ENTEROKINASE IN INFANCY.*

A. E. AUSTIN, M.D.

(*From the Medical Chemistry Laboratory of Tufts College and the Chemical Laboratory of the Medical Department of the University of Texas.*)

Our early knowledge of enterokinase is obtained from Pawlow [11] who used intestinal secretion in all cases instead of extracts of the alimentary tract. Ellinger and Cohn,[6] however, used extracts of human jejunum, and found them active. Otto Cohnheim,[3] in his work on "The Physiology of Digestion and Nutrition" (page 39), says that enterokinase is a specific product of the epithelial cells of the small intestine and is only produced by them when the pancreatic juice enters the intestine. Furthermore, he states (on page 120) that the enterokinase is soluble in dilute alcohol and will endure heating to 70° C. without harm, and since it is generally regarded as an enzyme it will not withstand boiling temperature. Bayliss and Starling [1] state that no substance but this activator can convert trypsinogen into active trypsin, that it is secreted chiefly in the upper part of the small intestine and that it is decomposed at a temperature of 40° C. Cohnheim [4] has further shown that an excess of intestinal extract can hinder the action of trypsin, in fact can stop it and cause an already active tryptic digestion to cease. Unfortunately, access to his original work could not be had and the review (in the *Jahresbericht für Thierchemie*) did not give the exact amount of the intestinal extract which produced the most active tryptic digestion. Hallion and Carrion [7] consider since the duodenum, the source of the production of the enterokinase, is much oftener affected than the pancreas, insufficient intestinal digestion is oftener due to the lack of the former than of trypsin. Carmelo Ciaccio [2] found enterokinase in the spleen and lymph glands in which it was very much increased during infections. I could find

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no reference to its increase or diminution in the duodenum its natural site, during infections.

Hamburger and Hekma[8] had an opportunity during the persistence of an intestinal fistula in a man to observe the intestinal secretion for a period of sixty-six days and came to the conclusion that enterokinase could not activate an unlimited amount of trypsinogen as Bayliss and Starling had contended; in fact that the combination of the two must take place within very narrow limits. From this they concluded that the activator was not a true ferment. Furthermore, they showed that it was not identical with erepsin which was destroyed at a temperature of 59° C. after two hours heating, while the kinase required three hours heating at 67° C. for its destruction. My original intention in this investigation was to prove or disprove the statement of Hallion and Carrion that the secretion of enterokinase was oftener deficient in intestinal disturbances than that of trypsin.

The only way open to me for study of this question was by means of extracts of human organs obtained at autopsy, since Cohnheim and others had found that very active digestive agents could be procured from these, perhaps not much lacking in efficiency when compared with results obtained from the true intestinal juices. My original intention was only partially carried out, for the work was begun in winter when intestinal disturbances in infants are much less common, but as so little is known about the presence of this coferment in infancy, it seemed worth while to examine organs of a number of children to determine at what age it appears and also some of the conditions pertaining to its extraction from infant organs. As the continuation of the work shows most of these individuals died from pneumonia, what effect this disease may have had upon the presence and activity of enterokinase cannot, of course, be stated.

At the autopsy the pancreas and duodenum were carefully separated from each other, wrapped individually in gauze and brought to the laboratory in as fresh condition as

possible. (My earnest thanks are due to Professor Leary of Tufts College for his aid in procuring these specimens.) Arrived at the laboratory, they were carefully minced by an Enterprise meat cutter, washed by running water until free from blood, since the latter, as is well known, has a tendency to inhibit tryptic digestion, and after the finely divided material was almost white, placed in glycerine; the duodenum in ten cubic centimeters of the extractive and the pancreas in thirty, where they were allowed to remain twenty-four hours. After this period they were strained through gauze and if the extract was not sufficient to equal ten and thirty cubic centimeters, respectively, the tissue was washed with enough glycerine to make up those amounts. It will be well to state at this point that C. Delezenne[5] claims that blood serum does not affect the action of trypsin, but, in even small amounts, does hinder the activation of the trypsinogen; in other words, possesses an energetic antikinase; in either case the washing of the tissue was justified. Physiological salt solution was used at first, but was found much less effective than the glycerine. Usually the extracts were of a pale straw color, though often slightly blood stained when the washing was insufficient. On account of the inability of salt solution to extract the ferment, it is not probable that much of the enzymes was lost by washing with water.

