CVI. DIGESTION IN THE COCKROACH.

II. THE DIGESTION OF CARBOHYDRATES.

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THE most recent account of the digestive processes of the cockroach is that given by Swingle [1925], who employed the Common Cockroach (*Blatta orientalis*) in his experiments and whose conclusions are briefly as follows. The salivary glands produce only an amylase. The hepatic caeca of the mid-gut produce an invertase, maltase, lipase and protease. The remainder of the mid-gut (stomach) secretes the same enzymes though less abundantly. The protease he divides into a "trypsin" and an "erepsin"; but the tests employed for erepsin were (1) the formaldehyde titration of a peptone digest, and (2) the production in this digest of nitrogenous compounds not precipitated by phosphotungstic acid; both of which tests would be given equally well by trypsin alone. The hind-gut does not secrete digestive enzymes. The greater part, if not the whole, of digestion takes place in the crop, the enzymes passing backwards from the salivary glands and forwards from the mid-gut. The crop is lined by a membrane of chitin and is generally regarded as playing no part in the secretion of digestive ferments; but Swingle claims that extracts of washed crops contain traces of invertase, maltase and lipase, and the same claim is made by Sandford [1918] for lipase. Swingle does not decide from his evidence whether these enzymes are formed in the cells of the crop or absorbed by them from the digestive cavity.

It may be mentioned here that the results of Swingle have been confirmed, but that, in addition, an invertase has been found in the salivary glands of the German Cockroach (*Blatella germanica*), though not in the American Cockroach (*Periplaneta americana*) and that small quantities of lactase have been demonstrated in extracts of the mid-gut. With regard to the enzymes of the crop, the results of Swingle have been repeated by his method. The reliability of these results depends, however, upon the successful washing of the crops, a procedure which is not easily carried out, since the crops contain earthy material which is often very difficult to remove; and it has been shown that if the gut is washed out thoroughly in the living animal by giving it water but no food for a fortnight or three weeks, extracts of the crop are free from maltase and invertase, while the other parts of the digestive system retain

their normal properties. These results conform to the more orthodox view that the crop plays only a passive rôle in digestion. The methods employed in these experiments will be described later when dealing with the enzymes in detail.

This paper will deal with those enzymes of the cockroach which are concerned in carbohydrate digestion, with a view to comparing them with the corresponding enzymes from other sources. It has been found [Wigglesworth, 1927] that these enzymes normally function in a medium rendered acid (p_H 5.0) by the action of micro-organisms. In the present communication it will be shown that they are adapted to work at this reaction; but that with this exception their properties are very similar to those of the enzymes with which they have been compared.

The most clear-cut distinctions between enzymes of a given kind are to be found in the effects of various ions upon their activity, and this part of the subject has received most attention here. The effects of substrate or enzyme concentration upon the kinetics of their action appear to be less important. Temperature effects are so dependent upon other conditions of the experiment that few clear comparative data are available, and this aspect of the matter has not been touched.

The comparisons which have been made are far from complete, but they are sufficient to indicate that the enzymes concerned in carbohydrate digestion in the cockroach are very similar to those of yeast and of higher organisms.

AMYLASE.

Amylases from various sources differ in their optimum p_H , in the effect of inorganic ions upon their amylolytic and saccharogenic activity [Sherman and Tanberg, 1916] and in the ratio between these two measurements of activity under a given set of conditions [Sherman and Schlesinger, 1913]. The salivary amylase of man is characterised by being inactivated by dialysis, and by having its activity (both amylolytic and saccharogenic) restored by the anions of salts in the following descending order: chlorides, bromides, nitrates, iodides, phosphates and sulphates. The optimum concentration of chloride is from 0.003 to 0.03 *N* [Willstätter, Waldschmidt-Leitz and Hesse, 1923]. The optimum p_H varies from 6.1 to 6.9, depending upon the salts present [Michaelis and Pechstein, 1914].

In the alimentary canal of the cockroach, amylase is confined to the salivary glands.

Methods.

Preparations of active enzymes. Stock preparations of enzymes from the cockroach have been obtained in two ways.

