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On the Salivary Amylase and Its Significance in the Caries Process.[1]

By

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Survey of the Literature.

A component of the saliva which has been most widely studied is the starch splitting enzyme, *amylase*, in earlier literature often known as diastase or ptyalin. Salivary amylase has been examined from the point of view of secretion and digestion physiology, of its chemistry and in relation to the caries process. The literature in this field is therefore somewhat dispersed and not easily surveyable. The first part of the present work represents an attempt to collect together the more important data on salivary amylase, with particular regard to intra-oral carbohydrate degradation and caries. Where no reference is given the information has been largely derived from BAMANN and MYRBÄCK (1941) or HOPKINS (1946).

Salivary amylase which is essentially derived from the parotid secretion, is α-amylase, and thus breaks the α-glucoside bonds of starch. In its effect it has proved to be identical with pancreatic amylase and α-amylase of malt. According to ANKER and VONK (1946) β-amylase also occurs in the saliva, but this is contradicted by MEYER (1950). Salivary amylase has been shown by preparation of the pure substance to be a homogeneous protein (MEYER, 1947), more specifically, of globulin type with isoelectric point about pH 4. Its starch hydrolysis passes rapidly through the high-

[1] This investigation forms part of co-ordinated work on dental caries sponsored by the Swedish Medical Research Council through its Sub-committee for Caries Research.

molecular amylo- and erythrodextrins to achroo-dextrin, and proceeds more slowly to maltose. (Erythro- and achroo-dextrins have of course been named from their colour reaction, or their absence of reaction with iodine.) Only to a small extent does the process go to glucose. The degradation seems to cease at a state of equilibrium when about 80 % of the starch has been converted to maltose, provided that no further fermentation removes the maltose formed (EVANS, 1912). The optimum pH value is about 6.8 (in the presence of chloride ions, otherwise lower; MYRBÄCK 1926). α-amylase is sensitive to high hydrogen ion concentrations and is rapidly destroyed at pH 3.3 for example; certain metallic ions, *e. g.* Cu and Hg, also inactivate salivary amylase (MYRBÄCK 1926), while F ions have no apparent effect (MCCLURE 1939). In salt-free solution amylase is inactive but the chlorine ions in 0.05 M sodium chloride are sufficient to activate it. The optimal phosphate concentration is 0.005 M. It is striking how well these experimentally determined optimum values agree with the average concentrations of the saliva. On the other hand, the optimum temperature of salivary amylase is reported as 46° C (EVANS, 1912) or 50° C (SCHNEYER, 1950), but in the immediate region of the body temperature the divergences from the optimum are small. The optimum starch concentration has been given as 3 % (EVANS, 1912).

The determination of the amylase content follows the general principle for the analysis of enzymatic activity — the measurement of its rate of action on the medium, in this case a starch solution. As an approximate measure of the rate of hydrolysis use has been made of the time required for the disappearance of opalescence (CARLSON and CRITTENDEN, 1910) or the time for a given reduction of the viscosity of the starch solution (MAYER, 1929, et alii). The commonest methods of analysis are however based either on the disappearance of the blue iodine-starch colour or on the liberation of reducing sugar. In both cases the saliva must be used greatly diluted so that the time of reaction is sufficiently long to permit a reliable observation of the digestion of the starch; the speed of reaction is proportional to the enzyme concentration.

In the iodine test a small quantity of saliva at body temperature is allowed to react with a starch solution whose phosphate and salt content, pH value and temperature have been standardized in the region of the optimal values. At equal time intervals samples

are mixed in a fixed proportion with an iodine solution. The time necessary to acquire a given shade is noted. The shade most easily defined is the reddish-violet erythrodextrin colour, which corresponds to a degradation of less than 30 % of the starch, chiefly to maltose. There is a linear relationship between digestion and time until about 30 % of the starch is split (EVANS, 1912). — Another procedure, deviced by WOHLGEMUTH (1908) and widely used, implies the mixing in definite proportions of 1 per cent starch solution and progressively diluted saliva. 1 Wohlgemuth unit (W. U.) is the number of cubic centimeters of starch solution that according to the iodine test has been transformed into dextrin by 1 cc saliva in 30 minutes.

The most accurate method of following the effect of the amylase is however to determine the quantity of liberated maltose. The common methods of analysis for reducing sugar will here of course give an error on account of the small amount of simultaneously liberated glucose and the slight reduction of the dextrins. The reaction rate in the saliva-starch mixture with approximate optimum conditions of temperature and ionic concentrations may be followed by determining the reducing capacity either in consecutive samples taken or in a single sample after a fixed time. In the latter case the time should be adjusted so that it falls within the linear part of the reaction curve. Various modifications of this method for determination of the salivary amylase have been described by BERTRAND 1906, EVANS 1912, WILLSTÄTTER and SCHUDEL 1918, PRINGSHEIM and GORODISKI 1923, DELHOUGNE 1927, WALKER and SHEPPARD 1935, FABER 1944, HAWK and BERGEIM 1937, BÁRÁNY 1947, HESS and SMITH 1948.

The amylase content of the saliva varies over a wide range, not only from one person to another (see below) but also for the individual. The amylase content rises with the rate of secretion and is thus higher after stimulation for example by chewing (CHITTENDEN and RICHARDS 1898, CARLSON and CRITTENDEN 1910, WALKER and SHEPPARD 1935, BAUER and MARTIN 1948). KOCH (1943) gives the range of 8—8,192 W. U. in slightly stimulated morning samples; 78 per cent of the cases were between 64 and 1,024 W. U. Stimulants as pilocarpine and Neu-Cesol (Merck) also increase the amylase activity as well as the flow of the saliva (GERNHARDT, 1933). The total protein content of parotid saliva rises in the same manner with the rate of secretion (CARLSON and CRITTENDEN 1910, BRAMKAMP 1936). BRAMKAMP

gives the range of variation of 11 mg protein nitrogen/100 ml at a secretion of 0.5 ml/min.—58 mg/100 ml at 2 ml/min. The total salt content of the saliva also increases with the rate of secretion: HEIDENHAIN's law (1868).

