THE EFFECT OF DEHYDRATION ON THE PANCREATIC AND INTESTINAL ENZYMES

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Enzymes in Dehydration—The purpose of these experiments is to compare the enzyme content of the pancreas and small intestine in dehydrated and normal rats, and to relate the results to clinical conditions which are associated with dehydration.

In 1931, Banting, Gairns, Lang, and Ross (1) made an extensive study of the enzyme content of the stools of infants suffering from acute intestinal intoxication (acute fermentative diarrhea), in which one of the outstanding symptoms is dehydration. They showed that the trypsin was markedly reduced in this disease, and if daily estimations were made, the curve obtained could be used as an index of the severity of the case. The amylase and lipase of stool were also reduced, although the results obtained were not so consistently low as in the case of trypsin.

A further study was made in this laboratory (2) of the intestinal enzymes in cases dying of acute intestinal intoxication, and the erepsin and phosphatase content of the small intestine was found to be lower in this condition than in cases in which death was due to other causes.

Severe dehydration is one of the most important clinical symptoms of this condition, and invariably results from the rapid loss of fluids due to the diarrhea and vomiting. Johnston, Brown, Tisdall, and Fraser (3) have shown that in cases of acute intestinal intoxication organisms of the colon-paratyphoid-dysentery group are found in the intestinal tract, the most important member of the group being Bacillus dysenteriae, Sonne. In this condition also there is invariably a state of acidosis varying from a mild to a severe degree (4), and a decrease in the ability of the tissues to
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tolerate injected glucose (5). The blood viscosity is greatly increased and there is a marked diminution in the blood flow in the peripheral capillaries. In acute intestinal intoxication severity of dehydration appears to be associated with a very low enzyme content of the stool. In order further to determine the association of these two conditions, it was thought desirable to produce a state of dehydration in animals, and to find what the resultant effect would be on the production of enzymes.

In these experiments 6 week-old albino rats were used. The method employed in dehydrating the animals was to place them in individual wire cages in an incubator at 37.5°. After 12 hours incubation they were removed and kept at room temperature for the succeeding 12 hours. This procedure was continued for from 3 to 4 days, depending on the vitality of the individual groups. An adequate normal diet was fed to the rats during this time, but water was withheld. Rats averaging 76 gm. in weight lost between 23 and 29 per cent of their weight after 4 days dehydration.

Although there are a number of different methods which might have been used for the determination of the enzyme content of the extracts, it was decided to follow the methods in use by the Willstätter (6) school of enzyme chemistry. These methods are better known and probably more widely used than others.

The technique for determining the amounts of the different enzymes present and the methods of calculating the units of activity are given in the experimental part. In all cases the results have been expressed in units of enzyme per gm. of tissue.

Pancreatic Enzymes

The rats were killed by a blow on the head and the pancreas was dissected out, weighed, and ground with sand and a volume of 25 per cent glycerol equal to 20 times the weight of the pancreas. After 18 hours extraction at room temperature, it was centrifuged, and the tryptic and amyloytic activity of aliquot samples determined.

Trypsin—Trypsin is the proteolytic enzyme of the pancreatic secretion. It acts on the proteins and semidegradation products of proteins coming from the stomach, to reduce them either to assimilable amino acids or simple peptides, which are further broken down by the action of intestinal erepsin.
TABLE I
Enzyme Content in Units per Gm. of Tissue of Normal and Dehydrated Rats