(i) Before dissection the insects are given nothing but distilled water for a week or a fortnight in order to clean out the alimentary canal. The gut is removed from the insect as previously described [Wigglesworth, 1927] and

divided up into salivary glands, crop and mid-gut. These parts are collected from about twenty *B. germanica* and ground up in a small glass mortar with a little washed sand and a drop of glycerol until a fine uniform emulsion is obtained. This is then made up to about 10 cc. with 50 % glycerol to which are added a few drops of 5 % thymol in alcohol. This will be referred to as a "glycerol extract."

(ii) Dissection is carried out as before but the tissues are ground up with sand and a drop of water and of 5 % thymol in alcohol. The resulting emulsion is dried at room temperature and powdered. This will be referred to as a "dry preparation."

Human ptyalin was obtained by holding in the mouth a mixture of equal parts of glycerol and water and filtering the mixture.

General method of experiment.

(i) *Amylolytic experiments.* The reactions are carried out in small test-tubes (100 mm. by 6 mm.), the total volume of fluid present being 1 cc., of which 0.5 cc. consists of a 2 % solution of soluble starch. A small pipette graduated in divisions of 0.025 cc. is placed in each tube at the outset of the experiment. A water-bath at 37° has been used throughout. At suitable intervals 0.025 cc. is withdrawn and run into 0.5 cc. quantities of very dilute (almost colourless) acidified iodine solution measured out into a series of uniform test-tubes. When the optimum of a series of conditions is sought, the method of Michaelis [1922] is adopted, *i.e.* the series of reacting mixtures is tested at an equal interval from the outset of the experiment and the one showing the greatest degree of change taken to represent the optimum. When the relative activity over a range of conditions is sought, the time taken to reach a suitable mauve tint in the fastest tube is noted. This tint is then used as an arbitrary end-point and the time taken by each mixture to reach this colour is observed. The reciprocals of these respective times are taken to represent velocity of action and, expressed as percentages of the fastest rate, are reported in the various experiments as "relative velocity." An additional check on the results has been obtained by noting the time taken to reach the achromic point. But for general use the former method has proved more satisfactory because in the slowly reacting mixtures the achromic point may never be attained, while in the rapidly reacting mixtures there is nothing to indicate whether it has been reached some time past.

Where two enzyme preparations are compared under the same series of conditions, the concentration of each is so adjusted before the experiment that it shows approximately the same activity as the other when both are at their respective optima. It is most convenient if the arbitrary end-point is reached in the fastest tube in about 15 minutes.

(ii) *Saccharogenic experiments.* The experiments are carried out precisely as above except that pipettes graduated in 0.1 cc. divisions are employed and the sugar produced in a given time is estimated by the method of Hagedorn

and Jensen [1922]¹. The concentration of enzyme is adjusted to give about 0.25 % of "glucose" in 15 minutes in the fastest mixture. The blank on such mixtures is so small that no preliminary precipitation is necessary. The amount of phosphate present in the phosphate buffer mixtures has been shown to have no effect on the reducing power of sugars as estimated by this method [cf. Visscher, 1926]. In these experiments it is assumed that the velocity is directly proportional to the amount of reducing sugar (expressed as "glucose") which is produced in a given time. This expression assumes that the hydrolysis of the starch is a linear function of time, an assumption which is probably only approximately true and which is only justified because no far-reaching conclusions are based upon it.

Results.

(i) *The effect of salts.* An experiment showing the effect of salts on cockroach amylase is given in Table I.

Table I.

Mixtures in reaction-tubes made up as follows: 0.1 cc. glycerol extract of salivary gland of *B. germanica* + 0.1 cc. 0.5 *M* solution of salt + 0.3 cc. distilled water + 0.5 cc. 2 % soluble starch.

Salt	Relative activity (amylolytic)
None added	30
Sodium sulphate	47
Sodium phosphate (p_H 6.8)	50
Sodium nitrate	74
Potassium iodide	80
Sodium bromide	100
Sodium chloride	100

This experiment shows that the amylolytic power of cockroach amylase is influenced by salts in the same way as ptyalin. In an experiment where the saccharogenic activity was measured, this was found to be influenced in the same way by the addition of salts.