Under constant conditions of stimulation the variations in the salivary amylase of any one person are said to be rather small (BAUER and MARTIN, 1948). Compare NØRBY's observation (1935) that the amylase content of the serum is fairly constant for and characteristic of individuals. KOCH (1943) however found great variations for the individual, and no correlation between serum and salivary amylase. It is interesting to note BOSCO's statement (cit. from KOCH, 1943) that smoking a cigarette greatly decreases the salivary amylase.

The amylase content for infants is considerably lower than for adults (MAYER, 1929), and in a group of people with an average age of 81 years MEYER and co-workers (1937) found an amylase content of the saliva that was only about 3 % of that in a group of 25 year-olds. It has not been possible to confirm any quantitative connection with carbohydrate supply with man nor any distinct qualitative relationship among the higher animals: primates and rodents have salivary amylase whilst many herbivorous animals have not, *e. g.* the horse and the goat; and carnivora also probably lack salivary amylase (CARLSON and CRITTENDEN 1910).

What is the real importance to the digestive process of the salivary amylase? Although the action of the amylase is very rapid, the digestion of the starch is but begun in the mouth. The continued action in the stomach is dependent on the secretion of hydrochloric acid and its penetration of the food. BERGEIM found (1926) that on the average 76 per cent of the starch in potato flour and 59 per cent in bread had been converted to maltose before the salivary amylase was inactivated by the hydrochloric acid. Certain earlier information (ROGER 1907, ROGER and SIMON 1907), according to which the salivary amylase is reactivated by the pancreatic secretion in the alkaline reaction of the small intestine, is firmly contradicted by BERGEIM (1926). On the other hand, the same author holds that it is possible that the salivary amylase has an indirect effect on the protein digestion of the stomach: MAXWELL and NAKAGAWA (cit. from BERGEIM, 1926) found that the protein hydrolysis of the digestive juices in vitro is delayed by starch but not by its hydrolysis products, probably on account of pepsin adsorption to the colloidal starch.

Pathological conditions are capable of reducing both the total secretion of the saliva and its amylase content. Dryness of the mouth in fever is a familiar occurrence. KOROPOW (1934) found in experiments on dogs that experimentally induced fever checked those centres which controlled secretion by conditioned reflexes; the direct reflexes were unchanged. CARLES and DELMAS-MARSALET (1924) discovered that the amylase content of the saliva diminished in infectious and cachectic states; the production of salivary amylase showed, further, an obvious parallel with the secretion of the digestive juices. FABER (1944) made a comparison between the amylase content and total protein of rest saliva in the normal case and in the case of xerostomia of various origins: congenital glandular aplasia, epidemic parotitis, chronic sialoadenitis, pernicious anaemia, etc. The amylase content of xerostomia patients was found to be strongly reduced but the protein content increased. The protein content was calculated on the basis of nitrogen determinations and it is possible that these also included submaxillary mucin, of which the content increases with reduced rate of secretion. The reduced amylase content in epidemic parotitis has been confirmed by WOLMAN and co-workers (1947); at the same time the amylase content of serum was generally increased.

In the case of persons with normal functioning of the stomach and intestines there were generally no disturbances in digestion of starch accompanying the falling off of the saliva (STRAUSS 1924); these would appear only with simultaneous intestinal hypermotility or certain dyspepsias.

The conception of some connection between the amylase content of the saliva and the caries incidence has been inspired by the knowledge of the part played by the enzymatic formation of acid in the caries process. The principal media for this acid formation on the dental surfaces are the starch and sugar from the food, and several investigators have for various reasons assigned the chief rôle to sugar (BUNTING, 1934, JAY and collab. 1936, WILSKA 1946). PROELL (1939) and WILSKA pointed out the striking parallel between the increase in the consumption of sugar ever since the beginning of the nineteenth century and the increase in caries incidence during the same time. The incidence of caries was, however, appreciable long before sugar became more than a rare spice, and the rapid conversion of starch to sugar and lactic acid in the mouth has also been proved (VOLKER and PIN-

KERTON 1947, NEUWIRT and KLOSTERMAN 1940, STEPHAN 1940, ERICSSON and HELLSTRÖM 1950). It is true that the oral bacteria have been proved capable of directly fermenting starch without influence of the salivary amylase (WOHINZ 1935, HANSEN, FOSDICK and EPPLE 1937) but this component can nevertheless be assumed to play the pricipal rôle in the starch degradation.

Attempts to establish a relationship between the amylase in the saliva and the caries frequency have however given very variable results, which can be seen in Table 1.

Table 1.

Reported correlation between salivary amylase activity and caries incidence.

Positive	Negative	No correlation
FLORESTANO and collab. 1941	PICKERILL 1924	HUBBELL 1933.
TURNER and CRANE 1944.	SVEJDA and BUDEJOVICE 1950.	DEAKINS and collab. 1941.
	SULLIVAN and STORVICK 1950.	BERGEIM and BARNFIELD 1945.
		BÁRÁNY 1947.
		HESS and SMITH 1948.

Moreover, the experiments of TURNER and CROWELL (1947) indicated that the high caries incidence was correlated to slow reduction of the blue starch colour but rapid dextrinizing.

Original Investigation.

The purpose of the experiments presented here has been to throw some light on the amylase activity of the saliva and the intra-oral splitting of starch of some subjects, and thus to constitute an introduction to a study of the part played by the salivary amylase in the caries process.

For the determination of the amylase activity the iodine test and analysis of liberated reducing substance have been used.

Iodine test. At a temperature of 37° C, 1 ml saliva reacted with 100 ml of a solution of one per cent analytically pure soluble starch and half per cent NaCl in 0.005 M phosphate buffer of pH 6.8. At half-minute intervals samples of 2 ml were taken from this digestion mixture and pipetted into test tubes containing

2 ml 0.002 N iodine solution in 0.2 per cent potassium iodide. The time required to attain a fixed shade of wine red (erythrodextrin colour) was noted. This shade is passed through most rapidly on the breaking down of starch and can consequently be used as a distinct end-point. 12 determinations in succession with this technique and with saliva of average splitting capacity gave a standard deviation of about 6 %.

