XXIII. THE EFFECT OF ALCOHOL ON THE DIGESTION OF FIBRIN AND CASEINOGEN BY TRYPSIN.

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THE behaviour of extracts of pancreas or pure pancreatic juice under different conditions has led various observers to conclude that the pancreas contains a number of proteolytic enzymes. It was shown by Fermi [1890] that after treatment with mercuric chloride, salicylic acid and various other substances, trypsin lost its power of digesting fibrin but would still digest gelatin. Vernon [1901] arguing from the varying sensitiveness of pancreatic extracts towards sodium carbonate, concluded that "trypsin" was really a mixture of enzymes of different degrees of stability, the more sensitive enzymes being destroyed first. Vernon only tested the digestive power of trypsin on raw fibrin in this connection however. In a later paper Vernon [1903, 2] states that pancreatic extracts contain an erepsin as well as trypsin. Pollak [1905] using different preparations of enzymes found that the relative amounts of serum and gelatin digested varied enormously in different cases. He also found that after treatment with hydrochloric acid trypsin lost its power of acting on serum, but was still about as active as ever on gelatin. Pollak concluded that extracts of pancreas contained in addition to trypsin (to which the action on serum was due) a special enzyme which acted only on gelatin. To this enzyme Pollak gave the name of glutinase.

According to Ascoli and Neppi [1908], however, this assumption of a special enzyme acting on gelatin is unjustified, as they find that slight variations in the reaction of the medium affect the digestion of different proteins to different degrees. Mays [1906] after a long series of experiments remarks that the presence of two proteolytic enzymes in pancreatic extracts can only be proved when it is possible to make a separation of the enzymes. It had been previously shown by Bayliss and Starling [1903] that pancreatic juice as secreted contains no trypsin (as tested on coagulated egg white), but contains a weak enzyme like erepsin. This has some action on caseinogen, but very slight. The erepsin has a slight action on fresh fibrin but practically none on fibrin which has been heated to 70°. It may here be mentioned that Long and Barton [1914] state that raw fibrin even when very carefully purified may soon become liquid owing to autolysis.

In later papers Fermi [1913, 1914] contests the theory that some proteolytic enzymes have a specific action, and maintains that all proteolytic enzymes have a general action on all proteins.

Slight differences of behaviour of trypsin towards different proteins under the same conditions have also been noted by Berg and Gies [1906], Porter [1910], Long and Hull [1917], but not much importance seems to have been attached to the facts. Others such as Glaessner and Stauber [1910] and Auerbach and Pick [1912] find differences between the proteolytic and peptolytic actions of trypsin, but in these cases possibly some of the action was due to the pancreatic erepsin also.

It seems to have been assumed, however, by all the authors quoted and by others such as Hedin [1905] that trypsin is the enzyme responsible for the digestion of fibrin and caseinogen, especially in experiments lasting only a few hours.

The action of alcohol on trypsin has been variously stated. Fermi and Pernossi [1894] using Mett's tubes filled with gelatin found that in presence of alcohol trypsin had more digestive action than in presence of water only. The percentage of alcohol used is not stated. Chittenden and Mendel [1896] found that the action of trypsin on fibrin was markedly inhibited by alcohol, but did not test the action on any other substrate. Dastre [1896] found that trypsin still digested fibrin and boiled albumin in presence of 15 to 20 per cent. of alcohol, while Gizelt [1906, 1, 2] states that 20 % alcohol totally inhibits trypsin. According to Bayliss [1915] trypsin will digest gliadin even in presence of 80 % alcohol, the action in this case being due to the trypsin in suspension. Vernon [1903, 1] noted that dilute alcohol had a considerable inhibitory effect on the digestion of raw fibrin by trypsin.

As dilute alcohol is frequently used in making extracts of various digestive organs, it is important to know how the digestive action is affected thereby.

EXPERIMENTAL DETAILS.

The experiments were carried out as described previously [Edie, 1914]. Ox fibrin after being finely minced and thoroughly washed was suspended in water and gradually heated to 85°. The fibrin was then pressed dry and preserved in glycerol and a little chloroform until required. The caseinogen was a 3 % solution in 1 % sodium carbonate. The pancreatic extracts were prepared by finely mincing sheep's pancreas and extracting with chloroform water for about a fortnight. The extract was then filtered and a little chloroform added as a preservative.

