the control period, the shift toward the alkaline side during the period of over-ventilation, and the subsequent shift to acidity when the alkali reserve and the ventilation fail to compensate for the trend toward acidity caused by increased katabolism. The value of 6.94 for the pH of arterial dog blood removed as soon as possible after death is in close agreement with previously reported observations[4] in which thinner membranes and a much more elaborate measuring equipment were used.

This equipment, used in conjunction with sturdy glass electrodes, should extend the usefulness of the glass electrode, as for example in the determination of soil acidity, and the measurement of hydrogen-ion activity in such materials as canned meats, vegetables and fruits by direct insertion of the electrode without separation of the liquid and solid material.

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SPECIAL ARTICLES

ISOLATION OF A CRYSTALLINE PROTEIN FROM PANCREAS AND ITS CONVERSION INTO A NEW CRYSTALLINE PROTEOLYTIC ENZYME BY TRYPSIN

KÜHNE and Heidenhain showed that the proteolytic enzymes of the pancreas are completely inactive in fresh pancreas or in freshly secreted pancreatic juice.[1] The enzymes become active when mixed with the enterokinase of the small intestine, as found by Schepowalnikow, or when the pancreas is allowed to stand in slightly acid solution. According to Vernon, activation may also be brought about by small amounts of active trypsin. The mechanism of this activation has been the subject of controversy for many years.

This note describes the isolation from fresh pancreas of an active crystalline protein which is converted by minute amounts of trypsin into a powerful proteolytic enzyme. This enzyme has also been obtained in crystalline form. The inactive protein has been called chymo-trypsinogen and the active protein chymo-trypsin.

Pancreas was removed from cattle immediately after slaughter and immersed in M/8 cold sulfuric acid. The pancreas was then minced and extracted for 24 hours at 5° C. with two volumes M/8 sulfuric acid. This extract has no measurable proteolytic activity but becomes highly active upon the addition of enterokinase or upon the addition of relatively large amounts of active trypsin. The addition of relatively small amounts of active trypsin does not cause activation. The extract contains a protein which is soluble in 0.4 saturated ammonium sulfate but insoluble in 0.7 saturated ammonium sulfate. This protein may be crystallized from 0.25 saturated ammonium sulfate by the addition of saturated ammonium sulfate and adjustment of the pH to about 5.0. It crystallizes in the form of elongated prisms. About 1 gm of crystalline material may be prepared from one beef pancreas. The protein prepared in this way can not be activated by enterokinase but becomes powerfully active upon the addition of a very small amount of crystalline trypsin[2] or of any crude trypsin solution. The crude extract and the mother liquor from the crystals, on the other hand, are completely activated by kinase but not by small amounts of trypsin. This apparent contradiction is due to the fact that crude extracts contain some material which inhibits trypsin so that small amounts of trypsin are completely inactivated. When kinase is added to such crude extracts sufficient active trypsin is formed to overcome the inhibiting effect and this active trypsin changes the chymo-trypsinogen to chymo-trypsin.

CONVERSION OF CHYMO-TRYPSINOGEN TO CHYMO-TRYPSIN

Three grams of crystalline chymo-trypsinogen were dissolved in 400 ml. M/30 pH 7.6 phosphate buffer, 1 mg of crystalline trypsin added and the solution kept at 5° C. The activity increased rapidly and after 24 hours had reached a constant value of about 1,000 times that of the trypsin added. The time rate of increase in activity is logarithmic and not auto-catalytic. This indicates that the chymo-trypsinogen can not be activated by chymo-trypsin and control experiments confirm this conclusion. No measurable hydrolysis of the chymo-trypsinogen occurred during activation. The active protein was precipitated from this solution by bringing to 0.7 saturated ammonium sulfate. The filter cake was dissolved in twice its weight of M/100 sulfuric acid, ammonium sulfate added to slight turbidity, and the pH adjusted to about 4.0 with sodium hydroxide. The solution was allowed to stand at 22°

[4] Carl Voegtlin, Floyd DeEds and H. Kahler, Public Health Reports, 45: 2223, 1930.

[1] For review of the literature see Carl Oppenheimer, "Die Fermente und Ihre Wirkungen," G. Thieme, Leipzig, fifth edition, Vol. II, p. 917.

[2] John H. Northrop and M. Kunitz, SCIENCE, 73: 262, 1931; *Jour. Gen. Physiol.*, 16: 267, 1932.

