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STUDIES ON THE UREASE ACTIVITY AND THE GLYCOLYTIC ACTIVITY OF ORAL MICROCOCCI

by

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It is well known that urea is decomposed by micro-organisms in saliva and in the dental plaque (*Hine* and *O'Donnell* 1943, *Kesel et al.* 1946, *Jenkins et al.* 1950, 1951, *Stephan* 1943, *Stephan* and *Miller* 1943, *Ballantyne et al.* 1951, *Clegg* and *Rae* 1956 and others). *Hine* and *O'Donell* found urease activity in the salivas of 92.8 per cent of 405 samples from 82 persons. Little, however, is known about the organisms responsible for this activity in the oral cavity. *Singer* (1951) isolated a micrococcus from saliva and showed that this organism was capable of splitting urea. He found that when it acted on glucose and urea simultaneously the pH of the medium gradually became more alkaline.

When studying a number of strains belonging to different types of oral micro-organisms, the present author found that urease activity was present in each of the six strains of oral micrococci investigated, but that in other types of organisms tested such activity was either absent altogether or too weak to be estimated by the methods used. *Jordan, Fitzgerald* and *Faber* (1956) found that of 109 strains of oral aciduric micrococci 76 per cent possessed the ability to split urea. Thus, oral micrococci may be of some importance for the urease activity of saliva and plaque material. Other organisms, however, may also be responsible.

The relative importance of oral micrococci for the urease activity found in plaque material can be estimated if a more precise knowledge of the urease activity of oral micrococci, the urease

activity of plaque material and the proportional distribution of micrococci in such material is acquired. The author has started a series of investigations in order to elucidate this problem.

It has been claimed that glucose is inhibitory to ammonia production in saliva (*Clegg* and *Rae*, 1956). There is evidence that high urea concentrations inhibit the urease activity of the oral flora (*Clegg* and *Rae*, 1956, *Jenkins* and *Wright*, 1951 and others) but detailed knowledge is lacking.

As early as 1899 *Burchard* quantitatively determined the ability of micrococci isolated from urine to decompose urea. He found that 180—1,200 mg of urea was decomposed by 1 mg of bacterial wet weight per hour. *Passmore* and *Yudkin* (1937) studied the effect of carbohydrates and allied substances on urease production by Proteus vulgaris. They used a titration method for the determination of urease activity and showed that the activity was directly proportional to the quantity of suspension present.

The purpose of the present investigation is,

(1) To determine the urease activity of oral micrococci *in vitro*.

(2) To study the influence on this activity of various factors, such as aerobiosis and anaerobiosis, the pH, the urea concentration, the concentration of phosphate buffer etc.

(3) To study the relationship between the urease activity and the glycolytic activity when the cells are acting on the two substances simultaneously.

METHODS

Preparation of suspensions

Six strains of oral micrococci were studied. These belonged to Staphylococcus [Staphylococcus albus (1—4, *Frostell*) and Staphylococcus aureus (5 and 6) or Micrococcus pyogenes var. albus and aureus]. The strains were typed according to the Manual of Bergey. The results of these investigations will be published later.

The strains were kept in culture on blood agar with weekly transfers. At the beginning of an experiment a tube with serum-dextrose broth was inoculated from the culture and incubated for 8 to 14 hours. The contents of this tube were transferred to

a centrifuge bottle containing 250 ml or 1,000 ml of either tomato-dextrose broth (*Frostell,* 1957) or Difco Brain-Heart Infusion. (Experiments and results, sections 4, 5, and 6). The volume of air in a flask was about 10 per cent of the volume of the broth. The bottles were closed with rubber stoppers. The bottles were incubated for 10 hours or 16 hours in a shaking machine. Suspensions were then prepared according to methods described earlier (*Frostell,* 1957).

The pH of the suspensions was controlled immediately after the last centrifuging, when the cells were suspended in a 10 mM phosphate solution. The pH of the suspension was set between 6 and 7 by adding, if necessary, minute amounts of 10 per cent hydrochloric acid or sodium hydroxide.

The wet weight was determined as described earlier (*Frostell,* 1957). In the present experiments the dry weight was determined as well. A few ml of the suspension were dried at 105° for 18 hours. The weight of the dried material was determined to the nearest tenth of a mg. A correction was made for the weight of the salts of the phosphate buffer.

Determination of activities

Urease activity

Three different methods with some modifications were used,

(1) The activity was determined as "alkali production" and calculated from the amount of acid required to keep the pH constant in a weakly buffered solution. It was expressed in moles (monovalent) per mg wet and/or dry weight per minute. The experiments were performed aerobically. This method was described earlier (*Frostell,* 1957). The values obtained by the method are considerably lower than those obtained if ammonia production is determined, because the ammonia is partly neutralized by carbonate formed during the hydrolysis of urea. Because of its simplicity, this method was preferably used when the author wanted to study the influence of various factors on the activity but considered it of minor interest to determine the urea decomposition quantitatively. In some of the experiments, however, the ammonia production was also determined.

(2) The experiments were performed aerobically or anaerobically and the activity was determined in moles of ammonia produced per mg wet and/or dry weight per minute.

In the first series, the experiments were performed in the following way,

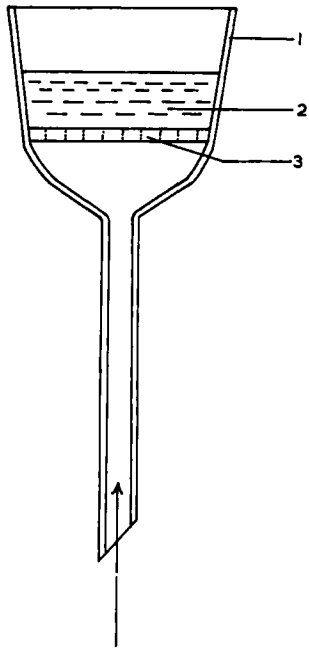

Fig. 1. Glass filter used in experiments performed according to method 2 A. Gas was driven through the filter as indicated by the arrow.

1. Opening of the filter was closed by a rubber stopper.
2. Bacterial suspension.
3. Filter proper.

(A) Into a sintered glass filter (Fig. 1) were pipetted 1.0 ml of suspension, 10.0 ml of a Ringer solution, 2.0 ml of a buffer solution (10 mM phosphate, pH 6.99) and 1.0 ml of a urea solution (2.0 gr/50.0 ml). The urea solution was added when the temperature of the mixture was 37.5°. Through the tube at the bottom of the filter a stream of N_2 was let through the solution. The gas had previously passed two cylinders with alkaline pyrogallic acid — each 60 cm of height — which were supposed to absorb traces of oxygen known to be present in the gas. It was shown by the Haldane method that the oxygen content of the gas was 0.17—0.30 volume per cent after the passage of the cylinders. The Ringer solution used in these experiments was

heated almost to boiling for about two minutes (and then cooled) immediately before use, in order to drive off oxygen. The opening of the filter was closed by a rubber stopper through which the electrodes of a pH-meter, a thermometer, two burettes (one for introducing alkali and one for introducing acid) and a gas outlet were inserted. The apparatus was placed in a thermostat chamber, the temperature of which was 38.0° ± 0.1° C. It was found that the temperature of the solution was about 0.5° C below the temperature of the thermostat chamber.

With these arrangements, experiments could be performed aerobically or anaerobically at constant temperature and constant pH even if there was a lively acid production or a strong alkali production in the solution. During the experiment acid or base could be added in varying amounts from burettes graded to one-hundredth of a ml through the tubes drawn through the rubber stopper.

At the end of an experiment a quantity of a 20 per cent $CuSO_4$ solution was added to stop the activity. The solution was transferred to a beaker and the concentration of ammonia in the solution was determined according to the method of *Ljungdahl* (1922), using a Parnas-Wagner apparatus. Either the whole of the solution or parts of it were used for the determination. In the latter case several determinations could be performed with one solution.

In some experiments samples were taken for determinations of the lactate concentration of the solution.

With these arrangements the alkali and ammonia production, as well as the acid and lactic acid production, could be determined during the same experiment, under aerobic or anaerobic conditions.