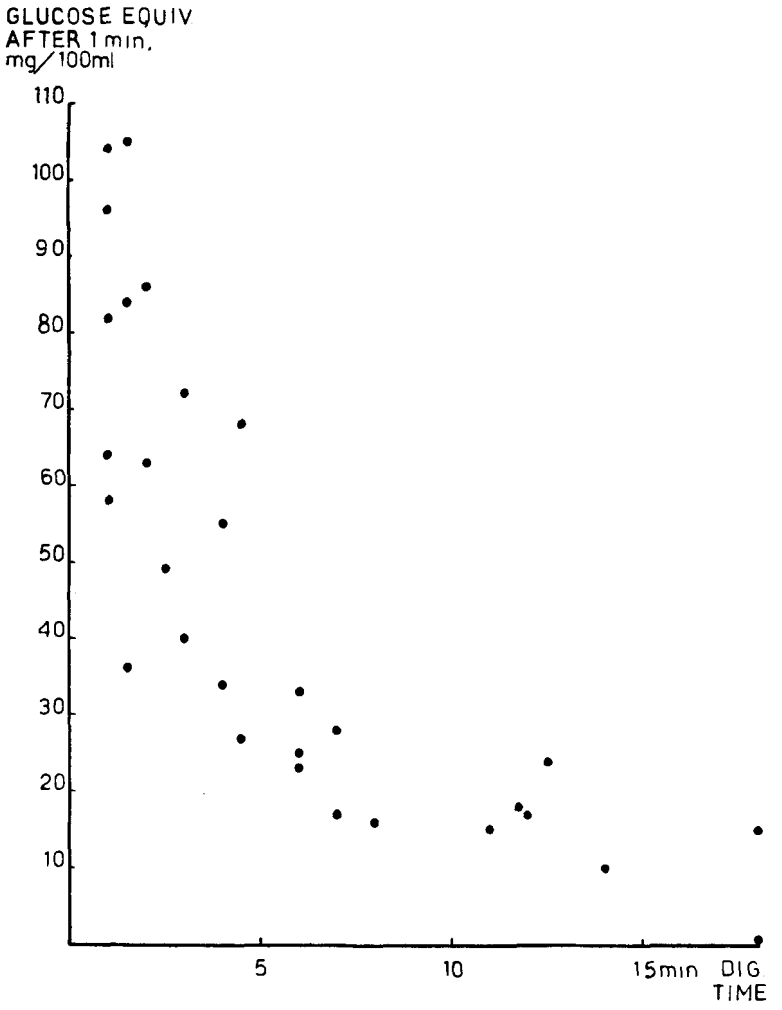
In a later version saliva, diluted 1 : 3, was added in the same proportion to the same starch solution, and corresponding reduction of the rate of the reaction obtained. 9 determinations in succession with this technique gave, with an average digestion time of 13.8 minutes, a stand. deviation of about 4.8 %.

Reduction test. In the above-mentioned mixtures of saliva and starch solution the content of reducing substance was determined after a given digestion time with the FOLIN-WU colorimetric method (1920). 1 ml of digestion mixture was pipetted into an ice-cold mixture of 8 ml water and 1 ml 0.1 N hydrochloric acid, thus arresting the enzymatic process and giving a 1 : 10 dilution. Of this liquid 2 ml was neutralized with 1—2 per cent soda solution, after which the FOLIN-WU technique was applied. The readings were taken in a WEKA compensation photometer. 10 determinations in succession of a solution of 5 mg glucose/100 ml gave a stand. deviation of $\pm$ 0.022 mg.

Comparisons of Iodine and Reduction Tests.

In 38 experiments where 1 ml undiluted saliva reacted with 100 ml starch solution in accordance with the above technique, the reduction capacity was determined on the appearance of the erythrodextrin colour. An average value corresponding to 99.58 $\pm$ 4.84 mg glucose/100 ml was obtained in this way. Converted into maltose equivalents this is an average value of 189.2 mg maltose/100 ml. Since the starch in the original reaction mixture corresponds to over 1,000 mg maltose/100 ml the hydrolysis at the erythrodextrin end-point is thus less than 20 per cent on the average; this iodine test falls in the linear phase of digestion.

In 30 of the above experiments the reduction capacity was also determined after 1 minute digestion periods. The results are apparent from diagram 1. The correlation coefficient of the results of both these test methods has been calculated at 0.78. There are grounds for assuming that the later applied method with a



Diagr. 1.
Relationship iodine test — 1 minute-reduction test.

1 : 3 dilution of the saliva before digestion gave a still closer correlation to the reduction test through the reduced determination error.

Examination of the Possible Effect of Oral Bacteria on the Digestion.

It is conceivable that the bacteria of the saliva influence the rate of splitting through their enzymes, especially in the stimu-

lated saliva, since paraffin chewing gives rise to a dispersion in the saliva of the bacterial coating of the teeth. On this account some tests were performed to determine any contingent influence of the bacteria on the salivary digestion of starch. As an antibacterial agent, toluene was used, this being effective — although not 100 % — and without notable influence on the enzymes of the saliva according to WEINMANN 1938. Some cultivations on blood agar from saliva with and without preliminary shaking with toluene also showed a practically complete elimination of the aerobic flora through the toluene.

Method.

1. Paraffin stimulated saliva was diluted with water 1 : 3 well mixed, divided equally into two, one part of which was shaken for 2 minutes with an equal quantity of toluene, and the other shaken for the same period without addition. The iodine test was performed with both saliva fractions and, in addition, the reducing capacity determined on obtaining the erythrodextrin colour.

2. The same as above except that the iodine test was carried out with only the untreated saliva fraction; the reducing capacity of this fraction was determined at the colour change, and with the toluene shaken fraction after the same digestion time.

