Synthesis and Breakdown of Glutamine by Various Micro-organisms

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SUMMARY: Glutamine is not required for staphylococcal growth, and was found to be synthesized by staphylococci from NH_3 and glutamic acid in the presence of glucose. The amount of glutamine found was, however, much less than that of the NH_3 reacting. Similar relationships were found in *Proteus morganii*, *Pr. vulgaris*, *Saccharomyces cerevisiae* and *S. ellipsoideus*. *Proteus morganii* was, however, outstanding in possessing a powerful glutaminase which could easily be separated from the cells; in this it differed markedly from *Pr. vulgaris*. Glutaminase activity was also demonstrated in yeasts, where its occurrence has been previously a matter of dispute.

Glutamine is essential for growth of certain β -haemolytic streptococci; it accelerates their main energy-yielding reaction, glycolysis, and it is hydrolysed to glutamic acid and NH_3 during this process (McIlwain, 1946*a, b*). Certain of these associations may be explained by the need of energy for the assimilation of glutamic acid and glutamine (Gale, 1947). This requirement (Taylor, 1947), like that for glutamine in growth (McIlwain, Fildes, Gladstone & Knight, 1939), is different in different microbial species. We have now studied the association between glucose, glutamine, glutamic acid and ammonia in several micro-organisms.

METHODS

Organisms. Staphylococcal strains comprised '35', from a case of osteomyelitis and 'E' from one of impetigo, and were grown under the conditions described for streptococci by McIlwain (1946*a, b*). Strains of *Proteus morganii* included No. 2818 of the National Collection of Type Cultures, and two freshly isolated from faeces; they were grown as described by McIlwain & Hughes (1945), but with 10^{-6} M pantothenate. *Pr. vulgaris* was grown as *Pr. morganii*; one strain (C) was from an infected wound, and the other had been maintained on laboratory media for many years. *Saccharomyces cerevisiae* and *S. ellipsoideus* were respectively Nos. 4614 and 7040 of the National Collection, No. 7040 being used shortly after its receipt from a distillery. They were grown in a mixture of inorganic and ammonium salts with 2 % glucose and 1–5 % of a bacteriological infusion broth. Bacteria were maintained on broth agar slopes and grown at 37°; yeasts, on malt-agar slopes and at 25° except where otherwise described.

Experimental procedure. The metabolic experiments were made and NH_3 and glutamine were determined by methods stated previously (McIlwain, 1946*a, b*; Roper & McIlwain, 1948). Yeasts were extracted mechanically and autolytically by the methods applied to bacteria (McIlwain, Roper & Hughes, 1948). The saline used was that of Krebs & Eggleston (1940).

RESULTS

Staphylococci

Growth. Glutamine (5×10^{-6} M to 5×10^{-4} M) was without effect on growth of the two strains under conditions in which the substance is necessary for streptococcal growth. Slight stimulation has been found in other strains (cf. Fildes & Gladstone, 1939), but was not sufficiently well defined to afford a subject for investigation. The sulphoxide and sulphone derived from methionine were without effect on the growth of strain 35, when present at 0.01 M in an amino-acid medium (Fildes & Richardson, 1937). (Inhibition is caused by the methionine derivatives in other bacteria and is related to their metabolism of glutamic acid.)

Table 1. *Reaction of staphylococci with glutamine and ammonium glutamate*

Strain of organism; dry wt. (mg.); duration of experiment (min.)	Substrates (μ mol.)	Change (μ mol.) in		
		Glutamine	NH ₃	CO ₂
'35'; 4.7; 80	Glutamine, 15	-2.0	+2.3	+1.8
	Glutamine, 15 and glucose, 200	-0.6	<0.1	+82
	Ammonium glutamate, 31	+0.1	<0.1	+1.0
	Ammonium glutamate, 31 and glucose, 200	0	-4.2	+82
'35'; 2.5; 120	Glutamine, 12.6	-2.2	+2.2	+1.2
	Glutamine, 12.6 and glucose, 200	<0.1	<0.1	+54
	Glucose, 200	<0.1	<0.1	+54
'35'; 2.1; 120	Ammonium glutamate, 95.5 and glucose, 200	<0.1	-6.5	+53
	Ammonium glutamate, 24.0 and glucose, 200	<0.1	-4.8	+54
'E'; 6; 80	Glutamine, 14.4	-0.9	+0.9	+0.8
	Glutamine, 14.4 and glucose, 200	<0.1	<0.1	+35
	Ammonium chloride, 15.1 and glucose, 200	<0.1	-1.0	+36