<table>
<thead>
<tr>
<th>Trypsin</th>
<th>Amylase</th>
<th>Epspin</th>
<th>Phosphatase</th>
</tr>
</thead>
<tbody>
<tr>
<td>pancreas</td>
<td>small intestine</td>
<td>pancreas</td>
<td>small intestine</td>
</tr>
<tr>
<td>Normal</td>
<td>Dehydrated</td>
<td>Normal</td>
<td>Dehydrated</td>
</tr>
<tr>
<td>20.9</td>
<td>11.3</td>
<td>139</td>
<td>49</td>
</tr>
<tr>
<td>34.2</td>
<td>15.2</td>
<td>183</td>
<td>83</td>
</tr>
<tr>
<td>24.7</td>
<td>19.0</td>
<td>191</td>
<td>104</td>
</tr>
<tr>
<td>34.2</td>
<td>15.2</td>
<td>180</td>
<td>47</td>
</tr>
<tr>
<td>22.8</td>
<td>15.2</td>
<td>161</td>
<td>43</td>
</tr>
<tr>
<td>20.9</td>
<td>21.0</td>
<td>180</td>
<td>59</td>
</tr>
<tr>
<td>24.7</td>
<td>17.1</td>
<td>126</td>
<td>71</td>
</tr>
<tr>
<td>36.1</td>
<td>19.0</td>
<td>145</td>
<td>94</td>
</tr>
<tr>
<td>11.3</td>
<td>134</td>
<td>100</td>
<td>22.2</td>
</tr>
<tr>
<td>22.8</td>
<td>145</td>
<td>97</td>
<td>28.7</td>
</tr>
<tr>
<td>13.3</td>
<td>143</td>
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</tr>
<tr>
<td>11.3</td>
<td>120</td>
<td>100</td>
<td>30.6</td>
</tr>
<tr>
<td>11.3</td>
<td>158</td>
<td>102</td>
<td>33.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>191</td>
<td>123</td>
</tr>
<tr>
<td>Average...</td>
<td>27.3</td>
<td>16.0</td>
<td>189</td>
</tr>
</tbody>
</table>

| 10.0 | 5.7 | 113 | 20.0 | 10.1 |
| 13.8 | 7.6 | 129 | 25.9 | 12.2 |
| 11.0 | 7.1 | 139 | 20.0 | 20.0 |
| 14.3 | 6.2 | 132 | 20.0 | 17.9 |
| 10.2 | 7.6 | 102 | 12.9 | 8.5 |
| 10.5 | 6.7 | 139 | 12.9 | 16.1 |
|        |        | 84 | 16.1 | 12.2 |
|        |        | 121 | 16.1 | 16.2 |
|        |        | 115 | 16.1 | 11.6 |
|        |        | 120 | 8.5 | 17.9 |
| Average... | 11.6 | 6.8 | 158 | 101 | 36.6 | 16.1 | 17.9 | 9.7 |

For trypsin, in the first section (8 normal and 15 dehydrated samples) the estimations were carried out on 0.5 cc. of extract, incubated 1 hour; in the second section (6 normal and 6 dehydrated samples) the estimations were carried out on 2 cc. of extract, incubated $\frac{1}{2}$ hour.
Effect of Dehydration on Enzymes

Estimations of trypsin were first made in duplicate with 0.5 cc. portions of the extract of pancreas; they were incubated for 1 hour. The average of these estimations shows that there were 16.6 units of trypsin per gm. of tissue in the fifteen dehydrated animals, as compared with 27.3 units in the eight normal animals.

Since the curve of tryptic digestion rises rather sharply at the outset and then flattens as the time increases, it was thought that a shorter incubation period would be preferable. A second series of estimations was carried out with 2 cc. of extract, incubated for 1/2 hour. Practically the same sort of result was obtained as in the first series of determinations. The dehydrated animals showed a definitely lower enzyme content than that shown by the normals; namely, an average of 6.8 as compared with 11.6 units (see Table I).

Amylase—Amylase is the carbohydrate-digesting enzyme of the pancreas. Its rôle in digestion consists in the breaking up of complex carbohydrates (polysaccharides) into simple diffusible sugars, such as maltose.

Forty-five estimations of amylase activity were carried out in duplicate on the same pancreatic extracts that were used in estimating tryptic activity.

The average amylase activity for the dehydrated animals (101 units per gm. of pancreas) is considerably less than that for the normal animals (158 units) (see Table I).

Intestinal Enzymes

The entire small intestine was removed from each rat, opened longitudinally, gently washed with tap water, dried between filter papers, and then weighed and ground with sand, and a volume of 25 per cent glycerol equal to 10 times the weight was added. The tubes were left 18 hours at room temperature for extraction, and the erepsin and phosphatase activity of aliquot samples was determined.

Erepsin—It has been shown that the mucous membranes of the small intestine elaborate a specific enzyme which is concerned in the hydrolysis of those intermediate protein degradation products called peptides, which are formed by the action of the gastric pepsin and the pancreatic trypsin on the food proteins.