Table 2.

Salivary amylase activity with and without previous shaking with toluene. Method 1.

Subject	Iodine test		Glucose equivalents	
	after toluene shaking, min.	without toluene shaking, min.	at erythrodextrin shade after toluene shaking, mg/100 ml	at erythrodextrin shade without toluene shaking, mg/100 ml
Y. E.	13	13	90	83
B. J.	4 3/4	4	135	148
»	29 1/2	28 1/2	137	133
»	28	28	106	108
E. E.	3	3	101	103
E. O.	15	13 1/2	139	142
I. H.	10 1/2	9 1/2	138	142
L. S.	4	4	73	48
L. J.	9 1/2	9	60	68

Table 3.

Salivary amylase activity with and without previous shaking with toluene. Method 2.

Subject	Iodine test, min.	Glucose equivalents	
		after toluene shaking, mg/100 ml	without toluene shaking, mg/100 ml
B. J.	15	81	93
L. S.	5	108	102
Y. E.	$7^1/_2$	70	77
E. O.	6	109	115
I. H.	$5^1/_2$	115	115

The results of these experiments are given in Tables 2 and 3. It is apparent from these tables that the toluene shaking was not accompanied by any definite changes in the starch digestion capacity of the saliva. Variations in the digestion period are not so great that they cannot be explained by the experimental error of the method itself, and this is also true in the majority of the reduction tests. The bacterial flora thus does not seem to have any demonstrable effect on the rate of splitting as far as can be ascertained with these methods.

The Salivary Amylase of a Number of Subjects.

The results of a number of iodine tests on the digestion capacity with saliva from 5 subjects are recorded in Table 4. The samples were taken from a number of laboratory personnel during the hours of work, some before and some after midday. All samples were taken at least $^3/_4$ hour after the previous meal. The paraffin stimulated saliva sample was taken immediately after that of the rest saliva; each consisted of 1—2 ml and was diluted 1 : 3 before the digestion test.

The higher amylase activity of the stimulated saliva is clear from the table; only in one case was the reverse the case.

Variations for each individual are substantial, but characteristic differences in the different persons can however be observed. E. E. has thus consistently higher amylase activity than B. J. and G. T., which is also evident in the experiments comparing iodine test and 1-minute reduction which were made on the saliva

Table 4.

Individual iodine tests.

Subject	Time	Digestion time, min.	
		unstim.	stim.
Y. E.	Before noon	8	—
		14	8½
		11	9
	After noon	9	9
		14	8½
		10½	7
		8½	6½
B. J.	Before noon	34	—
		31	18
	After noon	12	5½—6
		9½	5—5½
		19	10
		12½	8
		17½	6—6½
E. E.	Before noon	10	8½
		10½—11	6
		5	5½
		8½	7½
	After noon	2½—3	2½—3
		4½	3—3½
E. O.	Before noon	9	9
		15½	5
		16½	9½
	After noon	10	5½
		10	6½
		15	8½
G. T.	Before noon	39	17½
		24	22
	After noon	25	20
Mean difference between correlated values of unstim. and stim. saliva ..		5.00 ± 0.93	

of two of these subjects (Table 5). The experiments with saliva from G. T. were discontinued when a single parotid tumour calling for operation was diagnosed. Connection between the tumour and the low amylase activity is not ruled out.

Table 5.

Iodine test and 1-minute reduction on two subjects with widely differing amylase activities. All samples taken between 10 and 12 a. m., without stimulation.

Subject G. T.		Subject E. E.	
Iodine test, min.	1-min. reduction, mg glucose/100 ml	Iodine test, min.	1-min. reduction, mg glucose/100 ml
7	28	1½	84
8	15.5	1	64.5
12	17	1½	—
10½	15	2½	—
6	33		
12½	24		
18	15		

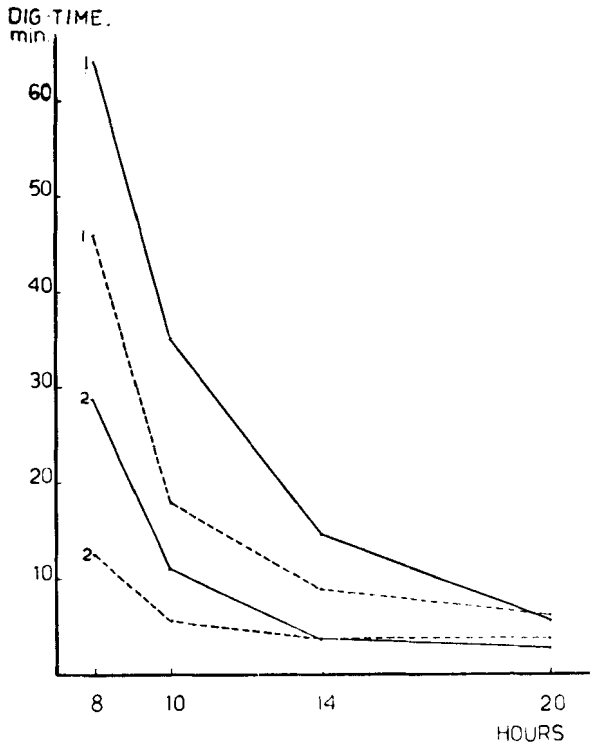
Diurnal Variation in Salivary Amylase.

Figures in Table 4 indicate a tendency towards higher amylase activity in the afternoon than in the morning. This observation gave rise to a number of experiments carried out to show the diurnal variation of the salivary amylase of some of the subjects. Samples, first of rest saliva and then of paraffin stimulated saliva, were taken at 8 a. m., 10 a. m., 2 p. m. and 8 p. m. The morning samples were taken on a fasting stomach and the others at least ¾ hr. after the previous meal. The saliva was diluted 1 : 3 and the iodine test performed. The results appear in diagrams 2—4. The digestion times are consistently longest in the morning and fall during the day to rise again in some series in the afternoon.

Intra-Oral Splitting of Starch.

