XLI. THE INACTIVATION OF ANIMAL AMYLASE BY PLANT PARALYSERS AND THE PRESENCE OF INACTIVATING SUBSTANCES IN SOLUTIONS OF ANIMAL AMYLASE.

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IN the preceding papers [1933] it was shown that in germinated and ungerminated cereals there are natural paralyzers of amylase, which we call "sistoamylase substances." Buckwheat malt contains many of these paralyzers, mostly in the leaves and roots. The sistoamylase adsorbs the plant amylase either in a state of solution or at the moment of being dissolved and so inactivates it.

Moreover we have found other substances, which counteract the sistoamylase and have named these substances "eleutoamylases." Any eleutoamylase added to the system sistoamylase-amylase prevents inactivation by the paralyser, or if the amylase has been already inactivated by the sistoamylase, the whole or a greater part of the inactivated amylase is put into action by the eleutoamylase. The most active eleutoamylases recognised by us are products of decomposition of protein found in peptone.

In the present paper the same problem is discussed in relation to animal amylase. Before anything else is suggested the following questions should be answered:

- (1) Is plant sistoamylase able also to inactivate animal amylase?
- (2) Does the system composed of plant sistoamylase and animal amylase react like the similar system containing plant amylase, the amylase of which can be reactivated by adding a suitable eleutoamylase?
- (3) Do there co-exist in the animal organism besides amylases sisto-substances characterised by properties similar to those of the plant sistoamylases?

EXPERIMENTAL.

The experiments were conducted on human saliva, collected on the day of starting experiments, and on Merck's pancreatic amylase. The saliva was diluted in the proportion 5:100, well mixed during 5 mins. and filtered. The solutions of pancreatic amylase were prepared by dissolving 0.25 g. of pancreatic amylase in 100 ml. of water at 20°, shaken during 5 mins. and then filtered. The leaves and roots of buckwheat-malt, germinated within 15 days at 15°, were taken as material containing the plant paralyser, the sistoamylase substance. This material was used in two different manners: well crushed undried, and dried in the apparatus of Faust-Heim at 35° and then reduced to powder. "Peptone siccum sine sale" (Merck) served as eleutoamylase. The p_H of Merck's soluble starch solution was adjusted by additions of phosphate buffers to 6.7, determined

potentiometrically. The amylolytic activities of the solutions of amylase were measured by their liquefying, dextrinising and saccharifying powers.

The liquefying power of saliva was determined with native starch according to the method of Lintner-Sollied [1902], but at 40° instead of 65°. The liquefying power of pancreatic amylase was established by the method of Pollak [1903] at 40°.

The dextrinising power. For pancreatic amylase a 2.5 % and for salivary amylase a 1 % solution of Merck's soluble starch was prepared, by addition of starch paste to boiling water, boiling the solution for a further 3 mins., and, after cooling it, adjusting the p_H . 50 ml. of this solution were warmed for 30 min. at 40°, afterwards 5 ml. of the enzyme solution to be tested were introduced. At intervals of 4 mins., 1 ml. of this system was taken and added to 1 ml. of iodine solution (0.8 g. iodine in 1 litre H_2O) and the colour obtained was observed. The dextrinising activity was measured by the time required for obtaining the following colours: violet, brownish red, light yellow.

The saccharifying power was investigated with the same solutions of soluble starch also at 40°; 5 ml. of the enzyme solution were added to 50 ml. of the solution of soluble starch and treated as before. After 15 mins. 10 ml. of $N/10$ NaOH were added, the mixture was cooled, and reducing sugars were determined in 30 ml. by the method of Willstätter and Schudel [1918]. The saccharifying power is expressed by the number of ml. of $N/20$ iodine reduced by 30 ml. of the liquid tested. The amount of iodine reduced by the control test was deducted.

I. SALIVARY AMYLASE.

(a) *Inactivation of salivary amylase by plant paralyser.*

In order to ascertain whether the plant paralyser also inactivates the amylase of saliva and in what degree, plant paralyser was taken from two sources: dried leaves and rootlets of buckwheat-malt (1) obtained a year ago and (2) obtained shortly before our experiments. The mixtures used were prepared by addition of the quantities of sistoamylase substance shown in Table I to 40 ml. of 5 % saliva. In each case (except No. 1) the solution was shaken mechanically for 8 mins. and then filtered, and the amylolytic activity of the filtrate was investigated (Table I).

Table I.

