

FURTHER STUDIES CONCERNING THE ESSENTIAL NATURE OF ANTITRYPTIC ACTIVITY OF NORMAL SERUM AND THE PHYSIOLOGIC FUNCTION OF PANCREATIC FERMENTS*

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A. REVIEW OF THE LITERATURE ON THE ANTITRYPTIC ACTION OF NORMAL SERUM

We have records of a great number of experimental observations on the antitryptic power of normal serum. The significance of this antitryptic power, however, has not yet been determined.

The power that normal serum possesses for preventing the action of trypsin, so far as I have been able to learn, was first definitely noticed by Hahn,¹ in 1897, who said that this action disappeared on heating after at from 65 to 70 C. Fermi and Pernossi,² in 1894, had noticed that trypsin rapidly disappeared after injection into the animal body, and that it was destroyed by contact with the tissues in vitro. On the significance of this power, Landsteiner,³ in 1900, stated that the antiaction of serum against trypsin was intimately connected with the albumin fraction, after coming down between half and full saturation of the serum with ammonium sulphate. Oppenheimer and Aron,⁴ in 1903, also observed that the resistance of serum to trypsin digestion depends on the configuration of the protein molecules; but, on the other hand, Glaessner,⁵ in 1903, contradicted Landsteiner's observation, and concluded that it was associated with the euglobulin, that is, the fraction precipitated by one-third saturation with ammonium sulphate. In 1904, however, Cathcart⁶ proved Landsteiner's observation, and also that globulin does not possess antitryptic action, that absolute specificity does not exist and that this antiaction is effected by heating for 30 minutes at 60 C. in the presence of alkali, and at about 70 C. in its absence. Delezenne and Pozarski,⁷ in 1903, found

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1. Hahn: *Berl. klin. Wchnschr.*, **34**: 1897.

2. Fermi and Pernossi: *Ztschr. f. Hyg.*, **18**: 1894.

3. Landsteiner: *Centralbl. f. Bakt.*, **27**, Nos. 10 and 11, Abt. 1, 1900.

4. Oppenheimer and Aron: *Hofmeisters' Beitr. z. chem. Phys. u. Path.*, **4**: 1903.

5. Glaessner: *Hofmeisters' Beitr. z. chem. Phys. u. Path.*, **4**: 1903.

6. Cathcart: *Jour. Physiol.*, **31**: 1904.

7. Delezenne and Pozarski: *Compt. rend. Soc. de biol.*, **55**: 1903.

that the serum preserved with chloroform soon lost its antitryptic action. Bayliss,⁸ in 1904, and Abderhalden and Gigon,⁹ in 1907 found that some of the products of tryptic activity possess the property of inhibiting the action of the ferment, the free amino-acids more than the polypeptids. Bayliss and Starling,¹⁰ in 1905, observed that the anti-enzyme seems only to inhibit enzyme action and not to destroy the enzyme itself. In 1906, Wells¹¹ considered why it was that certain body tissues were not digested by their own enzymes but by the enzymes of one another. He goes on to state that in some way the growth of anti-enzymes in the cells and tissue fluids are greatly impeded by the serum. Wiens,¹² in 1907, believed that the anti-ferment in serum was a true antibody, but that the antigen is a ferment liberated by the polymorphonuclear cells. In 1908, Jochmann and Kantorowicz¹³ found that antitrypsin is thermolabile and loses its action by heating for thirty minutes at 60 C. Bergmann and Bamberg¹⁴ found that the antitrypsin in normal serum is constant. Eisner,¹⁵ in the same year, studied the inhibiting action of serum against various ferments and concluded that it possessed a special affinity for trypsin, but Weil¹⁶ disproved his observation in 1910. Mayer,¹⁷ in 1909, obtained almost the same result as that of Bayliss and Starling in 1905, thus opposing the view of Delezenne,¹⁸ in 1901, who stated that the antitryptic action of the blood is due to an antikinase. He believed that the antibody acted on the trypsin. Döblin,¹⁹ however, in 1909, stated that the antitryptic action of normal serum is not a true immune antibody, but is colloid and thermolabile. In the same year, Schwartz²⁰ found that the antitryptic activity of serum is due to the lipoids of serum which partially inactivated the serum by extracting it with ether; and the serum can be reactivated by the addition of lecithin. On the contrary, Cobliner,²¹ in 1910, assumed that lipoids are not active agents. Dried serums extracted with ether, chloroform or petroleum ether do not lose their antitryptic activity. Rosenthal,²² in this year, believed that the antitryptic action of normal serum is due to protein cleavage products, and its increase in carcinoma

8. Bayliss and Starling: Arch. d. sc. biol., 1904, Suppl. 9.

9. Abderhalden and Gigon: Ztschr. f. physiol. Chem., **53**: 1907.

10. Bayliss and Starling: J. Physiol., **32**: 1905.

11. Wells: J. M. Res., **10**: 1906.

12. Wiens: München. med. Wchnschr., **54**: 1907.

13. Jochmann and Kantorowicz: Ztschr. f. klin. Med., **66**: 1908.

14. Bergmann and Bamberg: Berl. klin. Wchnschr., 1908, No. 30.

15. Eisner: Ztschr. f. Immunitätsf., Orig., 1, 1908-1909.

16. Weil: Am. J. M. Sc., **139**: 1910.

17. Mayer: Biochem. Ztschr., **23**: 1909.

18. Delezenne: Compt. rend. Soc. de biol., **53**: 1901.

19. Döblin: Ztschr. f. Immunitätsf., **4**: 1909.

20. Schwartz: Wien. klin. Wchnschr., **22**: 1909.

21. Cobliner: Biochem. Ztschr., **25**: 1910.

22. Rosenthal: Berl. klin. Wchnschr., **47**: 1910.

and other diseases with cachexia can be thus explained; he also found that this activity decreases during fastings, and increases during digestion. These facts were proved by Franz and Jarisch²³ in 1912. Meyer,²⁴ in 1911, does not believe that protein cleavage products are the inhibiting agents. He believes that antitrypsin is a true antibody, and that the ferment of tissue cells acts as antigen. In this year,

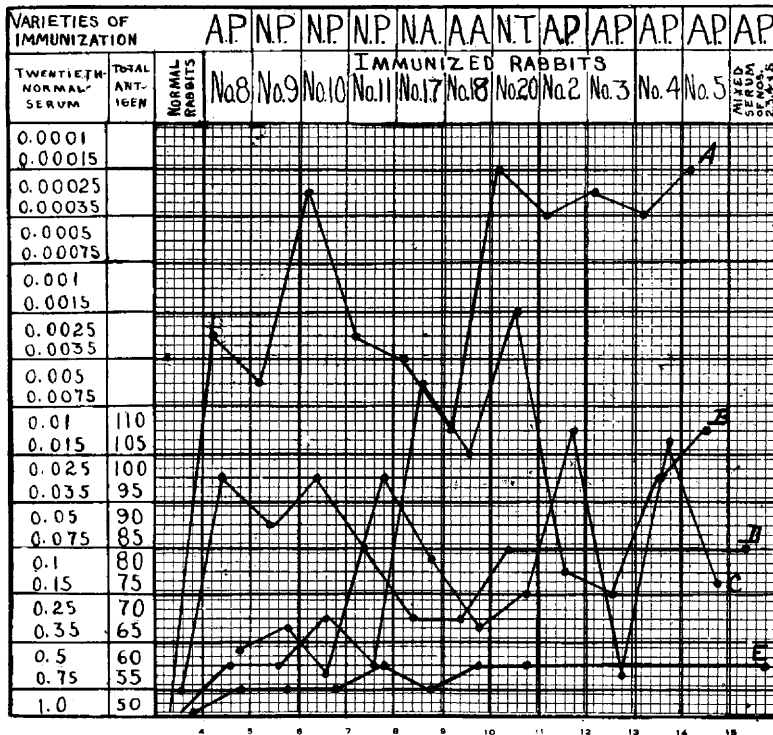


Chart showing the relation between the production of antibodies and the antitryptic action of serum. A. P.=alcoholic pancreatin; N. P.=native pancreatin; N. A.=native amylopsin; A. A.=alcoholic amylopsin; N. T.=native trypsin; A line=precipitation; B line=complement deviation; C line=total amount of antigens; D line=complete digestion of fibrin; E line=partial digestion of fibrin.