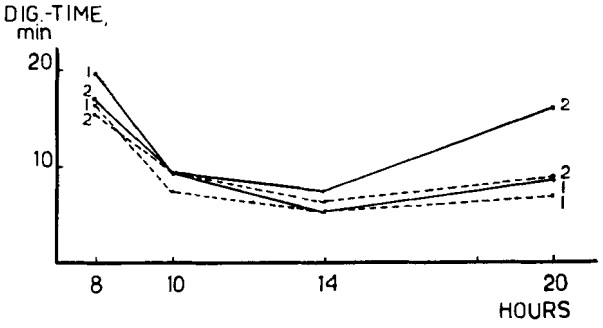
Starch splitting in the mouth has been investigated with a number of subjects. As sources of starch two of the most important starch-containing foods were used — bread (white bread) and boiled potato.

First of all the time was determined between the ingestion of the starch-containing foods and the disappearance of the blue colour with iodine on the buccal side of the interdental spaces. The determinations were performed in the morning at least ¾ hr. after the previous meal. After the persons had been eating bread or potato for 2 minutes a drop of iodine solution was applied with a platinum loop at equal intervals in the interdental spaces,



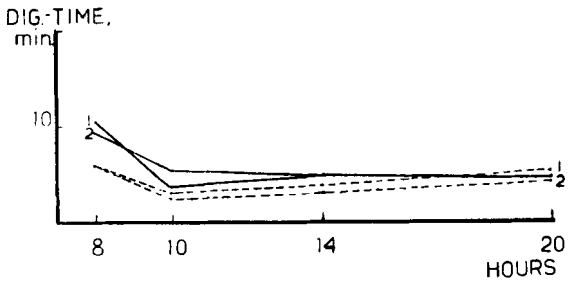
Diagr. 2.

Diurnal variation of salivary amylase activity. Subject B. J. Unstimulated (————) and paraffin-stimulated saliva (— — —), samples taken on two different days (1 and 2).



Diagr. 3.

Diurnal variation of salivary amylase activity. Subject L. S. The same marking of curves as in diagr. 2.



Diagr. 4.

Diurnal variation of salivary amylase activity. Subject Y. E. The same marking of curves as in diagr. 2.

Table 6.

Intraoral iodine test.

Subject	Blue colour last observed, minutes			
	Bread		Potato	
G. E.	19	18	1 1/2—2	3—3 1/2
B. J.	42	116	2—3	3 1/2
Y. E.	36	40	2—3	1 1/2
E. A. H.	100	102	7—8	3
I. H.	12	33	1/2	1/2

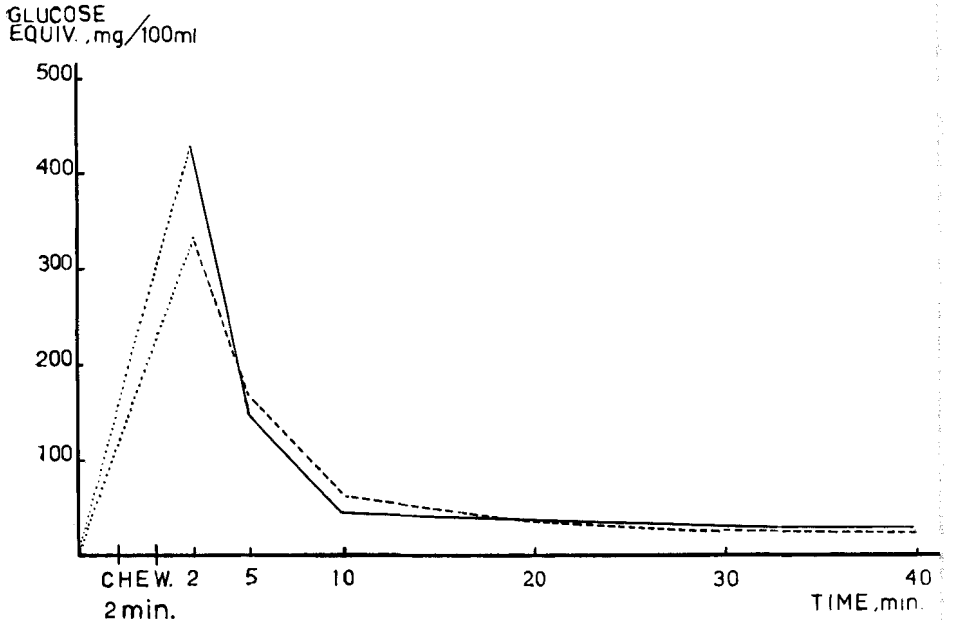
where food remains were either observable or suspected. The time of the last observed blue colour was noted. The time intervals were 1 minute after bread and 1/2 minute after potato. The iodine solution was 0.002 N in 0.2 per cent potassium iodide. Each test was made in duplicate on different days. The results are collected in Table 6. It appears that the iodine colour could be observed in only one case more than 3 1/2 minutes after ingestion of potato, while in the case of bread the period was many times longer. After potato, moreover, the iodine colour was always very weak. The results of these tests are in agreement with what could be observed with the naked eye, namely coatings in a number of interdental spaces a considerable time after consuming bread but not after chewing potato.

With the same subjects supplemented by four others the glucose clearance of the saliva was determined after bread and potato consumption in the same way as in the above-described iodine test. Before chewing, a sample of 1 ml of unstimulated saliva was taken; 2, 5, 10, 20, 30 and 40 minutes after finishing

Table 7.

Reducing sugar in saliva after ingestion of bread, potato, and cane sugar.

