The Fate of Amylase Introduced into the Body of Warm-Blooded Animals.

On the Relation between the Diastase Preparations Containing the
Amylolytic Ferment Given per Os or Hypodermically and Its
Amount Excreted in the Urine, Proportionate to That
in the Blood-Serum; and Also the Facts Observed
in the Course of These Experiments Con-
cerning the Blood-Sugar Content.

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The diastatic ferment in the urine has long been studied since C o h n h e i m¹⁾ in 1863 observed its presence in the urine. M a g e n d i e²⁾ in 1846 proved the human blood to be amylolytic. Although the ferment was known to be present, its study made little progress until W o h l g e m u t h³⁾ in 1908 succeeded in estimating accurately this ferment.

W o h l g e m u t h,⁴⁾ after the ligation of the duct of the pancreas in a dog, found both in the urine and the blood an increased amount of the ferment, which, however, very rapidly became normal again. In 1909⁵⁾ he found in four cases of kidney disease and two cases of diabetes a little decrease of diastatic content in the urine.

B e n c z u r,⁶⁾ following W o h l g e m u t h's original method, examined the serum and urine of fifty cases and discovered that there was no change in the value of the diastatic content. In one case of occlusion of the pancreatic duct, however, he found a diastatic content much exceeding normal, and he also found a high content in nine out of sixteen cases of nephritis.

On the other hand, V y n h a u s e n⁷⁾ in 1910 found in the urine of forty diabetics and thirty-two cases of nephritis a large decrease in diastatic content. R o s e n t h a l⁸⁾ in 1911, using the modified W o h l g e m u t h method, found the decreased diastatic ferment of the urine in seven cases of chronic parenchymatous nephritis.

Marino⁹⁾ in 1911, following a modified method of his own, found a great decrease in diastatic ferment of the urine in nephritis.

Loeper and Fiçai,¹⁰⁾ 1907, after ligating the Wirsung duct of a dog, found, both in the urine and the blood, a decreased amount of the ferment.

In another field, Kussmaul¹¹⁾ in 1874 tried to use diastase preparations in the medical treatment of diabetics. Although he hypodermically injected the diastase preparation, he found no change in the blood and in the urine. But he succeeded in decreasing the elimination of sugar in the urine by means of the intravenous injection of a lesser quantity of the same substance.

So it will be interesting to investigate the value of the diastatic content of the blood and the urine after the diastase preparations are given both per os and injected hypodermically.

METHOD.

The substratum used consisted of a 1 per 1000 solution of soluble starch, the starch being Kahlbaum's special "Lösliche Stärke." The amount of this (0.1 grm.) was weighed out and 100 c.c. of distilled water were added. The suspension was heated to boiling and kept boiling for fifteen minutes with stirring. The solution was then allowed to cool, and distilled water was added in order to make it up to 100 c.c. For safety a fresh solution was prepared daily. When urine was to be examined, the urine passed in the twenty-four hours was collected and the amylase content of the day's mixed specimen was determined. The product of this value and the volume of urine passed gave the total amount of amylase passed each day. So from the above specimen each definite quantity of urine was pipetted into a series of test tubes, in decreasing quantities.

To each tube 2 c.c. of the starch solution were then added; the tubes were mixed by shaking and were placed in a water-bath at 38° C.; at the end of thirty minutes the tubes were removed from the bath and were plunged into cold water to arrest digestion. Upon adding a few drops of Lugol's solution to each tube a beautiful graduation of color was produced. The tubes in which no blue color is seen at all are naturally free from starch, whereas those colored purple show the presence of this substance. That tube which stands at the lowest in the non-blue set and at the same time next to the first purple one

is taken as the limit ("Limes"); that is to say, it contains just enough amylolytic ferment to change 2 c.c. of 1/1000 starch solution.

Reduction to units is done as follows: X = number of c.c. of urine required to digest 2 c.c. of starch solution (1:1000) in thirty minutes at 38°C . $D_{30'}^{38^{\circ}}$ = number of amylolytic units per c.c. of urine, determined under these conditions. Then $D_{30'}^{38^{\circ}} = \frac{2}{X}$.

The method I used for the determination of the amylase value of blood-serum was the same as that used for urine, aside from using normal saline solution in place of distilled water. The solution given per os consisted of 5 grms. of these preparations in 50 c.c. of ordinary water. 2 c.c. of 1 per cent of the diastase preparations were hypodermically injected. The "Taka-diastase" is made by Sankyo & Co. of Tokyo and "pancreatin" by Parke, Davis & Co.

The blood-sugar content was estimated after Bang by puncture of the ear-lobe every morning.