(B) Since it was found in the first series of experiments that the bacterial suspension often foamed out of the outlet tube when using the method just described, the arrangements were modified as follows,

In a beaker closed with a rubber stopper were pipetted 5.0 ml of a dilute phosphate buffer (10 mM) (or physiological saline), 1.0 ml of suspension and 1.0 ml of a urea and/or 1.0 ml of a glucose solution. The same electrodes, thermometer and tubes as

described under 2 A were drawn through the rubber stopper. The difference between these experiments and those described before was that — during experiments performed anaerobically — gas was let in *over* the surface of the solution in the beaker through the outlet tube. The pH was kept constant by the addition of alkali or acid from burettes graded to 0.01 ml.

(3). In some experiments the ammonia production was determined by aeration and titration and calculated as moles of ammonia produced per minute by 1.0 mg of dry or wet weight. This method was used only a few times, when several experiments were to be performed simultaneously with one suspension.

In a tube of Pyrex glass were pipetted 8.0 ml of a 0.2 M phosphate buffer solution, 1.0 ml of bacterial suspension and 1.0 ml of a urea solution (6.25 g/50 ml). The tube was kept in a machine under continuous shaking in a waterbath, the temperature of which was 38.0° ± 0.1. The experiment was allowed to go on for a certain length of time, after which it was stopped by adding 1.0 ml of a 20 per cent $CuSO_4$ solution. The solution was made alkaline by the addition of potassium hydroxide, and air — which had previously passed through a tube containing 10 ml of a 0.1 N HCl solution in order to be free from traces of ammonia — was sucked through the tube for 40 minutes into a tube containing 10.0 ml of a 0.01 N HCl solution. The solution was titrated against sodium hydroxide and the ammonia production was determined as the difference between alkali consumption by test tube and a control containing 10.0 ml of 0.01 N HCl. Five or six tubes could be used simultaneously.

This method was used because several tests could be performed simultaneously. The experiments were performed aerobically and the buffer concentration was heavy so as to allow only minute changes of the pH during the experiment. This method often failed in the hands of the author.

In some of the experiments of this type the ammonia determination was carried out according to the method of *Ljungdahl* (1922). Aliquots of 1.0 ml were taken to the Parnas-Wagner apparatus for ammonia determination and two or more determinations of ammonia concentration were performed. The results obtained by this method were more accurate than those obtained by aeration of the ammonia.

Glycolytic activity

This activity was determined either as "acid production", i.e. the total amount of "acid" produced, or as "lactic acid production". This acid was expressed in moles per minute per mg wet or dry weight and was calculated either from the amounts of alkali required to keep the pH constant in a weakly buffered solution during glycolysis or from the difference in the lactate concentrations of the solution determined before and after the experiment.

Lactate concentration was determined according to the method of *Barker* and *Sumnerson* reported by *Umbreit et. al.* (1945). Before and after every experiment, samples were taken *in triplo* (0.2 ml) and transferred to tubes containing $CuSO_4$.

Errors of the methods

Ammonia determination

Ten determinations of the same ammonium hydroxide solution were performed with the Parnas-Wagner apparatus. The error of the method was determined according to the formula

$$s = \pm \sqrt{\frac{\Sigma(X-M)^2}{n-1}} \quad \ldots\ldots (1)$$

The error was determined at ± 2.15 per cent (variation coefficient). However, this figure expresses only one of many possible errors. The author has performed 12 identical experiments with a suspension of Staphylococcus albus (1) according to method 2 B (gas let in over the surface). The error of the method of determination of "alkali production" was ± 4.9 per cent (variation coefficient). The error of the determination of the ammonia production was ± 3.6 per cent (variation coefficient). The errors of the determinations of wet or dry weights are not included in these figures.

The error of the determination of alkali or acid production from the amounts of acid or base required to keep the pH constant was also determined from a series of double experiments

performed during this and other investigations. The formula used was

$$s = \pm \sqrt{\frac{\Sigma (X_p - M_p)^2}{n - k}} \quad \ldots\ldots (2)$$

The error was determined at 6.7 per cent (variation coefficient).

Lactate determination

During this investigation all lactate determinations were performed *in triplo*. Thus it is possible to calculate the error of the method very accurately from the figures of the investigation itself according to formula 2. In a few instances one of the values obtained was considerably higher than the other two. These values were considered to be due to contamination and were excluded. They are not incorporated in the error of the method. The error of the lactate determination method has been calculated at 5.0 per cent (error of the mean of 3 determinations $= \frac{s}{\sqrt{3}}$).

Chemicals

All chemicals used in this investigation were analytically pure. The acids and bases used were prepared from concentrated standard samples (with one exception) according to the directions given by the manufacturers. Thus they have never been further diluted. As standards for the production of acid and ammonia, 0.100 and 0.0100 N solutions of HCl (Titrisol) served throughout this investigation.

EXPERIMENTS AND RESULTS

1. Anaerobiosis and alkali production from urea by Staphylococcus albus

Four experiments were performed with different suspensions of Staphylococcus albus (1). The arrangements described under method 2 A (gas driven through the solution) were used. The pH was kept constant at pH 6.5 ± 0.2 by the continuous addition of

small amounts of 0.1 N HCl. Nitrogen gas was driven through the solution for 15—30 minutes. The nitrogen was then replaced by air for 15—30 minutes and lastly nitrogen was again used. Fig. 2, is representative of the results obtained. No influence on alkali production from urea by changes of oxygen tension was found.

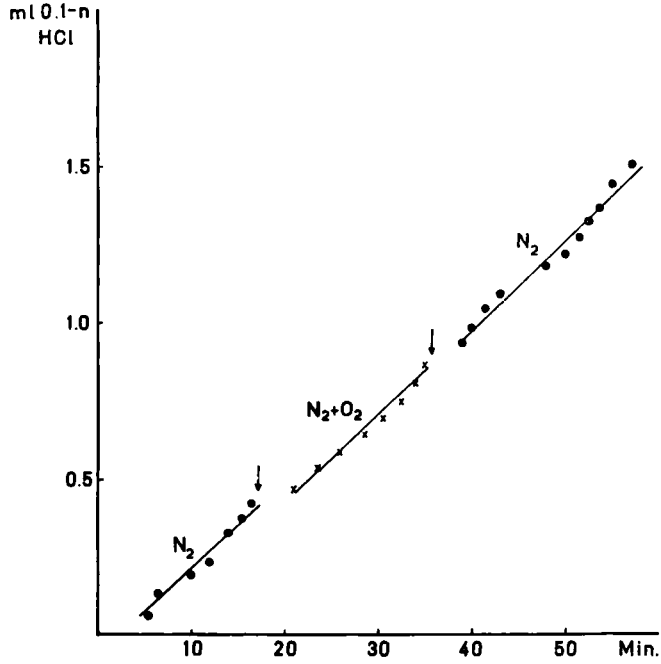

Fig. 2. Alkali production from urea by a suspension of Staphylococcus under anaerobic and aerobic conditions.

2. Localization of the enzyme

Two experiments were performed aerobically according to method 1 with different suspensions of Staphylococcus albus (1). The cells of the suspensions were centrifuged for 5 minutes at 2,000 r.p.m. and for 10 minutes at 4,000 r.p.m. The supernatant fluid was removed and the cells were suspended to the original volume. Experiments were performed with the supernatant fluid and the suspension separately at pH 6.5.

