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

reverse it. Preliminary experiments utilizing the manometric technique of Zamecnik *et al.* (*8*) for the determination of lecithinase have yielded identical results.

These results suggest that Ca^{++} activates but is not essential for the activity of lecithinase whereas Zn^{++}, Co^{++}, and Mn^{++} activate and are essential for the activity of the enzyme. They can be explained on the basis that Zn^{++}, Co^{++}, and Mn^{++} are preferentially chelated over Ca^{++} (*9*) and thus remain chelated, even in an excess of Ca^{++}.

The action of EDTA in protecting mice from the toxin is undoubtedly due to its ability to chelate metal ions, inasmuch as it no longer protects when an "essential" ion is present in excess. The protection afforded by EDTA and the reversal of this protection by an "essential" ion are rather dramatic. The mice that received the toxin plus EDTA manifested no ill effects and no decrease in their normal activity. In those mice in which the protective action of EDTA was reversed by either Zn^{++}, Co^{++}, or Mn^{++}, the pattern of death was identical with that of the controls that received toxin alone. They all died in the same time interval, and had an extensive area of inflammation at the site of injection.

The action of the toxin is not confined to the injection site. Therefore the EDTA exerts more than a local effect. An experiment was performed in which the toxin and EDTA were injected into different sites on opposite sides of mice, and five of the ten mice survived. The greater amount of protection that resulted when the toxin and EDTA were injected simultaneously indicates that inhibition of the toxin at the site of injection is involved in this enhanced protection. A study of the dosage levels of EDTA and Ca-EDTA that are required to protect animals when they are given by intravenous and other routes has been started.

EDTA is not toxic for animals and human beings until an amount is given that induces a hypocalcemic state with resultant tetany (*10*), but Ca-EDTA can be given in large quantities without any apparent toxicity and has been used clinically in cases of lead poisoning (*11*). The possible use of Ca-EDTA in the therapy of gaseous gangrene is obvious.

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5. Supported in part by a grant from the Purdue Research Foundation and grant G-3444 of the National Institutes of Health, U.S. Public Health Service.
6. Kindly supplied by Lederle Laboratories Division, American Cyanamid Company.
7. Borate-buffer was made up as follows: (i) 1.25 g of boric acid was dissolved in 100 ml of 0.16*M* NaCl, and (ii) 1.9 g of Borax was dissolved in 100 ml of 0.16*M* NaCl; 10 ml of ii mixed with 90 ml of i to give a *p*H of 7.6. The final *p*H of the mixtures used for injection was 6.9 to 7.1.
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Simplotite, New Calcium Tetravanadite from the Colorado Plateau

Simplotite, $CaV_4O_9 \cdot 5H_2O$, is a new mineral found in several vanadium-uranium mines on the Colorado Plateau (*1*). The mines are located in the Salt Wash sandstone member of the Morrison formation of late Jurassic age. The new mineral is named in honor of J. R. Simplot (*2*), former owner of the Peanut Mine, Montrose County, Colo., where the material used for this description was collected.

Simplotite occurs as hemispherical aggregates of dark-green, platy crystals. It is found in comparatively unoxidized ore and is associated with montroseite, paramontroseite, vanadiferous silicates, uraninite, and coffinite. At the Peanut Mine it occurs as coatings on fractures in the ore-bearing sandstone with duttonite, $VO(OH)_2$ (*3*), melanovanadite, native selenium, and an undescribed vanadium oxide.

The color of simplotite varies from nearly black, in coarse aggregates, to yellow-green, in thin flakes. It is biaxial negative; 2V is about 25°; and dispersion is $r > v$, weak and crossed. $X = b$ (yellow); Y (green); $Z \wedge c = +58°$ (green); $\alpha = 1.705 \pm 0.002$, $\beta = 1.767 \pm 0.002$, $\gamma = 1.769 \pm 0.002$. The specific gravity, as measured by immersion in a mixture of bromoform and acetone, is 2.64 ± 0.02.

A microchemical analysis made by one of us (R. M.) on approximately 70 mg of simplotite showed the following composition: CaO, 11.6 percent; V_2O_4, 67.7 percent; V_2O_5, 0.5 percent; H_2O, 18.4 percent; and insoluble material, 0.5 percent; total, 98.7 percent. Qualitative spectrographic analysis by Katherine E. Valentine of the original material indicated the presence of Mg and Al in amounts of 0.1 to 0.5 percent and Na, K, and Fe in amounts 0.05 to 0.1 percent.

Simplotite is monoclinic and pseudotetragonal. It has a very easy micaceous cleavage on (010) and is very soft. The unit-cell constants were determined by M. E. Mrose of the U.S. Geological Survey as follows: $a_0 = 8.39 \pm 0.02$ A, $b_0 = 17.02 \pm 0.02$ A, $c_0 = 8.37 \pm 0.02$ A, $\beta = 90°25' \pm 5'$; $a:b:c = 0.4929:1:0.4918$. The space group is C $2/m$; the cell contents are 4 $(CaV_4O_9 \cdot 5H_2O)$; and the calculated specific gravity is 2.65.

Simplotite was first found in 1952 by Alice D. Weeks and other members of the U.S. Geological Survey field party at the Sundown claim, San Miguel County, Colo. The sample used for this description was collected by one of us (C. H. R.) in the course of a detailed study of the mineralogy, geochemistry, and geology of the Peanut Mine. The mineral has been identified in the following three other mines: the Shattuck-Denn lease on Club Mesa and the J. J. Mine in Paradox Valley, both in Montrose County, Colo., and the Vanadium Queen Mine at La Sal Creek, San Juan County, Utah.

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Relation of Aromatic Amino Acids to Excretory Pattern of Schizophrenics

It has been shown that schizophrenics generally excrete a number of aromatic compounds that appear little, if at all, in normal urine (*1, 2*). The appearance of these abnormal metabolites may be related to the production in schizophrenia of some hallucinogen. Because of the well-known limitations on human biosynthesis of aromatic compounds, such abnormal metabolites are most probably derived from the dietary aromatic amino acids, phenylalanine, tyrosine, and tryptophan. Limiting the dietary intake of one or all of these aromatic amino acids should provide evidence concerning the origin of these aromatic compounds. With this in mind, we fed a