A dry preparation of cockroach salivary gland was extracted with distilled water for 24 hours and the extract dialysed in a small collodion sac, prepared by the method of Gates [1922], against several changes of distilled water for 24 hours. The 2 % starch solution was similarly dialysed. In the absence of added salt the preparation so treated was almost inactive. A comparison of amylolytic activity at various concentrations of sodium chloride was then made. These results are given in Fig. 1 and show an effect identical with that described in the case of human ptyalin. It is of interest to note that the amount of enzyme present in each of these mixtures was equivalent to one-fiftieth of the salivary gland of the cockroach.

¹ Since Hagedorn and Jensen make no reference to the fact and as I have found so many workers unacquainted with it, I may mention here that the *N*/200 solution of sodium thiosulphate will remain perfectly stable in ordinary distilled water for many weeks if this be made just alkaline with sodium hydroxide. Thus the titre of a solution of thiosulphate in distilled water against standard potassium iodate changed from 18.0 to 22.4 in 17 days, whereas a similar solution made just alkaline with sodium hydroxide changed from 18.1 to 18.2 in 2 months.

(ii) *The effect of hydrogen ion concentration.* In considering the "adaptation" of an enzyme to its environment, the precise concentration of hydrogen ions necessary for its optimal activity is of less importance than its ability to function over that range of p_H which it is liable to encounter in its sphere of action. It is on this account that in the experiments here reported more attention has been paid to the relative activity of the enzymes over a wide range of p_H than to the determination of the absolute optimum. Moreover, differences in activity around the optimum are so slight that a more reliable basis of comparison is afforded by considering the p_H at which activity is reduced by 50 %, irrespective of any theoretical interpretations [Michaelis and Davidsohn, 1911] which may be put upon this figure.

Fig. 1. Effect of sodium chloride on dialysed amylase from the salivary gland of the cockroach.

The glycerol extracts prepared as above described are practically unbuffered and the required hydrogen ion concentration has been obtained by taking in each tube 0.1 cc. of glycerol extract (after dilution if necessary), 0.1 cc. 1 % sodium chloride and 0.3 cc. of buffer. 0.5 cc. of 2 % soluble starch is added at the last moment. The phthalate and phosphate buffer mixtures of Clark and Lubs have been used. (Borate buffers cannot be employed in the presence of glycerol because of the production of the highly dissociated glyceroboric acid.) The p_H has been re-determined colorimetrically at the end of the experiment and shown to be unchanged.

Under these conditions the optimum p_H for cockroach amylase was found to be about 5.9 and in the case of human ptyalin about 6.5.

The relative activity over a wide range of p_H is shown in Fig. 2, which was obtained from a preparation from *Blatella germanica*. From this it will be seen that whereas the cockroach amylase still shows 50 % activity at p_H 4.8, human ptyalin is almost inactive at this reaction. The cockroach enzyme is much more tolerant of acid, a finding which was to be expected from the conditions obtaining in the gut of this insect [Wigglesworth, 1927]. The relative difference in activity in the alkaline direction is less striking. Twelve hours after the conclusion of this experiment digestion was still incomplete

in the more acid mixtures. The p_H in the various mixtures was brought back to about 6.4 with potassium hydroxide and acetic acid, and 0.5 cc. of 2 % starch added. In the tubes at p_H 4.0 both amylases had been destroyed. At less acid reactions the enzymes had been partially destroyed, that of the cockroach being the more resistant. Clearly the diminished activity in the acid region is due partly to enzyme destruction and partly to a reversible inactivation.

Fig. 2.

Fig. 2. p_H -activity curves (amyloclastic) for amylase. A, Salivary amylase of *Blatella germanica*. B, Human ptyalin.

Fig. 3.

Fig. 3. p_H -activity curves for amylase. A, Salivary amylase of *Periplaneta americana*. B, Human ptyalin.

Fig. 3 shows a similar experiment with an amylase preparation from the salivary gland of *Periplaneta americana*. Owing to an error in calculation the human preparation was nearly four times as active as the cockroach preparation at about the optimum; yet the activity of the cockroach amylase falls off far less rapidly in the acid direction.

Fig. 4 is a comparison of the two p_H -activity curves in an experiment where sugar production was taken as a measure of activity. This gives exactly the same results as the amyloclastic experiments.