A representative result is presented in Fig. 3. The urease ac-

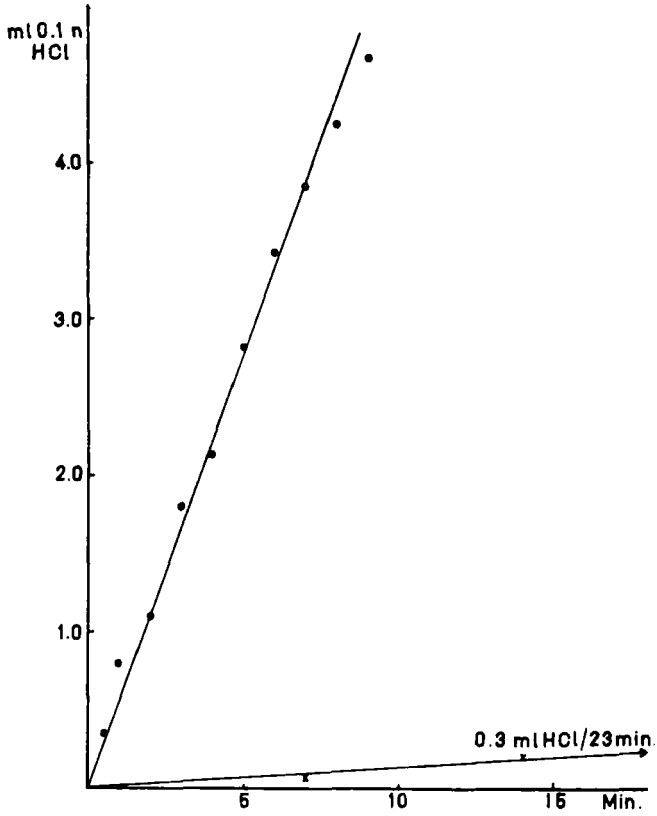

Fig. 3. Alkali production from urea by a suspension of Staphylococcus. Location of the enzyme.
.——.——.——. The cells centrifuged and resuspended.
+——+——+—— The supernatant solution.

tivity appeared to be confined to the cells. The low activity obtained with the supernatant fluid is considered to be due to a low concentration of cells in the supernatant fluid.

3. Effect of pH on the urease activity of Staphylococci

Changes of pH in suspensions containing urea

In order to show the changes of pH which occurred in a suspension of staphylococcus when acting on urea, a few pilot experiments were made. To a bacterial suspension prepared in the

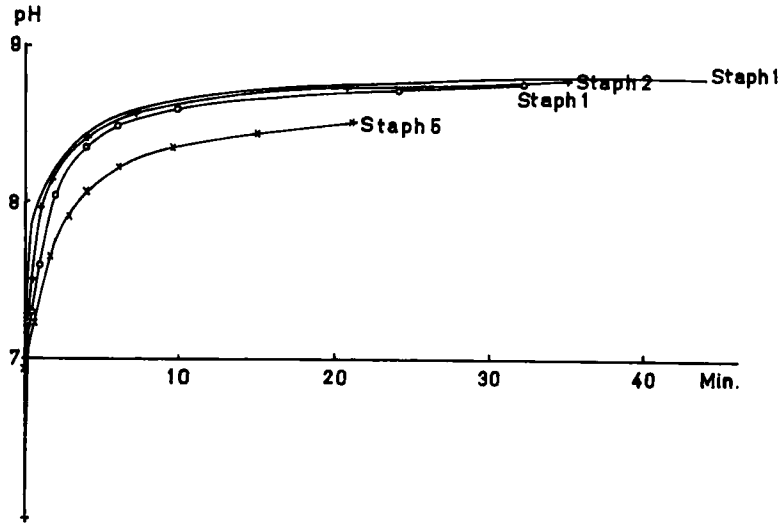

Fig. 4. Alkali production from urea by suspensions of oral staphylococci. Changes of pH of suspensions after addition of urea.

usual manner, the temperature of which was 37.5°, ± 0.2, a small amount of urea was added in dry substance, the suspension being stirred by a machine-driven glass stirrer. The pH was read at short intervals. No acid was added. The curves obtained are shown in Fig. 4. During the first minutes a rapid pH increase occurred. When the suspension became more alkaline the pH changed more slowly. Since the buffer capacity of the suspensions was weak this indicated that the activity was very low at pH values over 8.5.

Determination of optimum pH for urease activity

Method 2 A (gas driven through the solution)

The experiments were performed anaerobically with a suspension of Staphylococcus albus (3) which was 24 hours old. The pH of the mixture of suspension, phosphate buffer and urea was set at pH 6.5 and 5.5 respectively. The pH was kept constant through the addition of 0.1 N HCl. (Fig. 5.)

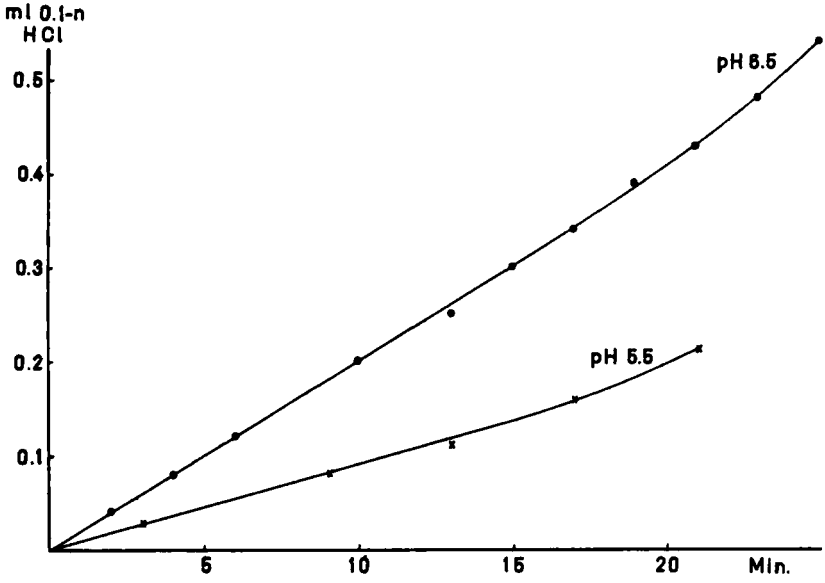

Fig. 5. Alkali production from urea by a suspension of Staphylococcus at different pH.

Method 3

One experiment was performed with Staphylococcus albus (1) and four with Staphylococcus aureus (5). Five or six tubes were used simultaneously in each experiment. The tubes contained 1.0 ml suspension, 8.0 ml of a buffer solution (phosphate buffer, Na_2HPO_4, KH_2PO_4, final PO_4-concentration 0.16 M) and 1.0 ml of a urea solution (6.25 g/50 ml). The pH of the different tubes had been set at various values between 5 and 9. The addition of an equal amount of alkali as produced during the experiment to buffers of the same strength caused pH-differences of a few tenth of a unit; in the range pH 5.0 to 6.0 the change was slightly greater. Ammonia production was allowed to go on for periods varying between ten and twenty minutes, the time, however, being the same for all tubes in one experiment. Alkali production was stopped by the addition of 1.0 ml of 20 per cent solution of $CuSO_4$. Ammonia was aerated into 0.01 N HCl.

A further experiment was performed with a suspension of Staphylococcus albus (1). Five tubes were used. Every tube con-

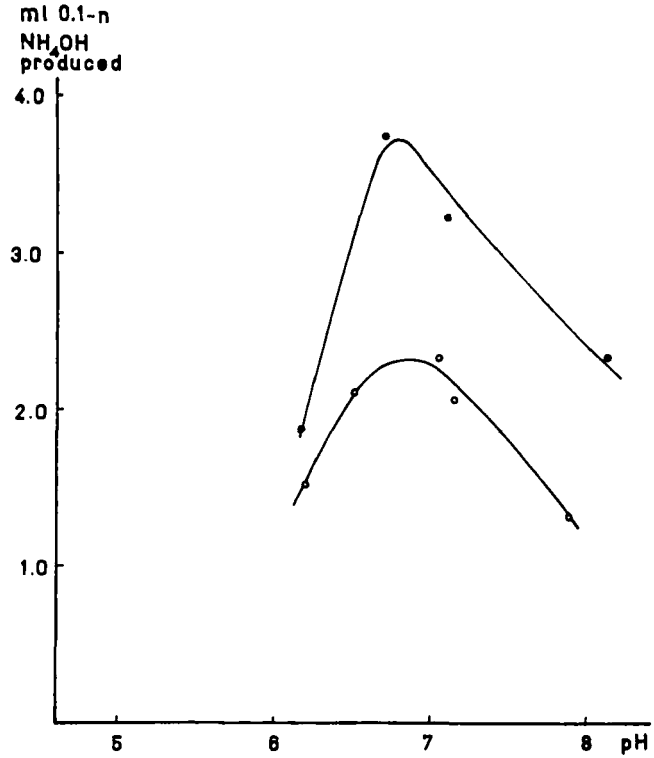

Fig. 6. Influence of pH on the urea decomposition by oral Staphylococci. NH_3 produced = ml of 0.10-n HCl required to neutralize the amount of ammonia produced by 1 ml of suspension during the experimental period.

tained 1.0 ml of suspension, 8.0 ml of a buffer solution (Na_2HPO_4 0.1 M and KH_2PO_4 0.1 M, final concentration 0.16 M) and 1.0 ml of a urea solution (6.25 g/50 ml). Ammonia production was allowed to go on for 15 minutes and was then stopped by the addition of 1.0 ml of a 20 per cent $CuSO_4$ solution. Amounts of 1.0 ml were taken to the Parnas-Wagner apparatus and two or more determinations were performed from every tube.