NORMAL AMYLASE CONTENT OF BLOOD-SERUM AND URINE.

As to the amount present in the serum of normal rabbits I have found it to be constant, viz. $D = 8$ units. In the urine of normal rabbits a value of from 4 to 32 units per c.c. has been found the average, though in the case of urine more variation might be expected than in the serum.

EXPERIMENTAL RESULTS.

The following experiments were carried out with a view to testing the influence of the concentration of the urine on the value of $D_{30'}^{38^{\circ}}$ per c.c. To test this point further I took two rabbits, collected the urine passed in twenty-four hours on five successive days in each case, and determined the amylase content of the day's mixed specimen and also occasionally that ferment of the blood-serum of the rabbit. From the sixth day an artificial diuresis was produced by giving the rabbit 50 c.c. of ordinary water.

The difference between the diastatic ferment in the urine for five days before and after giving ordinary water was $2150 - 2020 = 130$, the amount of diastatic ferment in the urine being increased (Table I).

The difference between the total diastatic ferment in the urine for five days before and after giving ordinary water was $2470 - 2328 = 142$, the amount of diastatic ferment in the urine being increased (Table II).

TABLE I.
Experiment I. Normal rabbit. 1.83 kilos.

Date	$D_{30'}^{38^{\circ}}$	Volume of urine c.c.	Total $D_{30'}^{38^{\circ}}$	Total amount of $D_{30'}^{38^{\circ}}$ for 5 days
Sept., 1922				
5	4	40	160	2020
6	4	95	380	
7	4	100	400	
8	4	100	400	
9	4	170	680	
After giving 50 c.c. of ordinary water.				
12	2	155	310	2150
13	4	165	660	
14	2	140	280	
15	4	150	600	
16	2	150	300	

TABLE II.
Experiment II. Normal rabbit. 1.8 kilos.

Date	$D_{30'}^{38^\circ}$	Volume of urine c.c.	Total $D_{30'}^{38^\circ}$	Total amount of $D_{30'}^{38^\circ}$ for 5 days
Sept., 1922				
5	4	67	268	2328
6	4	145	580	
7	4	110	440	
8	4	180	720	
9	4	80	320	
After giving 50 c.c. of water.				
12	2	150	300	2470
13	4	155	620	
14	2	155	310	
15	4	160	640	
16	2	200	400	

While the urine volume increases, as with the two rabbits in Experiments I and II, after giving water D tends to decrease inversely.

After all, the total amount of amylase for five days before giving 50 c.c. of water is almost the same as that following it. We now proceed to experiments in which diastase preparations were given per os and hypodermically. The amylolytic power of the diastase preparation is shown in Table III.

TABLE III.				
$D_{30'}^{38^\circ}$	of 1 c.c. of	1 per cent of	taka-diastase	250
"	"	1	" pancreatin	25
"	"	10	" taka-diastase	2500
"	"	10	" pancreatin	250

The diastatic ferment in the daily urine was determined on five or ten successive days; occasionally that of the blood-serum and the blood-sugar content of the rabbit were also estimated before giving the diastatic preparation.

Experiment III. Normal rabbit. 1.79 kilos.

We determined the amylolytic power of the urine for five days and then gave per os 5 grms. of taka-diastase dissolved in 50 c.c. of water to find out the amylolytic power of the urine (Table IV).

TABLE IV.

Date	Urine				Blood
	$D_{30'}^{38^\circ}$	Volume c.c.	Total $D_{30'}^{38^\circ}$	Total amount of D for 5 days	$D_{30'}^{38^\circ}$
April, 1922					
14	8	160	1280		
15	4	140	560		
16	4	150	600		
17	4	145	580		
18	4	150	600	3620	8
After giving taka-diastase per os.					
20	4	180	720		
21	16	90	1440		
22	8	250	2000		
23	8	200	1600		
24	8	210	1680	7440	8

The difference between the total diastatic ferment in the urine for five days before and after giving taka-diastase was 3820, the amount of diastatic ferment in the urine being increased. The total amount of the diastatic ferment of the taka-diastase given per os for five days was 6250.

So we concluded that about half of the diastatic value given per os was excreted in the urine in five days.

Experiment IV. Normal rabbit. 1.75 kilos.

Five grms. of taka-diastase dissolved in 50 c.c. of water were given per os to the rabbit five successive days, and the amylolytic power of the urine and the blood-sugar was determined (Table V).

The difference between the total diastatic ferment in the urine for five days before and after giving taka-diastase was 7080, the amount of diastatic ferment in the urine being increased. The total amount of the diastatic ferment of taka-diastase given per os for five days was 6250.