C. over night and about 2 gm of a crystalline protein in the form of plates appeared. The activity of this preparation is about one third that of the previously described crystalline trypsin with respect to the digestion of hemoglobin or casein. It is much less active than trypsin in liquefaction of gelatin but much more active in clotting milk. It does not clot blood and contains no amylase or lipase activity. The digestion of casein is carried much further than by the crystalline trypsin. The enzyme is evidently quite distinct from the trypsin previously isolated and may represent the "pancreatic rennet" of Vernon.

The chymo-trypsinogen has been recrystallized ten times and shows constant optical activity and constant proteolytic activity after activation by trypsin. Some samples showed a very slight proteolytic activity without activation which was equivalent to about 1/5000 of that of the activated material. This trace of activity is variable and is probably due to the presence of a small amount of active material.

The chymo-trypsin has been recrystallized three times and all fractions show constant optical activity and constant proteolytic activity as measured by digestion of hemoglobin, casein or gelatin, or rennet action.

There is reason to believe, therefore, that these preparations represent pure proteins, and that the proteolytic activity is a property of the protein molecule.

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DEER AS CARRIERS OF ANAPLASMOSIS

THE possibility that deer might serve as a reservoir for anaplasmosis has been considered for some time. Through the assistance of Mr. Ronald P. Harville, the Division of Fish and Game furnished two deer—one, a southern black-tailed buck, *Odocoileus columbianus scaphiotus;* the other, a Rocky Mountain mule deer, *Odocoileus hemionus hemionus* (Raf.). Both these animals were obtained in areas from which no anaplasmosis has been reported in cattle.

The southern black-tailed deer, number 1, was brought to the laboratory in a very weak condition. It was heavily infested with ticks, of which a large number were removed and identified as *Dermacentor occidentalis, Dermacentor parumapertus* and *Ixodes ricinus californicus.* After removal of the ticks, the animal speedily recovered, but a microscopic examination of this deer's blood made some time afterwards revealed a few bodies resembling *Anaplasma.* To prove the animal a carrier, 5 cubic centimeters of its blood were injected into calf 2642 on December 6, 1932. No symptoms, blood changes nor marginal bodies were observed in the calf. Later, it received blood containing *Anaplasma* from carrier cow 454, and promptly developed the disease, showing mild symptoms and blood changes. Deer 1, therefore, was not proved to be a carrier at this time.

Immediately after this deer was bled for the injection of calf 2642 on December 6, it received 10 cubic centimeters of blood from cow 265, which later succumbed to anaplasmosis. Deer 1 never developed any symptoms or blood changes characteristic of the disease.

On March 22, 1933, 13 cubic centimeters of blood were taken from this deer and injected into bull 133. This animal exhibited none of the symptoms or blood changes observed in anaplasmosis. Later, it was infected with blood from carrier cow 2603 and developed a mild case.

On July 1, 1933, 20 cubic centimeters of blood were taken from deer 1 and inoculated into cow 842. This cow presented symptoms and blood changes typical of anaplasmosis and eventually recovered.

In the blood of the mule deer, number 2, no *Anaplasma* were observed; consequently, it was assumed that this animal was not a carrier. On April 15, 1933, 20 cubic centimeters of blood were injected into this deer from cow 2607, which died of the disease shortly afterwards. As a control, cow 2603 received 10 cubic centimeters of blood from 2607 at the same time. On May 3, 1933, numerous marginal bodies were observed in the blood of control cow 2603. The infection ran a severe course, but the cow recovered. Deer 2 never developed symptoms of anaplasmosis, and, of several blood examinations, no marginal bodies were noted except in one instance and they were too few to be regarded as other than suspicious.

To determine whether deer 2 would transmit the infection, blood was taken from it on May 24, 1933, and injected into cow 2615. This cow exhibited a typical blood picture; numerous marginal bodies were observed, the red corpuscle count dropped from 6,160,000 to 1,440,000 and other changes, such as anisocytosis and punctate basophilia, were noted. The symptoms were characteristic: constipation, rapid pulse and respiration, and progressive weakness, terminating in death on June 28, 1933. The autopsy showed the usual lesions: icterus in the subcutaneous tissue and peritoneum; pale, viscous blood; petechial hemorrhages on the epicardium; markedly enlarged and jam-like spleen; slightly icteric liver; a gall bladder distended with thick, dark-green bile; and mucus-covered fecal pellets in the colon. From all these findings, therefore, the diagnosis of anaplasmosis is conclusive.