The digestion was carried on at 37° in small flasks, a small measured quantity of chloroform being added to exclude bacterial action in every case. When fibrin was used, the amount of digestion was estimated by filtering off the undissolved fibrin and determining the nitrogen in the filtrate by a Kjeldahl determination. When caseinogen was the substrate, the amount of

digestion was found by precipitation with tannic acid and subsequent estimation of the nitrogen in the filtrate. Controls showed that the sodium carbonate alone had no digestive action whatever either on fibrin or caseinogen. The following are typical results showing the effect of dilute alcohol on the digestion of fibrin and caseinogen by trypsin.

					Digestion in cc. of N/10 nitrogen
1.	(a)	1 c.c. trypsin, 20 c.c. 10 % alcohol, 20 c.c. 1 % Na_2CO_3	...	...	4.6
	(b)	1 c.c. trypsin, 20 c.c. water, 20 c.c. 1 % Na_2CO_3	...	...	17.1
		1.4 g. fibrin added. Digestion 3 hours.			
	(a)	1 c.c. trypsin, 20 c.c. 10 % alcohol, 20 c.c. caseinogen	...	...	23.8
	(b)	1 c.c. trypsin, 20 c.c. water, 20 c.c. caseinogen	...	...	23.6
		Digestion 1 hour.			
2.	(a)	1 c.c. trypsin, 20 c.c. 12 % alcohol, 20 c.c. 1 % Na_2CO_3	...	...	4.6
	(b)	1 c.c. trypsin, 20 c.c. water, 20 c.c. 1 % Na_2CO_3	...	...	13.8
		1 g. fibrin. Digestion 2.75 hours.			
	(a)	1 c.c. trypsin, 20 c.c. 12 % alcohol, 20 c.c. caseinogen	...	...	24.1
	(b)	1 c.c. trypsin, 20 c.c. water, 20 c.c. caseinogen	...	...	24.3
		Digestion 1.25 hours.			
3.	(a)	1 c.c. trypsin, 20 c.c. 10 % alcohol, 20 c.c. 1 % Na_2CO_3	...	...	3.8
	(b)	1 c.c. trypsin, 20 c.c. water, 20 c.c. 1 % Na_2CO_3	...	...	14.1
		1.3 g. fibrin. Digestion 2 hours.			
	(a)	1 c.c. trypsin, 20 c.c. 10 % alcohol, 20 c.c. caseinogen	...	...	27.9
	(b)	1 c.c. trypsin, 20 c.c. water, 20 c.c. caseinogen	...	...	27.0
		Digestion 1.25 hours.			
4.	(a)	1 c.c. trypsin, 20 c.c. 10 % alcohol, 20 c.c. 1 % Na_2CO_3	...	...	8.1
	(b)	1 c.c. trypsin, 20 c.c. water, 20 c.c. 1 % Na_2CO_3	...	...	23.6
		1.5 g. fibrin. Digestion 2.25 hours.			
	(a)	1 c.c. trypsin, 20 c.c. 10 % alcohol, 20 c.c. caseinogen	...	...	30.0
	(b)	1 c.c. trypsin, 20 c.c. water, 20 c.c. caseinogen	...	...	29.3
		Digestion 1 hour.			
5.	(a)	1 c.c. trypsin, 20 c.c. 8 % alcohol, 20 c.c. 1 % Na_2CO_3	...	...	5.1
	(b)	1 c.c. trypsin, 20 c.c. water, 20 c.c. 1 % Na_2CO_3	...	...	12.1
		1 g. fibrin. Digestion 3 hours.			
	(a)	1 c.c. trypsin, 20 c.c. 8 % alcohol, 20 c.c. caseinogen	...	...	30.6
	(b)	1 c.c. trypsin, 20 c.c. water, 20 c.c. caseinogen	...	...	30.6
		Digestion 1.25 hours.			
6.	(a)	1 c.c. trypsin, 20 c.c. 8 % alcohol, 20 c.c. 1 % Na_2CO_3	...	...	4.9
	(b)	1 c.c. trypsin, 20 c.c. water, 20 c.c. 1 % Na_2CO_3	...	...	12.1
		1 g. fibrin. Digestion 3 hours.			
	(a)	1 c.c. trypsin, 20 c.c. 8 % alcohol, 20 c.c. caseinogen	...	...	27.7
	(b)	1 c.c. trypsin, 20 c.c. water, 20 c.c. caseinogen	...	...	28.0
		Digestion 1 hour.			
7.	(a)	1 c.c. trypsin, 20 c.c. 6 % alcohol, 20 c.c. 1 % Na_2CO_3	...	...	11.5
	(b)	1 c.c. trypsin, 20 c.c. water, 20 c.c. 1 % Na_2CO_3	...	...	20.3
		1 g. fibrin. Digestion 3 hours.			
	(a)	1 c.c. trypsin, 20 c.c. 6 % alcohol, 20 c.c. caseinogen	...	...	22.2
	(b)	1 c.c. trypsin, 20 c.c. water, 20 c.c. caseinogen	...	...	22.0
		Digestion 1 hour.			

