AMYLOLYTIC ADAPTATIONAL CHANGES IN MAMMALIAN SALIVA

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Until now it has not been clear why the saliva in some animals has a very high amylolytic activity, while in others it has practically none (G. Sticker, 1899 [5]; D.A. Biryukov, 1935 [1]).

Some light has been thrown on this as yet unsolved problem by investigations in which the effect of a carbohydrate and a protein diet on the amylase content of human and canine saliva has been studied. In cases where amylolytic activity is high on a mixed diet, there is a noticeable increase or decrease on changing to a carbohydrate or a meat diet respectively. If on a mixed diet there is no amylase in the saliva, then none is found on a protein diet either, but on returning to a carbohydrate diet even on the 4-7th day amylase is found in considerable quantities [2,3,7]. These results help to explain why amylolytic activity is poorly-developed in the saliva of carnivores and well-developed in many herbivores.

It is possible to make one further deduction, whose confirmation is the subject of the present work. Most physiological investigations on this subject are to some extent unsatisfactory, as the tests for amylolytic activity were carried out on only one of the many possible substrates, namely starch. Generally, at any rate as far as phosphorylase is concerned (J. Sumner and G. Somers [8]), this enzyme is completely specific both for starch and glycogen.

In a previous communication [4] we showed that canine gastric juice taken after feeding meat, causes a greater breakdown of animal than of plant protein, while juice secreted by the same animal when fed bread, on the contrary is more effective in hydrolyzing protein of plant than of animal origin. (We have referred to the breakdown of substrates of animal and plant origin as zoolytic and phytolytic activity, respectively.) It remains to find whether it is possible for glands causing hydrolysis of polysaccharides to show the same kind of adaptation as for gastric glands which cause partial hydrolysis of proteins. Our method has been to compare the extent of the breakdown of starch and glycogen under similar conditions. Evidently, if such an adaptation is possible, then it will be most clearly shown by comparing the properties of the saliva of animals with different natural diets.

EXPERIMENTAL METHOD

Fistulae were placed in the ducts of the parotid and submaxillary salivary glands and the amylolytic activity of the saliva determined in the following animals: 3 monkeys, 1 fox, 3 fox cubs, 8 guinea pigs, 3 rats, and 4 cats. In addition, an examination was made of the saliva from the parotid gland in 24 human subjects fed a normal diet.

In 1950-1953 we determined the amylolytic activity using Wohlgemuth's method (parallel determinations using 0.1% starch and 0.1% glycogen in a phosphate buffer at pH 6.5 or 7.2). Subsequently, simultaneous

determinations were made using the method of Wohlgemuth and Smith-Roe [6]. The latter method is very precise. The amylolytic activity is determined by the reduction of the initial concentration of substrate caused by the hydrolysis, (concentration was measured using a photoelectric colorimeter with a red light filter for starch and a blue for glycogen). As distinct from the classical method, a 0.3% solution of starch was used. The same concentration was also used in the case of glycogen. There was usually a good agreement between the two methods.

EXPERIMENTAL RESULTS

In the saliva from both the parotid and submaxillary glands in guinea pigs, there was a considerably greater amylolytic activity with respect to starch than to glycogen. In the parotid saliva of guinea pigs, the phytolytic activity varied between 320-1280 Wohlgemuth units, while for glycogen the corresponding figures were 160-640 units; in each experiment the second figure was 2-8 times less than the first (in most of the experiment it was 4 times less). In guinea pigs the phytolytic activity of the saliva of the submaxillary glands is also 2-4 times greater than the zoolytic, but the general level of amylase in it is somewhat greater (1280-2560 Wohlgemuth units for starch). Saliva from both the parotid and the submaxillary glands was taken during feeding on beetroot, carrot and grass, and from the parotids during spontaneous secretion. It is interesting to note that the spontaneously secreted saliva which represents the natural state of the gland is more strongly phytolytic than zoolytic.

The amylolytic activity of the parotid secretion in rats is even greater (5000-20,000 Wohlgemuth units for starch). The phytolytic activity in these animals is 2-4 times greater than the zoolytic. The amylolytic activity was determined using the method of Smith-Roe [6], and this confirmed that the phytolytic activity was greater than the zoolytic. This was found both for saliva taken during feeding and that stimulated by pilocarpine.

A marked preponderance of phytolytic over zoolytic activity was also found in monkey saliva (Macaque rhesus). The amylolytic activity of the parotid secretion of these animals varied from 10,000-100,000 Wohlgemuth units for starch, and the submaxillary secretion – between 4000-16,000 units. In most of the experiments the phytolytic activity was 2-4 times greater than the zoolytic. In some cases however neither Wohlgemuth's method nor that of Smith-Roe revealed any difference in the rate of digestion of starch and glycogen. The zoolytic and phytolytic activities were equal or very nearly so. The reason for this is not clear, but the occurrence was by no means exceptional. Any systematic error was excluded by making on each sample a measurement with Wohlgemuth's method and, in two optimal dilutions with Smith-Roe's method.

Thus, in this group, fed on an either exclusively or chiefly vegetable diet, amylase hydrolyzed starch more than glycogen.