It was now necessary to select a method for the determination of the digestive power of the ferment extracted; a method was desired which would enable me to compare the organs of the different individuals with fair accuracy, yet not one requiring enormous expenditure of time. Such an one had been devised by Loehlein[10] for quantitative trypsin determination, a method first used for pepsin determination by Vohlhard, which only required that the addition of hydrochloric acid be deferred until the digestion was completed. The principle of this procedure, as is well known, depends upon the fact that the acid combines both with the undigested protein as well as with the digested proteose, but the latter passes through the filter paper when the former is precipitated by sodium sulphate, and the acidity of the filtrate when measured by tenth normal sodium hydrate is

increased by every increase in the digested protein (or proteose) and hence in trypsin. Therefore, for comparative purposes the number of cubic centimeters of the sodium solution represents the amount of digestive enzyme present.

The only objection to this method that at once appears to one is whether the degree of alkalinity is sufficient for the energetic proteolytic power of the trypsin; this can be answered by the experience of Kantiz,[12] who found a solution containing from one-seventieth to one two-hundredth normal hydroxyl ions to be the best suited for this purpose; the solution recommended by Loehlein, however, contains one eighty-fifth normal hydroxyl ions and hence is well within the limit.

At first the casein gave some trouble in determining the end reaction with the filtrate because it contained some fat which gave a yellow color that obscured the change from a colorless to a pink solution. The casein was extracted several times with ether and then dried, whereupon a perfectly colorless solution ensued. Phenolphthalein was always used for an indicator.

The points to be first established were whether the stock solution would undergo an autodigestion; whether the pancreatic extract had any digestive power or not; whether this power was magnified by the addition of intestinal extract, and finally if the combination of pancreatic and intestinal extract were actually the cause of any increase of digestion which might occur beyond that taking place in the stock solution itself. Hence four digestions were prepared; the first consisting of one hundred cubic centimeters of the stock solution alone, diluted to three hundred; the second of one hundred of the stock solution, ten cubic centimeters of pancreatic extract and water to three hundred; the third of the same amount of casein solution, the same amount of pancreatic extract and five cubic centimeters of intestinal extract with water to three hundred; the fourth of exactly the same ingredients and proportion except that the extracts were cooked in a test-tube placed in a water bath which was kept briskly boiling for thirty minutes. This certainly will destroy any trypsin present and should render any kinase

inert, if it partakes of the nature of an enzyme as it is supposed to do. All digestions were saturated with chloroform and a layer of toluol poured over them to avoid any possible suspicion of bacterial action, and I may say that, as far as odor may inform one of such action, these digestions remained bacteria free. They were all placed in a brood oven at a temperature of 37° C., where they remained twenty-four hours. After the digestion was completed, in pursuance of the method of Loehlein, eleven cubic centimeters of normal hydrochloric acid were added and a saturated solution of sodium sulphate until the total reached four hundred cubic centimeters. A clear and colorless filtrate always resulted, except as stated above, upon filtration, two hundred cubic centimeters of which were always titrated with tenth normal sodium hydrate and the double of this factor (equal to the proteose in four hundred) used as the factor in the table which follows. Under remarks is stated the time elapsing after death when the autopsy was performed, the age of the child and the disease from which it died.

No.	Casein alone.	Pancreatic alone.	Amount Digested.	Pancreatic and Intestinal.	Amount Digested.	Pancreatic and Intestinal Cooked.	Amount Digested.	Remarks.
1	56.8	75	38.2	106	49.2	58.8	2	9 months old. Pneumonia.
2	57.2	106	48.8	110	52.8	64	6.8	18 months old. 24 hours P.M.
3	59.6	96	36.4	96.4	36.8	66	6.4	2 months old. 3 hours P.M.
4	59.6	107.2	47.6	105.4	45.8	69.6	10	1.5 months old. 6 hours P.M.
5	56.8	102.2	45.4	104.8	48	72.2	15.4	3 months old. 5 hours P.M.
6	55.8	69.8	14	85.4	28.36	55.7	1	4 months old. 14 hours P.M.
7	57.2	130.4	73.2	134.4	77.2	58.2	2	3 months old. 5 hours P.M.
8	54.6	101	46.4	101.6	47	57.9	3.2	11 months old. 5 hours P.M.
9	58.6	82.4	23.8	92	33.4	63.9	5.2	12 months old. 15 hours P.M.