						Digestion in cc. of $\bar{N}/10$ nitrogen
8.	(a)	1 c.c. trypsin, 20 c.c. 6 % alcohol,	20 c.c. 1 % Na_2CO_3	...	...	12.5
	(b)	1 c.c. trypsin, 20 c.c. water,	20 c.c. 1 % Na_2CO_3	...	...	22.2
		1 g. fibrin. Digestion 3 hours.				
	(a)	1 c.c. trypsin, 20 c.c. 6 % alcohol,	20 c.c. caseinogen	...	...	25.0
	(b)	1 c.c. trypsin, 20 c.c. water,	20 c.c. caseinogen	...	...	24.6
		Digestion 1 hour.				
9.	(a)	5 c.c. trypsin, 20 c.c. 16 % alcohol,	20 c.c. 1 % Na_2CO_3	...	...	7.1
	(b)	5 c.c. trypsin, 20 c.c. water,	20 c.c. 1 % Na_2CO_3	...	...	18.2
		1 g. fibrin. Digestion 2 hours.				
	(a)	5 c.c. trypsin, 20 c.c. 16 % alcohol,	20 c.c. caseinogen	...	...	31.6
	(b)	5 c.c. trypsin, 20 c.c. water,	20 c.c. caseinogen	...	...	31.9
		Digestion 1 hour.				
10.	(a)	5 c.c. trypsin, 20 c.c. 13 % alcohol,	20 c.c. 1 % Na_2CO_3	...	...	14.0
	(b)	5 c.c. trypsin, 20 c.c. water,	20 c.c. 1 % Na_2CO_3	...	...	25.2
		1.3 g. fibrin. Digestion 3 hours.				
	(a)	5 c.c. trypsin, 20 c.c. 13 % alcohol,	20 c.c. caseinogen	...	...	34.5
	(b)	5 c.c. trypsin, 20 c.c. water,	20 c.c. caseinogen	...	...	34.6
		Digestion 1.25 hours.				
11.	(a)	5 c.c. trypsin, 20 c.c. 14 % alcohol,	20 c.c. 1 % Na_2CO_3	...	...	13.7
	(b)	5 c.c. trypsin, 20 c.c. water,	20 c.c. 1 % Na_2CO_3	...	...	26.8
		1.2 g. fibrin. Digestion 2.5 hours.				
	(a)	5 c.c. trypsin, 20 c.c. 14 % alcohol,	20 c.c. caseinogen	...	...	32.5
	(b)	5 c.c. trypsin, 20 c.c. water,	20 c.c. caseinogen	...	...	32.5
		Digestion 1 hour.				
12.	(a)	5 c.c. trypsin, 20 c.c. 14 % alcohol,	20 c.c. 1 % Na_2CO_3	...	...	10.8
	(b)	5 c.c. trypsin, 20 c.c. water,	20 c.c. 1 % Na_2CO_3	...	...	24.6
		1 g. fibrin. Digestion 2 hours.				
	(a)	5 c.c. trypsin, 20 c.c. 14 % alcohol,	20 c.c. caseinogen	...	...	30.0
	(b)	5 c.c. trypsin, 20 c.c. water,	20 c.c. caseinogen	...	...	30.1
		Digestion 1 hour.				

These experiments are sufficient to show that alcohol, when present in percentages varying from 3 to 7, has a very marked inhibitory effect on the digestion of fibrin by trypsin but no such effect on the digestion of caseinogen. The amount of fibrin digested under these conditions varied from about 25 to 50 % of the amount digested in absence of alcohol, the proportion varying somewhat with different trypsin solutions and with varying percentages of alcohol. In no case was there any appreciable difference in the amount of caseinogen digested, beyond the limits of experimental error.

With higher percentages of alcohol the digestion of fibrin was in some cases entirely stopped, a fair amount of caseinogen still being digested, however.