Subject	Food ingested	Glucose equivalents, mg/100 ml						
		before	after					
			2 min.	5 min.	10 min.	20 min.	30 min.	40 min.
Y. E.	Bread	3.0	200	78.7	58.7	53.7	43.8	38.8
	Potato	14.5	300	72.5	55	60	36.3	55
	Sugar	8.1	107.5	36.3	37.5	32.5	32.5	20.0
B. J.	Bread	1.0	688	513	115	39	6.0	0
	Potato	1.6	750	575	79	63	51	48
	Sugar	0.4	80	32.5	14.0	7.0	5.0	11.3
E. A. H.	Bread	11.0	275	112.5	53.7	27.5	28.7	32.5
	Potato	1.9	725	91.3	24.3	8.5	12.0	6.5
	Sugar	7.7	85.0	105	49	23	36.5	
I. H.	Bread	0.5	275	61.3	22.5	30	17.5	17.5
	Potato	1.6	262.5	78.3	28.3	22.5	7.5	15.0
G. E.	Bread	—	500	217.5	10.0	10.0	5.0	6.3
	Potato	23.6	595	150	55.5	55.0	54.3	50.0
	Sugar	0.4	351.3	148.8	39.0	36.3	45.0	
E. E.	Bread	1.25	287.5	141.3	57.5	6.3	0	0
	Potato	12.1	587.5	167.5	50.0	47.5	27.5	33.8
	Sugar	8.5	69.3	50.0	49.0	34.3	43.8	
I. L.	Bread	17.1	262.5	120	72.5	52.5	40	41.2
	Potato	2.0	87.5	62.5	57.0	53.8	47.5	33.0
L. S.	Bread	3.9	300	197.5	120	42.5	32.5	23.7
	Potato	0.9	412.5	115.8	29.0	10	13.3	14.5
E. O.	Bread	11.0	175	55	46.3	37.5	40.0	22.5
	Potato	0.3	150	22.3	13.0	5.5	5.5	0
Mean values	Before	6.01						
	Bread		329.22	166.31	61.80	33.22	23.72	20.28
	Potato		430.00	148.36	43.46	36.20	28.32	28.42
	Sugar		138.62	74.52	37.70	26.62	32.56	



Diagr. 5.

Mean values of reducing substance in saliva after 2 minutes supply of boiled potato (————) or white bread (— — —).

chewing 0.2 ml samples were taken. The saliva was immediately pipetted in $1^1/_2$ times its volume of 1 per cent trichloracetic acid to precipitate the protein. After centrifuging, these samples taken after ingesting starch were diluted 10 and 100 times and determination of the reducing substance performed on each by the FOLIN-WU method. The results, calculated as glucose, appear in Table 7. In the table there have been included some analysis series which were carried out with cane sugar for the purpose of comparison; the same method was used but a piece of lump sugar was sucked by the subjects for 2 minutes. The average values of the determinations after bread and potato consumption are to be found in Diagram 5. All the individual glucose clearance curves agree in type with these average curves.

The content of reducing sugar is, as would be expected, always highest at the first determination after carbohydrate ingestion. It falls off rapidly during the first 10 minutes, slower during the next half hour and after 40 minutes generally remains at a level somewhat higher than the original. Potato has in most

cases provided the highest immediate concentration, and sugar in all cases the lowest; after 10 minutes the differences are to a great extent smoothed out.

Discussion.

The results presented here confirm the observations of earlier investigators regarding the connection between saliva stimulation and amylase activity. The differences between individuals have also been apparent, in spite of strong variations between the different analyses from the same person. In one respect, this variation has been found to conform to a law among the persons examined: the amylase activity is lowest in the morning, rising steeply during the early hours of the day until, towards the afternoon, only minor changes are apparent. This shows the necessity for standardizing the times at which samples are taken in comparative examinations of different persons.

Behind the investigation of the intra-oral splitting of the starch in the two important types of food, bread and potato, there lay the conception of a possible essential difference between their rate of splitting in the oral cavity. In experiments in vitro with these foods mixed with saliva, no definite difference in splitting and acid production were demonstrable (ERIKSEN and SCHULERUD, 1941; WESTIN, 1949).

Significant chemical and physical differences appear however between these two important carbohydrate sources. Bread contains, in round figures, 50 per cent starch against 20 per cent for potato. Modern baking technique with addition of bromate retains the gluten content in the bread (JØRGENSEN, 1945) resulting in a sticky consistency (WOHINZ, 1935; NYROP, 1943). Potato lacks gluten and has a looser consistency on boiling on account of the cellulose membranes from the cells. Theoretically one would expect a stronger adhesion of the bread remains to the dental surface, a slower diffusion of saliva into these remains, and a slower and more protracted course of oral splitting of the bread than the potato starch. Observations of the intra-oral iodine tests thus also point in this direction just as do the high sugar concentrations in the saliva immediately after ingestion of potato. On the other hand, consumption of bread does not result in any appreciably prolonged dispersal of reducing sugar in the saliva than potato or cane sugar. It is interesting to confirm in the case

of cane sugar the strikingly low salivary glucose values after 2—5 minutes, possibly depending on the slower splitting through bacterial saccharase than starch splitting by salivary amylase, more rapid removal with the strongly stimulated saliva or a combination of both these factors.

Summary.

A survey of literature provides a resumé of such knowledge of salivary amylase that would appear of particular significance to the intra-oral splitting of carbohydrates and, thus, to caries.

Reference is made to various reports relating to a correlation between the activity of the salivary amylase and the caries incidence.

The author has investigated the action of the salivary amylase, using iodine tests and determinations of the reducing substances liberated from starch. The two methods yield consistent results. The splitting of the starch has been proved independent of the salivary bacteria.

The experiments have confirmed the observations of previous workers in respect to variation of the amylase activity between individuals and the dependence on the stimulation of the saliva. A distinct diurnal variation was demonstrated.

A study was made of the clearance of reducing hydrolysis products after ingestion of bread and potato — the two most important sources of starch: comparisons were made, in some cases, with the clearance after consumption of cane sugar. The results appear in Table 7 and Fig. 5, and are commented on in the concluding discussion.

Zusammenfassung.

In einer Literaturübersicht werden solche Data bezüglich der Speichelamylase zusammengefasst, welche von besonderer Bedeutung für intraorale Kohlehydratspaltung und für die Karies zu sein scheinen. Schwankende Angaben werden angeführt bezüglich einer Korrelation zwischen der Amylaseaktivität des Speichels und der Kariesanfälligkeit.