It will be seen that almost the total amount of the diastatic value given per os was excreted in the urine in five days; or rather some-

what more is excreted than is given per os. This may be explained by the fact that the diastase in the urine is in a more favorable medium. The blood-sugar is almost unchanged.

TABLE V.

Date	Urine				Blood	
	$D_{30'}^{38^\circ}$	Volume c.c.	Total $D_{30'}^{38^\circ}$	Total amount of D for 5 days	$D_{30'}^{38^\circ}$	Sugar %
March, 1922						
31	8	180	1440			0.098
April						
1	8	200	1600			
2	4	190	760			
3	8	170	1360			
4	4	120	480	5640	8	
After giving taka-diastase per os.						
5	16	210	3360			
6	16	170	2720			
7	16	140	2240			
8	16	200	3200			
9	8	150	1200	12,720	8	0.101

Experiment V. Normal rabbit. 1.82 kilos.

The normal amylolytic power of the urine and the normal blood-sugar are determined. Then 5 grms. of pancreatin dissolved in 50 c.c. of water are given per os to the rabbit for eight successive days, and this result is compared with those of the normal rabbit (Table VI).

TABLE VI.

Date	Urine				Blood	
	$D_{30'}^{38^\circ}$	Volume c.c.	Total $D_{30'}^{38^\circ}$	Total amount of D for 8 days	$D_{30'}^{38^\circ}$	Sugar %
June, 1922						
7	8	120	960			
8	16	125	2000			
9	16	180	2880			
10	16	120	1920			0.096
11	8	125	1000			
12	16	150	2400			
13	8	170	1360		8	
14	16	90	1440	13,960		0.094
After giving pancreatin per os.						
16	8	210	1680			
17	8	130	1040			
18	8	150	1200			
19	32	120	3840			
20	16	160	2560			
21	16	135	2160			
22	16	135	2160			0.107
23	8	80	640	15,280	8	

The difference between the total diastatic ferment in the urine for eight days before and after giving pancreatin was 1320, the amount of diastatic ferment in the urine being increased. The total amount of the diastatic ferment of the pancreatin given per os for eight days was 1000. So we see that almost the total amount of the diastatic value given per os was excreted in the urine in eight days.

Experiment VI. Normal rabbit. 1.85 kilos.

After determining the amylolytic power of the normal urine and the normal blood-sugar for ten days, we gave per os to the rabbit 5 grms. of pancreatin dissolved in 50 c.c. of water for the purpose of examining the variation of the amylolytic power and the blood-sugar (Table VII).

TABLE VII.

Date	Urine				Blood	
	$D_{30'}^{38^\circ}$	Volume c.c.	Total $D_{30'}^{38^\circ}$	Total amount of D for 10 days	$D_{30'}^{38^\circ}$	Sugar %
June, 1922						
7	16	75	1200			
8	16	125	2000			
9	16	90	1440			
10	16	90	1440			
11	8	100	800			
12	16	125	2000			0.100
13	8	140	1120			
14	16	140	2240			
15	16	130	2080			
16	16	90	1440	15,760	8	
After giving pancreatin per os.						
28	8	170	1360			
29	8	100	800			
30	8	130	1040			
July						
1	16	180	2880			
2	16	180	2880			
3	16	180	2880			
4	16	120	1920			0.118
5	8	75	600			
6	16	120	1920			
7	8	150	1200	17,480	8	

The difference between the total diastatic ferment in the urine before and after giving pancreatin for ten days was 1720, the amount of diastatic ferment in the urine being increased. The total amount of the pancreatin given per os for ten days was 1250. So the result is that almost the total amount of the diastatic value given per os was excreted in the urine in ten days. The blood-sugar is found to increase slightly.

Experiment VII. Normal rabbit. 1.75 kilos.

After we had determined the urine and the blood of the normal rabbit daily for five days, we dissolved 5 grms. of taka-diastase in 50 c.c. of water and gave it per os to the rabbit for five successive days in order to determine the amylolytic power of the urine and the blood-sugar (Table VIII).

TABLE VIII.

Date	Urine				Blood	
	$D_{30'}^{38^\circ}$	Volume c.c.	Total $D_{30'}^{38^\circ}$	Total amount of D for 5 days	$D_{30'}^{38^\circ}$	Sugar %
June, 1922						
7	16	50	800			0.09
8	8	110	880			
9	16	120	1920			
10	16	80	1280			
11	4	90	360	5240	8	0.103
		After giving taka-diastase.				
28	16	100	1600			
29	16	100	1600			
30	16	80	1280			0.103
July						
1	8	40	320			
2	32	80	2560	7360	8	

The difference between the total diastatic ferment in the urine for five days before and after giving taka-diastase was 2120, the amount of diastatic ferment in the urine being increased. The total amount of taka-diastase given per os for five days was 6250. So one-third of the diastatic value given per os was excreted in the urine in five days. The blood-sugar content was almost the same before and after the administration of taka-diastase.