It was of interest to determine whether the greater resistance of the cockroach amylase to acid was due to differences inherent in the enzyme or to some associated factor; for it is well known that the optimum p_H of amylases is markedly affected by the presence of salts [Michaelis and Pechstein, 1914]. This point was tested in the experiment given in Table II. Preparations of ptyalin (A) and of amylase from the German Cockroach (B) were adjusted to have the same activity at their p_H optima. Mixtures were made up as shown in the table and their activity compared at p_H 4.4. The "inactivated"

enzymes were left for 5 minutes in a boiling water-bath and were shown to be completely inactive.

Table II.

Mixture	Relative activity
0.1 cc. B + 0.1 cc. "inactivated" A + 0.1 cc. 1 % NaCl + 0.2 cc. phthalate buffer p_H 4.4 + 0.5 cc. 2 % starch	100
0.1 cc. B + 0.1 cc. 50 % glycerol: the rest as before	100
0.1 cc. A + 0.1 cc. "inactivated" B: the rest as before	12.5
0.1 cc. A + 0.1 cc. 50 % glycerol: the rest as before	Very slight

A similar result has been obtained in the case of the amylase from the American Cockroach, and would seem to indicate that the ability of the cockroach enzyme to function in a more acid medium is due in part at least to some associated substance which is not destroyed by heating at 100° for 5 minutes.

Fig. 4.

Fig. 4. p_H -activity curves (saccharogenic) for amylase. A, Salivary amylase of *Blatella germanica*. B, Human ptyalin.

Fig. 5.

Fig. 5. Hydrolysis of sucrose by cockroach invertase.

(iii) *Ratio of amyloclastic to saccharogenic activity.* Preparations of cockroach amylase and human ptyalin were adjusted to have about the same activity at p_H 6.6; and, preparing the mixtures in the usual manner, their amyloclastic and saccharogenic activities were compared at p_H 6.6 and 8.0. The ratio of amyloclastic to saccharogenic activity was arbitrarily expressed as $\frac{1}{t} : x$; where t is the time in minutes required to reach the arbitrary end-point of starch hydrolysis and x is the reducing sugar (expressed as g. "glucose" %) produced in 15 minutes. The results are shown in Table III.

Table III.

Enzyme	p_H	Ratio of amyloclastic to saccharogenic activity
Cockroach	6.6	1 : 2.5
"	8.0	1 : 2.3
Human ptyalin	6.6	1 : 3.2
"	8.0	1 : 3.3

Sherman and Schlesinger [1913] found that this ratio (expressed in other units) differed widely in various enzymes, ranging from 9 : 1 to 2 : 7. They found that whereas preparations of pancreatic amylase showed a more or less constant ratio, the ratio in the case of plant amylase preparations was extremely variable. Although a long series of experiments would be necessary to establish the point completely, the above results suggest that by this criterion human and cockroach amylases are similar in nature.

INVERTASE.

Of the enzymes which hydrolyse sucrose, that obtainable from yeast has been the most closely studied. This enzyme is reversibly inactivated by alkali, and the alkaline branch of the p_H -activity curve is regarded by Michaelis and Davidsohn [1911] as the dissociation-residue curve of a weak-acid enzyme which is active only in the form of undissociated molecules. The acid branch of the curve is similarly interpreted by Myrbäck [1926]; but in this region there is in addition the factor of irreversible destruction of the enzyme. The activity of yeast invertase is inhibited by heavy metals, by high concentrations of sodium chloride and by glycerol among other substances.

Euler and Svanberg [1921] studied the invertase of the human jejunum and found that it was active in a more alkaline medium than yeast invertase; and Euler and Myrbäck [1921] showed that this human invertase was more sensitive to heat.

Sarin [1923] observed that the invertase from the gut of the honey-bee was inhibited by glycerol. Nelson and Cohn [1924] made a careful comparison between the invertase of honey and of yeast. They found certain differences in the kinetics of the reaction in relation to substrate concentration, but the optimum concentration of sucrose for both enzymes was about 4 %. The optimal range of p_H was 3.5–5.0 in the case of yeast and 5.5–6.3 in the case of honey invertase, the same relative change in p_H having a greater effect upon the latter enzyme.

The distribution of invertase in the alimentary tract of the cockroach has already been described.

Methods.

