Comparative Physiology. — The presence of α- and β-amylase in the saliva of man and in the digestive juice of Helix pomatia. II. Polari­metric determinations. By L. ANKER and H. J. VONK. (From the Laboratory of Comparative Physiology University of Utrecht.)
(Communicated by Prof. A. de KleyN.)

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In our previous communication (6) we mentioned that two types of amylase may be distinguished which behave differently in the following points: 1° the limit unto which they are able to split up the starch-molecule, 2° the rapidity with which the jodine-test of the unattacked starch disappears during the reaction, 3° the stereoisomeric form of the maltose which is set free in the reaction by both of the enzymes.

The last mentioned fact has been discovered by KUHN in 1924. Maltose may appear in two forms (called α- and β) the first of which causes a stronger positive rotation than the second. These forms are unstable: when they are present together in aqueous solution they are converted into each other, so that an equilibrium-mixture is formed, which contains 36 % α-maltose and 64 % β-maltose. This transition (called mutarotation) is strongly accelerated by an excess of OH-ions (e.g. addition of soda). If originally an excess of α-maltose is present, the mutarotation may be called negative, in the reverse case the mutarotation is positive.

KUHN observed in the amylolytic cleavage of starch by amylase of different origin in some cases a negative in other a positive mutarotation. In the first case maltose was set free in the α-form, in the second in the β-form. He therefore distinguished two types of amylases: an α- and a β-amylase. Further research showed that the α-amylase was identical with ÖHLSSON’S dextrinogen-amylase, the β-amylase with ÖHLSSON’S saccharogen amylase. The names α- and β-amylase are mostly used nowadays.

Whereas in the vegetable kingdom both types of amylases occur as well together as separately, the animal amylases are — as far as investigated — nearly always of the α-type. Only relatively small amounts of β-amylase occurring together with the α-amylase have been observed by PURR in the pancreatic juice of the pig and by ANKER and VONK in the saliva of man and in the digestive juice of Helix pomatia.

The most conclusive evidence for recognising an amylase as belonging to the α- or to the β-type is undoubtedly the primary appearance of maltose in the α- resp. in the β-form. As far as we know this criterion has never been applied to the action of saliva on starch and glycogen 1), so that we

1) These experiments have been performed by KUHN for the amylases of malt, taka and pancreas. (Cf. also SAMEC (3) p. 132—134.)
thought it important to follow the course of this reaction polarimetrically. The same experiments were carried out with the digestive juice of Helix pomatia (as far as the material allowed) which has neither been investigated with the polarimetric method. Simultaneously the course of these reactions has been followed by the determination of the change in reduction when tested with an alkaline copper-solution in order to compare the results of these methods. KÜHN has applied the same procedure in his researches on the cleavage of starch by the α-amylase of malt and of the pancreas. In using these methods also an impression can be obtained of the limit unto which the hydrolysis may proceed.

I. The cleavage of starch by saliva.

a. Method and calculation.

The reaction-mixture contained 250 cm$^3$ of a 1 % solution of soluble starch (KAHLBAUM), buffered by KH$_2$PO$_4$ and Na$_2$HPO$_4$ at a pH of 6.8 to which 3 to 4 cm$^3$ of filtered saliva were added. The saliva had been collected after a thorough cleaning of the mouth (like in our previous communication), this time to prevent that small amounts of reducing sugars arising from rests of food would disturb the result of the determinations. The reaction took place in an ERLENMEYER flask of 300 cm$^3$ at a constant temperature of 25° C.

At fixed times three portions were taken simultaneously from the mixture. Of the first portion the rotation was determined immediately. To the second portion 2n soda was added 2) to stop the reaction and to cause a rapid course of the mutarotation. The rotation of this sample was determined after 15 min. After correction of the observed value for the dilution by the addition of soda, it can be compared with the directly measured value of the first sample. The comparison of these two values gives an answer to the question whether a negative or a positive mutarotation is present, viz. whether the amylase for the largest part consists of the α- or the β-type. In the third portion the reduction was determined by means of the method of LUFF, as indicated by SCHOORL (4).