Weiberg and Ruenstein²⁵ found that ultraviolet rays destroyed the antitryptic substance in human serum. Rubenstein²⁶ also observed that the antitryptic power disappeared by heating at 70 C. In 1912, Abderhalden²⁷ found that during pregnancy there developed in the

23. Franz and Jarisch: Wien. klin. Wchnschr., **25**: 1912.

24. Meyer: Folia serolog., **7**: 1911.

25. Weiberg and Rubenstein: Compt. rend. Soc. de biol., **71**: 1911.

26. Rubenstein: Compt. rend. Soc. de biol., **71**: 1911.

27. Abderhalden: München. med. Wchnschr., **59**: 1912.

blood specific enzymes for digesting placenta proteins. The blood of a pregnant woman shows constantly an increased antitryptic power. In 1914, Bronfenbrenner²⁸ observed that any normal serum placed in contact with "sensitized" placenta acquired the same property. Therefore, it might be surmisable that the Abderhalden reaction would be composed of two phases: the one specific for the sensitization of placenta, the other nonspecific for the autodigestion of the serum as a result of the presence of sensitized placenta; and he²⁹ also said that the specificity of the Abderhalden reaction depends not as Abderhalden believed, on the presence of specific enzymes, but on the presence in the blood of pregnant women of a specific antibody that combines with placenta antigen and thus sets free the only proteolytic enzymes which is always present in the normal serum. In 1913, Kirchheim³⁰ concluded that the antiferment merely prolongs the action of the trypsin and does not destroy it, confirming Bayliss and Starling's experiments. He also found chloroform reduced the inhibiting action of the serum as Delezenne and Pozarski's experiments proved. Sugimoto,³¹ in 1913, observed that there was a decrease in the antitryptic strength of serum after the serum had been extracted with ether and benzol. He concluded that the lipoids were the active constituents. Confirming Schwartz, he believed also that a complete extraction of fat and the dissociation of the lipoid-protein combinations will cause a removal of the ferment inhibiting action. In this year we have a critical discussion on the significance of antitryptic action of normal serum by Kämmerer,³² Kämmerer and Aubry,³³ and Rosenthal.³⁴ Kämmerer contradicted Rosenthal's theory and asserted that the globulin fraction is inhibited like the albumin fraction. This antitryptic action is affected at 56 C. Rosenthal was opposed to Kämmerer's theory, but in 1914, Jobling and Petersen³⁵ observed that the ferment-inhibiting substances of the serum are lipoids corresponding to Schwartz's and Sugimoto's observation, and that they are in fat solvents, and lose their ferment inhibiting action when heated with serum at 70 C., similar to Rubenstein's observation.

On the antitrypsin in pathologic serum, Brieger and Trebing,³⁶ in 1908, found that it increases in carcinoma and sarcoma, and this was proved by Bergmann and Meyer.³⁷ Bergmann and Bamberg¹⁴

28. Bronfenbrenner: *Proc. Soc. Biol. and Med.*, **12**: 1914, No. 1.

29. Bronfenbrenner: *Biochem. Bull.*, **4**: 1915.

30. Kirchheim: *Arch. f. exper. Path. u. Pharmakol.*, **73**: 1913.

31. Sugimoto: *Arch. f. exper. Path. u. Pharmakol.*, **74**: 1913.

32. Kämmerer: *München. med. Wchnschr.*, **60**: 1913.

33. Kämmerer and Aubry: *Biochem. Ztschr.*, **48**: 1913.

34. Rosenthal: *München. med. Wchnschr.*, **60**: 1913.

35. Jobling and Petersen: *J. Exper. Med.*, **19**: 1914.

36. Brieger and Trebing: *Berl. klin. Wchnschr.*, 1908, Nos. 22, 29 and 45.

found antitrypsin not only in carcinoma and sarcoma, but present in acute infections and chronic diseases such as pneumonia, typhoid fever, tuberculosis, syphilis, chronic anemia, basedow, amebic dysentery, pancreas necrosis of men, and various sicknesses.

On the significance of antitryptic action of normal serum, therefore, we have at present no definite agreement. This important subject stimulated my interest and I have followed it up in the experiments to be described.

B. ON THE INHIBITION OF NORMAL SERUM AGAINST PANCREATIC DIGESTION

EXPERIMENT 1.—*The Inhibiting Action of Normal Ox, Sheep, Rabbit and Guinea-Pig Serum against Fibrin Digestion by Pancreatin.*—For this examination the following materials were used: (a) Half-normal serum of 5 oxen, 5 sheep, 8 rabbits and 6 guinea-pigs; (b) one c.c. of a 1 per cent. pancreatin salt solution filtered through a Berkefeld filter added to every tube, which contained half-normal serum; (c) carmin-stained oxfibrin about the size of an apple seed.

TABLE 1.—INHIBITING POWER AGAINST PANCREATIN DIGESTION OF NORMAL RABBIT SERUM

Results in 24 hours at 38 C. with 1 c.c. of 1 per cent. pancreatin

Half-Normal Serum, C.c.	Rabbit 1	Rabbit 2	Rabbit 3	Rabbit 4	Rabbit 5	Rabbit 6	Rabbit 7	Rabbit 8
0.35	0	0	±	0	0	±	0	0
0.25	++	++	+++	+++	+++	+++	++	+++
0.15	+++	++++	++++	++++	++++	++++	+++	++++
0.1	Comp.	Comp.	Comp.	Comp.	Comp.	Comp.	Comp.	Comp.
0.075	Comp.	Comp.	Comp.	Comp.	Comp.	Comp.	Comp.	Comp.

In this and following tables 0 denotes a negative for fibrin digestion; ± = trace (very slight digestion); + = slight digestion (partial); ++ = medium digestion; +++ = marked digestion; Comp. = complete digestion; ++++ = almost complete digestion.

TABLE 2.—THE INHIBITING POWER AGAINST PANCREATIN DIGESTION OF NORMAL OX SERUM

Results in 24 hours at 38 C. with 1 c.c. of 1 per cent. pancreatin

Half-Normal Serum, C.c.	Ox 1	Ox 2	Ox 3	Ox 4	Ox 5
0.35	0	0	0	0	0
0.25	0	0	0	0	0
0.15	++	+++	++	+++	+++
0.1	++++	++++	++++	+++	++++
0.075	Comp.	Comp.	Comp.	Comp.	Comp.

TABLE 3.—THE INHIBITING POWER AGAINST PANCREATIN DIGESTION OF NORMAL SHEEP SERUM

Results in 24 hours at 38 C. with 1 c.c. of 1 per cent. pancreatin

Half-Normal Serum, C.c.	Sheep 1	Sheep 2	Sheep 3	Sheep 4	Sheep 5
0.35	0	0	0	0	0
0.25	0	0	0	0	0
0.15	+++	+++	+++	++	+++
0.1	++++	Comp.	++++	+++	Comp.
0.075	Comp.	Comp.	Comp.	Comp.	Comp.

TABLE 4.—THE INHIBITING POWER AGAINST PANCREATIN DIGESTION OF NORMAL GUINEA-PIG SERUM

Results in 24 hours at 38 C. with 1 c.c. of 1 per cent. pancreatin

Half-Normal Serum, C.c.	Pig 1	Pig 2	Pig 3	Pig 4	Pig 5	Pig 6
0.35	0	0	0	0	0	0
0.25	0	0	0	0	0	0
0.15	0	0	0	0	0	0
0.1	++	+++	++	++	+++	++
0.075	+++	+++	++++	+++	++++	+++
0.05	Comp.	++++	Comp.	Comp.	Comp.	++++
0.035	Comp.	Comp.	Comp.	Comp.	Comp.	Comp.

