTA) (*4*), a metal-chelating agent, was used as a calcium-binding agent in the manner described in the previous paragraph. On a number of occasions this compound was able to prevent death following the injection of a lethal dose of organisms, but the results are not reproducible at will. This variability is thought to be due to marked variations in the virulence of the test organism, and further studies are being carried out to see whether it is possible to establish conditions whereby protection by EDTA can be readily demonstrated.

However, it has been found that EDTA consistently protects mice against a lethal dose of *C. perfringens* type A toxin, and this report deals with the work that has been carried out thus far with the toxin (*5*).

In the experiments described here, 0.5 ml of a 1 to 4 dilution of a toxic culture filtrate (*6*) was injected intracutaneously into white mice that weighed 20 to 25 g. This dose contained 2 M.L.D. and killed mice within 10 hours. In the initial experiments, groups of ten mice were injected with the following mixtures: (i) 1 vol of toxin plus 3 vol of borate-buffer (BB) (*7*) and (ii) 1 vol of toxin plus 1 vol of BB plus 2 vol of 0.68-percent EDTA in BB. All the animals in the first group died, and none in the second group died.

In an attempt to reverse the protective effect of EDTA, $CaCl_2$ was added to an EDTA-BB solution until a precipitate formed on the addition of sodium oxalate, indicating the presence of free Ca^{++}. The calcium-saturated EDTA protected mice to the same extent that EDTA alone did. However, when Zn^{++}, Co^{++}, and Mn^{++}, which also activate lecithinase (*8*), were added to the toxin-EDTA mixture, the toxicity was restored.

These metal ions were tested for their ability to reverse the protective action of EDTA by injection of the following mixtures into groups of ten mice, in addition to those previously mentioned: 2.5 vol of toxin plus 5 vol of 0.68-percent EDTA plus 1.5 vol of BB plus 1 vol of 0.135*M* solutions of $CaCl_2$, $ZnCl_2$, $CoCl_2$, and $MnCl_2$, respectively.

The amount of metallic salt that was added in each case was not quite twice the amount that could be chelated by the EDTA present. The mice that received the toxin-EDTA and toxin-EDTA-$CaCl_2$ mixtures survived, whereas those that received toxin alone or the toxin-EDTA plus either $ZnCl_2$, $MnCl_2$, or $CoCl_2$, died. The various salts used, the EDTA, and the mixtures of EDTA and the salts had no apparent effect on mice when they were injected separately.

When the effects of these salts were tested for their ability to reverse the *in vitro* inhibition of lecithinase with the lecithovitellin test, the results paralleled those obtained *in vivo*. EDTA inhibited the lecithovitellin reaction. An excess of either Zn^{++}, Co^{++}, or Mn^{++} reversed this inhibition, and an excess of Ca^{++} did not