All of these children died of pneumonia with the exception of number two, of enteritis; number six and seven, of empyema; number eight, of heart disease, and number nine, of rachitis.

The first thing that attracts our attention is that the pancreatic extract alone contains apparently an active trypsin as shown by the marked increase of the digestive product with that extract over that of the casein solution alone; as the latter was subjected to exactly the same conditions of temperature and underwent the same manipulations as the former, no other deduction is permissible. That the ferment in the glycerine was in its active form is very doubtful unless an active agent is found in the pancreas which according to the authorities quoted is very doubtful. How then did it become activated? We have several possibilities to consider: first, there is the statement of Hammersten[13] that pancreas or an infusion of it has the proferment converted to the ferment by the action of air and water; further, there is the bare chance that the pancreas and the intestine were brought in contact with each other at the autopsy, which is very doubtful on account of the care employed to avoid this at my request; again, it is very reasonable to suppose that the alkali used for the solution of the casein when brought in contact with the proferment may render it active. The first view is probably the correct one, since active trypsin extracts of the pancreas were made long before the kinase was ever known.

Upon the addition of the intestinal extract it is noticed that in many instances, not in all, there is a marked increase in the amount of proteoses as measured by the alkali used. For instance, in number one there is an increase of eleven cubic centimeters while the control shows only an increase of two over the casein alone. Number six also shows an excess of fourteen while the control shows only one; number seven gives four, but as the control has a digestion of two this result is too near the limit of error to place any dependence upon it.

In two instances the strong evidence of digestion with cooked extracts is inexplicable; all the extracts were

subjected to the same temperature, at least 98° C., as shown by an inserted thermometer and for the same period, thirty minutes, and all digestive action should have been destroyed. Since the degree of error does not approach, except in two instances, the increase of the results from the mixed extracts over the pancreas alone, I think we may conclude that this shows the presence of a kinase. Age apparently has no effect upon the results; as good a digestion was obtained with the organs of an infant of one and a half months old as one eighteen, while both are surpassed by one of three months. No distinctive effect can be seen either from variations of the time elapsing between death and the autopsy, at least within twenty-four hours; two equally good results were obtained with organs removed six and twenty-four hours after death.

As these results were not as distinctive as could be wished, it was decided to prepare another series in which the intestinal extract alone should be cooked; if kinase were present and is destroyed by heat, we would have more emphatic evidence of its action in the digestion with the double extracts. Hence the same routine was followed except that the intestinal extract was cooked thirty minutes and after cooling added to the mixture which contained the casein and the pancreatic extract. This formed one control, while the casein solution alone formed the other. It was found necessary to use the latter in every case since the casein solution differed in its titer from one period to another, while the specimen which had remained in the brood oven for twenty-four hours differed in its titer from the original stock solution in that the former always required one or two more cubic centimeters of the alkali than the latter; this can only be explained by the supposition that an autolytic process was going on, though this was avoided as much as possible by cooking the stock solution for a moment when it was made.

COOKED INTESTINAL EXTRACT AS CONTROL.

No.	Casein alone.	Pancreatic alone.	Amount Digested.	Pancreatic and Intestinal.	Amount Digested.	Pancreatic and Intestinal Cooked.	Amount Digested.	Remarks.
10	58.2	121.4	63.2	120.4	62.2	128.4	70.2	12 months old. 8 hours P.M.
11	59.6	89.4	29.8	120	60.4	90.8	31.2	14 months old. 24 hours P.M.
12	59.6	103.8	42.2	107.6	48	105.6	46	5 months old. 8 hours P.M.
13	56.2	141.2	85	140	83.8	140	83.8	11 years old. 2 hours P.M.
14	56.4	72.2	15.8	115.4	59	72	15.6	4 months old. 10 hours P.M.

Three of these children died of pneumonia, numbers eleven, twelve, and fourteen; number ten died of rachitis and number thirteen of pericarditis. Yet no distinction based upon the character of the disease can be discovered.