The results in Tables 1 to 4, inclusive, show that normal animal serum has an inhibiting power against pancreatin digestion of oxfibrin, but the extent of this inhibiting power differs appreciably in the different animals. In my experiment 0.35 c.c. of half-normal rabbit serum inhibited completely fibrin digestion with 1 c.c. of a 1 per cent. pancreatin, 0.25 c.c. of a ox and sheep half serum and 0.15 c.c. of a guinea-pig half serum.

On the neutralization of the inhibiting power of normal serum against trypsin, Achalme and Stevenin,³⁸ in 1911, stated that 0.01 or 0.015 c.c. of trypsin neutralized 0.006 c.c. of human serum, 0.015 c.c. of rabbit serum and 0.01 of guinea-pig serum. My experiments with regard to the amount of pancreatin necessary for the neutralization of the inhibiting power of normal rabbit, ox, sheep and guinea-pig serum against pancreatin differ very much from those of Achalme and Stevenin in their results, as shown in the foregoing tables: 0.35 c.c.

38. Achalme and Stevenin: Compt. rend. Soc. de biol., 1911, **70**, No. 12.

of rabbit half serum, 0.25 c.c. of ox and sheep half serum and 0.15 c.c. of guinea-pig half serum were neutralized completely by 1 c.c. of a 1 per cent. pancreatin. Therefore, 0.1 c.c. of rabbit serum is necessary to neutralize 0.006 of pancreatin, 0.1 c.c. of ox and sheep serum neutralizes 0.008 of pancreatin, and 0.1 c.c. of guinea-pig serum neutralizes 0.013 of pancreatin. The inhibiting power of guinea-pig serum, therefore, is the strongest among the serums, such as ox, sheep and rabbit serum, the rabbit serum being the weakest.

EXPERIMENT 2.—*Effect of Heat on the Inhibition of Normal Ox, Sheep, Rabbit and Guinea-Pig Serum against Fibrin Digestion by Pancreatin.*—The technic for fibrin digestion by pancreatin in this experiment was just the same as in Experiment 1, but the quantity of 1 per cent. pancreatin used was 0.35 c.c. to each tube.

TABLE 5.—THE EFFECT OF HEAT ON THE INHIBITION OF NORMAL RABBIT SERUM AGAINST PANCREATIC DIGESTION

Results in 24 hours at 38 C. with 0.35 c.c. of 1 per cent. pancreatin

Half-Normal Serum, C.c.	Unheated Serum	Heated Serum, 56 C. 30'	Heated Serum, 65 C. 30'
1.0	0	0	Comp.
0.75	0	0	Comp.
0.5	0	±	Comp.
0.35	0	++++	Comp.
0.25	0	Comp.	Comp.
0.15	0	Comp.	Comp.
0.1	+	Comp.	Comp.
0.075	Comp.	Comp.	Comp.
0.05	Comp.	Comp.	Comp.

TABLE 6.—THE EFFECT OF HEAT ON THE INHIBITION OF NORMAL OX AND SHEEP SERUM AGAINST PANCREATIC DIGESTION

Results in 24 hours at 38 C. with 0.35 c.c. of 1 per cent. pancreatin

Half-Normal Serum, C.c.	Unheated Serum	Heated Serum, 56 C. 30'	Heated Serum, 65 C. 30'	Heated Serum, 70 C. 30'
1.0	0	0	0	Comp.
0.75	0	0	0	Comp.
0.5	0	0	0	Comp.
0.35	0	0	+++	Comp.
0.25	0	0	Comp.	Comp.
0.15	0	++	Comp.	Comp.
0.1	0	Comp.	Comp.	Comp.
0.075	+	Comp.	Comp.	Comp.
0.05	+++	Comp.	Comp.	Comp.
0.035	Comp.	Comp.	Comp.	Comp.

TABLE 7.—THE EFFECT OF HEAT ON THE INHIBITION OF NORMAL GUINEA-PIG SERUM AGAINST PANCREATIC DIGESTION

Results in 24 hours at 38 C. with 1 per cent. pancreatin

Half-Normal Serum, C.c.	Unheated Serum	Heated Serum, 56 C. 30'	Heated Serum, 65 C. 30'
1.0	0	0	+
0.75	0	0	Comp.
0.5	0	0	Comp.
0.35	0	0	Comp.
0.25	0	0	Comp.
0.15	0	0	Comp.
0.1	0	Comp.	Comp.
0.075	0	Comp.	Comp.
0.05	0	Comp.	Comp.
0.035	++++	Comp.	Comp.
0.025	Comp.	Comp.	Comp.

Tables 5, 6 and 7 show that the inhibiting action of normal serum against pancreatin digestion is affected by heating. It was fairly affected by heating for thirty minutes at 56 C., and lost its power at 65 or 70 C., corresponding to the results of Hahn, Cathcart, Rubenstein, Rosenthal and Jobling and Peterson, but not to those of Jochmann-Kantorowicz, Kämmerer, and Kämmerer and Aubry. The extent of the effect of heating, however, differs on the different species of animals, according to the tables; that is, rabbit serum has the weakest resistance against heating compared with ox, sheep, and guinea-pig serum; guinea-pig serum has the strongest resistance to heating; ox and sheep serum are almost equal in this respect and have a stronger resistance than rabbit serum, but weaker than guinea-pig serum. It may be said that the antitryptic substance of normal serum is fairly thermostabile, and also that the quantity of this substance seems to be constant.

C. PRECIPITATION OF NORMAL RABBIT SERUM BY PANCREATIN SOLUTION

According to current literature, we have two views on the essential nature of the antitryptic substance of normal serum, namely, that of a true immune antibody, according to Wiens, Eisner, and Mayer, and of nonspecificity, according to Cathcart, Döblin and Rosenthal. We also have three views of the production of so-called antitrypsin in normal serum. According to Landsteiner the antitryptic action is intimately connected with the albumin fraction, which is approved by Cathcart and Rosenthal. According to Glaessner and Kämmerer

the antitryptic action is associated with globulins. The third view is that the ferment inhibiting substance of the serum are the lipoids, as advocated by Schwartz, Sugimoto, and Jobling and Petersen.

The interesting question as to whether or not the antitryptic substance is a true antibody, and what the source of this substance is, invited my further attention. If this antitryptic substance is a true antibody it must have immune reactions, so that I carried out the following experiment for the determination of immune reactions.

EXPERIMENT 3.—*Determination of Immune Reactions.*—For this experiment the serums of forty-one normal rabbits were employed; for the precipitation, trypsin (Central Scientific Co.), pancreatin (Parke, Davis & Co.), and amylopsin (Digestive Ferments Co.) were used as antigen. The antigens were employed in 1 per cent. solution (dissolved in 0.85 per cent. sodium chlorid solution), filtered through a Berkefeld filter. I obtained four positive results out of a total of forty-one cases, as shown in Table 8.

TABLE 8.—PRECIPITATION OF NORMAL RABBIT SERUM BY TRYPSIN

Dilution of Half-Normal Serum, C.c.	Rabbit A			Rabbit B			Rabbit C			Rabbit D		
	Tryp- sin	Pan- cre- atin	Amy- lop- sin	Tryp- sin	Pan- cre- atin	Amy- lop- sin	Tryp- sin	Pan- cre- atin	Amy- lop- sin	Tryp- sin	Pan- cre- atin	Amy- lop- sin
1.0	+	—	—	+	—	—	++	—	—	+	—	—
0.75	+	—	—	+	—	—	+	—	—	+	—	—
0.5	+	—	—	+	—	—	+	—	—	+	—	—
0.35	±	—	—	—	—	—	±	—	—	±	—	—
0.25	—	—	—	—	—	—	—	—	—	—	—	—

— = negative; + = very slight positive; ± = trace.
41 cases: 37 cases negative; 4 cases positive.