The activity of invertase has been measured by the same method as the saccharogenic activity of amylase, 3 % sucrose being used as substrate. The concentration of enzyme has usually been adjusted to give rise to about 0.25 % reducing sugar (measured as "glucose") in 1 hour.

Under these conditions the hydrolysis of sucrose has been shown to be practically a linear function of time (Fig. 5), and the activity of the enzyme may be regarded as directly proportional to the amount of reducing sugar produced at the end of half an hour. This relation obtains around the optimal p_H of the enzyme and on the alkaline side of this; but on the acid side (see

p_H 4.2 and 5.2 in Fig. 5) the activity soon begins to diminish as the result of irreversible destruction of the enzyme and the relative activity will apparently fall off as the experiment proceeds. In other words, in short experiments with an active preparation the optimal zone will extend further in the acid direction. Hence the importance of so adjusting the concentration of the enzymes to be compared that they act at approximately the same rate.

Yeast invertase was prepared by grinding up fresh yeast with 50 % glycerol and a little fine sand. Human invertase was prepared by extracting for a week, with 50 % glycerol, the mucous membrane removed from the jejunum and then filtering.

The various preparations were incubated for 15 hours with a 0.5 % solution of glucose. There was no change in reducing power. None of the preparations contained glycolytic enzymes.

Fig. 6.

Fig. 6. p_H -activity curves for invertase. A, Mid-gut invertase from *Blatella germanica*. B, Invertase from human jejunum. C, Invertase from yeast.

Fig. 7.

Fig. 7. p_H -activity curve for invertase from the salivary gland of *Blatella germanica*.

(i) *The effect of salts, heavy metals and glycerol.* Low concentrations of sodium chloride have no effect upon the activity of cockroach invertase. A concentration of 1 % has an inhibitory action (Table IV).

Table IV.

Invertase from mid-gut of <i>B. germanica</i> .	
NaCl concentration (g. %)	Relative activity
Nil	100
0.1	100
1.0	61

That glycerol when present in a concentration above 10 % has a strong inhibitory effect in spite of the increased quantity of enzyme present is shown in Table V.

Table V.

Glycerol extract of mid-gut of *P. americana*.

cc. of enzyme preparation	% glycerol	Relative activity
0.2	10	100
0.4	20	88

Cockroach invertase is extremely sensitive to silver nitrate. This is shown in Table VI.

Table VI.

Equal quantities of invertase (glycerol extract) from mid-gut of *B. germanica*.

Concentration of silver nitrate	Relative activity
Nil	100
0.0001 <i>N</i>	46.5
0.001 <i>N</i>	0

In all the above respects cockroach invertase behaves like yeast invertase.

(ii) *The effect of hydrogen ion concentration.* The methods employed were the same as those already described in connection with amylase. It was shown that at p_H 6.0 the activity was the same whether phosphate or phthalate buffer was used.

Fig. 6 shows p_H -activity curves for the invertase from yeast, from the cockroach and from the human jejunum. The curve for the cockroach enzyme is very similar to that obtained by Nelson and Cohn [1924] for honey invertase. The curves for yeast invertase and human invertase agree with those of Michaelis and Davidsohn [1911] and Euler and Svanberg [1921] respectively. It is clear that cockroach invertase is well adapted to work at the range of reaction (p_H 4.4–6.2) commonly found in the crop. As was noted by Nelson and Cohn in the case of honey invertase the zone of p_H over which it is active is more restricted than that of the other two enzymes.

At the end of this experiment the tubes were left in the water-bath for about an hour and then the p_H of the mixtures at 4.2 and 8.0 was adjusted to 5.8 by the addition of traces of 5 % caustic potash and of glacial acetic acid respectively (*vide infra* in connection with maltase). These mixtures were incubated for a further period of 30 minutes; and it was found that whereas the mixture previously at p_H 8.0 showed 100 % activity, that which had stood at p_H 4.2 was almost inactive, *i.e.* the enzyme is stable but inactive on the alkaline side of its optimal zone, while it is highly labile on the acid side—a behaviour identical with that of yeast invertase.

Fig. 7 shows the p_H -activity curve of invertase from the salivary gland of the German Cockroach. This curve falls off a little more rapidly in the acid direction but in other respects is the same as that described above.