The values obtained by the polarimeter and the determination of the reduction were drawn up in curves showing the relation of cleavage and time. In order to compare the curves obtained for the polarimetrical observations and these of the reduction with each other, and to calculate the limit unto which the cleavage proceeds, some constants of the starch-solutions had to be determined previously. Firstly the percentage of amylose, viz. of digestible substance. This value was determined by boiling the starch-solution after adding hydrochloric acid (until the solution contained 2.5 % HCl) on a waterbath for 3 hours, neutralizing and determining the reduction. By bringing this reduction in relation to the weight of the starch which the solution contained, a percentage of 86.5 for the amount of

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2) An amount of 5 cm$^3$ 2n soda for a sample of 20 cm$^3$. 
amylose in the dry substance was found. Secondly the reductive power of the starch-solution itself had to be determined. It varied in the various experiments from 0.89 to 1.20 cm³ of 0.1035 n thio-sulfate per 10 cm³ solution.

The reduction-value which was observed in the samples of the reaction-mixture taken at different times after the beginning of the experiment, consists of the reduction caused by the amount of maltose, already formed at this time and the reduction power of the unaltered starch still present. As for a definite amount of starch which is split up a certain amount of reductive power has disappeared, which is replaced by the reduction of the maltose which has been formed, the amount of maltose present at a certain time can be calculated in mg.

In order to compare this amount with that which had been found polarimetrically, the rotation was calculated which is equivalent to the amount of maltose found by means of reduction-determinations, assuming that the maltose is present in the state of an equilibrium-mixture of α- and β-maltose. To this rotation had to be added the rotation-value of the unhydrolysed starch. For this purpose the specific rotation of the dissolved starch was determined before the beginning of each set of experiments.

For the specific rotation of the equilibrium-mixture of maltose the value \( \alpha_D = 137.9^\circ \) was taken, which is generally used in the literature on starch-chemistry. As substrate we used Amylum solubile of SCHERING-KAHLBAUM. The length of the polarimeter-tube was 2 dm. Pipettes had been carefully calibrated.

Assuming that the course of the reaction follows indeed this simple scheme, the values of the curves determined by reduction and by polarisation (after taking away the mutarotation by means of soda) should coincide. This is not strictly the case. At the beginning of the reaction a fairly strong deviation is seen, which diminishes with the proceeding of the reaction. Similar deviations have been observed by KUHN and SAMEC for the cleavage of starch by the α-amylase of malt and pancreas. They must be ascribed to the fact that the reaction-mixtures contain besides starch and maltose also dextrines, the reduction as well as the rotation of which are unknown. Consequently they cannot be taken into account in the calculations. Most of these dextrines are gradually split up into maltose during the reaction, as is known, by the action of the α-amylase. So it is clear that the divergences between the polarimetric values and those of the reduction disappear in the course of the reaction, as may be seen from our results.

b. Results. Fig. 1 shows the results of an experiment in which the cleavage of starch by saliva has been studied. Curve a represents the direct polarimetric observations, curve b shows the polarimetric values which are obtained after the mutarotation has finished. The values of curve c have been obtained by calculating the rotation-values, which an
equilibrium mixture of maltose would show, from the determined reduction values.

In comparing curves a and b it may be seen that (with the exception of slight deviations at the beginning and at the end of an experiment) there is a distinct negative mutarotation, so that we may conclude that the majority of the saliva-amylose consists of the a-type. The nearly coinciding of the curves b (rotation of the equilibrium-mixture) and c (rotation of equilibrium-mixture calculated from the reduction), is a good control for the exactitude of the polarimetric as well as the reductometric determinations.