These results show that the anti-enzyme-like substance of normal serum seems to have a special affinity for trypsin, according to Eisner's view, and also the quantity of this so-called antitrypsin is constant.

According to Landsteiner, I treated the four positive serums for fifteen minutes in a half saturation with ammonium sulphate, and tested it again for precipitation. I could not find any precipitation at all. The precipitation of the untreated serum with ammonium sulphate for trypsin should be only a pseudoprecipitation. If, again, the albumin fraction has an important significance in the inhibiting action of serum, the serum of rabbits and guinea-pigs treated with ammonium sulphate might lose the inhibiting action.

With this point in view I examined the serum treated with ammonium sulphate ($(\text{NH}_4)_2\text{SO}_4$) for the inhibiting action of pancreatin on fibrin digestion, but could find only a very slight decrease, as shown in Tables 9 and 10.

TABLE 9.—COMPARISON OF THE INHIBITING POWER OF RABBIT SERUM TREATED AND UNTREATED WITH AMMONIUM SULPHATE AGAINST THE FIBRIN DIGESTION OF PANCREATIN

Result in 24 hours at 38 C. with 1 c.c. of 1 per cent. pancreatin

Half-Normal Serum, C.c.	Rabbit A		Rabbit B		Rabbit C		Rabbit D	
	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated
0.5	0	0	0	0	0	0	0	0
0.35	0	+++	0	Comp.	0	+++	0	++++
0.25	++	Comp.	+++	Comp.	++	Comp.	++	Comp.
0.15	++++	Comp.	Comp.	Comp.	+++	Comp.	+++	Comp.
0.1	Comp.	Comp.	Comp.	Comp.	Comp.	Comp.	Comp.	Comp.

Control: 1. $(\text{NH}_4)_2\text{SO}_4$ ($\frac{1}{2}$ Sat.) + pancreatin + fibrin = complete digestion.
 2. NaCl (0.85 per cent.) + pancreatin + fibrin = complete digestion.

TABLE 10.—COMPARISON OF THE INHIBITING POWER OF GUINEA-PIG SERUM TREATED AND UNTREATED WITH AMMONIUM SULPHATE AGAINST THE FIBRIN DIGESTION OF PANCREATIN

Result in 24 hours at 38 C. with 1 c.c. of 1 per cent. pancreatin

Half-Normal Serum, C.c.	Untreated	Treated
0.15	0	0
0.1	±	Comp.
0.075	++++	Comp.
0.05	Comp.	Comp.

The results in Tables 9 and 10 show that the so-called antitrypsin of normal serum is not a true antibody as Wiens, Eisner and Mayer believed, but acts like a "pseudo-antiferment," or especially like a "pseudo-antitrypsin" in this instance, and the albumin fraction of serum has not an important significance for the antitryptic action as Landsteiner, Cathcart, Döblin and Rosenthal believed, but it seems to have more or less relation to the antitryptic power of the serum.

D. THE RELATION BETWEEN THE ANTITRYPTIC ACTION OF NORMAL SERUM AND ANTICOMPLEMENT

Believing that a factor of the antitryptic action of normal serum would also be associated with complement, I tried to find out the relation of the antitryptic action to the anticomplement. For this experiment I employed the serums of two rabbits and a normal guinea-pig.

First I examined the inhibiting action of the rabbit serum against the fibrin digestion of pancreatin, the precipitation for guinea-pig serum and the complement fixation; after that I immunized the rabbits

TABLE 11.—INHIBITING ACTION OF THE SERUM OF RABBITS A AND B BEFORE AND DURING IMMUNIZATION WITH NORMAL GUINEA-PIG SERUM

Result in 24 hours at 38 C.

Half-Normal Serum, C.c.*	Before Injection		During Immunization	
	Rabbit A	Rabbit B	Rabbit A	Rabbit B
0.5	0	0	0	0
0.35	0	0	+	++
0.25	++	+	Comp.	Comp.
0.15	+++	+++	Comp.	Comp.
0.1	Comp.	Comp.	Comp.	Comp.

* One c.c. of 1 per cent. pancreatin was added to each tube, and a small piece of carmin-stained oxifibrin was used as in the first experiment.

TABLE 12.—PRECIPITATION OF THE SERUM OF RABBITS A AND B BEFORE AND DURING IMMUNIZATION WITH GUINEA-PIG SERUM

Serum, C.c.*	Before Injection		During Immunization	
	Rabbit A	Rabbit B	Rabbit A	Rabbit B
1.0	0	0	++++	+++++
0.5	0	0	+++	++++
0.25	0	0	++	+++
0.1	0	0	+	++
0.05	0	0	+	++
0.025	0	0	+	+
0.01	0	0	0	+
0.005	0	0	0	±

* One c.c. of a 20 times dilution of guinea-pig serum as antigen was used for this examination.

TABLE 13.—COMPLEMENT FIXATION OF THE SERUM OF RABBITS A AND B BEFORE AND DURING IMMUNIZATION WITH NORMAL GUINEA-PIG SERUM

Rabbit Serum, C.c.*	Before Injection		During Immunization	
	Rabbit A	Rabbit B	Rabbit A	Rabbit B
1.0	None	None	Complete	Complete
0.5	None	None	Complete	Complete
0.25	None	None	Almost complete	Complete
0.1	None	None	Partial	Almost complete
0.05	None	None	Trace	Partial
0.025	None	None	None (complete hemolysis)	None (complete hemolysis)

* 1. Rabbit serum was inactivated for thirty minutes at 56 C. 2. Complement employed, 0.5 c.c. of a 10 per cent. guinea-pig serum. 3. Five per cent. sheep blood 0.5 c.c. + 0.2 per cent. antisherp serum, 0.5 c.c.

with normal guinea-pig serum and tested the serum again for the inhibiting action against pancreatic digestion, the precipitation for guinea-pig serum and the complement fixation with the immunized rabbit serum. These experiments are as follows:

EXPERIMENT 3.—Protocols of Experiment 3. Rabbit A: Feb. 8, 1918. Body weight, 2,500 gm. Bled and tested with this serum for precipitation for guinea-pig serum, complement fixation, and inhibiting action against fibrin digestion of pancreatin.

After these examinations I injected 5 c.c. of a 10 times dilution of guinea-pig serum (0.1 c.c. of 10 times dilution of guinea-pig serum hemolyzed completely 0.5 c.c. of 5 per cent. sheep red blood corpuscles suspension).

February 9. Body weight, 2,450 gm. Injected 5 c.c.

February 10. Body weight, 2,420 gm. Injected 5 c.c.

February 11. Body weight, 2,420 gm. Injected 10 c.c.

February 13. Body weight, 2,410 gm. Injected 15 c.c.

February 22. Body weight, 2,600 gm. Bled on the ninth day after the last injection and tested for the precipitation of guinea-pig serum, the complement fixation, and the inhibition against the fibrin digestion of pancreatin.

Rabbit B: Feb. 8, 1918. Body weight, 2,450 gm. This case was treated the same as Rabbit A. Injected 5 c.c. (10 guinea-pig serum).

February 9. Weight, 2,400 gm. Injected 5 c.c.

February 10. Weight, 2,400 gm. Injected 5 c.c.

February 11. Weight, 2,350 gm. Injected 10 c.c.

February 13. Weight, 2,350 gm. Injected 12 c.c.

February 22. Weight, 2,200 gm. Bled the ninth day after the last injection and tested for

The results shown in Tables 11, 12 and 13 indicate that the anti-complement to the complement of guinea-pig serum and the "anti-pseudo-antitrypsin" were surely formed in the immunized rabbit serum, and the antitryptic action of the immunized rabbit serum was decreased.

In 1902, Korschun³⁹ found that antiantilab-ferment was formed in serum by the injection of normal horse serum, which contains an antilab, into normal horse; this observation relates to the chemical reaction. I suppose that the action of the antilab ferment of normal horse serum, decreased by the production of the antiantilab, would also probably be associated with the production of anticomplement, as in my experiments.