The results of all these experiments were consistent. Representative curves are given in Fig. 6. Optimum activity was found very near to pH 7. Apparently the top of the curve is to be found on the acid side of neutrality. The activity fell rapidly on both sides of the optimum pH.

4. Phosphate concentration and urease activity

Two experiments were performed with suspensions of Staphylococcus albus (1) in the stable phase. Method 1 was used, and alkali and ammonia production were determined. The experiments were performed aerobically, i.e. no gas was let in.

1.0 ml of bacterial suspension, 8.0 ml of buffer (final concentration either 0.16 M or 0.05 M) and 1.0 ml of a urea solution (6.25 g/50 ml) were used in every experiment. After about 10 minutes the experiment was stopped by adding 1.0 ml of a 20 per cent $CuSO_4$ solution. Two experiments of this kind were thus performed with every suspension, viz. one experiment with a low phosphate buffer concentration and one with a high buffer concentration. After this, a third experiment was performed with a high urea concentration. The results of the urea experiments will be presented in section 5 below.

No influence of phosphate buffer concentration was found. (Fig. 8). The difference in ammonia production velocity between experiments with different phosphate concentrations was less than 5 per cent; in one experiment the higher value was connected with a high buffer concentration, in the other experiments with a low buffer concentration.

5. Effect of urea concentration

Method 1

Four experiments were performed. Urea concentrations were set at 0.017, 0.084, 0.17, and 0.33 M (at the beginning of the experiment). Alkali production was allowed to go on for 10—20 minutes at pH 6.5.

Method 3

(A) A suspension of Staphylococcus albus (1) which was 24 hours old was used. Urea concentrations in the different tubes were set at 1.7, 3.2, 5.3, 8.9, and 10.04 M. Ammonia production was allowed to go on for 15 minutes at pH 6.96, after which it was stopped by the addition of $CuSO_4$. The solutions were made alkaline and air was driven through the tubes for 40 minutes, the ammonia being absorbed by 0.01 N HCl.

(B) In another experiment a suspension of Staphylococcus albus (1) which was 30 hours old was used. Five tubes were used and the urea concentrations were set at 0.37, 1.48, 2.96, 4.44, and 5.92 M. The ammonia production was kept going for 15 minutes. Samples of 1.0 ml were taken to the Parnas-Wagner apparatus, 2—5 samples from every tube. Since it was found that urea was destroyed in the apparatus by the heat when the concentrated urea-mixture was boiled, giving a continuous flow of ammonia by distillation, several determinations were made of the quantity of ammonia produced by urea alone at a distillation time of exactly 5 minutes with the different concentrations. The variations of these results were about ± 10 per cent. The quantities of ammonia produced by urea alone in the different concentrations were subtracted from the values obtained with the different test tubes, the differences being regarded as produced by the bacteria.

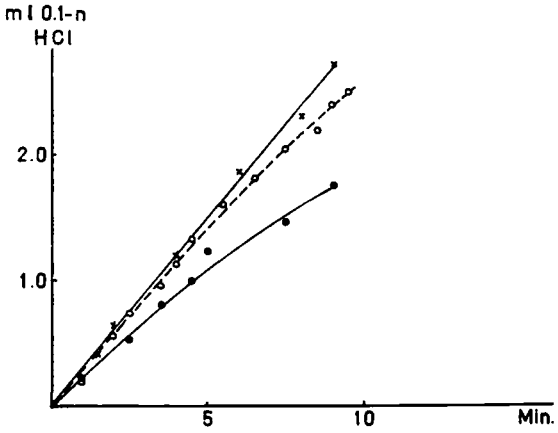

Fig. 7. Alkali production from urea by Staphylococcus. Influence of phosphate and urea on the urea decomposition by a suspension of Staphylococcus. A concentration of 5 M urea partially inhibits urea decomposition.

+——+——+—— 0.16 M phosphate buffer, 0.21 M urea.
°— —°— —°— — 0.01 M phosphate buffer, 0.21 M urea.
.——.——.——.—— 5 M urea. Phosphate concentration 0.01 M.

The results of the experiments are given in Figs. 7 and 8. Maximum activity was obtained at concentrations ranging from about 0.2 M to about 3 M. Even at concentrations of 5 M and more there was still some activity. For reasons given above the

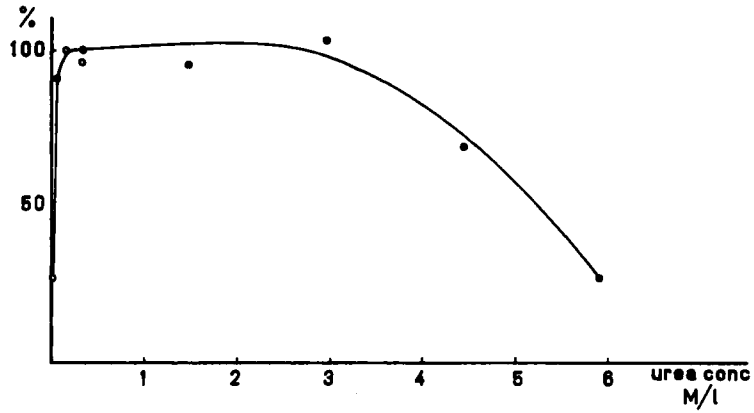

Fig. 8. Influence of urea concentration on the alkali production from urea by a suspension of Staphylococcus.
° Method 1, pH 6.50.
. Method 3(B), pH 6.96.

values of ammonia production in tubes containing concentrated urea must be considered approximate. The lower limit of maximum activity is to be found at a urea concentration of about 0.16 M. The results obtained with method 3 (A) showed 6 per cent activity at 8.9 M but no activity at 10 M.

Fig. 7 illustrates 2 experiments performed with method 1 [see under (4)] in which the urea concentration was set at 5 M. It will be seen that the activity gradually decreased, indicating that the cells slowly poisoned by the high urea concentration.

6. Influence of ammonium concentration

Two series of experiments were performed with Staphylococcus albus (1). The suspensions were 24—26 hours old (stable phase). Method 1 was used. The experiments were performed aerobically (no gas was let in) at pH 6.5. In the first series ammonium concentrations were 0.0, 0.2, and 0.63 M at the beginning of alkali production. In the second series the concentrations were 0.0, 0.17, 0.67, 1.8, and 0.0 M. In these experiments the volume of the solution varied between 10 and 23.5 ml. It was found that changes of the volume within these ranges did not significantly influence the results.

A third series of experiments was performed with Staphylococcus aureus (5). NH_4 concentrations were set at 0.0, 0.33, 0.55, and 1.4 M by adding NH_4Cl. The volumes of this series varied between 6.5 and 8.5 ml.

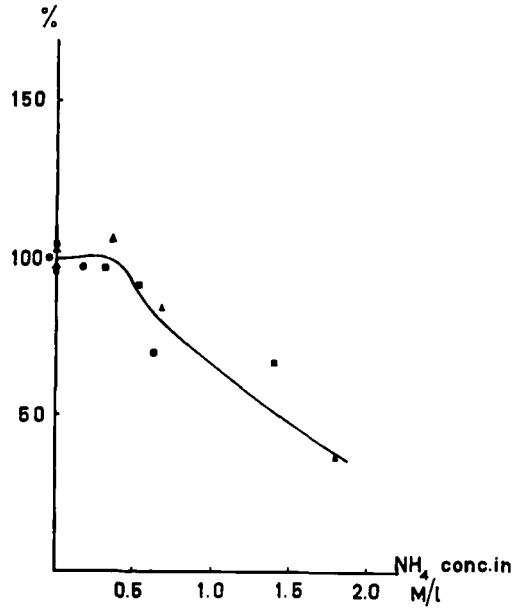

Fig. 9. Influence of ammonium ion concentration on the alkali production from urea by suspensions of Staphylococci.

