II). Supernatant I and II were mixed and kept in a dry ice-box. The extracts obtained by the above procedure from several groups of infected brains were combined. This mixture was precipitated with 1.6 M ammonium sulfate at pH 7.0. The mixture was centrifuged and the precipitate discarded. The supernatant was then reprecipitated at pH 5.6 with 2.3 M ammonium sulfate and left for two hours in the refrigerator. The centrifuged precipitate was suspended in physiological saline and dialyzed in a Cellophane tube against saline for three days. The saline was changed every few hours. The dialyzed solution was brought to a pH of about 4.3 with n/10 acetic acid and the centrifuged precipitate discarded. To the clear, colorless supernatant n/100 acetic acid was very carefully added, drop by drop, until a first precipitate appeared. This precipitate was examined under a polarizing microscope and was found to consist partly of birefringent matter. One of these conglomerates was separated, washed in n/1000 acetic acid and dissolved in a small amount of dilute NaOH. It dissolved with difficulty. It proved highly infective for mice, producing typical paralytic symptoms of poliomyelitis after intracerebral inoculation in 14 to 72 hours. Another group of mice treated with omission of the ether extraction and with slight modifications of the above-mentioned method gave a somewhat better yield of the crystalline material. The data on this latter method are, however, still incomplete.

Another conglomerate was separated under the polarizing microscope and an x-ray diffraction photo of the wet material was taken by Dr. Fankuchen. It showed, in addition to some undifferentiated low angle scattering, a distinct though diffuse halo at an angle corresponding to about 4.5 Å. A halo of this character seems to be characteristic of protein material.[5, 6]

As encouraging as these data are it must be stressed that there is no evidence and no claim that the crystalline material obtained by this procedure represents the poliomyelitis virus. The possibility that the virus is adsorbed on the protein can not be excluded.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

ON A NEW PROTEASE FROM PILEUS MEXICANUS[1]

Pileus mexicanus is an arboreous plant about eight meters high, belonging to the family of the Caricaceae; it grows wild in different states of Mexico (Morelos, Guerrero, Colima, Campeche y Yucatan), and is commonly known as "cuaguayote." From fruits and leaves a latex is obtained that has great activity, similar to that of papaya. The latex, collected by making longitudinal incisions on the fruits, clots rapidly, becoming brownish yellow in color.

After drying the latex in vacuum at 45° C it becomes easy to pulverize, yielding a white powder, similar to pulverized papain.

The enzymatic activity was determined by the milk-clotting method (Balls and Hoover)[2] and by titulation with alcoholic KOH (Willstätter, Waldschmidt-Leitz, modified by Balls).[3] Table I shows the activity obtained.

TABLE I

Mg of enzyme	Activator	pH	Clotting time in seconds	Milk-clotting units	cc of alcoholic KOH
1	None	4.6	60	1.00	....
1	Cystein 0.05 M.	4.6	50	1.20	....
5	None	4.7	..	....	1.10
5	H_2S	4.7	..	....	1.35

The corresponding values obtained by Balls for raw papain[4] in milk-clotting units are 1.11 after activation with Na CN and 1.09 non-activated per mg of latex. By titulation with alcoholic KOH, the maximum activity obtained by Balls on 5 mg of raw papain is 1.00 cc after activation with H_2S. These values show that this enzymatic preparation has a slightly superior activity to papain.

Similarly to other enzymes of the papain type, it is activated by HCN, H_2S and cystein, and rendered inactive by H_2O_2 and I_2. However, the papain clots the citrated blood, while the protease from Pileus does not. Its antihelminthic power was tried on *Ascaris lumbricoides, Macracanthorhynchus hirudinaceus, Oxyurus equi* and an Ankylostomid, being strongly positive in all cases.

The control specimens, in boiled enzyme, remained alive eight hours after the experiment was begun.

[1] Syn. *Jacaratia mexicana* (Sessé et Moc. ex D.C.); *Pileus heptaphyllus* (Sessé et Moc.), Ramirez; *Leucopremna mexicana* (Sessé et Moc.), Stanley.

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TABLE II

ANTIHELMINTHIC ACTIVITY ON ASCARIS LUMBRICOIDES FROM INTESTINE OF THE PIG. (pH 5, BUFFERED WITH CITRIC ACID AND DISODIC PHOSPHATE, AT 40° C)

Enzyme concentration	2 hours	4 hours	8 hours	24 hours
1 per cent.	An ulcer attaining body cavity	Partial digestion	Intense digestion	Total digestion
0.5 " "	Several ulcers	Incipient digestion	Partial digestion	Total digestion
0.1 " "	No change	Several ulcers	Incipient digestion	Partial digestion
0.05 " "	No change	No change	Several ulcers	Partial digestion

The fresh latex and dry weight relation is 30 per cent., while in papain it is only 20 per cent.

Pileus mexicanus is quite abundant in Mexican tropical regions, making its industrialization possible, to compete with papain. Methodic breeding of the plant would be an important source of the enzyme. We propose the name of "mexicain" for this enzyme.

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FUNGICIDAL VALUE OF THE SALICYLATES

THE problem of finding suitable substitutes for copper fungicides is becoming increasingly important. In recent discussions, prominent mention has been made of such organic compounds as phenothiazine, tetramethyl thiouram disulfide and ferric dimethyl dithio carbamate. However, one of the major needs for copper fungicides is in the control of various downy mildew diseases (caused by species of *Phytopthora, Peronospora* and *Pseudoperonospora*), and information as to possible copper substitutes in this field appears to be lacking. During the past ten years, the Bureau of Plant Industry, in cooperation with the state experiment stations of Georgia, South Carolina, North Carolina and Maryland, has conducted an extensive search for sprays effective against the blue mold or downy mildew disease of tobacco. The organic compounds mentioned above have been tested along with numerous others. Most promising results have been obtained with the salicylates, practically all of which were more or less effective. The best of these compounds so far tested has been bismuth subsalicylate, used at the rate of 1½ pounds, plus 1 pound of Vatsol O.T.C. (sodium dioctyl sulfosuccinate) in 100 gallons of water. With the aid of the wetting agent, the subsalicylate makes a quick and stable suspension, and the spray adheres very well to tobacco leaves. This spray used against blue mold has given excellent control, with strong residual protection after spraying was discontinued, and no plant injury. It has been superior to the regular copper oxide-oil in all three respects, and the copper oxide-oil has, in turn, been much superior to bordeaux mixture. The second best of the salicylate mixtures so far developed has been benzyl salicylate, one fourth pound dissolved in 1 gallon of cottonseed or soybean oil, emulsified and diluted to 100 gallons. This mixture has been very effective, but has occasionally caused plant retardation, and it does not have quite the residual protection of the previous. Salicylic acid and zinc salicylate at the rate of one half pound dissolved in 1 gallon oil, emulsified and diluted to 100 gallons, have been effective fungicides, but likely to cause plant injury. Materials showing some promise are butoxyethyl salicylate, dinitrosalicylic acid and salicyl salicylic acid, all at the one half pound rate in oil. So far, most of the salicylates do not appear to be critical materials, but difficulties regarding availability and price may be expected. It would seem most important to find out as soon as possible what fungicides can be used against each specific disease, and it would not be surprising if very much improved spray treatments would ultimately result.

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