E. APPEARANCE OF A TRYPTIC ENZYME IN NORMAL URINE

Enzymes of various kinds have been isolated from normal urine. Among these may be mentioned pepsin (Brücke,⁴⁰ Sahli⁴¹); trypsin (Kühn,⁴⁰ Bendersky,⁴⁰ Tesulli,⁴⁰ Sahli⁴¹); diastatic ferment (Cohn-

39. Korschun: Hoppe-Seyler's Ztschr. f. physiol. Chem., **36**: 1902.

40. Brücke: Kühn, Bendersky, Tasuli, Cohnheim, Grützner, Holovtschiner, Helwes, Hopkins, Neumeister and Boas: Huppert-Neubauer, Ed. 10, 1908, p. 599.

41. Sahli: Pflüger's Arch. f. d. ges. Physiol., **36**: 1885.

heim⁴⁰), and lab ferment (Grützner,⁴⁰ Holovtschiner,⁴⁰ Helwes⁴⁰). Among these proteolytic enzymes in normal urine, Hopkins⁴⁰ could not prove trypsin, Neumeister⁴⁰ pepsin in rabbit urine, and Boas⁴⁰ lab ferment at all; and Matthes,⁴² in 1903, stated that pepsin appears in normal human and dog urine but not trypsin. Schoenborn,⁴³ in 1910, stated that pepsin, diastase and rennin all have been found in normal urine, but trypsin is chiefly a trypsinogen, especially abundant after a meat diet.

EXPERIMENT 4.—*Tryptic Enzyme in Normal Urine.*—I wanted to know whether or not a tryptic ferment appears in normal urine and I experimented with the urine of nine normal rabbits. The colorimetric method for the digestion of the carmin-stained ox fibrin was used and the urine was aseptically taken from the bladder and centrifugalized before the examination.

TABLE 14.—TRYPTIC ACTION OF NORMAL RABBIT URINE
Results in 24 hours at 38 C.

Dilution Used,* C.c.	Rabbit 1	Rabbit 2	Rabbit 3	Rabbit 4	Rabbit 5	Rabbit 6	Rabbit 7	Rabbit 8	Rabbit 9
1.0	0	0	0	Comp.	0	0	Comp.	0	0
0.75	0	0	0	Comp.	0	0	Comp.	0	0
0.5	0	0	0	Comp.	0	0	Comp.	0	0
0.35	0	0	0	Comp.	0	0	++	0	0
0.25	0	0	0	0	0	0	+	0	0
0.15	0	0	0	0	0	0	0	0	0
0.1	0	0	0	0	0	0	0	0	0

* The urine was filtered aseptically through Berkefeld filter. A piece about the size of an apple seed of the carmin-stained ox fibrin was added to each tube containing the urine, the contents covered with toluol and incubated for twenty-four hours.

Table 14 shows that a tryptic enzyme may appear in a small amount in normal rabbit urine in a few cases (two in nine cases).

F. THE APPEARANCE OF ANTITRYPSIN IN NORMAL URINE

Döblin,⁴⁴ in 1909, found antitrypsin in normal urine (positive results in four of thirty cases) with the casein method, but Müller and Kolaczek,⁴⁵ in 1907, could find no antitrypsin in normal urine. In my experiments I could find no antitrypsin in normal rabbit urine; I therefore made the following experiment:

EXPERIMENT 5.—*Determination of Antitrypsin in Normal Rabbit Urine.*—For this experiment I employed the urine of nine normal rabbits obtained from the bladder and centrifugalized before the examination. I used a 1 per cent.

42. Matthews: Arch. f. exper. Path. u. Pharmacol., **49**: 1903.

43. Schoenborn: Ztschr. f. Biol., **53**: 1910.

44. Döblin: Ztschr. f. Immunitätsf. u. exper. Therap. **4**: 1909.

45. Müller and Kolaczek: München. med. Wchnschr., 1907, No. 8.

trypsin (Central Scientific Co.) in salt solution, filtered through a Berkefeld filter, and for the test for inhibiting action to fibrin digestion I used 0.2 c.c. of a 1 per cent. pancreatin (Parke, Davis & Co.) in salt solution, filtered through Berkefeld filter. I could obtain no positive result for the precipitation of the immune reaction, nor any positive reaction for inhibition against pancreatic digestion. (A piece about the size of an apple seed of carmin-stained oxfibrin in 1 c.c. of urine was completely digested with 0.2 c.c. of a 1 per cent. pancreatin.)

G. THE ESSENTIAL NATURE OF THE ANTITRYPTIC ACTION OF NORMAL SERUM

Considering my foregoing experiments, the essential nature of the antitryptic action of normal serum can not be explained in a simple manner, but it is reasonable to suppose that there are existing non-specific "pseudo-antiferments," especially "pseudo-antitrypsin" in this instance. As a product of the physiologic processes of tissue cells these "pseudo-antiferments" not only inhibit the autodigestion of tissue cells, but also foreign ferments outside of the body.

The increase of the antitryptic power of the serum in malignant diseases such as carcinoma and sarcoma, or acute and chronic diseases such as pneumonia, typhoid fever, tuberculosis, syphilis, chronic anemia, etc., can be explained by the increase of "pseudo-antiferments" in order to prevent the autodigestion of tissue cells or foreign ferments. These "pseudo-antiferments," therefore, are of important significance for the antiferment-like action of serum.

The reason for this opinion is that the essential nature of the antitryptic action is a nonspecific "pseudo-antiferment" and that the antitrypsin-like substance of normal serum is fairly thermostable, and sometimes takes the place of the "pseudoprecipitation" by trypsin; that the production of antipseudo-antitrypsin, as well as the quantity of antitrypsin-like substance, is constant and the normal serum also has the inhibiting action against enzymes of various kinds as observed by many workers.

As the secondary factors, the albumin fraction and the complement of the serum are associated with it, because the antitryptic action is stronger in the serums of animals which have a strong complement, like guinea-pig serum, and also stronger in the serums which have an abundance of serum albumin, such as ox, sheep, and guinea-pig serums, as well as decreased by the production of anticomplement, and also by the treatment of the serum with ammonium sulphate.

H. THE FATE OF THE ENZYME PANCREATIN INTRODUCED INTO THE BLOOD STREAM OF NORMAL RABBITS

1. *The Fixation of Pancreatin Injected as Antigen.*—The statement is often made in regard to the origin of antibodies that antibodies are formed in the blood itself by the leukocytes or the hemopoietic organs.

Up to a recent time, however, we have no conclusive evidence of that, but Hektoen and Carlson,⁴⁶ in 1909, found that the antigens injected intravenously, at least in some animals, are quickly removed from the blood or in some way so changed that the antigenic property is lost, and antibodies are not produced in the blood. The removal or change of the antigen takes place within three to six hours. Antibodies in active immunization are produced outside of the blood stream. According to Luckhardt and Becht's⁴⁷ observation in 1911, the production of the antibodies with "immune" tissue, specific antibodies with "immune" spleen were obtained, while "immune" heart muscle, liver, bonemarrow and lymph gland did not give positive results. They concluded that the spleen takes a significant part in the fixation of antigen. Luckhardt and Becht, however, found the production of antibody resulted even in asplenic animals. In 1912, Pettit and Carlson⁴⁸ observed that the soluble and insoluble antigens are fixed outside of the blood stream. Recently, Kyes⁴⁹ has shown that certain endothelial cells of the liver and spleen are constantly active in phagocytizing red blood corpuscles from the circulating blood stream; these he designates as "hemophages." This interesting investigation was trustworthily proved by Cary,⁵⁰ Berry and Melick⁵¹ in this laboratory. On the possibility of the activity of these hemophages, Kyes explained that foreign erythrocytes, certain bacteria and some colloids injected into the blood stream are rapidly taken up and digested by the hemophages, so that the hemophages are definitely concerned in antibody production.