The results of all these experiments are given in Fig. 9. At concentrations higher than 0.5 M a decrease in alkali production was found.

7. Effect of length of incubation period on urease activity

The effect of this factor was not studied in detail, but it was found that in a series of experiments in which the incubation period was about 16 hours the mean activity of 6 suspensions of Staphylococcus albus (1) was $44.7 \cdot 10^{-9}$ moles of alkali per minute per mg wet weight.

In another series of experiments with the same strain the incubation period was about 10 hours. The mean urease activity in the absence of glucose was $365 \cdot 10^{-9}$ moles of alkali per minute per mg wet weight in this series. The same substrate (tomato-dextrose broth) was used in the two series. Thus, with

an incubation period of 10 hours, the mean activity was about 8 times as great as with the longer incubation period. The weight of the bacterial mass obtained from 1 litre of broth was many times greater in the first series than in the second. The differences in bacterial masses and urease activities indicate that the cells have passed the logarithmic growth phase, when the incubation period is as long as 16 hours.

INFLUENCE OF UREA HYDROLYSIS AND GLYCOLYSIS ON EACH OTHER

When urease activity is determined at constant pH according to method 1, and the amounts of acid required to keep the pH constant are plotted against time, the curve obtained under appropriate experimental conditions will be a straight line. If, on

Fig. 10. Alkali production from urea by a suspension of Staphylococcus in the absence and in the presence glucose.

the addition of glucose, the acid production influences alkali production, the curve will be changed and take a new direction, being either a straight line if the sum activity is constant or a curved line if the influence manifests itself gradually.

A suspension of Staphylococcus albus (1) which was 48 hours old (stable phase) (*Frostell*, 1957) was used. An experiment was performed according to method 1. After 7 minutes glucose was added (dry substance, about 25 mg) and alkali production was allowed to go on for another 7 minutes. (Fig. 10). The effect of the presence of glucose on alkali production from urea, if any, was very slight. Thus either the acid production from glucose was only some per cent of the alkali production from urea or else acid production was almost completely inhibited. Another possible explanation is that alkali production from urea is stimulated in the presence of glucose, this stimulation being masked by a possible acid production from glucose.

In order to study the relationship between glycolysis and urea hydrolysis, if any, the following two series of experiments were performed,

(1). Suspensions of Staphylococcus albus (1) were used. Incubation time was about 16 hours. The experiments were performed according to method 2 and, in most cases, as soon as possible after that the suspensions were prepared. Thus with few exceptions the experiments were performed in the labile phase (*Frostell*, 1957). Table 1.

To the glass filter were applied: 10.0 ml of a Ringer solution, 2.0 ml of a buffered Ringer solution, 1.0 ml of bacterial suspension and, when the temperature was 37.0° C, 1.0 ml of a urea solution (2.4 g/50 ml Aq. dest.).

The pH was kept at 6.5 ± 0.2 through the addition of 0.0263 N HCl. Alkali production was allowed to go on for about 15 minutes. The electrodes were removed and the activity was stopped by the addition of 5.0 ml 0.1 N HCl and 0.2 ml 20 per cent $CuSO_4$. The solution was transferred *in toto* to a beaker, the electrodes being thoroughly rinsed with distilled water.

Immediately after the electrodes were rinsed, a new glass filter was inserted in the apparatus and the experiment was repeated with the sole difference that now 1.0 ml of a solution of glucose

Table 1. Urease activity of Staphylococcus albus (1) in the absence and in the presence of glucose expressed in 10^{-9} M per minute per mg wet weight or dry weight.

Experiment No.	ww/ml	dw/ml	Without glucose				With glucose				Comments
			Alk.		NH_3		Alk.		NH_3		
			ww	dw	ww	dw	ww	dw	ww	dw	
1 a		17.2		298		388		306		305	labile phase
b				139		181		312		*	stable phase
2	191	19.4	36	345	39	378	50	483	50	481	labile phase
***				M:321.5		M:383		M:394.5		M:393	
3	320		37		45		39		46		—»—
4 a	280		22		22		11**		12**		—»—
b			17		19						—»—
c			12		13						—»—
5 a	403		25		27		24		27		—»—
b			20		22		19		28		—»—
c			18		23		20		25		—»—
***			M:30.0		M:33.2		M:31.0		M:33.7		
6 a	342		48				48				labile phase
b			49				48				—»—
c			46				49				stable phase
7 a	308		100				104				—»—
b			106				97				—»—
Mean (of first value obtained)			44.7				46.0				

Alk = alkali production
NH_3 = ammonia production
ww = per mg wet weight
dw = per mg dry weight
ww/ml = wet weight per ml
dw/ml = dry weight per ml

* Ammonia determination failed.

** This experiment was performed after exp. 5 c, i.e. late in the labile phase.

*** Means given within the table refer to groups of experiments in which either the activity per mg dry weight or the ammonia production was determined.

(1.25 g/50 ml Aq. dest.) was added. Thus the cells acted on urea and glucose simultaneously.

After about 15 minutes the experiment was stopped in the same way as the first experiment and the contents of the filter were transferred to another beaker.

In a third experiment, 1.0 ml of the suspension acted on glucose only, the proportions of the other constituents being the same, but no urea was added. The ammonia content of the two beakers was determined according to the method of *Ljungdahl* (1922). The whole content of a beaker was used for one determination. 0.1 N HCl was used for the absorption of the ammonia distilled.

Alkali production per minute per mg wet weight and/or dry weight was determined from the amount of acid required to keep the pH at 6.5 ± 0.2. Ammonia production was calculated from the figures obtained at the determination of the ammonia content of the beakers.

Several experiments were performed with some of the suspensions; in these experiments the time was noted at which the different experiments started, making it possible to study the effect of instability of the suspensions. The results of this series of experiments are given in Table 1.

The results lend no definite support to the opinion that alkali or ammonia production from urea is generally influenced by the presence of glucose. In experiments Nos. 2 and 3, however, alkali and ammonia production was considerably higher in the presence of glucose.

The results of the determination of the acid production from glucose revealed that this production was very low under the conditions of the experiments. The mean activity of 3 experiments was 2.6×10^{-9} M per mg wet weight per minute, which amounts to 8.3 per cent of the alkali production of the same suspensions. In the other experiments the acid production was too low to be determined by the method used.

(2). Suspensions of Staphylococcus albus (1—4) and aureus (5—6) were used. The experiments were performed according to method 2 B (gas let in over the surface). The incubation period was 10 hours. The suspensions were 24—48 hours old and thus the tests were made in the stable phase (*Frostell,* 1957).

Three different experiments were performed with every suspension: —

(A). Into the glass container were poured 5.0 ml of physiological saline and 1.0 ml of bacterial suspension. The saline solution was heated almost to boiling before use in order to drive off oxygen and was put into a refrigerator until the temperature of the solution was less than 37°. The container was placed in the apparatus, the temperature of the waterbath being 38.0° ± 0.1. Nitrogen gas was let in over the surface for at least 10 minutes, after which the temperature of the solution was 37.5° ± 0.3. Then 1.0 ml of a glucose solution was added through the gas outlet tube and the acid production was allowed to go on for 10—15 minutes, the pH being kept constant at 6.5 ± 0.2 by addition of 0.01 N NaOH. The activity was stopped by the addition of 0.2 ml of a 20 per cent $CuSO_4$ solution. 0.2 ml was then taken *in triplo* for lactic acid determination.

(B). Into a glass container were poured 5.0 ml of a 10 mM phosphate solution and 1.0 ml of bacterial suspension. Before use the phosphate solution was treated in the same way as the saline solution in experiment A. Nitrogen gas was let in over the surface for 10 minutes, after which 1.0 ml of a urea solution (2.4 g/50 ml) was added. Alkali production was allowed to go on for 10—15 minutes and the pH was kept constant at 6.5 ± 0.2 by the addition of 0.1 N HCl. The experiment was stopped by the addition of 0.2 ml of the same $CuSO_4$ solution. The whole content of the glass container was then transferred to the Parnas-Wagner apparatus for ammonia determination.