						Digestion in c.c. of $\bar{N}/10$ nitrogen
13.	(a)	1 c.c. trypsin, 20 c.c. 25 % alcohol,	20 c.c. 1 % Na_2CO_3	...	...	3.1
	(b)	1 c.c. trypsin, 20 c.c. water,	20 c.c. 1 % Na_2CO_3	...	...	27.2
		1 g. fibrin. Digestion 3 hours.				
	(a)	1 c.c. trypsin, 20 c.c. 25 % alcohol,	20 c.c. caseinogen	...	...	20.0
	(b)	1 c.c. trypsin, 20 c.c. water,	20 c.c. caseinogen	...	...	23.7
		Digestion 1 hour.				

						Digestion in cc. of N/10 nitrogen
14.	(a)	1 c.c. trypsin, 20 c.c. 25 % alcohol, 20 c.c. 1 % Na_2CO_3	...	...	...	4.8
	(b)	1 c.c. trypsin, 20 c.c. water, 20 c.c. 1 % Na_2CO_3	...	...	...	25.2
		0.8 g. fibrin. Digestion 3 hours.				
	(a)	1 c.c. trypsin, 20 c.c. 25 % alcohol, 20 c.c. caseinogen	...	...	...	32.8
	(b)	1 c.c. trypsin, 20 c.c. water, 20 c.c. caseinogen	...	...	...	37.9
		Digestion 1.75 hours.				
15.	(a)	1 c.c. trypsin, 20 c.c. 50 % alcohol, 20 c.c. 1 % Na_2CO_3	...	...	...	0.0
	(b)	1 c.c. trypsin, 20 c.c. water, 20 c.c. 1 % Na_2CO_3	...	...	...	23.6
		1 g. fibrin. Digestion 3 hours.				
	(a)	1 c.c. trypsin, 20 c.c. 50 % alcohol, 20 c.c. caseinogen	...	...	...	5.3
	(b)	1 c.c. trypsin, 20 c.c. water, 20 c.c. caseinogen	...	...	...	25.2
		Digestion 1 hour.				
16.	(a)	1 c.c. trypsin, 20 c.c. 50 % alcohol, 20 c.c. 1 % Na_2CO_3	...	...	...	0.0
	(b)	1 c.c. trypsin, 20 c.c. water, 20 c.c. 1 % Na_2CO_3	...	...	...	23.4
		1.2 g. fibrin. Digestion 3 hours.				
	(a)	1 c.c. trypsin, 20 c.c. 50 % alcohol, 20 c.c. caseinogen	...	...	...	4.6
	(b)	1 c.c. trypsin, 20 c.c. water, 20 c.c. caseinogen	...	...	...	28.2
		Digestion 1 hour.				

These experiments show that in presence of 25 % of alcohol the digestion of fibrin by trypsin is entirely inhibited, while digestion of caseinogen still proceeds to a limited extent. In presence of 12 % alcohol the amount of fibrin digested is from 10 to 20 % of the control, while the caseinogen digested amounts to about 85 % of the control.

Trypsin is well known to be very unstable under some circumstances, and it was considered possible that contact with dilute alcohol for some time might lead to an actual destruction of that part of the enzyme molecule which digests fibrin. The following experiments were carried out to test such a theory.

						Digestion in c.c. of N/10 nitrogen
17.	(a)	20 c.c. trypsin, 15 c.c. 15 % alcohol	} kept at 37° C. for 3 hours			
	(b)	20 c.c. trypsin, 15 c.c. water				
		2 c.c. of (a), 40 c.c. 0.5 % Na_2CO_3	...	...	...	31.2
		2 c.c. of (b), 40 c.c. 0.5 % Na_2CO_3	...	...	...	31.0
		1.3 g. fibrin. Digestion 3 hours.				
18.	(a)	20 c.c. trypsin, 5 c.c. 30 % alcohol	} kept at 37° C. for 3 hours			
	(b)	20 c.c. trypsin, 5 c.c. water				
		2 c.c. of (a), 40 c.c. 0.5 % Na_2CO_3	...	...	...	18.2
		2 c.c. of (b), 40 c.c. 0.5 % Na_2CO_3	...	...	...	18.3
		1.2 g. fibrin. Digestion 2 hours.				
19.	(a)	40 c.c. trypsin, 10 c.c. 30 % alcohol	} kept at 37° C. for 3 hours			
	(b)	40 c.c. trypsin, 10 c.c. water				
		1 c.c. of (a), 40 c.c. 0.5 % Na_2CO_3	...	...	...	14.0
		1 c.c. of (b), 40 c.c. 0.5 % Na_2CO_3	...	...	...	14.2
		1 g. fibrin. Digestion 3 hours.				
20.	(a)	15 c.c. trypsin, 10 c.c. 15 % alcohol	} kept at 37° C. for 3 hours			
	(b)	15 c.c. trypsin, 10 c.c. water				
		2 c.c. of (a), 40 c.c. 0.5 % Na_2CO_3	...	...	...	17.4
		2 c.c. of (b), 40 c.c. 0.5 % Na_2CO_3	...	...	...	17.0
		1 g. fibrin. Digestion 2.75 hours.				