Kyes,⁵² by his study, in 1916, on the natural resistance of the pigeon to the pneumococcus, has shown that phagocytosis of fixed tissue cells is not only most active in the hemophages of liver and spleen, but exists in the fixed tissue cells of lung, bonemarrow, kidney, pancreas and intestine, although its activity is very weak. Bartlett and Ozaki,⁵³ in 1917, also stated that *Micrococcus aureus* introduced into the blood stream of dogs is stored up in relatively great numbers in the lung capillaries and is rapidly ingested by morphonuclear leukocytes, but in considerable numbers in wandering cells and fixed cells of the spleen and liver, and also in small numbers, or not at all, in the blood, bonemarrow, kidney, intestinal wall, heart muscle and skeletal muscle. It seems to me that they⁵⁴ are surely proving Kyes' observation by

46. Hektoen and Carlson: Tr. Chicago Path. Soc., **8**: 1909, No. 1.

47. Luckhardt and Becht: Tr. Chicago Path. Soc., **8**: 1911, No. 6.

48. Pettit and Carlson: J. Infect. Dis., **10**: 1912, No. 1.

49. Kyes: Internat. Monatschr. f. Anat. u. Physiol., **31**: 1914.

50. Cary: J. Infect. Dis., **17**: 1915, No. 2.

51. Berry and Melick: J. Immunol., **1**: 1916, No. 1.

52. Kyes: J. Infect. Dis., **18**: 1916, No. 3.

53. Bartlett and Ozaki: J. Med. Res., **35**: 1917, No. 3.

54. Bartlett and Ozaki: J. Med. Res., **37**: 1917, No. 1.

their study on phagocytosis in vivo under various conditions, because the bacteria employed by them were phagocyted in great numbers by the fixed tissue cells of liver and spleen.

According to Ozaki,⁵⁵ the accumulation of bacteria in the spleen, such as occurs in experimental bacteremia, depends on the vital activity of the cells and the mechanical filtration of bacteria by the spleen, which is not an important factor. The kidney, on the other hand, may be a much more effective filter than the spleen. Recently, Okazaki⁵⁶ found by his study on the fate of the starch granules injected into the rabbit's vein that some colloidal substance such as starch was phagocyted by some endothelial cells such as those of lung capillaries and Kupffer's cells, or some giant cells.

The following experiments on the fate of the pancreatin introduced into the blood stream of rabbits might explain the significant rôle of the physiologic function of tissue cells against foreign colloids, as well as the fixation of antigens by tissue cells.

EXPERIMENT 6.—Fate of Pancreatin Introduced Into the Blood.—For this experiment I employed fifty-three rabbits, which were injected with pancreatin intravenously. I also used nine other rabbits for control examinations. The experimental rabbits were killed with chloroform at intervals in order to determine the fate of pancreatin injected as antigen; that is, to find out where the antigen was fixed, or if it was fixed, whether or not it had a timely relation.

With this point in view, seven rabbits were killed thirty minutes after the injection, 10 rabbits at one hour, 7 at one and a half hours, 6 at two hours, 3 at two and a half hours, 2 at three hours, 1 at five hours, 1 at six hours, 1 at twelve hours, 3 at twenty-four hours, 2 at thirty-eight hours, 5 at forty-eight hours, 3 at fifty-four hours, 1 at sixty hours, and 1 at seventy-two hours, and also 9 for control.

As an antigen to be injected, 1 per cent. native pancreatin filtered through a Berkefeld filter and 2 per cent. alcoholic pancreatin were used.

Glycerin extracts of the rabbits' organs, such as bone-marrow, spleen, liver, kidney, suprarenal capsule and lung, were made. The examination was then carried out on the following material:

1. Half-normal serum. 2. Ten per cent. organ glycerin extracts. This latter extract was prepared in the following way: The organs were cut into small pieces with scissors and ground up by mixing with sea sand in a mortar and then extracted with 50 per cent. glycerin solution. This extract, after being kept for twenty-four hours at room temperature, was mixed with a small amount of chloroform to avoid putrefaction, following Salkowski's⁵⁷ suggestion. Finally, this extract was filtered through a porcelain filter.

Method of Examination.—The method of carmin-stained fibrin digestion was employed to obtain an indication of the presence of antigen in three tissue extracts and serum. The tissue extracts and serum were examined in graded dilution with a physiologic sodium chlorid solution to the amount of 2 c.c. in every tube, and a small piece of fibrin the size of an apple seed and the solution covered with toluol and then incubated for twenty-four hours at 38 C. To the fibrin digestion of the tissue extract there was added 1 drop of tenth-normal sodium hydroxid to each tube. During this examination aseptic precautions were observed as far as possible.

55. Ozaki: J. Med. Res., **36**: 1917, No. 3.

56. Okazaki: The Sei-i-Kwai Med. J. (Tokio), **36**: 1917, No. 10.

By this method the following results were obtained: In 7 rabbits killed thirty minutes after injection the antigen was found in the bone-marrow in 6 cases, in the spleen in 5 cases, in the liver and lung in 2 cases, in the kidney in 1 case and in the suprarenal capsule in 4 cases. In 10 rabbits, killed after one hour, antigen was found in the bone-marrow and liver in 7 cases, in the spleen in 6 cases, in the kidney in 4 cases, in the suprarenal capsule in 5 cases and in the lung tissue in 3 cases; no antigenic reaction was found in 1 of 10 cases. In 7 rabbits, killed after one and a half hours, the antigen was found in the bone-marrow in 5 cases, in the spleen and liver in each of 2 cases, in the kidney in 2 cases, in the suprarenal capsule in 4 cases and in the lung in 1 case. In 6 rabbits, killed after two hours, the antigen was found in the bonemarrow in 4 cases, in the spleen in 3 cases, in the liver in 5 cases, in the kidney and suprarenal capsule in 2 cases, while there was no antigenic reaction in any extract in 1 of 6 cases.

In 3 rabbits, killed at two and a half hours, antigen was found only in the bonemarrow and liver of 1 case each; in 2 rabbits, killed at three hours, in only the bonemarrow, liver and kidney of each case; in 1 rabbit, killed at five hours, antigen was found in the bonemarrow and spleen; in 1 rabbit, killed after six hours, it was found in the bonemarrow, spleen, liver, kidney and suprarenal capsule; in 1 rabbit, killed at twelve hours, it was found in the bonemarrow, spleen, liver and kidneys; in 3 rabbits, killed at twenty-four hours, it was found in the bonemarrow and spleen in each case, in the liver in 1 case, and in the kidney in 2 cases; in 2 rabbits, killed at thirty-eight hours, it was found in the bonemarrow, spleen, kidney and suprarenal capsule of each case, and in the liver in 1 case; in 5 rabbits, killed at forty-eight hours, it was found in the bonemarrow and spleen in each case, and in the liver and kidney in 3 cases; in 3 rabbits, killed at fifty-four hours, 1 rabbit killed at sixty hours, and also 1 at seventy-two hours, no antigenic reaction was found.

Of the antigenic reaction of the serum, it was positive in 6 of 7 animals, which were killed at thirty minutes after injection; in 8 of 10 cases killed at one hour, in 5 of 7 cases killed at one and a half hours, and in 4 of 6 cases killed at two hours, while a negative result was obtained in other cases; also, at the same time, the inhibiting action of the serum against pancreatic digestion was examined in order to determine the presence of the antigen in the serum, concerning which I obtained the following results: the inhibiting action of the serum of rabbits killed within two hours after the injection was markedly decreased compared with that of normal serum, while no decrease of this power was found in cases in which the rabbit was killed after two hours.

As a control, tissue extracts and serum of nine rabbits were carefully examined for pancreatic action, but no positive results were found.