(C). Phosphate solution and bacterial suspension were added to a third glass container in the way described under B. Nitrogen gas was let in over the surface and after ten minutes 1.0 ml of glucose solution and 1.0 ml of urea solution were added. As the acid production in these experiments was low, and the alkali production was heavy, the pH was kept constant by addition of 0.1 N HCl. The experiment was allowed to go on for 10—15 minutes and was then stopped by addition of 0.2 ml of a $CuSO_4$ solution. Samples were taken *in triplo* for lactic acid determination and the rest of the contents of the glass container was used for ammonia determination.

Lactic acid concentration before the start of the experiment

was determined by diluting 1.0 ml of bacterial suspension with 5.0 ml of physiological saline and taking 0.2 ml of this suspension *in triplo* to tubes containing $CuSO_4$.

Thus lactic acid production and ammonia production were determined under different experimental conditions, viz. (A). When the cells were acting on glucose alone, (B). when the cells were acting on urea alone, and (C). when the cells were acting on glucose and urea simultaneously.

Ammonia determination was performed as soon as possible immediately after experiment C. Seven such determinations were performed, in the following order: on 2 controls of distilled water, on 1.0 ml of urea solution, on the whole contents of the glass containers A, B, and C and, lastly, on 1 control of distilled water.

The container A never gave ammonia. The urea solution gave a small amount of ammonia in some experiments (a few per cent of total ammonia content). In these cases corrections were made for ammonia values obtained for containers B and C.

Experiment A was performed with the cells suspended in saline solution, since it was impossible to determine the acid production from the amount of alkali required to keep the pH constant if a more heavily buffered solution was used, owing to the low activity compared with urease activity. It was found that the difference in buffer concentration between experiments A and B and C did not influence the lactic acid production.

"Acid production", as defined above, under "Determination of Activities", was calculated from the amounts of alkali required to keep the pH constant. Lactic acid production was calculated from the difference between the initial and the end concentrations of lactate and the volumes. "Alkali production" was calculated from the amount of acid required to keep the pH constant and ammonia production activity from ammonia produced. For experiment C a correction was made for the amount of solution used for lactic acid determination (0.6 ml). All values were calculated in moles per minute per mg wet and dry weight.

Double experiments were made with some suspensions.

The results are given in Tables II and III. If the figures given in Table II are examined (alkali production calculated from the amount of acid required to keep the pH constant) it will be seen

Table 2. Urease activity of Micrococci in the absence and in the presence of glucose expressed in 10—9 M per minute per mg wet weight and dry weight.

Strain	ww/ml	dw/ml	Without glucose				With glucose			
			Alk.		NH_3		Alk.		NH_3	
			ww	dw	ww	dw	ww	dw	ww	dw
Staph. alb. (1)										
a)	98	22.2	423	1,870	611	2,700	630 (652)	2,750 (2,850	721	3,190
b)	—	—	444	1,950	—	—	582 (604)	2,560 (2,700	—	—
Staph. alb. (1)	90	20.8	241	1,100	244	1,060	229 (—)	990 (—)	*	*
Staph. alb. (2)	111	20.8	217	1,160	400	2,160	212 (230)	1,130 (1,230	438 —	2,310**
Staph. alb. (3)	123	22.1	292	1,630	400	2,200	259 (264)	1,440 (1,470)	348	1,950**
Staph. alb. (4)	146	32.7	370	1,650	472	2,130	378 (391)	1,690 (1,750	527	2,350
Staph. alb. (4)	104	—	135	—	152	—	120	—	126	—
Staph. aur. (5)	76	16.0	147	700	170	806	118 (133	560 (630)	157	746
Staph. aur. (6)										
a)	61	16.3	187	699	218	815	197 (222)	737 (831)	262	980
b)	—	—	189	707	232	868	—	—	—	—
Mean (of first value obtained)			251	1,258	346	1,801	268	1,328	368	1,921

Alk. = alkali production determined in moles required to keep pH constant.
NH_3 = production of ammonia
ww = wet weight in mg
dw = dry weight in mg
ww/ml = bacterial wet weight per ml of suspension
dw/ml = bacterial dry weight per ml of suspension

* Ammonia determination failed
** No correction for urea decomposition (error estimated at 2 per cent)

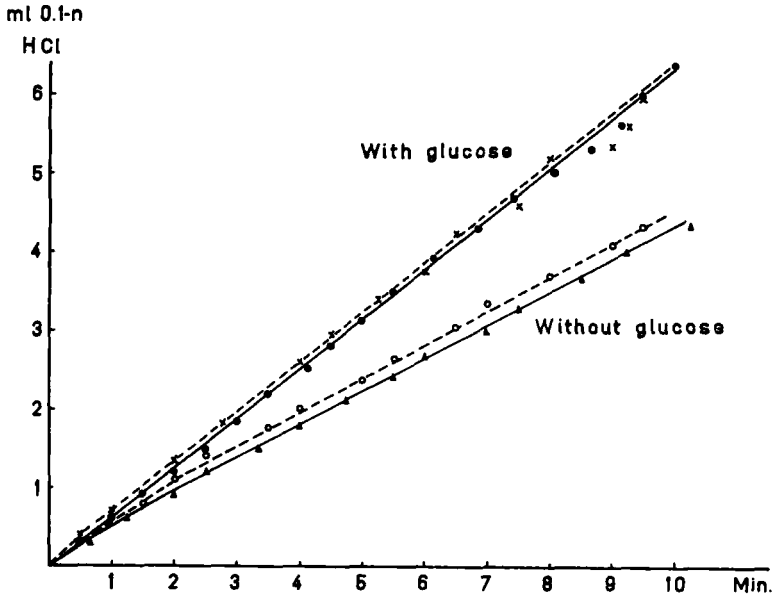

Fig. 11. Alkali production from urea by a suspension of Staphylococcus. Stimulation of activity in the presence of glucose. The experiments with and without glucose were performed alternately.

that in most of the experiments the difference in alkali production between experiments with and without glucose is very small. However, in one of the experiments with Staphylococcus albus (1) it is obvious that alkali production is stimulated in the presence of glucose. This fact is clearly seen, since two series of experiments were performed alternately with the same suspensions on the same occasion. It must be remembered that these figures are calculated from curves constructed from about 20 different readings (Fig. 11. Table II). Figures in brackets express alkali production calculated with regard to the acid production in experiments A.

Lactic acid production from glucose was generally lower in the presence of urea than when the cells acted on glucose only. The inhibition, however, was far from complete, and in one experiment lactate production appeared to be higher in the presence of glucose. Even in some pilot experiments no inhibition was found.

Table 3. Acid production from glucose by Micrococci in the presence and in the absence of urea expressed in 10^{-9} M per minute per mg wet weight and dry weight.

Strain	ww/ml	dw/ml	Without urea				With urea	
			Total		Lactate		Lactate	
			ww	dw	ww	dw	ww	dw
Staph. alb. (1)								
a)	98	22.2	21.6	95.3	12.8	56.7	10.1	44.5
							9.6	42.5
b)* ..								
Staph. alb. (2)	111	20.8	18.4	98.0	18.1	97.0	11.2	59.8
							11.6	61.9
Staph. alb. (3)	123	22.1	4.6	25.6	4.1	22.8	3.1	17.3
Staph. alb. (4)	146	32.7	13.2	59.0	7.1	31.6	12.5	55.8
Staph. alb. (4)	104	—	1.99	—	1.70	—	1.46	—
Staph. aur. (5)	76	16.0	14.5	68.9	11.5	54.6	11.1	52.7
Staph. aur. (6)	61	16.3	25.0	93.5	18.9	70.8	16.6	62.1
Mean (of first value obtained)			13.9	73.4	10.6	55.6	9.4	48.7

ww = wet weight
dw = dry weight
Total = Total acid production as calculated from the amount of alkali required to keep the pH constant.
Lactate = Lactate production.

* lactate determination failed.

The acid production in moles produced from glucose per mg wet or dry weight amounted to only 5.5 per cent of alkali production from urea by the same suspensions expressed in a similar way (mean of 7 experiments).