No. of solution	Additions g.	Sacchari-fying power ml. <i>N/20</i> I	Dextrinising power			Liquefying power Lintner-Sollied method 1 g. starch is liquefied by 5 % saliva, ml.
			Violet	Brownish red	Light yellow	
			was obtained with iodine in mins.			
1	None	1.8	20	40	220	1.8
2	0.1 sistoamylase 1	0.1	110	180	Not obtained in 240 mins.	2.4
3	0.3 "	0.1	Without change after 240 mins.			Not liquefied by 4 ml.
4	0.5 "	0.1	"			
5	0.7 "	0.1	"			
6	0.1 sistoamylase 2	0.1	190	Not obtained after 240 mins.		
7	0.3 "	0.1	Without change after 240 mins.			"
8	0.5 "	0.1	"			"
9	0.7 "	0.1	"			"

These experiments demonstrate that a plant paralyser also inactivates salivary amylase: 0.3 g. of sistoamylase substance is sufficient to inactivate completely 40 ml. of 5 % saliva in 8 mins. The dried sistoamylase substance obtained shortly before the experiment possesses the inactivating power in a higher degree than that obtained a year ago.

[illegible]

The amylolytic action of the filtrates was then investigated.

Table III.

No. of solution	Sacchari-fying power ml. <i>N</i> /20 I	Dextrinising power			Liquefying power Lintner-Sollied method 1 g. of starch is liquefied by 5 % saliva, ml.
		Violet	Brownish red	Light yellow	
		was obtained with iodine in mins.			
1	1.6	60	90	240	1.8
2	0.1	Without change after 4 hours			Not liquefied by 4 ml.
3	0.1		"		"
4	0.1		"		"
5	0.1		"		"
6	0.1		"		"
7	0.1		"		"
8	0.0		"		"
9	1.7	24	42	120	1.2
10	1.0	26	54	Not obtained	1.3
11	0.6	48	90	"	1.4
12	0.6	90	174	"	1.7
13	1.7	24	40	124	0.9
14	1.1	34	48	218	1.1
15	5.8	9	12	42	0.3

The dried plant paralyser inactivates the amylolytic action of salivary amylase so strongly that even prolonged contact of peptone with adsorbed amylase does not lead to an elution of the ferment, and such a system does not exhibit amylolytic activity. After treatment with undried plant paralyser on the other hand peptone reactivates the salivary amylase. These results are in agreement with those obtained on plant amylase.

It remains to be established whether the addition of peptone to the system undried sistoamylase + salivary amylase, the amylolytic action of which is nearly nil, actually leads to elution of amylase or whether a small part of the amylase undergoes no adsorption and gives the corresponding intensive amylolytic action with peptone. To this end, the following solutions were prepared. To 40 ml. of 5 % saliva was added 1 g. undried sistoamylase, the mixture shaken for a definite time and filtered during 6 mins. The nature of the sistoamylase added and the times of shaking are shown below.

No. of solution	Duration of malting in prep. of sistoamylase days	Duration of shaking mins.
1	11	8
2	11	12
3	11	27
4	11	57
5	25	8
6	25	27
7	25	57

Two series of experiments were made by establishing the dextrinising and saccharifying powers of each of the above solutions after filtration. The first series (*a*) without any addition, the next (*b*) with the addition of 0.2 g. peptone to 50 ml. of 1 % soluble starch solution. If peptone does not elute but only increases the amylolytic activity of the inactivated solutions, the numbers of series "*b*" must agree with the results in solutions 9–14 in Table III.

In Table IV we see that the young undried sistoamylase substance produced in 11 days' malting adsorbs the salivary amylase quantitatively. But the

Table IV.

No. of solution	Sacchari-fying power ml. N/20 I	Dextrinising power			Liquefying power Lintner-Sollied method 1 g. of starch is liquefied by 5 % saliva, ml.
		Violet	Brownish red	Light yellow	
		was obtained with iodine in mins.			
1 a	0.0	Without change after 4 hours			Not liquefied by 4 ml.
1 b	0.0		"		"
2 a	0.0		"		"
2 b	0.0		"		"
3 a	0.0		"		"
3 b	0.0		"		"
4 a	0.0		"		"
4 b	0.0		"		"
5 a	0.0		"		"
5 b	0.1		"		"
6 a	0.0		"		"
6 b	0.1	120	180	Not obtained	"
7 a	0.1	Without change after 4 hours			"
7 b	0.1	110	160	Not obtained	"

undried sistoamylase substance produced in 25 days also adsorbs the amylase at first very strongly; however, shaking the system for a long time has the effect that a small quantity of the sistoamylase with adsorbed amylase passes into solution. This dissolved system does not itself show any amyolytic action, but when peptone is added, as in the experiment with the solutions of starch of series "b," this system begins to act, the action of the sistoamylase being eliminated.