Summarizing these facts, the enzyme, pancreatin, introduced into the blood stream was probably phagocyted by the fixed tissue cells of the body, and seemed to keep its activity during two days, while the antigen in the serum seemed to exist for two hours after the injection, and after that would be phagocyted by fixed tissue cells. The antigen seemed to be markedly phagocyted in the hemopoietic organs within one hour after injection, although it was more or less fixed by other organs.

2. *The Excretion of Pancreatin Injected as Antigen.*—As I have already shown, the urine of some normal rabbits contains a small amount (0.35, or 0.25 in the lowest active dilution) of some tryptic enzyme. In my foregoing experiment it was positive in two of nine rabbits. I carried out the following experiment with a view of finding out whether or not pancreatin introduced into the blood stream is excreted into the urine; if so, how much is excreted.

EXPERIMENT 7.—*Excretion of Pancreatin in Urine.*—For this experiment I employed the urine taken directly from the bladder of fifty rabbits into which I had injected the pancreatin, and also that of nine normal rabbits. This urine was centrifugalized before examination. The method for the pancreatic digestion of the urine was the same as that for serum.

The urines in six of fifty animals injected and seven of the nine normal specimens were negative. The amount of pancreatin excreted in the urine in the remaining forty-four cases is not large as compared with that of normal urine. I assume, therefore, that the pancreatin is phagocyted by the tissue cells. The excreted pancreatin was found in the urine in from thirty minutes to fifty-four hours after the injection.

I. ANTITRYPTIC ACTION OF IMMUNE SERUMS AND TISSUE EXTRACTS WITH PANCREATIC FERMENTS

I wanted to know the relation between the production of antibodies and the antitryptic action of serums and tissue extracts from animals immunized with pancreatic ferments. For this purpose rabbits were immunized to a higher degree with both unmodified and alcohol modified pancreatin, trypsin, and amylopsin, with which I carried out the experiments. The pancreatic ferment solutions employed were made by the following procedure:

a. Unmodified pancreatic ferment solutions. Commercial pancreatin (Parke, Davis & Co.), trypsin (Central Scientific Co.) and amylopsin (Digestive Ferments Co.) in powders were added to physiologic sodium chlorid solution in the amount of 1 per cent. by weight. After repeated shaking the solutions were filtered through hard-pressed filters and finally through Berkefeld candles. The resulting filtrate was clear and sterile.

TABLE 15.—THE ANTITRYPTIC ACTION OF TISSUE EXTRACTS OF IMMUNIZED RABBITS. RESULTS IN TWENTY-FOUR HOURS AT 38 C.

10% Tissue Extract, C.c.	Varieties of Immunization												Control, Eight Normal Rabbits		
	Pancre- atin		Pancre- atin		Pancre- atin		Pancre- atin		Pancre- atin		Amylop- sin			Tryp- sin	
	Rabbit 2	Rabbit 3	Rabbit 4	Rabbit 5	Rabbit 6	Rabbit 8	Rabbit 9	Rabbit 10	Rabbit 11	Rabbit 17	Rabbit 18	Rabbit 20		Rabbit 18	Rabbit 20
Bone Marrow	+	0 Comp.	0 Comp.	0 Comp.	0 Comp.	0 Comp.	0 Comp.	0 Comp.	0 Comp.	++ Comp.	+	++ Comp.	++ Comp.	++ Comp.	
	Comp.	Comp.	+++ Comp.	0 Comp.	++ Comp.	Comp.	0 Comp.	0 Comp.	Comp.	+++ Comp.	++ Comp.	+++ Comp.	+++ Comp.	+++ Comp.	
	Comp.	Comp.	Comp.	Comp.	++ Comp.	Comp.	++ Comp.	+++ Comp.	Comp.	Comp.	Comp.	Comp.	Comp.	Comp.	
	Comp.	Comp.	Comp.	Comp.	++ Comp.	Comp.	++ Comp.	+++ Comp.	Comp.	Comp.	Comp.	Comp.	Comp.	Comp.	
	Comp.	Comp.	Comp.	Comp.	Comp.	Comp.	Comp.	Comp.	Comp.	Comp.	Comp.	Comp.	Comp.	Comp.	
Spleen	+	0 Comp.	0 Comp.	0 Comp.	0 Comp.	++ Comp.	0 Comp.	0 Comp.	Comp.	± Comp.	++ Comp.	++ Comp.	++ Comp.	++ Comp.	
	+	+	+	± Comp.	++ Comp.	Comp.	0 Comp.	0 Comp.	Comp.	Comp.	++ Comp.	++ Comp.	++ Comp.	++ Comp.	
	+	+++ Comp.	+++ Comp.	Comp.	++ Comp.	Comp.	Comp.	± Comp.	Comp.	Comp.	Comp.	Comp.	Comp.	Comp.	
	Comp.	Comp.	Comp.	Comp.	++ Comp.	Comp.	Comp.	+++ Comp.	Comp.	Comp.	Comp.	Comp.	Comp.	Comp.	
	Comp.	Comp.	Comp.	Comp.	Comp.	Comp.	Comp.	Comp.	Comp.	Comp.	Comp.	Comp.	Comp.	Comp.	
Liver	0	0 Comp.	0 Comp.	0 Comp.	0 Comp.	0 Comp.	0 Comp.	0 Comp.	+	+	+	0 Comp.	0 Comp.	0 Comp.	
	0	0	0	0	0	+	0	0	+	+	++ Comp.	+	+	+	
	± Comp.	+	± Comp.	+	0	+	0	0	+	+	++ Comp.	++ Comp.	++ Comp.	++ Comp.	
	++ Comp.	+	++ Comp.	++ Comp.	0	Comp.	++ Comp.	0	+++ Comp.	+++ Comp.	Comp.	+++ Comp.	+++ Comp.	+++ Comp.	
	++ Comp.	+	++ Comp.	++ Comp.	+	Comp.	++ Comp.	+	+++ Comp.	+++ Comp.	Comp.	+++ Comp.	+++ Comp.	+++ Comp.	
Suprarenal Capsule	+	Comp.	+++ Comp.	Comp.	Comp.	Comp.	0	+++ Comp.	Comp.	Comp.	Comp.	Comp.	Comp.	Comp.	
	Comp.	Comp.	+++ Comp.	Comp.	Comp.	Comp.	0	Comp.	Comp.	Comp.	Comp.	Comp.	Comp.	Comp.	
	Comp.	Comp.	Comp.	Comp.	Comp.	Comp.	+	Comp.	Comp.	Comp.	Comp.	Comp.	Comp.	Comp.	
	Comp.	Comp.	Comp.	Comp.	Comp.	Comp.	++ Comp.	Comp.	Comp.	Comp.	Comp.	Comp.	Comp.	Comp.	
	Comp.	Comp.	Comp.	Comp.	Comp.	Comp.	++ Comp.	Comp.	Comp.	Comp.	Comp.	Comp.	Comp.	Comp.	
Kidney	0	0 Comp.	0 Comp.	0 Comp.	0 Comp.	0 Comp.	0 Comp.	0 Comp.	+++ Comp.	+	++ Comp.	+	++ Comp.	++ Comp.	
	± Comp.	± Comp.	0 Comp.	0 Comp.	++ Comp.	Comp.	0 Comp.	0 Comp.	Comp.	Comp.	Comp.	Comp.	Comp.	Comp.	
	+	+	+	0	++ Comp.	Comp.	0 Comp.	0 Comp.	Comp.	Comp.	Comp.	Comp.	Comp.	Comp.	
	+	+	+	+	++ Comp.	Comp.	+	0	Comp.	Comp.	Comp.	Comp.	Comp.	Comp.	
	++ Comp.	++ Comp.	++ Comp.	+	++ Comp.	Comp.	++ Comp.	++ Comp.	Comp.	Comp.	Comp.	Comp.	Comp.	Comp.	