						Digestion in cc. of N/10 nitrogen
21. (a)	15 c.c. trypsin,	10 c.c. 15 % alcohol	} kept at 37° C. for 3 hours			
(b)	15 c.c. trypsin,	10 c.c. water				
	2 c.c. of (a),	40 c.c. 0.5 % Na ₂ CO ₃	...	...	...	16.3
	2 c.c. of (b),	40 c.c. 0.5 % Na ₂ CO ₃	...	...	...	16.2
	1 g. fibrin. Digestion 2.75 hours.					

No destruction whatever of the trypsin is caused by the action of 6 % alcohol, although the digestive action of the enzyme is reduced to 30 % or less of the normal amount by the presence of this proportion of alcohol.

A solid substrate such as fibrin might be rendered less digestible by prolonged treatment with concentrated alcohol, owing to the hardening thus brought about. Alcohol of under 30 %, however, could hardly be supposed to have such an effect, and a few experiments showed that after treatment with dilute alcohol fibrin was no less digestible by trypsin than previously.

						Digestion in c.c. of N/10 nitrogen
22. (1)	Fibrin + 10 % alcohol	} kept at 37° C. for 3 hours				
(2)	Fibrin + water					
	1 c.c. trypsin,	40 c.c. 0.5 % Na ₂ CO ₃ ,	1 g. fibrin (1)	...	...	19.3
	1 c.c. trypsin,	40 c.c. 0.5 % Na ₂ CO ₃ ,	1 g. fibrin (2)	...	...	18.1
	Digestion 2.5 hours.					
23. (1)	Fibrin + 10 % alcohol	} kept at 37° C. for 19 hours				
(2)	Fibrin + 10 % alcohol					
	1 c.c. trypsin,	40 c.c. 0.5 % Na ₂ CO ₃ ,	1 g. fibrin (1)	...	...	22.9
	1 c.c. trypsin,	40 c.c. 0.5 % Na ₂ CO ₃ ,	1 g. fibrin (2)	...	...	20.2
	Digestion 3 hours.					

The fibrin which was to be treated with alcohol in these experiments was first washed with alcohol in order to remove any adherent moisture. It will be seen that after treatment with 10 % alcohol fibrin is apparently slightly more readily attacked by trypsin than previously.

The action of trypsin on fibrin and on caseinogen is affected by dilute alcohol to such different degrees that it is reasonable to suppose either that there are two enzymes concerned in the digestion of these proteins or that different groups of the same enzyme molecule take part in the hydrolysis of the different proteins. In the latter case the groups which digest fibrin are very much more easily inhibited by alcohol than the groups which digest caseinogen.

The theory that different side chains in the molecule of an enzyme are responsible for different functions is used to explain the zymoid modification of enzymes. Some observers also, for example, Nencki and Sieber [1901], hold that the behaviour of pepsin and rennin under varying conditions can best be explained on the theory that only one enzyme is concerned here, with different side chains responsible for the proteolytic and milk coagulating functions. Vernon [1903, 1] also considers this probable in the case of the milk coagulating and proteolytic actions of trypsin.

Hitherto it has apparently been assumed that one enzyme "trypsin" is responsible for the digestion of fibrin and caseinogen by pancreatic extracts.

In this case the function is the same (hydrolysis of a protein to form simpler products), but it would seem that different side chains may be necessary for the hydrolysis of different proteins.

SUMMARY.

Alcohol when present to the extent of 3 % and upwards markedly inhibits the action of trypsin on fibrin. The digestion of caseinogen by trypsin is not affected until the concentration reaches 10 %. The action of alcohol is not due to the destruction of the trypsin, since on suitable dilution of the mixture of trypsin and alcohol the digestion of fibrin is as great as in the control.

Fibrin is not rendered less digestible by contact with dilute alcohol, but seems to be slightly more readily dissolved by trypsin than previously.

If "trypsin" is a single enzyme the digestion of fibrin and caseinogen is probably carried on by different side chains, those digesting fibrin being much more readily affected by alcohol than the others.

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