MALTASE.

Maltase has not been so extensively studied in the past as invertase. The maltase of yeast was shown by Michaelis and Rona [1913] to have a narrow p_H range of 6.1–6.8 and to be very sensitive to changes in reaction outside this zone, whether in the acid or the alkaline direction, and to be in general

a far more labile enzyme than invertase. It is readily adsorbed by kaolin, while invertase is not, and is readily extracted from this adsorbate with secondary alkali phosphate [Willstätter and Kuhn, 1921]. Its action is inhibited by glycerol and by sodium chloride above 0.2 *N* concentration. The properties of the maltase of the succus entericus do not seem to have been closely studied.

In the cockroach, maltase occurs only in the secretion of the mid-gut.

Methods.

Quantitative measurements of maltase activity have been made by the method of Hagedorn and Jensen (as described above), using 0.25 % maltose as substrate. By adding measured quantities of a standard solution of glucose to measured quantities of this substrate, a graph was prepared from which the reducing power of the mixtures in g. glucose %, as calculated from the table of Hagedorn and Jensen, could be converted into "percentage hydrolysis" of the maltose substrate. Serial experiments were usually continued until about 60 % hydrolysis had occurred in the fastest tube. In experiments on the p_H range of the enzymes, the p_H has been plotted against this "percentage hydrolysis." When such a large fraction of the substrate is hydrolysed, this percentage can give only an approximate indication of the "relative velocity" of action.

The first attempts to demonstrate maltase in the glycerol extracts of cockroach organs were unsuccessful. It seemed probable that the enzyme had been adsorbed by the ground sand used in the preparation. The extract was therefore poured off and the sediment shaken for about 2 hours at 30° with a few cc. of phosphate buffer p_H 6.8. This extract was very rich in maltase. In the experiment given in Fig. 8, for example, the enzyme equivalent to one-hundredth of a cockroach was present in each mixture and this effected 60 % hydrolysis of 1 cc. of 0.25 % maltose in an hour. Much of this activity was lost in the course of about a week at room temperature. The experiments recorded below were made upon such an extract or upon a similar extract made from the "dry preparations" of tissues.

For experiments on yeast maltase, an aqueous extract of dried yeast was employed. In the case of human maltase, the same preparation as for invertase was used, but this was suitably diluted to obviate the inhibitory action of the glycerol.

Table VII.

Test mixture	Percentage hydrolysis after 1 hour
No added salt or glycerol	37.0
0.1 % sodium chloride	37.5
1.0 % sodium chloride	8.0
10 % glycerol	0.0

(i) *The effect of sodium chloride and glycerol on cockroach maltase.* This effect is shown in Table VII. The action of maltase is partially inhibited by

1 % sodium chloride and completely inhibited by 10 % glycerol. It is more susceptible to glycerol than is invertase. In this it resembles yeast maltase; and this fact affords a further reason for the failure to demonstrate maltase in the glycerol extracts of cockroach tissues.

(ii) *Effect of hydrogen ion concentration on cockroach maltase.* Since the extracts containing maltase were made with phosphate buffer, special methods had to be adopted to obtain the required p_H . The experimental mixtures were made up as follows: 0.1 cc. of enzyme preparation, 0.2 cc. of buffer at the required p_H and 0.2 cc. of distilled water. A duplicate series of mixtures was made up in uniform tubes as follows: 0.2 cc. of the same series of buffers as before, and 0.3 cc. of distilled water. Suitable and identical amounts of appropriate indicators were added to the two series of mixtures; and the mixtures containing enzyme were adjusted to the same p_H as the standards without altering their volume appreciably in the following manner. The ends of two capillary tubes were drawn out into hair-like capillaries and filled with 0.5 *N* HCl and 0.5 *N* NaOH respectively, to such a level that when dipped in liquid the reagent would flow gently out. The test mixtures were stirred cautiously with one or other of these capillaries until the colour matched that of the standard. 0.5 cc. of 0.5 % maltose was then added and the experiment continued as previously described.

Fig. 8. p_H -activity curves for maltase. A, From mid-gut of *Periplaneta americana*. B, From human jejunum. C, From yeast.