We must suppose, that on account of the structure of the molecules of starch (more precisely, amyloses) the latter are attacked in preference to the more branched molecules of glycogen. However, even for the substances we have tested, such a conclusion is doubtful, because firstly, the ratio of phytolytic to zoolytic activity varies within fairly wide limits, and secondly, because in certain cases the zoolytic activity is not less than the phytolytic. All this suggests that besides the quantitative variation of the amylolytic properties of saliva (greater or lesser concentrations of enzyme), qualitative changes are also possible and are shown by a change in the preferential action with respect to starch or glycogen.

Considerable variability in the properties of the enzymes were found in cat and fox saliva, but this occurred in addition to the well-known preponderance of zoolytic action. In fox cubs it was not possible to measure the amylase concentration, because there was not enough saliva to use in concentrations less than 1 in 10, and in greater dilutions no amylolytic activity could be observed. In adult foxes phytolytic activity did not exceed 10 Wohlgemuth units, while the zoolytic varied between 40-80 units.

In cats, the concentration of amylase varied between 20-160 Wohlgemuth units for starch (the latter figure occurred only exceptionally). In most experiments these zoolytic properties exceeded the phytolytic by 2-4 times, more rarely the two activities were equal, and still more rarely the phytolytic activity preponderated.

Thus our results confirmed the previous observations on the low amylolytic activity of cat and fox saliva. But in this low degree of activity it remains qualitatively distinct in its action on starch and glycogen from that of the saliva of herbivores.

The hardest task we encountered was that of classifying human saliva correctly. We give here the variation in amylolytic properties (in Wohlgemuth units) of one of the groups we investigated (see Table).

TABLE

Amylolytic Properties of Human Saliva

Subject	Activity		
	phytolytic	zoolytic	ratio
1	5,120	2,560	2:1
2	2,560	2,560	1:1
3	40,960	10,240	4:1
4	1,024	16,384	1:16
5	1,024	8,192	1:8
6	2,048	2,048	1:1
7	16,384	8,192	2:1
8	8,192	8,192	1:1
9	4,096	4,096	1:1
10	16,384	32,768	1:2
11	32,768	3,192	4:1
12	4,096	1,024	1:2
13	4,096	2,048	2:1
14	4,096	2,048	2:1
15	32,768	8,192	4:1
16	32,768	32,768	1:1
17	16,384	8,192	2:1
18	16,384	8,192	2:1
19	32,768	16,384	2:1
20	4,098	512	8:1
21	32,768	16,384	2:1
22	32,768	8,192	4:1
23	32,768	8,192	4:1
24	4,096	1,024	1:2

Thus from these results alone it can be seen that there are three variants of the relationship between phytolytic and zoolytic activity in human saliva: there can be an equality of the two, or else a preponderance of either one or the other. It is clear that in most cases this difference is far greater than any possible experimental error. Late the same variants were found using the method of Smith-Roe.

Quite apart from the problem of what causes these differences, we were concerned to show that there is apparently a variation in the specific activity of the saliva of different individuals. The properties of the saliva of a single individual may vary within wide limits. The ratio of phytolytic to zoolytic activity depends to a considerable extent, though not entirely, on the type of diet. We carried out three experiments to determine the effect of a change from a mixed to a purely vegetable diet * (in 1953 – 2 people were maintained on a

* The participants in these experiments, students and scientific workers, were fed on an arbitrary vegetarian diet, which excluded meat products and extracts.

vegetable diet for 4 days, and in 1954 – 2 people on the same diet for 5 days, and in 1955 – 3 people on the same diet for 8 days), and in all these experiments a sharp reduction in the zoolytic activity was found, as well as an irregular change in the phytolytic activity, i.e. the reaction was adapted to the quality of the current diet.

Thus, as regards the biological aspect of these results, it appears that the enzyme properties of the saliva of different mammals are adapted to the diet: the action is chiefly phytolytic in herbivores and zoolytic in carnivores. Together with the adaptations characteristic of the species there are also marked individual adaptations (experiments on human subjects). Thus in some cases the saliva preferentially breaks down starch, and in others glycogen. This could be brought about either by the enzyme properties remaining unchanged while those of the medium in which it acts becoming altered, or with changes in the property of the enzyme itself. When it is remembered that the monkey and human saliva for the estimation of amylase was diluted hundreds or thousands of times in buffered solutions, it is clear that variation of the medium in which the enzyme acts would not exceed the limits of experimental error. Therefore preponderance of zoolytic or phytolytic properties of the saliva are likely to be due to changes in the specific activity of the saliva amylase.

SUMMARY

Saliva of herbivorous animals (rats, guinea pigs and monkeys) splits starch (phytolytic effect), more actively than glycogen (zoolytic effect), while the saliva of carnivores (cats and foxes) on the contrary splits glycogen more actively than starch. The specific activity of saliva of man shows wide variations in different individuals. In one group there is a prevalence of zoolytic activity, in another phytolytic, while in certain persons both activities are equal. The data that were obtained are considered to be an adaptive mechanism of the enzymatic activity to the quality of food. The adaptive mechanisms not only common to the same species were revealed but, likewise, those which appear with the change of the diet.

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