The general shape of our curves agrees with those published by KUHN for the a-amylose of malt-extract. We repeated the experiment shown in fig. 1 seven times with different samples of saliva and with approximately the same results. In two of these experiments a sharp deviation was observed in the beginning of the reaction. This deviation has been represented in fig. 1 by the dotted line. It is also present in KUHN's curves. It is absent in the curves of fig. 1, probably because of the rapid course of the reaction in this experiment, where the saliva proved to be very active. In the two cases where this anomaly was observed by us, the amylose-activity was apparently low. In the rapid course of the experiment represented in fig. 1 the phenomenon took probably place between two observations. The exact causes of this phenomenon are unknown.

Although the curves b and c in fig. 1 show a very good agreement, in some of our experiments (which are not represented here for lack of room)
the curve c is running somewhat more beneath the curve b. After SAMEC these curves meet if the starch is totally hydrolysed. Both KUHN and SAMEC mention deviations of those curves to both sides, without trying to give any explanation of this phenomenon. We think it possible that the deviations of KUHN and SAMEC are due to the fact that they probably did not determine the specific rotation of the dissolved starch for each experiment separately. This value which we determined for each experiment appeared to be somewhat variable according to the way in which the solution had been prepared. The same is true for the reduction of the starch-solution itself.

Without going into theoretical considerations concerning the mechanism of the enzymatic reaction or on the structure of starch we may make the following remark on the limit unto which the starch is hydrolysed by both types of amylase. Conclusions drawn from finding a certain cleavage-limit are of small value, if the nature of the substrate is not exactly known. Starch occurs chiefly in two forms which are split up by both types of amylase to a different degree. (For extensive data in this respect see the work of SAMEC (3)). It depends on the relative amounts of these forms in the substrate to which degree the latter is hydrolysed by a certain amylase. Some substrates consist already partly of cleavage-products of starch, e.g. the so-called LINTNER-starch. Our substrate was hydrolysed by amylase for about 80 %. This agrees with the values formerly found by VONK and BRAAK in this laboratory for the same substrate.

II. The cleavage of glycogen by saliva.

The amount of glycogen at our disposal being very limited we chiefly
restricted our investigation to the difference in rotation which could be observed before and after the mutarotation. Here too we found a negative mutarotation which confirms the fact already found by other investigators, that the type of maltose which is set free, does not depend on the character of the substrate, but is determined by the enzyme by which the sugar is set free.

Moreover it must be observed that a certain amount of saliva hydrolyses a definite amount of starch more rapidly than the same amount of saliva hydrolyses the same amount of glycogen. From some determinations of reduction-values we got the impression that the percentage of the saccharification in the case of glycogen is lower than in that of the starch.

We repeated the experiments with glycogen five times with the same results. One of these experiments is represented in fig. 2.

III. The cleavage of starch by the digestive juice of Helix pomatia.

Owing to the great amount of snails which is necessary for the collection of a few cm$^3$ juice from the crop of the snail we could only perform two experiments, one of which is represented in fig. 3. In both experiments we found a strongly negative mutarotation, so that by far the largest amount of the amylase of Helix must belong to the $\alpha$-type. Such in accordance with the fact that we found in our previous communication by means of the diffusion-method only a small amount of $\beta$-amylase. It is remarkable, that the amylase of the juice of Helix which contains a lot of enzymes (in
rather large amounts), which are capable of hydrolysing $\beta$-hexosides, belongs for the greater part to the $\alpha$-type.

**Summary.**

1. In the cleavage of starch- and glycogen by the saliva of man and also in the cleavage of starch by the digestive juice of Helix the maltose is set free in the $\alpha$-form. In both of these digestive juices the amylase must therefore consist for the largest part of $\alpha$-amylase. This agrees with the results of our previous communication (obtained with diffusion methods) where only very small amounts of $\beta$-amylase together with large amounts of $\alpha$-amylase were found.

2. The results of the polarimetric determinations (after the mutarotation) nearly agree with the determinations by means of sugar-titration.

3. Starch-solutions (of Amylum solubile KAHNBAUM) are hydrolysed by human saliva for about 80%.

**LITERATURE.**