In eigenen Untersuchungen wurde die Wirkung der Speichel-

amylase teils durch den Jodtest, teils durch Analyse der abgespalteten Menge reduzierender Substanz bestimmt. Gute Übereinstimmung zwischen den Resultaten der beiden Methoden wurde gefunden. Die Stärkespaltung des Speichels war unabhängig von dessen Bakterienflora.

Die Beobachtungen früherer Untersucher über die Schwankungen der Amylaseaktivität zwischen Individuen wurden bestätigt, wie auch die Abhängigkeit von der Stimulation. Eine deutliche Tagesvariation wurde auch gefunden.

Die Clearance der Speichel von reduzierenden Spaltprodukten wurde studiert nach Zufuhr der beiden wichtigen stärkereichen Nahrungsmittel Brot und Kartoffel, in einigen Fällen mit derselben Clearance nach Rübenzuckerzufuhr verglichen. Die Resultate sind in Tab. 7 und Diagr. 5 zusammengefasst und in einer abschliessenden Diskussion näher erörtert worden.

Résumé.

L'auteur a revu les données de la littérature relative à l'amylase salivaire et d'importance dans le clivage intraoral d'hydrate de carbone et dans la genèse de la carie. En particulier, on cite les rapports concernant la correlation entre l'activité de l'amylase salivaire et la fréquence de la carie.

Les investigations faites par l'auteur se rapportent à l'action de l'amylase salivaire: elles mesurent cette action de l'amylase par le test à l'iode et par détermination de la substance réductrice libérée d'amidon. Les deux méthodes s'accordent bien. On a montré que le clivage de l'amidon est indépendant de la flore bactérienne de la salive.

Les essais confirment les observations d'auteurs antérieurs sur la variation entre les individus de l'activité de l'amylase, et sa dépendance de la stimulation de la salivation. Une variation diurne se manifeste nettement.

On a examiné l'élimination des produits réductifs de l'hydrolyse résultant de l'ingestion de pain et de pommes de terres — les sources d'amidon les plus importantes —, et on a fait, dans quelques cas, une comparaison avec l'élimination qui suit la consommation de sucre de canne. Le tableau 7 et le graphique 5 rassemblent les résultats discutés dans la conclusion.

References.

ANKER, L., and VONK, H. J.: Presence of α- and β-amylase in the saliva of man and in the digestive juice of Helix pomatia. Proc. Koninkl. Nederland. Akad. Wetenschapp. 49: 677, 1946. (Chem. Abstr. 1946: 7255.)

BAMANN, E., and MYRBÄCK, K.: Die Methoden der Fermentforschung. Leipzig 1941.

BÁRÁNY, F.: Resistance to caries in relation to certain properties of saliva. Acta Med. Scand. 127: 370, 1947.

BAUER, CH. W., and MARTIN, W. F.: The stability and activity of salivary amylase. J. Am. Pharm. Ass. 37: 189, 1948.

BERGEIM, O.: Intestinal chemistry. III. Salivary digestion in the human stomach and intestines. Arch. Int. Med. 37: 110, 1926.

BERGEIM, O., and BARNFIELD, W. F.: Lack of correlation between dental caries and salivary amylase. J. Dent. Res. 24: 141, 1945.

BRAMKAMP, R. G.: The protein content of human parotid saliva. J. Biol. Chem. 114: 369, 1936.

CARLES, J., and DELMAS-MARSALET, P.: Variations du pouvoir amylolytique de la salive au cours d'états pathologiques divers. C. Rend. Soc. Biol. 91: 42, 1924.

CARLSON, A. J., and CRITTENDEN, A. L.: The relation of ptyalin concentration to the diet and to the rate of secretion of the saliva. Am. J. Physiol. 26: 169, 1910.

CHITTENDEN, R. H., and RICHARDS, A. N.: Variations in the amylolytic power and chemical composition of human mixed saliva. Am. J. Physiol. 1: 461, 1898.

DEAKINS, M., CHEYNE, V. D., BIBBY, B. G., and VAN KESTEREN, M.: Significance of salivary analyses upon small groups of subjects. J. Dent. Res. 20: 161, 1941.

DELHOUGNE, F.: Untersuchungen über die Speichelsekretion. D. Arch. Klin. Med. 154: 305, 1927.

ERICSSON, Y., and HELLSTRÖM, I.: Salivens mjölksyrehalt efter kolhydrattillförsel. (The lactic acid content of the saliva after carbohydrate ingestion.) Sv. Tandl.-Tidskr. Festskr. 115, 1950.

ERIKSEN, A., and SCHULERUD, A.: Syredannelse av kullhydratrik kost under innvirkning av saliva. Odont. Tidskr. 49: 326, 1941.

EVANS, C. L.: The amyloclastic property of saliva. J. Biol. Chem. 44: 191, 1912.

FABER, M.: Protein and amylase in the saliva from normal individuals and from patients with xerostomia. Acta Med. Scand. 118: 457, 1944.

FABIAN, G.: Physiologie und Pathologie der Speichelsekretion des Menschen in ihrer Beziehung zur Magenfunktion. Halle 1938.

FLORESTANO, H. J., FABER, J. E., and JAMES, L.: Studies on the relationship between diastatic activity of saliva and incidence of dental caries. J. Am. Dent. Ass. 28: 1799, 1941.

FOLIN, O., and WU, H.: A simplified and improved method for determination of sugar. J. Biol. Chem. 41: 367, 1920.

GEDDES, W. F.: The amylases of wheat and their significance in milling and baking technology. Adv. Enzymology 6: 415, 1946.

GERNHARDT, A.: Über die Einwirkung des Pilocarpins und des Neu-Cesols auf den Amylasegehalt des menschlichen Mundspeichels. Z. klin. Med. 123: 16, 1933.

GORLIN, R. J.: Variations in salivary protein concentration. J. Dent. Res. 27: 603, 1948.