DISCUSSION

Different methods were used for the determination of the urease activity because different methods proved to be suitable for different purposes. Methods 2 A and B were used in most cases and proved to be the best in the hands of the author. Method 2 A (gas driven through the solution) was used by preference in experiments in which it was desired to change the gas pressure of the test solution rapidly, for example, when changing

from aerobic to anaerobic conditions. However, if the nitrogen gas contains even traces of oxygen this method is not suitable for anaerobic experiments for obvious reasons. Since it is known that the method used by the author to free the gas from oxygen is not 100 per cent effective, "anaerobic" in this paper means "having very low oxygen tension".

The activity was stopped by the addition of 20 per cent $CuSO_4$. Different amounts of the solution were used in the different methods. This was due to the fact that when the copper sulphate concentration was high there was a foaming of the solution in the Parnas-Wagner apparatus, whereby alkali went over to the acid and destroyed the experiment. Instead the pH was lowered to 1.5 by adding 0.1 N HCl in all experiments when the low copper sulphate addition was used (0.2 ml). (This was also performed in order to prevent evaporation of NH_3.) However, urease is very sensitive to copper sulphate and the concentration obtained in these experiments (1/1500 weight/volume) is more than sufficient to stop the activity immediately.

The ammonia determinations were always performed immediately after the alkali production experiments, because it was found that ammonia sometimes "disappeared" slowly from the solution in spite of the fact that the pH was about 1.5. It was thought that this was due to formation of organic or inorganic nitrogen compounds from NH_3 which at the subsequent determination of the ammonia content did not give off ammonia.

When determining the error of method 1 the author used a suspension with low activity per volyme because the suspension had to be diluted so that many experiments could be performed. When the activity is low the pH changes rather slowly unless the buffer capacity is very low and it will then be difficult to determine the moment when the experiment should be stopped. When the activity is lively and the buffer capacity is in correspondense with it (i.e. the change of pH is about 0.2 per minute), the error of the method may be kept lower.

The meaning of the expressions "acid production" and "alkali production" must be clearly understood. When urea is decomposed in the presence of phosphate buffer, ammonia and carbon dioxide are formed (*Sumner,* 1951). At pH-values below neutrality the ammonia is retained as ammonium ion in the solu-

tion and the carbon dioxide is partly in the form of carbonate ion. The CO_2 will evaporate and cause loss of acidity. The rapidity of this loss is dependent upon several factors, among which the pH and the concentrations of carbon dioxide in the solution and in the gas over the surface are the most important. Thus "alkali production" is alkali production under the conditions of the experiments and may not be directly transferred to conditions in the oral cavity. In spite of this, the results give a clear indication of the possibilities of alkali production by the organisms in question in saliva and in the dental plaque. The ammonia production activity is less informative in this respect because the alkali production from ammonia is partly balanced by the "acid production" in the form of carbonate production.

At pH-values over 7, ammonia will evaporate rapidly. If the ammonia is to be determined by methods 1 or 2 at such pH values the system must be closed so that the NH_3 evaporated may be led from the outlet tube to a tube containing acid. When the author had to perform experiments at pH-values over 7, method 3 was used.

Many methods are available for the determination of ammonia. The Ljungdahl method was chosen since it proved to be practicable and rapid and since a very high degree of accuracy was of little value in experiments, in which the biologic variation is so great.

The values of acid production from glucose determined by methods 1 or 2 may be too low owing to the possibility that volatile acids may be produced which may evaporate. When the experiments are performed anaerobically, however, the amount of volatile acids produced from glucose is usually very small (*Strålfors*, 1950 a. o.).

The results of the experiments on the possible influence of oxygen tension on urease activity in micro-organisms are in accordance with what was to be expected. The author has not, however, been able to find any reports of investigations dealing with this factor.

The experiments on the localization of the enzyme are in agreement with known facts. The slight activity found in the supernatant fluid was considered to be due to organisms that were

present in this fluid. Since it is well known that urease is an intracellular enzyme it was considered of little value to study this question further.

The strains tested in this investigation raised the pH to between 8.5 and 8.8 in 30 to 40 minutes. This is of interest in relation to the results of *Stephan,* 1943, who found pH-values over 9 in the dental plaque. It may be that certain strains may raise the pH to higher values than those obtained in the present investigation. There may be other urea-hydrolysing organisms than micrococci in the dental plaque with ability to cause very high pH-values. It may also be that the antimony electrode used by *Stephan* gave values that were too high (*Brudevold & Thompson,* 1954).

Maximum activity was found near to pH 7, which is in agreement with earlier investigations on pure urease solutions. From the results of this investigation it is not possible to determine exactly where the top of the curve is to be found.

Jenkins & Wright (1951) claim that urease activity is inhibited in the presence of phosphate (0.15 M). This was not confirmed by the present investigation. In the experiments by *Jenkins & Wright,* however, a number of varying factors are present at the same time.

In the present study, urease activity was not inhibited by concentrations of urea as high as 3 M (5.1 per cent) but at concentrations of 5 M (8.5 per cent) the cells were slowly poisoned. *Clegg* and *Rae* (1956) claim that 10 per cent urea added to saliva causes inhibition of ammonia production. Thus their finding is in agreement with the results of the present investigation.

The experiments by *Kesel et al.* (1946), *Jenkins* and *Wright* (1951), and *Clegg* and *Rae* (1956) on the possible relationship between ammonia production from urea and lactate production in saliva were performed under such circumstances that only a few conclusions can be drawn from their results. The incubation period was several days in the cases for which this period is mentioned in the papers. This means that any effect exerted by a certain factor may have affected the growth of the organisms. Very little is known of the changes in the pH during such extended experiments.

The difference in lactate production found in some of the present experiments between suspensions in the presence and in the absence of urea must be considered with caution. These complicated experiments were performed under artificial conditions. The suspensions were in the stable phase (*Frostell*, 1957), i.e. they were about 24 hours old. It has been found earlier (*Frostell*, 1957) that in suspensions of lactobacilli the acid production is sometimes slight at the beginning of the experiments and gradually becomes more lively and stable. Such experience has been gained with other micro-organisms than lactobacilli in rare cases. Variation in the acceleration of lactate production may lead to differences in the results of experiments with the same suspension. The experimental period has been kept short, a factor which increases the risks of error due to this phenomenon. However, in some experiments the difference in lactate production in the presence and in the absence of urea is rather obvious and cannot be explained by experimental difficulties alone.

The mechanism of a possible inhibition of the lactate production is obscure, but may be explained in several different ways. The presence of urea may exert an inhibiting action on the enzymes responsible for glucose degradation. The increasing ammonium ion concentration during the experiment may have the same effect. Since the author has found no inhibition of the lactate production by suspensions of lactobocilli in the presence of concentrations of urea or ammonium ion likely to occur in the glucose-urea experiments it seems improbable that these ways of explaining the inhibition are correct.

In several experiments in this investigation there was a tendency to obtain higher values for urea hydrolysis in the presence of glucose than in the absence of this substance. The difference between the means is not statistically significant, however, although in some experiments the difference is obvious and beyond doubt. A tentative explanation of these findings is offered. When the cell is acting simultaneously on glucose and urea, the urea degradation dominates quantitatively and large amounts of alkali are produced. The pH of the protoplasm of the cell becomes alkaline and optimum pH for lactate production is not brought about. Hence lactate production is "inhibited". On the other

hand, when glycolysis is going on at the same time as urea hydrolysis, several organic acids are formed which prevent the alkalinity from reaching the high level that is present when only urea hydrolysis is going on. Hence urea hydrolysis is going on in a medium the pH of which is nearer to optimum pH than if no sugar degradation occurs and alkali production is "stimulated". It is, however, hard to explain why this suggested pH-effect was demonstrable in only a few experiments.

SUMMARY

Urease activity of six strains of micrococci from the oral cavity have been studied under different experimental conditions, using a titration method presented earlier by the author. This method permits determination of alkali and acid production by microorganisms at constant pH.

It was found that the urease activity was not affected by changes in oxygen tension or moderate changes in phosphate buffer concentration. Optimum pH was found on the acid side of neutrality. Maximum urease activity was found at urea concentrations between 0.16 and about 3 M; activity was, however, still present at urea concentrations as high as 8.9 moles. It was found that the cells were slowly poisoned by urea at concentrations of 5 M.