II. PANCREATIC AMYLASE.

(a) *Inactivation of pancreatic amylase by plant paralyser.*

To ascertain whether the plant sistoamylase is able to inactivate pancreatic amylase, the following experiments were made. Solutions of 0.25 g. pancreatic amylase in 100 ml. of water were shaken mechanically for 5 mins. and then various quantities of sistoamylase were added, the solutions shaken for 8 mins. and filtered. The amyolytic power of the filtrates was then examined (Table V).

Table V summarises the results.

Table V.

No. of solution	Additions g.	Sacchari-fying power ml. N/20 I	Dextrinising power			Liquefying power Pollak method 0.3 g. starch is liquefied by 5 % saliva, ml.
			Violet	Brownish red	Light yellow	
			was obtained with iodine in mins.			
1	None	3.9	80	150	Not obtained	0.5
2	0.1 sistoamylase 1	1.7	150	240	"	0.7
3	0.3 "	0.2	Without change after 4 hours			Not liquefied by 3 ml.
4	0.5 "	0.1		"		"
5	0.7 "	0.1		"		"
6	0.1 sistoamylase 2	0.7	Nearly without change after 4 hours			1.0
7	0.3 "	0.1		"		Not liquefied by 3 ml.
8	0.5 "	0.1	Without change after 4 hours			"
9	0.7 "	0.0		"		"

These results show that the plant sistoamylase also inactivates pancreatic amylase. 0.3 g. of the sistoamylase preparations inactivates 100 ml. of 0.25 % pancreatic amylase in 8 mins.

(b) *The inactivation of pancreatic amylase by sistoamylase in presence of peptone.*

(1) *Peptone an antisistoamylase substance to pancreatic amylase.* The effect of peptone on the sistoamylase-pancreatic amylase system was investigated in a similar manner, filtered solutions of 0.25 g. pancreatic amylase in 100 ml. water being used and shaken for 8 mins. after the additions had been made.

Table VI.

No. of solution	Additions	Sacchari-fying power ml. N/20 I	Dextrinising power			Liquefying power Pollak method 0.3 g. starch is liquefied by 5 % saliva, ml.
			Violet	Brownish red	Light yellow	
			was obtained with iodine in mins.			
			Without change after 4 hours			
1	None	3.9	98	156	Not obtained	0.4
2	0.3 g. dried sistoamylase	0.1	Without change after 4 hours			Not liquefied by 3 ml.
3	„ + 2 g. peptone	16.6	20	30	85	0.2
4	2 g. peptone	16.8	18	28	78	0.1

This table shows that only a very small quantity of free amylase is present in the solution of pancreatic amylase. Besides this, there is about four times as much amylase inactivated by a specific paralyser. The latter can be put out of action by peptone. We see further that the peptone acts also as anti-substance to added plant paralyser, just as in the case of the salivary amylase.

(2) *Peptone an eleutoamylase substance to pancreatic amylase.* In order to prove that peptone has the capacity of eluting the pancreatic amylase from the system pancreatic amylase + plant paralyser, the amylolytic activity of the following solutions was examined:

No. of solution												
1	0.25 g. of pancreatic amylase + 100 ml. of water 5 mins. mechanically shaken and filtered											
2	As above	Afterwards	+ 0.3 g. dried sistoamylase.	Shaken	8 mins. and then	+ 2 g. of peptone, eluted	10 mins. and	„				
3	„	„	+ 0.3 „	„	8 „	„	30 „	„				
4	„	„	+ 0.3 „	„	8 „	„	60 „	„				
5	„	„	+ 1.0 g. undried	„	8 „	„	„	and				
6	„	„	„	„	8 „	+ 2 g. of peptone, eluted	10 mins. and	„				
7	„	„	„	„	15 „	„	10 „	„				
8	„	„	„	„	30 „	„	10 „	„				
9	„	„	„	„	60 „	„	10 „	„				
10	„	„	„	„	8 „	„	30 „	„				
11	„	„	„	„	15 „	„	30 „	„				
12	„	„	„	„	„	+ 2 g. of peptone,	„	„				

These experiments (Table VII) show that dried sistoamylase adsorbs the pancreatic amylase much more strongly than undried. We see also that the adsorption of the pancreatic amylase is the more complete, the longer the undried sistoamylase acts, so that it becomes more and more difficult to elute the amylase.