Experiment VIII. Normal rabbit. 1.72 kilos.

After we injected daily for five days 2 c.c. of 1 per cent of pancreatin hypodermically in the back of the normal rabbit, the amylolytic power of the urine and blood-sugar was determined (Table IX).

The difference between the total diastatic ferment in the urine for five days before and after the injection of pancreatin was 320, the amount of diastatic ferment in the urine being increased. The total amount of the pancreatin injected for five days was 250. Almost the total amount of the diastatic value injected was excreted in the urine in five days. The blood-sugar increased slightly.

TABLE IX.

Date	Urine				Blood	
	$D_{30'}^{38^\circ}$	Volume c.c.	Total $D_{30'}^{38^\circ}$	Total amount of D for 5 days	$D_{30'}^{38^\circ}$	Sugar %
July, 1922						
26	8	110	880			0.103
27	16	140	2240			
28	8	120	960			
29	16	115	1840			
30	16	30	480	6400	8	0.101
After injection of pancreatin.						
August						
4	8	180	1440			
5	8	110	880			
6	16	160	2560			
7	8	120	960			
8	8	110	880	6720	8	0.137

Experiment IX. Normal rabbit. 1.82 kilos.

2 c.c. of 1 per cent of taka-diastase were injected hypodermically in the back for five days. Then the urine and the blood were treated in the same way as in Experiment VIII. (Table X.)

TABLE X.

Date	Urine				Blood	
	$D_{30'}^{38^\circ}$	Volume c.c.	Total $D_{30'}^{38^\circ}$	Total amount of D for 5 days	$D_{30'}^{38^\circ}$	Sugar %
July, 1922						
6	4	46	184			0.100
7	4	94	376			0.101
8	4	85	340			
9	4	104	416			
10	4	156	624	1940	8	
After injection of taka-diastase.						
12	8	30	240			
13	8	145	1160			
14	4	170	680			
15	4	140	560			0.105
16	4	140	560	3200	8	

The difference between the total diastatic ferment in the urine for five days before and after the injection of taka-diastase was 1260, the amount of diastatic ferment in the urine being increased. The total amount of taka-diastase injected for five days was 2500. So about half of the total amount of the diastatic value injected was excreted in the urine in five days.

Experiment X. Normal rabbit. 1.69 kilos.

On five successive days 2 c.c. of 1 per cent of taka-diastase were injected hypodermically in the back of the rabbit and the amylolytic power of the urine and the blood-sugar of the rabbit was determined with a view to comparing it with those of the normal rabbit before the injection (Table XI).

TABLE XI.

Date	Urine				Blood	
	$D_{30'}^{38^\circ}$	Volume c.c.	Total $D_{30'}^{38^\circ}$	Total amount of D for 5 days	$D_{30'}^{38^\circ}$	Sugar %
July, 1922						
6	4	108	432			0.108
7	4	112	448			0.108
8	4	117	468			
9	4	120	480			
10	4	120	480	2308	8	
After injection of taka-diastase.						
12	16	44	704			
13	8	100	800			
14	4	125	500			
15	4	130	520			0.09
16	4	170	680	3204	8	

The difference between the total diastatic ferment in the urine for five days before and after giving taka-diastase for five days was 896, the amount of diastatic ferment in the urine being increased. The total amount of taka-diastase given hypodermically for five days was 2500. About one-half of the diastatic value given hypodermically was excreted in the urine in five days. From the above researches I learned that the diastatic ferment increased in the urine by almost the total amount of the given diastatic value when pancreatin was administered per os and when it was injected hypodermically. Moreover when taka-diastase was given per os the diastatic ferment was found to increase by one-half of the given diastatic value in the urine. But when it was given hypodermically almost all the injected amylase was excreted in the urine.

CONCLUSIONS.

(1) The value of the diastatic ferment, i. e. amylase, varies from time to time in the course of a day; and the total amount of the same ferment excreted in a day does not remain constant.

(2) Whenever the amylolytic ferment is introduced into the

body, whether per os or injected hypodermically, the amount of the same ferment increases in the urine.

(3) The level of the amylolytic ferment in the blood-serum always remains constant. The amount of the same ferment determined in the serum shows the nearest or rather the same value whether this ferment is given per os or hypodermically.

(4) The blood-sugar content was found to increase slightly whenever pancreatin was given either per os or hypodermically.

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