Urease activity of highly active suspensions of micrococci was of the order of 260×10^{-9} M/min./mg wet weight when urease activity was expressed as moles of alkali produced under the conditions of the experiments. If the activity was expressed in moles of NH_3 the same value was about 350×10^{-9}. Acid production of the same strains was about 15×10^{-9} M/min./mg. Thus acid production amounted to only 5.5 per cent of alkali production by the same strains.

When the cells of the suspensions acted on glucose and urea simultaneously, neither glycolysis or urea hydrolysis was seriously affected as a rule. This means that the cell is capable of performing maximum urease activity and maximum glycolytic activity simultaneously. However, it was shown that sometimes urease activity is markedly increased in the presence of glucose.

Acid production determined as lactate production was generally somewhat lower in the presence than in the absence of glucose. This tendency could not be explained by experimental difficulties.

The author offers a tentative explanation of these findings. When urea hydrolysis is going on, large amounts of alkali are perhaps set free within the cell, changing the pH of the protoplasm to the alkaline side of neutrality, and thereby optimum acidity for glycolysis is not obtained. Consequently, glycolysis is "inhibited". On the other hand, when glycolysis is going on at the same time as urea hydrolysis, a number of acids and intermediary acid products may be set free within the cell, and so hydrogen ion concentration will be kept closer to neutrality than if the cell acts on urea alone. Consequently, urease activity is "stimulated" by the presence of glucose. It is, however, difficult to explain why these effects are demonstrable in only a few experiments.

RESUME

RECHERCHES SUR L'ACTIVITE DE L'UREASE ET L'ACTIVITE GLYCOLYTIQUE

L'activité de l'uréase de six colonies de microcoques de la cavité orale a été étudiée dans différentes conditions expérimentales, en utilisant une méthode précédemment exposée par l'auteur, méthode qui permet la détermination de la production de l'alcali et de l'acide à un degré constant de pH.

On a trouvé que l'activité de l'uréase n'était pas affectée par les changements de la tension en oxygène ou par des changements modérés dans la concentration de la solution tampon de phosphate. On a trouvé que le pH optimum était sur le versant acide du point de neutralité. L'activité maximum de l'uréase a été trouvée à des concentrations d'urée variant entre 0,16 et environ 3 M. L'activité était cependant encore présente à une concentration d'urée aussi élevée que 8,9 M. On a trouvé ques les cellules étaient lentement empoisonnées par l'urée à des concentrations de 5 M.

L'activité de l'uréase de suspensions hautement actives de microcoques était de l'ordre de 260×10^{-9} M par minute

par milligramme de poids à l'état mouillé quand l'activité de l'uréase était exprimée en molécules d'alcali produites dans les conditions de l'expérience. Si l'activité était exprimée en molécules de NH_3 cette valeur était d'environ 350×10^{-9}. La production acide des mêmes colonies était d'environ 15×10^{-9} M/min./mg. Ainsi la production acide s'élevait à seulement 5,5 pour cent de la production d'alcali dans les mêmes colonies.

Quand les cellules des suspensions agissaient sur le glucose et l'urée simultanément, ni la glycolyse ni l'hydrolyse de l'urée n'étaient sérieusement affectées en général. Ceci indique que la cellule est capable de produire un maximum d'activité de l'uréase et un maximum d'activité glycolytique simultanément. Cependant, il a été montré que parfois l'activité de l'uréase est notablement augmentée en présence de glucose. La production acide déterminée comme production d'acide lactique était généralement un peu plus basse en présence qu'en son absence (de glucose). Cette tendance ne pouvait pas être expliquée par les difficultés expérimentales.

L'auteur présente une théorie pour expliquer ces constatations. Quand l'activité de l'uréase est en cours, de larges quantités d'alcali sont libérées à l'intérieur de la cellule, déplaçant le pH du protoplasme vers le versant alcalin du point de neutralité, et ainsi l'acidité optimum pour la glycolyse n'est pas obtenue. En conséquence, la glycolyse est "inhibée". Au contraire, quand la glycolyse est en cours en même temps que l'hydrolyse de l'urée une quantité d'acides et de produits acides intermédiaires est libérée à l'intérieur de la cellule, et ainsi la concentration ionique de l'hydrogène sera gardée plus près de la neutralité que si la cellule agit uniquement sur l'urée. L'activité de l'uréase est donc "stimulée" par la présence du glucose. Il est cependant difficile d'expliquer pourquoi ces effets ne sont démontrables que dans quelques expériences seulement.

ZUSAMMENFASSUNG

UNTERSUCHUNGEN ÜBER DIE UREASE- UND GLYKOLYTISCHE AKTIVITÄT EINIGER MIKROKOKKEN DER MUNDHÖHLE

Die Ureaseaktivität von sechs Mikrokokkenfamilien aus der Mundhöhle ist unter verschiedenen Versuchsbedingungen untersucht worden. Dabei gelangte eine Methode zur Anwendung, die

der Autor schon früher veröffentlicht hat, und die die Bestimmung der Basen- und Säurebildung durch Mikroorganismen bei konstantem pH erlaubt.

Man fand, dass die Ureaseaktivität durch Änderung des Sauerstoffdruckes oder durch mässige Änderung der Konzentration des Phosphatpuffers nicht beeinflusst wurde. Der optimale pH-Wert lag auf der sauren Seite des Neutralpunktes. Die maximale Ureaseaktivität erreichte man bei Ureakonzentrationen von 0,16 bis ungefähr 3 Mol/l. Die Ureaseaktivität war jedoch noch bei so hohen Ureakonzentrationen wie 8,9 Mol vorhanden. Man fand. dass die Zellen bei Konzentrationen von 5 Mol/l allmählich durch Urea vergiftet wurden.

Die Ureaseaktivität stark aktiver Mikrokokkus-Suspensionen entspricht der Grössenordnung 260×10^{-9} m/Min./mg Feuchtgewicht, wobei die Ureaseaktivität, die unter den Versuchsbedingungen erzeugt, in Alkalimol ausgedrückt wurde. Der entsprechende Wert für die Aktivität ausgedrückt durch NH_3-mol ergab 350×10^{-9}. Die Säureproduktion derselben Mikrokokkenfamilien betrug etwa 15×10^{-9} M/Min./mg. Die Säurebildung erreichte also nur 5,5 % der Alkalibildung derselben Familien.

Wenn die Zellen der Suspensionen gleichzeitig auf Traubenzucker und Urea einwirkten, wurde in der Regel weder die Glykolyse noch die Hydrolyse nennenswert beeinträchtigt. Das bedeutet, dass die Zelle fähig ist, zur gleichen Zeit maximale Urease- und maximale glykolytische Aktivität auszuüben. Jedoch wurde gezeigt, dass die Ureaseaktivität bei Anwesenheit von Traubenzucker manchmal bedeutend zunimmt. Die Säurebildung in Form von Milchsäure ist im allgemeinen bei Anwesenheit von Traubenzucker etwas niedriger als beim Fehlen desselben. Diese Tendenz konnte nicht auf experimentelle Schwierigkeiten zurück geführt werden.

Der Autor hat eine Theorie zur Deutung dieser Ergebnisse aufgestellt. Wenn Ureaseaktivität vorliegt, werden innerhalb der Zelle grosse Mengen von Alkali frei, die eine pH-Verschiebung des Protoplasmas auf die alkalische Seite des Neutralpunktes herbeiführen, wodurch der optimale pH-Wert für die Glykolyse nicht erreicht wird. Infolgedessen wird die Glykolyse gehemmt. Wenn dagegen die Glykolyse gleichzeitig mit der Ureahydrolyse

stattfindet, werden innerhalb der Zelle eine Anzahl von Säuren und intermediären Säureprodukten frei. Hierdurch nähert man die Wassenstoffionenkonzentration dem Neutralpunkt auf die gleiche Weise, als wenn die Zelle alleine auf Urea einwirkte. Infolgedessen wird die Ureaseaktivität durch die Anwesenheit von Traubenzucker "angeregt". Es ist jedoch schwer zu erklären, warum diese Resultate nur in wenigen Experimenten erzieht werden konnten.

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