Upon examination of the figures, it is readily seen that eleven and fourteen show a marked increase of the digestive products with the combined extracts over that of the pancreas alone, while twelve has less increase, but when compared with the cooked intestinal extract still shows evidence of the effect of a kinase. How otherwise are we to interpret these results than that there is some agency which activates the pancreatic extract, in other words, a true kinase obtained from the intestine? Now when this latter is heated, we would expect to obtain the same results as with the pancreas alone. If we note the column containing these figures, we shall find that this expectation is fulfilled. In the first case there is apparently a marked discrepancy, but as this is so entirely unlike the others it must be regarded as a mistake of technic.

As before, no law of amount can be based either upon the age of the child or upon the time of the autopsy after death. Equally large quantities of kinase can be found in infants of four months as in those of fourteen, while almost

as much of this substance is found at an autopsy ten hours after death as one twenty-four hours.

The increase of digestive power of the child of eleven years, however, is noticeable; whether this is only relative or absolute I cannot say; unfortunately the organs were not weighed, so that no comparison can be made between the actual amount of tissue and its digestive power.

Another point remained to be investigated before we could accept these results as absolutely demonstrating the presence of a kinase; could not the increase of digestion noted when intestinal extract was added be due to proteolytic power of the intestinal secretion itself? True, all authorities are generally agreed that this secretion possesses no power to modify proteins nor fats, but it is still possible that the erepsin may act upon the casein, the only protein which it will affect; hence it was thought advisable to repeat these experiments with this variation, that the control contain cooked pancreatic extract added to uncooked intestinal extract; other than this modification, the digestions were of the same character as before. In this way it is very easy to demonstrate whether the intestinal extract has digestive power per se.

COOKED PANCREATIC EXTRACT AS CONTROL.

No.	Casein alone.	Pancreatic alone.	Amount Digested.	Pancreatic and Intestinal.	Amount Digested.	Pancreatic and Intestinal Cooked.	Amount Digested.	Remarks.
15	60	91.2	30.8	97	36.6	80.6	20.2	24 days old. 24 hours P.M.
16	55	86	31	113.6	58.6	54	0	2 years old. 24 hours P.M.
17	53.4	91	37.6	92.2	38.8	54	.6	7 months old. 24 hours P.M.
18	51.8	72.8	21	85	33.2	51	0	15 months old. 6 hours P.M.

In this list, numbers fifteen and seventeen died of pneumonia, sixteen of measles, and eighteen of empyema. As

can be seen, no significance can be attached to the cause of death as affecting the digestive power of the extracts. We have marked evidence of a kinase in three instances bearing in its amount no relation to the age of the infant nor to the time of the autopsy elapsing after death. That this increased power of the tryptic digestion is due to the kinase and not to a proteolytic action of a possible erepsin in the casein is readily shown by the fact that the intestinal extract when mixed with cooked pancreatic extract exhibits no action whatever; in only one instance is this not true, in the first where we are obliged to account for this apparent action in some other way since it is so contrary to the other three.

Of the antikinase which has been mentioned by Cohnheim and others, I have seen no evidence; if it were present, then the pancreatic digestions alone should have accomplished more than the mixed digestion. Upon a perusal of these figures no instance of that nature can be found. It is of course possible that with smaller portions of the intestinal extract better results might have been obtained, but at least there is no case in which the antikinase was able to nullify the effect of the pancreas infusion. Whether in the instances where there was no increase of the action of the two extracts over that of the pancreas alone, antikinase played any part cannot of course be told; the presumption is rather that there was no kinase under these conditions. It is interesting to note also that as early as twenty-four days after birth there was present both trypsin in the pancreas and probably a kinase in the intestine, though the evidence of the latter is not as good as could be wished.

Of these eighteen children we may say, with all fairness, that seven possessed intestines in which kinase was being secreted while two more had fair but less absolute evidence of the possession of the same ferment.

In closing, based upon this work, we may claim that the following conclusions are justifiable:

(1.) That from the intestines of some infants, not all, from twenty-four days of age on, a kinase can be isolated

which follows all the conditions laid down for that substance when present in the natural intestinal secretion.

(2.) That the disease from which the child dies (apart from intestinal disturbances which I have not yet had the opportunity to study), the age of the child and the period of the autopsy after death do not modify the amount of this coferment in any way which I have been able to discover.

(3.) As far as these experiments go, no evidence of an antikinase was discovered. In every case the digestion with pancreas and intestinal extract never produced less digestive products than the pancreas alone, though often equal amounts.

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