HANSEN, H. L., FOSDICK, L. S., and EPPLE, C. F.: The action of mouth organisms on certain carbohydrates. J. Am. Dent. Ass. 24: 1611, 1937.

HEIDENHAIN, R.: Beiträge zur Lehre von der Speichelabsonderung. Stud. physiol. Inst. Breslau 4: 1, 1868.

HEMMETER, J. C.: Die Wirkung der Totalexstirpation sämtlicher Speicheldrüsen auf die sekretorische Funktion des Magens beim Hunde. Bioch. Zschr. 11: 238, 1908.

HESS, W. C., and SMITH, B. T.: The salivary amylase activity of carious and non-carious individuals. J. Dent. Res. 27: 593, 1948.

HOPKINS, R. H.: The actions of the amylases. Adv. Enzymology 6: 389, 1946.

HUBBELL, R. B.: The chemical composition of saliva and blood serum of children in relation to dental caries. Am. J. Physiol. 105: 436, 1933.

JAY, P., HADLEY, F. P., BUNTING, R. W., and KOEHNE, M.: Observations on relationship of Lactobacillus acidophilus to dental caries in children during experimental feeding of candy. J. Am. Dent. Ass. 23: 846, 1936.

JØRGENSEN, H.: Studies on the nature of the bromate effect. Copenhagen 1945.

KOCH, W.: Beiträge zur Physiologie des gemischten Nüchternspeichels des Menschen. Z. ges. exp. Med. 113: 649, 1943.

KOROPOV, W. M.: Über die Sekretionstätigkeit der Speicheldrüsen im Fieberzustand. Arch. Exp. Pathol. 175: 156, 1934.

MAYER, W. B.: A comparison of the amylase concentration in the saliva of infants and adults. Bull. Johns Hopkins Hosp. 44: 246, 1929.

MCCLURE, F. J.: Effect of fluorides on salivary amylase. Pub. H. Rep. 54: 2165, 1939.

MEYER, J., GOLDEN, J. S., STEINER, N., and NECHELES, H.: The ptyalin content of human saliva in old age. Am. J. Physiol. 119: 600, 1937.

MEYER, K. H., et al.: Purification et cristallisation de l'α-amylase de salive. Experientia 3: 455, 1947.

MEYER, K. H., DUCKERT, F., and FISCHER, E. H.: Sur la liquéfaction de l'empois d'amidon par l'α-amylase humaine. Helv. Chim. Acta 33: 207, 1950.

MYRBÄCK, K.: Über Verbindungen einiger Enzyme mit inaktivierenden Stoffen. I und II. Hoppe-Seylers Z. 158: 160, 1926 and 159: 1, 1926.

NEUWIRT, I., and KLOSTERMAN, J. A.: Demonstration of rapid production of lactic acid in oral cavity. Proc. Soc. Exp. Biol. Med. 45: 464, 1940.

NYROP, E.: Ernährung und Zahnkaries. D. Zahn-, Mund- u. Kieferheilk. 10: 19, 130, 157, 1943.

NØRBY, G.: Om amylasen i blod og urin. Copenhagen 1935.

PICKERILL, H. P.: A note on the etiology of caries of the teeth. N. Zeal. Med. J. 23: 485, 1924.

PRINGSHEIM, H., and GORODISKI, H.: Über die Sekretion und Aktivität der Speichelamylase. Bioch. Zschr. 140: 175, 1923.

ROGER, H.: Action du suc gastrique sur la salive. C. Rend. Soc. Biol. 62: 1021, 1907.

ROGER, H., and SIMON, L.-G.: Action synergique de la salive et du suc pancréatique. C. Rend. Soc. Biol. 62: 1070, 1907.

SCHNEYER, LEON H.: Effects of temperature on salivary amylase activity. J. Dent. Res. 29: 696, 1950.

STEPHAN, R. M.: Changes in hydrogen ion concentration on tooth surfaces and in carious dentin. J. Am. Dent. Ass. 27: 718, 1940.

STRAUSS, L.: Über den Einfluss der Ausschaltung des Mundspeichels bei Magen- und Darmkranken. Arch. f. Verd.-krankh. 33: 163, 1924.

SULLIVAN, J. H., and STORVICK, C. A.: Correlation of saliva analyses with dental examinations of 574 freshmen at Oregon state college. J. Dent. Res. 29: 165, 1950.

SVEJDA, J., and BUDEJOVICE, C.: Decoloration of blue starch solution as a test for caries susceptibility. J. Dent. Res. 29: 698, 1950.

TURNER, N. C., and CRANE, E. M.: A relationship between dental caries and saliva. Science 99: 262, 1944.

TURNER, N. C., and CROWELL, G. E.: Dental caries and tryptophane deficiency. J. Dent. Res. 26: 1, sid. 99, 1947.

VOLKER, J. F., and PINKERTON, D. M.: Factors influencing oral glucose clearance. J. Dent. Res. 26: 225, 1947.

VOLKER, J. F., and PINKERTON, D. M.: Some observations on the fermentation of carbohydrates by saliva. J. Dent. Res. 25: 172, 1946.

WALKER, F., and SHEPPARD, L.: A study of the variations in the saccharogenic power of human saliva. Am. J. Physiol. 111: 192, 1935.

WEINMANN, J.: Weitere Untersuchungen über die Eigenproteolyse im Speichel. Z. Stomatol. 36: 95, 1938.

WESTIN, G.: Personal communication.

WILSKA, A.: En fysiologs syn på kariesproblemet. Odont. Tidskr. 54: 296, 1946.

WOHINZ, R.: Über Versuche zur Kenntnis der primären Vorgänge bei der Zahnkaries. D. Zahn-, Mund- u. Kieferheilk. 2: 68, 1935.

WOHLGEMUTH, J.: Über eine neue Methode zur quantitativen Bestimmung des diastatischen Ferments. Bioch. Z. 9: 1, 1908.

WOLMAN, I. J. et collab.: Amylase levels during mumps. The findings in blood and saliva. Am. J. Med. Sc. 213: 477, 1947.

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