Table VII.

No. of solution	Sacchari-fying power ml. N/20 I	Dextrinising power			Liquefying power Pollak method 0.3 g. starch is liquefied by 5 % saliva, ml.
		Violet	Brownish red	Light yellow	
		was obtained with iodine in mins.			
1	3.9	98	156	Not obtained	0.4
2	0.1	Nearly without change after 4 hours			Not liquefied by 3 ml.
3	0.4	210	Not obtained		2.8
4	0.7	180	220	Not obtained	2.0
5	0.1	Nearly without change after 4 hours			Not liquefied by 3 ml.
6	11.1	28	41	106	0.1
7	9.0	35	48	135	0.1
8	7.0	45	60	192	0.15
9	6.3	52	73	216	0.2
10	12.0	24	46	94	0.1
11	8.6	36	53	192	0.15
12	16.8	18	28	78	0.1

DISCUSSION.

Our researches on animal amylase give us not only an explanation of its relations to the natural plant paralyser, our sistoamylase substance, but also explain the manner in which different systems of animal amylase act under the influence of eleuto-substances.

We have ascertained that animal amylase, similarly to vegetable amylase, shows, depending on its origin and conditions of action, different mutual ratios of the three functions of amylolytic activity, the liquefying, dextrinising and saccharifying powers.

We see further that animal amylase under the influence of a plant paralyser is inactivated. This inactivation is of different grade depending on the sistoamylase substance, on the time of its activity and on the origin of the amylase. It is to be noticed that dried sistoamylase substances have a greater power of adsorption than undried. The degree of adsorption increases with the time of action of the paralyser on the amylase.

The most interesting result of our investigation is that in salivary and pancreatic amylases substances inactivating the amylase are found. The result of this is that only a part of amylase is able to act, *e.g.* in a solution of salivary amylase only about 1/3 and in a solution of pancreatic amylase only about 1/4–1/5 of the total amylase is free. It can be deduced that natural paralyzers of amylase, sistoamylase substances, occur along with the amylase in the animal, just as in the vegetable, organism. Peptone acts towards the plant paralyser in the same way as to the paralyser found in the solutions of animal amylase. Peptone added to the system plant sistoamylase + animal amylase prevents permit inactivation of the amylase, and also has the power of reactivating the amylase already inactivated by the sistoamylase substance originally present. The adsorption of animal amylase by an animal sisto-substance seems not to be so strong as the adsorption in the system plant sistoamylase + animal amylase. From the first system, especially in solutions of pancreatic amylase, the amylase is freed with more facility, and its amylolytic activity is restored. From the second system the amylase is freed with more difficulty, and in the system dried plant sistoamylase + animal amylase the amylase is so strongly adsorbed that it can only be eluted with difficulty by adding peptone. It is an interesting fact that peptone does not act on all the functions of the amylase equally. As

shown by our experiments, the saccharifying power is maximum on addition of 0.5 g. of peptone to 40 ml. of 5 % solution of salivary amylase. The dextrinising and liquefying powers show the best activity when 1–2 g. peptone are added to the same quantity of solution.

The question of the paralysing of amylase was treated for the first time by Wolff and Fernbach [1906], who introduced into the literature the term anti-amylase for the agent obtained by subcutaneous immunisation of rabbits to malt amylase. This phenomenon has been the subject of numerous researches by different workers. These investigations show that the anti-amylase acts as an inhibitor only on vegetable amylase. Abderhalden and Wertheimer [1922] not only question its paralysing action, but reject in general the existence of this anti-amylase. Kiselew [1928] distinguishes another kind of inactivation in the adsorption of amylase by vegetable plasma, coagulated by warming. Oparin and Kurssanow [1929] showed that in these cases the result was not due to the specific action of the plasma but to the inhibiting action on amylase caused by the presence of tannin.