51 estimations of the ereptic activity of extracts of small intes-
tine were made in duplicate. The average of the figures for the dehydrated animals (16.1 units) is less than half the average value for the normal rats (36.6 units).

**Phosphatase**—Intestinal phosphatase, which is sometimes called nucleotidase, has to do with the hydrolysis of the organic compounds of phosphoric acid contained in the food. Such compounds include the phosphoric esters of sugars and related substances, the more complex nucleotides which have been studied extensively by Levene and Dmochowski (7), and the phosphorylated fats, lecithin and cephalin, the enzymic hydrolysis of which has recently been investigated by King (8).

Thirty-eight estimations of the phosphatase activity of the intestinal extracts were made in duplicate. The average of the results for the dehydrated rats (9.7 units) is found to be almost half that of the normal rats (17.9 units).

**EXPERIMENTAL**

**Trypsin**—Activation of the trypsin solution by enterokinase is necessary before its activity can be measured. The enterokinase solution is prepared by dissolving 2 gm. of dried intestinal mucosa in 100 cc. of 0.05 N NH₄OH. After the mixture has been kept for 2 hours at 37.5°, solution should be complete. As enterokinase is unstable in the presence of ammonia, however, it is necessary to rid the solution of ammonia by evacuation at the water pump for 1 hour. The solution should be stored in the ice box and made fresh each week.

For the determination of the tryptic activity the pancreas is extracted with dilute glycerol. A measured amount is activated for 30 minutes at 37.5° with 0.3 cc. of the enterokinase solution. Water is added to 5 cc., and 0.3 gm. of casein (5 cc. of 6 per cent solution at pH 8.9) is added, together with 2 cc. of N NH₄-NH₄Cl (1:2) buffer of pH 8.9. Four tubes are put up in this way; two of them are kept at 37.5° for exactly 60 minutes, and the other two are poured into 50 cc. of hot alcohol, which stops the enzyme action immediately. The tubes are washed out with an additional 50 cc. of hot alcohol, which has been made alkaline to thymolphthalein with a few drops of 0.2 N KOH. The mixture is titrated with 0.2 N KOH made up in alcohol. At the end of the 60 minute incubation period the other two tubes are treated in a similar manner.
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The increase in the titratable acidity is measured by the difference between the potassium hydroxide used in the 0 and 60 minute tubes, and is taken as the measurement of the trypsic activity.

The unit of trypsin is defined as that amount of enzyme which will increase under these conditions the acidity of the mixture an amount equal to 1.05 cc. of 0.2 N KOH.

Amylase—Amylase activity is determined by estimating the amount of maltose liberated from starch by the pancreatic extract. The maltose formed is estimated by determining its reducing power against hypoidote by the Willstätter-Schudel method. In alkaline solution maltose reacts with iodine, with quantitative oxidation of the aldehyde group to carboxyl according to the equation, R·CHO + I₂ + 3NaOH → R·COONa + 2NaI + 2H₂O. Excess iodine is added over and above that necessary to give complete oxidation of the maltose, and the residual iodine is determined by titration with thiosulfate.

In four cylinders are measured 25 cc. of freshly prepared 1 per cent starch solution; 10 cc. of phosphate buffer, pH 6.8; 1 cc. of 0.2 N NaCl; 0.5 cc. of enzyme solution; and 0.5 cc. of water. The enzyme action in two of the cylinders is stopped by the addition of 3 cc. of 9 N acid. The other two tubes are incubated for the stated period of 1 hour, and the enzyme action is stopped by the addition of the same amount of acid.

10 cc. portions are titrated with N NaOH to phenolphthalein to determine the amount of alkali necessary to neutralize the acid and the phosphate buffer. This amount is usually fairly constant from one determination to the next, and the titration may hence be usually omitted. 10 cc. portions are treated with the same amount of alkali in 50 cc. glass-stoppered flasks, and 2 cc. of 0.1 N I₂ together with 3 cc. of 0.1 N NaOH are added. The flasks are kept for 15 minutes at 20°. 1 cc. of N acid is then added to liberate the remaining iodine, which is now titrated with 0.02 N thiosulfate, 3 drops of soluble starch solution being added for indicator. The difference between the thiosulfate titration in the 0 time and the ½ hour tests is taken as the measurement of the amylase activity, 1 cc. of 0.02 N thiosulfate being equal to 3.4 mg. of maltose.