Total amount of tissue extract in each tube made, 2 c.c. with 0.85 per cent. NaCl.
 Control test for glycerin: 1 c.c. of a 50 per cent. glycerin + 1 c.c. of 0.85 per cent. NaCl + 0.2 c.c. of 1 per cent. pancreatin + fibrin = complete digestion.
 Comp. = complete digestion of fibrin; 0 = no digestion of fibrin (complete inhibition).

b. Alcohol modified pancreatic ferment solutions. A 10 per cent. solution of the same ferments was made in physiologic sodium chlorid solution, and after thorough shaking was filtered through hard-pressed paper. To the resulting filtrate was added 20 volumes of absolute alcohol and the mixture allowed to stand thirty minutes, resulting in the formation of a white precipitate. The mixture was then centrifugalized and the sediment rapidly resuspended in an amount of salt solution making the concentration 2 per cent. relative to the amount of pancreatic ferment powders originally employed.

EXPERIMENT 8.—*The Antitryptic Action of Immune Serum.*—I employed the serums of eleven immunized rabbits and eight normal rabbits for this experiment. For the determination of antitryptic action I used the method of fibrin digestion, as explained before, but I employed 0.35 c.c. of 1 per cent. native pancreatic solution filtered through a Berkefeld filter as antigen in this instance. I obtained the results shown in the chart; that is, the extent of the digestion of fibrin by pancreatin is markedly decreased in the immune serums as compared with the normal serums, although there is not so much difference in the partial digestions. These results indicate that the antitryptic power of serum is increased by immunization by pancreatin and trypsin, but there is no marked increase of this power in immunization by amylopsin.

EXPERIMENT 9.—*The Antitryptic Action of Immune Tissue Extracts.*—I used tissue extracts from twelve immunized rabbits and twelve normal rabbits (glycerin extracts as before) for determining inhibition against fibrin digestion by pancreatin, employing 0.2 c.c. of a 1 per cent. native pancreatin as a digestive agent in this instance.

The results obtained were as shown in Table 15. The tissue extracts of the eight normal rabbits had no inhibiting action against the digestion of fibrin with 0.2 c.c. of 1 per cent. pancreatin, while the tissue extracts of the immunized rabbits showed a marked inhibiting action against pancreatin, especially noticeable in the liver, kidney, spleen and bonemarrow; only a trace of inhibiting action was found with suprarenal capsule of the rabbits immunized with the pancreatin and none with the suprarenal capsule of rabbits immunized with amylopsin.

These results indicated that liver, kidney, spleen and bonemarrow of rabbits immunized with pancreatin and trypsin exhibited antitryptic power, signifying the production of antibodies against the antigen.

J. CHANGES IN QUANTITY OF GLUCOSE IN SERUM AND LIVER AFTER IMMUNIZATION WITH PANCREATIN

In 1916 Schäfer⁵⁸ asserted that when the pancreas is totally removed, grape sugar is no longer stored in the liver. This seemed to me a very interesting fact, because I also found a change in the quantity of grape sugar in serum and of glycogen in the liver in my experiments on immunization by pancreatin. I felt that if animals

57. Salkowski: Deutsch. med. Wchnschr., 1888, No. 16.

58. Schäfer: The Endocrine Organs, 1916, p. 2.

could be immunized with pancreatin there might be some change in the quantity of grape sugar in the serum, as well as of glycogen in the liver, so that I experimented with nine rabbits to ascertain the point.

EXPERIMENT 10.—*Quantitative Estimation of Glucose in Serum and Liver After Immunization by Pancreatin by Reduction with Fehling's Solution.*—For this experiment I used a 10 per cent. liver extract (with 50 per cent. glycerin) digested for twenty-four hours, and a half-normal serum tested within two hours after bleeding.

For a control I employed liver extract and half-normal serum of five normal rabbits. The results are shown in Table 16.

TABLE 16.—GRAPE SUGAR OF SERUM AND GLYCOGEN OF LIVER IN IMMUNIZED RABBITS

Number of Rabbit	Varieties of Immunization	Reduction of Fehling's Solution (1 C.e.)	
		10% Liver Extract (1 C.e.)	Half Serum (1 C.e.)
2	A. P.	+ (4)*	+
3	A. P.	+ (5)	±
4	A. P.	+ (5)	±
5	A. P.	0	±
6	A. P.	+ (4)	+ (2)
8	A. P.	+	+ (2)
9	N. P.	+ (4)	+ (2)
10	N. P.	+	+ (2)
11	N. P.	+ (5)	+ (3)
17	N. A.	+ (5)	+ (2)
18	A. A.	+ (5)	+ (3)
20	N. T.	+ (5)	+ (3)
5 normal rabbits, control		+ (6)	+ (4)

* The figure in parentheses following the + indicates the degree of reduction: + = slight positive; + (2) = ++, etc.; ± = trace; 0 = negative.

Table 16 shows that the quantity of grape sugar in the serum was markedly decreased in four cases and moderately decreased in five cases. The quantity of glycogen in the liver also was markedly decreased in three cases, moderately in three cases, and very slightly decreased in three cases after immunization with pancreatin. The decrease in the quantity of grape sugar in the serum and of glycogen in the liver is probably due to the fact that the antipancreatin affects the external secretion of the pancreas. In the immunization with amylopsin and trypsin a slight decrease is shown in the quantity of grape sugar in the serum and glycogen in the liver, as compared with the control cases, although only a few cases were tested.

SUMMARY AND CONCLUSIONS

1. Normal rabbit, guinea-pig, sheep and ox serum have an inhibiting action on pancreatic digestion.

2. The extent of the inhibiting action differs in different species of animals, but the quantity of the inhibiting substance is constant in each in relation to the others.

3. One c.c. of rabbit serum neutralizes 0.006 of pancreatin, and 0.1 c.c. of ox and sheep serum neutralizes 0.008 of pancreatin; also 0.1 c.c. of guinea-pig serum neutralizes 0.013 c.c. of pancreatin.

4. The inhibiting action of normal rabbit, guinea-pig, sheep and ox serums against pancreatic digestion is affected by heating; that is, fairly decreased at 56 C., and is lost by heating for thirty minutes at from 56 to 70 C., so that this inhibiting substance is fairly thermostabile.

5. Some normal serums show a precipitation with trypsin, but this phenomenon disappears on treating the serum with ammonium sulphate.

6. The inhibiting action of normal serum is decreased in a slight degree on treating the serum with ammonium sulphate.

7. I conclude from my various experiments that normal serum contains "pseudo-antiferment" (especially "pseudo-antitrypsin" in this instance).

8. The inhibiting activity of the serum also is decreased more or less by the production of anticomplement and antipseudo-antiferment (especially antipseudo-antitrypsin in this instance).

9. A tryptic enzyme appears in small amount in normal rabbit urine.

10. No antitrypsin was found in normal rabbit urine, and no antitryptic action against pancreatic digestion.

11. On the essential nature of the inhibiting power of normal serum against pancreatin, I propose that there exists nonspecific "pseudo-antitrypsin" as the product of physiologic function of tissue cells, so that this "pseudo-antiferment" is of important significance for the antiferment action of serum, and also, as secondary factors, the albumin fraction and the complement of serum are associated with it.

12. Pancreatin introduced into the blood stream of rabbits is fixed by some of the fixed tissue cells, and seems to keep its activity during two days.

13. The possibility of tissue cells phagocytizing pancreatin seems marked in the hemopoietic organs within one hour after injection, although pancreatin is also fixed by other organs.

14. The antigen (pancreatin) exists in the blood stream for two hours after injection.

15. The antigen (pancreatin) introduced into the blood stream is excreted into the urine in small amount.

16. The antitryptic power of the serum is increased by immunization by pancreatin and trypsin, and also by amylopsin, though in a slight degree.

17. The tissues of rabbits immunized with pancreatin and trypsin have a strong antitryptic power, especially the liver, kidneys, spleen, and bonemarrow; and also in a slight degree after amylopsin.

18. The quantity of grape sugar in the serum, and of glycogen in the liver, is decreased by immunization with pancreatin; and also in a slight degree after trypsin and amylopsin.

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