Fig. 8 shows the "percentage hydrolysis" of maltose by the three enzymes plotted against p_H . These curves give only an approximate indication of the relative activity at the different reactions. The curve for cockroach maltase (with an optimal zone from 5.0 to 6.2) falls off more rapidly than that for invertase in the acid direction, but it is active over the greater part of the p_H range found normally in the crop of the insect. The curve for yeast agrees

well with that obtained by Michaelis and Rona [1913]. Human maltase is active in the acid direction as far as is the cockroach enzyme, but it is much more resistant than the latter on the alkaline side.

LACTASE.

Relatively few comparative studies have been made upon lactase. The lactase of yeast is said to have its optimum p_H at 7.0.

The presence of lactase was demonstrated in extracts from the mid-gut of the cockroach, but not in those from salivary glands or crop. The formation of glucosazone and the increase in reducing power of a lactose solution, as measured by the method of Hagedorn and Jensen, were used as qualitative tests. The enzyme was obtained by extracting "dry preparations" of enzymes from the mid-gut with phosphate buffer at p_H 6.8. For the p_H -activity curve given in Fig. 9, the methods described under maltose were employed, both for the calculation of the percentage hydrolysis and for the adjustment of the p_H . The enzyme was far less active than the maltase in the same preparations. For example, in this experiment the enzyme equivalent of one-tenth of a cockroach was employed in each mixture and these were incubated for 15 hours. The range of p_H over which it is active is similar to that of the enzymes described above, the optimal zone being from 5.0 to 6.4.

Fig. 9. p_H -activity curve for lactase from mid-gut of *Periplaneta americana*.

This lactase was shown to be completely inhibited by 10 % glycerol.

Neither the yeast preparations nor those from the human jejunum contained lactase.

DISCUSSION.

Although, in the experiments here described, not all the known properties of the various enzymes have been compared, yet these experiments are sufficient to indicate the existence of a very close similarity between the

enzymes of the cockroach and those from yeast and from man. As was to be expected from the reaction of the crop contents of the cockroach, the enzymes of this insect are most active over an acid range from about p_H 5.0 to 6.5; and this range is similar for all the enzymes except amylase, in which it extends in the alkaline direction to p_H 7.0. It should be noted that the conditions under which this enzyme works are peculiar in that besides acting with the other enzymes in the crop, the saliva is expelled from the mouth of the insect and mixed with the food before it is ingested.

The results have been got from very small quantities of material; but the good agreement with the results of others, obtained when dealing on the same scale with the other enzymes, is evidence that they are reliable.

SUMMARY.

1. The salivary glands of the cockroach secrete an amylase and in *Blatella germanica* (though not in *Periplaneta americana*) an invertase. Extracts of the crop, when thoroughly freed from food contents, do not contain enzymes acting on carbohydrates. The stomach and hepatic caeca secrete invertase, maltase and small quantities of lactase.

2. The amylase of the cockroach is inactivated by dialysis and is affected by salts in the same way as is human ptyalin. The ratio of amylolytic to saccharogenic activity is similar to that of human ptyalin. The p_H -activity curves for these two enzymes have been compared. Under the same conditions cockroach amylase is more resistant to acid (optimum p_H 5.9). This is shown to be due in part at least to some associated factor which is not destroyed by heating at 100° for 5 minutes.

3. Cockroach invertase has been compared with yeast invertase and human jejunal invertase. It is partially inhibited by 1 % sodium chloride, 20 % glycerol and 0.0001 *N* silver nitrate; and completely inhibited by 0.001 *N* silver nitrate. The p_H -activity curve for cockroach invertase shows an optimal range from 5.0 to 6.2 which is intermediate between the ranges for yeast invertase and human jejunal invertase.

4. Cockroach maltase has been compared with the maltase of yeast and the human jejunum. It is partially inhibited by 1 % sodium chloride and completely inhibited by 10 % glycerol. It is adsorbed by powdered sand, but can be extracted from this with phosphate buffer p_H 6.8. The p_H -activity curve shows an optimal range from 5.0 to 6.2, which is more acid than the range in the maltase of either yeast or human jejunum.

5. Cockroach lactase is completely inhibited by 10 % glycerol and shows an optimal zone of p_H from 5.0 to 6.4.

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