LX. A NOTE ON THE QUESTION OF THE IDENTITY OF GASTRIC RENNIN AND PEPSIN.

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MUCH has been written on the question of the identity of gastric rennin and pepsin, and two theories have been brought forward. One, first associated with Pavlov [Pavlov and Parastschuk, 1904], is that pepsin and rennin are identical, Savjalov [1905] and Gewin [1907] holding that coagulation is the first stage in the digestion of milk by pepsin. The identity theory is based on the parallelism between the behaviour of the proteolytic and milk coagulating actions of gastric extracts under different conditions. The other theory is that the enzymes are different. There are two possibilities here, one, put forward by Nencki and Sieber [1901] and others, being that "pepsin" consists of a large molecule with different side chains, one set of which digests protein in acid solution, while another set is responsible for the coagulation of milk in neutral solution. A second possibility is that there are two distinct enzymes involved in the two functions. This is the theory which has been mainly developed by Hammarsten [1908]. It is difficult to distinguish experimentally between these two possibilities, and indeed the present state of our knowledge of the constitution of enzymes renders a distinction hardly practicable. Both depend on the fact that by suitable treatment the two actions can be separated from one another, so that a solution may be obtained which coagulates milk but has no proteolytic action, and on the other hand it is also possible to obtain an active proteolytic solution which has no milk coagulating properties.

Porter [1911] in support of Hammarsten's theory found that various commercial preparations, while coagulating milk, were actually anti-peptic. A full discussion of the subject is given by Oppenheimer [1913].

When investigating the development of enzymes from foetal life onwards I tested the properties of extracts of stomachs of young rabbits and compared these with similar extracts from adult animals. The differences found are recorded in this note.

According to Oppenheimer, pepsin is already present in the stomach of rabbits before birth, while according to Gmelin [1902] rennin is absent from the stomach of new-born animals. Other observers find that rennin develops

very rapidly after birth. Rakoczy [1910, 1911] and Van Hasselt [1910] found that in the case of calves the rennin disappeared rapidly during the first month after birth, while the pepsin increased greatly. The youngest animal employed by Rakoczy was apparently nine days old, but Van Hasselt does not state the exact age of the animals he used. In my experiments the stomachs of rabbits were taken as soon as possible after birth, washed out thoroughly and ground up with twice their weight of water. A little chloroform was added and after three days the liquid was filtered off and the filtrate tested. The stomachs of adult rabbits were tested in exactly the same way. The extracts were subjected to no further treatment with acid, sodium chloride or other substances such as were used by Hammarsten and others with a view to destroying the proteolytic or milk coagulating action as the case might be.

In the coagulating experiments 1 cc. of extract together with 5 cc. of milk was kept in a water-bath at 37° and examined every five minutes. Coagulation was considered complete when the test tube could be inverted without disturbance of the contents. Experiments were always repeated three times at least.

The proteolytic experiments were carried out on fibrin which had been finely minced, thoroughly washed and heated to 85°. 1 cc. extract + 40 cc. N/20 HCl + 1 g. fibrin were kept at 37° for a certain time and then filtered, the nitrogen being determined in the filtrate by Kjeldahl's method. Controls (HCl + fibrin) were also done and allowance was made for the amount of nitrogen originally present in the extracts.

The results are expressed as the number of cc. of decinormal nitrogen obtained from the fibrin digested.

Results of experiments:

A. New-born rabbits (each represents a different litter):

No.	Fibrin digested in 2 hours	Coagulation time
1	0.0 cc.	12 minutes
2	"	18 "
3	"	30 "
4	"	12 "
5	"	13 "
6	"	22 "

B. Adult rabbits:

No.	Fibrin digested in 1 hour	Coagulation time
1	4.6 cc.	No coagulation in 2 hours
2	8.0	" " 2 "
3	9.3	" " 1 hour
4	23.8	" " 2 hours
5	8.4	" " 2 "

These results show the very wide differences which exist between the gastric extracts prepared from young and adult rabbits respectively with regard to their content of rennin and pepsin.

In some cases the extract from young rabbits' stomachs was incubated with acid and fibrin for 18 hours without any appreciable amount of digestion taking place.

At the end of every coagulation experiment, in the case of the adult extracts, a few drops of active rennin were added to the mixture of extract + milk. Coagulation now always took place, proving, if need be, that the absence of coagulation at first was not due to lack of calcium or other deficiency in the milk, but to lack of rennin in the extract. These facts, so far as they go, seem to argue against pepsin and rennin being identical. The extracts were exactly similar in mode of preparation and it is unlikely that one set would contain any inhibitory substance (for example removable by dialysis) which would be absent from the other. No stomach, either of young or adult rabbit, was an exception to the rule that in young animals we get rennin but no pepsin, and as the animal reaches adult life the rennin entirely disappears but pepsin is now found in the stomach.

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