

pancreas is pretreated with sodium hydrate, the same type of inactivity results as is observed in like treatment of liver. This inactive pancreatic extract can readily be activated, however, by the addition of enterokinase. Furthermore, when liver, which has become active in alkaline medium by standing is treated with 0.4 per cent. sodium hydrate, inactivity which occurs when fresh liver is so treated is not produced.

In contrast to the effect of alkaline treatment upon the proteolytic enzymes of liver and pancreas is its effect upon the alkaline digesting enzyme of the polymorphonuclear leucocyte. It has been observed that the leucoprotease of the polymorphonuclear leucocyte maintains its activity when kept continuously in alkaline medium, and is able to effect proteolysis after treatment with sodium hydrate. From this observation it seems probable that leucoprotease exists in the cell in an active form.

The work outlined leads to the following conclusions. Autolysis of fresh normal liver progresses much more favorably in acid than in alkaline medium. Allowing liver to stand, and treating liver with weak acetic acid call into activity an enzyme which shows marked proteolysis in alkaline medium. This enzyme exists in the cell in an inactive form. Liver probably contains two proteolytic enzymes, one acting in acid and the other in alkaline medium. The inhibitory effect of alkali upon liver autolysis is referable to the preservation of the zymogen condition of the enzyme which acts in alkali. The maintenance of the equilibrium of the proteolytic enzymes of liver must be intimately dependent upon the preservation of tissue neutrality. The fact that leucoprotease is active after pretreatment with alkali suggests that this enzyme exists in the cell in an active form.

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Enzymes and antienzymes of the blood serum with certain degenerative changes in the liver.

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Since the liver undergoes advanced degenerative changes with chloroform poisoning it is possible that proteolytic enzymes are set

free and perhaps temporarily accumulate in the blood. Studies of Dr. Dochez (these *Proceedings*, p. 97) furnish evidence that the normal liver contains two enzymes: (a) The well known autolytic enzyme which causes proteolysis with greatest activity in a weakly acid medium, and (b) an enzyme which after activation with acid (like trypsin formed from trypsinogen of the pancreas) digests with greatest activity in a weakly alkaline medium.

The normal blood serum contains enzymes which digest proteins. Serum undergoes slight autolysis in the presence of an acid medium (0.2 per cent. acetic acid) but fails to autolyze in a neutral or alkaline medium (0.2 per cent. sodium carbonate). Nevertheless Hedin has shown that the globulin fraction of the serum obtained by one-third saturation with ammonium sulphate contains an enzyme which digests protein in alkaline or neutral media but is inactive in acid. The albumin fraction of the serum obtained by complete saturation with ammonium sulphate after removal of the globulin contains a thermo-labile antibody which inhibits the action of the enzyme associated with the globulin fraction, so that the mixture of globulin and albumin in the whole serum causes no proteolysis.

In our experiments the attempt has been made to determine if changes occur in the enzymes or antienzymes of the blood serum during the course of chloroform poisoning.

The following experiment, representing a considerable number, illustrates the increased proteolysis in acid which occurs at the end of three days as the result of daily administration of chloroform — at a time when there is advanced necrosis of the liver and temporary diminution or complete absence of the coagulability of the blood.

Autolysis of 3 c.c. of Blood Serum in Acid.

	First Day.	Fourth Day.	Fifth Day.
Control	1.0	1.1	1.8
After 5 days at 37° C.	3.7	5.05	4.9

No noteworthy increase of proteolytic activity in the presence of neutral or alkaline media is produced by poisoning with chloroform.

Since there is close analogy between degenerative changes in the liver and post-mortem autolysis, the attempt has been made to determine if with chloroform poisoning there is any loss of the

power of the serum to inhibit the autolysis of fresh liver tissue. The inhibiting action of serum of animals receiving chloroform when tested with normal liver or with liver of poisoned animals does not differ from that of serum obtained from normal animals.

It is noteworthy that fresh liver tissue autolyses with considerable activity in acid but is almost entirely inactive in alkali ; liver treated for a time with acid acquires the ability to digest, like trypsin, in weak alkali. In order to test by a convenient method the ability of serum of animals receiving chloroform to inhibit an enzyme which digests in the presence of alkali the enzyme of polynuclear leucocytes was employed. A progressive increase of the anti-enzymotic action of blood serum has been found after administration of chloroform. The following experiment will represent many which have been performed.

The proteolysis caused by 20 mgr. of leucoprotease acting during five days at 37° C. is represented by 17.25 c.c. N/10 H₂SO₄. The figures obtained on different days with the same enzyme plus 0.5 c.c. of blood serum from an animal receiving chloroform daily are as follows :

<i>1st Day.</i>	<i>4th Day.</i>	<i>7th Day.</i>	<i>10th Day.</i>
13.75 c.c.	12.45 c.c.	5.25 c.c.	2.9 c.c.

Jochmann and Kantorowicz have shown that the anti-enzyme of blood serum is not specific for either trypsin or enzyme of leucocytes, for serum which exerts increased inhibition upon one has a parallel action upon the other. A series of comparative tests indicate that the serum which, as the result of chloroform poisoning exhibits increased power to inhibit the action of leucoprotease, exhibits a parallel increase of its power to inhibit the action of that enzyme of the liver which after treatment with acid digests in the presence of alkali.

The parallel between the enzymes and anti-enzymes which have been mentioned is indicated in the following scheme :

Enzymes in Liver.	Enzymes of Serum.	Anti-action of Serum.
1. Digesting in acid.	1. Digesting in acid. Increased by chloroform.	1. Alkaline reaction.
2. Digesting in alkali (Made active by treatment of fresh liver with acid)	2. Digesting in alkali (Contained in globulin fraction and inhibited by antibody of albumin).	2. Antienzyme inhibiting trypsin, leucoprotease and perhaps enzyme of liver. Increased by chloroform.

Chloroform which produces profound degenerative changes in

the liver increases in the blood serum an enzyme which like that of the fresh liver digests in acid; the alkalinity of the blood serum is capable of inhibiting this enzyme. A second enzyme capable of digesting in alkali is perhaps discharged by the degenerating liver; the blood serum acquires an increased ability to inhibit the action of similar enzymes. It is probable that increase of this antibody is the means by which the body protects itself from its own enzyme.

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A preliminary note upon experimental lobar pneumonia with a demonstration of specimens.

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Soon after the relationship of the pneumococcus to lobar pneumonia was established, a great many attempts were made to produce lobar pneumonia in animals. As early as 1888 Gama-léia claimed to have succeeded in producing pneumonia regularly in sheep and dogs by injecting pneumococci directly into the lung through the chest wall; but he failed when he made the injections into the trachea. From then until 1903 the attempts of all investigators have been successful in producing a pneumonia in only a relatively small number of experiments, and the inflammation has been usually of the lobular type. Wadsworth in 1903 was able to produce pneumonia with regularity by intratracheal injection only in immunized rabbits.

Dogs were used in our experiments. Under anesthesia a small stomach tube (as used in the intra-tracheal method of artificial respiration by Meltzer and Auer) is introduced through the larynx into a bronchus and from 5 to 10 c.c. of a broth culture of a very virulent pneumococcus injected through the tube. The animals quickly recover without untoward results. Until now fifteen animals have been so treated. Of these four are under observation; nine have been killed at various periods of time after the injection—from one to six days; and two have died. All of the eleven animals which came to autopsy had pneumonia with consolidation of from one-half of one lobe to complete consolida-