The most interesting researches in this direction are those of Lesser [1919; 1921] and of Kerner and Lesser [1919], who proved that amylase of the liver is partly inactivated by adsorption, and that the retardation of the decomposition of glycogen may take place not only *in vivo*, but may also be reproduced experimentally by the addition of some adsorbent to the solution. The development of the details of this question is given by Przyłęcki *et al.* [1927]. Lesser showed, using adrenaline as stimulant, that the amylase of the liver can be freed, so that the active concentration of the ferment increases by elution of the inhibitor. Truszkowski [1928] finds that amylase can be inactivated by adsorption on lipoids to the extent of about 67 % of that present in the solution. The amylolytic action of the inactivated amylase can be restored almost quantitatively by the addition of an appropriate quantity of propyl or butyl alcohol.

The activating influence of peptone and of amino-acids is described by Effront [1900] and by Pozerski [1902]. Oppenheimer's [1927] opinion is that here we have to do with a change of p_H , though it is not excluded that these substances may have a real stimulating influence, as was proved by the researches of Sherman *et al.* [1921; 1922] and Rockwood [1924], who took the p_H into consideration. Oppenheimer supposes that this phenomenon can be explained on the ground that peptone protects the amylase from inactivation by rise of temperature.

According to our experiments the so-called phenomenon of activation of animal amylase is to be explained quite differently. We have shown that the whole of the amylase is not active, but only a portion of it. Very often a large portion of it is inactivated by adsorption by different accompanying substances, which are called by us sistoamylase substances. If peptone is added to a system of sistoamylase-amylase the action of the sistoamylase is excluded, the active concentration of amylase increases by elution, and in this case, we often find in the solution several times more free amylase, which according to its quantity will develop a correspondingly greater activity. The increase of amylolytic activity when different substances are added to the solution of animal amylase is nothing else than an elution of amylase from the system sistoamylase-amylase, in which a part of the amylase was adsorbed by the accompanying adsorbent. We are inclined to believe that various phenomena of the protection of amylase from inactivation, as for instance by moderate use of temperature, are in reality complete or incomplete hindrances of adsorption of the amylase by substances already present or formed during the treatment.

SUMMARY.

Experiments on salivary and pancreatic amylase lead us to the following results.

1. Plant sistoamylase inactivates not only plant but also animal amylase. Dried sistoamylase inactivates amylase more powerfully than undried.

2. In the place of origin of amylases as well as in their solutions natural paralyzers, the so-called animal sistoamylases, are also found. The system sistoamylase-amylase is thus formed, in which the amylase is partly inactivated. Usually about 1/3 of the salivary and about 1/5 of the pancreatic amylase is not adsorbed.

3. In the system plant or animal sistoamylase-animal amylase, in which the amylase is inactivated, the amylase can be reactivated by the aid of eleuto-substances of which one of the best known by us is peptone. Certain forms of dried sistoamylase are of such adsorbing power that the amylase cannot be eluted by the use of peptone.

4. We explain the increase of amylolytic action of amylase on the addition of certain substances as an elution of amylase, which in this manner is rendered free to act.

REFERENCES.

- Abderhalden and Wertheimer (1922). *Fermentforsch.* 6, 286.
Chrząszcz and Janicki (1933). *Biochem. Z.* 260, 354; 263, 250; 264, 192; 265, 260.
Effront (1900). *Les diastases.* (Paris.)
Kerner and Lesser (1919). *Biochem. Z.* 102, 284.
Kiselew (1928). *Dziennik d. Bot. Tagung*, 23.
Lesser (1919). *Biochem. Z.* 102, 304.
—— (1921). *Biochem. Z.* 119, 108.
Lintner-Sollied (1902). *Z. ges. Brauw.* 26, 329.
Oparin and Kurssanow (1929). *Biochem. Z.* 209, 181.
Oppenheimer (1927). *Die Fermente*, 701. (Thieme, Leipzig.)
Pozerski (1902). *Malys Jahresber.* 460.
Pollak (1903). *Woch. Brau.* 20, 595.
Przyłęcki, Niedzwiecka and Majewski (1927). *Biochem. J.* 21, 1025.
Rockwood (1924). *J. Amer. Chem. Soc.* 46, 1641.
Shermann and Caldwell (1921). *J. Amer. Chem. Soc.* 43, 2469.
—— (1922). *J. Amer. Chem. Soc.* 44, 2926.
—— and Walker (1921). *J. Amer. Chem. Soc.* 43, 2461.
Truszkowski (1928). *Biochem. J.* 22, 767.
Willstätter and Schudel (1918). *Ber. deutsch. chem. Ges.* 51, 780.
Wolff and Fernbach (1906). *Woch. Brau.* 23, 743.