The amylase unit is that amount of enzyme which will liberate 5 mg. of maltose under the above conditions.
Example—For six normal rats in which 0.5 cc. of a 1:20 extract was used, 22.4 mg. of maltose were formed. The 0.5 cc. contains therefore 22.4/5 units of amylase, and 1 gm. of tissue contains 22.4/5 \times 20/0.5 = 179.2 units.

Erepsin—Ereptic activity is determined by estimating the hydrolysis of the dipeptide glycylglycine by a measured amount of the enzyme solution. Erepsin acts on dipeptides to liberate free amino and carboxyl groups, the increase in either of which may be taken as a measurement of the enzyme activity. In the present method the carboxyl groups are measured by the Willstätter method, which takes advantage of the fact that the carboxyl group of the amino acid may be quantitatively titrated by alkali if the titration be carried out in alcohol. 5 cc. of 0.2 M (2.62 per cent) glycylglycine adjusted to pH 7.8 are added to 5 cc. of 0.1 M phosphate buffer of pH 7.8 and 0.5 cc. of intestinal extract. The volume is then made up to 15 cc. and the mixture is incubated for exactly 18 hours. It is then poured into 50 cc. of hot alcohol and the tube washed out with 50 cc. more of the hot alcohol (the alcohol should previously have been made just alkaline to thymolphthalein with KOH). Determinations are carried out in duplicate with 0 time controls.

The erepsin unit is that amount of enzyme which under the conditions of the experiment will liberate carboxyl groups equivalent to 1 cc. of 0.2 N KOH.

Example—0.5 cc. of 1:10 extract of rat intestine liberated carboxyl groups equivalent to 2.0 cc. of 0.2 N KOH. 1 gm. of tissue contains therefore 2.0 \times 10/0.5 = 40 units.

Phosphatase—The method used is that described by Kay (9). 5 cc. of Sørensen’s glycine buffer (pH 8.9 at 37°), 5 cc. of 0.3 per cent sodium-β-glycero phosphate, 0.5 cc. of the extract (adjusted to the same pH), and 2 drops of chloroform are added to each of four test-tubes. 2 cc. of 25 per cent trichloroacetic acid are added immediately to two of the tubes, and the other two are closed with well fitting rubber stoppers and incubated exactly 2 hours at 37.5° in a water thermostat, after which 2 cc. of the trichloroacetic acid are added to each. The mixtures are filtered through ash-free filter papers, and the inorganic phosphate estimated in 10 cc. of the filtrate by the colorimetric procedure described by King (1932) (10).
The phosphatase unit is defined as that amount of enzyme which will liberate 1 mg. of phosphorus under these conditions. Example—0.448 mg. of phosphorus was liberated under the conditions of the experiment. The 0.5 cc. of intestinal extract represents, therefore, 0.448 unit of phosphatase. Since a 1:10 extract was used, then 1 gm. of tissue (equivalent to 10 cc. of extract) contains 0.448 \times 10/0.5 = 8.96 units.

DISCUSSION

An examination of the figures for the activity of the four enzymes (see Table I) shows that there is considerable individual variation between rats which had been similarly treated. This is particularly true in the case of amylase, in which the activity of the pancreatic extract from seventeen normal rats varies from 126 to 191 units, and the activity of extracts from twenty-eight dehydrated rats varies from 43 to 139 units. These variations are probably only to a minor extent due to errors in technique, as duplicate estimations gave an average experimental error of only 3 per cent.

The more likely explanation is that there was considerable variation in the amount of digestive activity in progress when the animals were killed. Some rats on autopsy were found to have stomachs greatly distended with food; others had a moderate amount of food in the stomach, while in others the stomach was practically empty. There also appeared to be more secretion and digestion products in the upper part of the small intestine in rats whose stomachs were distended with food, indicating a more active digestive process probably associated with an increased secretion of enzymes.

SUMMARY

Rats which have been subjected to prolonged dehydration by dry heat show a decrease in the production of both pancreatic and intestinal enzymes.

The diminished enzyme production in dehydrated animals is probably comparable to that found in dehydrated infants suffering from acute intestinal intoxication.
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