Experiments were performed anaerobically at pH 7.6 and 37° with the substrates and amount of organism indicated, in 3 ml. of bicarbonate saline. The changes recorded are in the complete metabolic mixtures without separation of organisms. Organisms were grown for 18–22 hr., washed twice with 0.9% NaCl, and suspended in that solution for use.

Anaerobic reactions with glucose. The reaction was examined only in order to find whether glutamine affected it, as is the case with streptococci. Acid was formed from glucose and determined (a) by evolution of CO₂ from bicarbonate which occurred at rates comparable to those observed in streptococci; and (b) as lactic acid, according to Friedemann & Graesser (1933). Table 1 includes experiments in which the rates were of 10–15 μ mol./mg. dry wt. of cells/hr. Glutamine had little or no effect on the rates, the maximum increase of some 8% being no more than that given by ammonium glutamate. The organisms were examined after 5, 18 and 30 hr. growth.

Action on glutamine and ammonium salts. Glutamine was decomposed with formation of NH₃ in the absence of glucose (Table 1), a phenomenon not found

in the exacting streptococci. The rate of decomposition was, however, not high; mean values for Q_{glut} , calculated from the data of Table 1, assuming the rate to be steady during the experiments, were -0.12 to $-0.44 \mu\text{mol./mg. dry wt./hr.}$, or about 1/5 to 1/10 of those observed in streptococci in the presence of glucose. The NH_3 formed was about equivalent to the glutamine decomposed; the organisms may thus be said to exhibit a small 'glutaminase' activity. (The term 'glutaminase' has been retained in describing such formation of glutamic acid and NH_3 from glutamine, though it may later be necessary to use a more specific name such as 'glutamine desamidase'. 'Glutaminase' has been avoided in describing the coupled breakdown of glutamine to the same products, which occurs, for example, during streptococcal glycolysis.) During this reaction the total CO_2 evolved from the bicarbonate containing media was small, being less than the change in glutamine. No reaction corresponding, for example, to glycolysis from stored carbohydrate, was therefore occurring.

In marked contrast to the findings with streptococci, glucose did not increase the quantity of ammonium glutamate formed from glutamine, but decreased it. The ratio of change in glutamine to change in CO_2 then became $<1:100$ and often $<1:500$ (Table 1), whereas the corresponding ratios with exacting streptococci were $1:5$ to $1:20$. Such decomposition of glutamine as did occur with staphylococci in the presence of glucose did not lead to accumulation of NH_3 .

Ammonia added as NH_4Cl or as ammonium glutamate also disappeared in the presence of glucose; the rate of reaction was then -0.7 to $-1.6 \mu\text{mol. NH}_3/\text{mg. dry wt./hr.}$ The glutamine formed was too small to be detected by the method of Vickery, Pucher, Clark, Chibnall & Westall (1935), but could be determined microbiologically. By such methods (cf. Roper & McIlwain, 1948) the strain 'E' in phosphate buffer of pH 7.6, with glucose (0.017 M) and ammonium L-glutamate (0.0053 M) for short periods (cf. McIlwain *et al.* 1948) synthesized and stored glutamine in the cells, usually at rates of $5\text{--}15 \mu\text{mol./mg. dry wt./hr.}$ In one case a rate of $50 \mu\text{mol./mg./hr.}$ was observed. These indicate minimum values only for the rate of glutamine synthesis.

Proteus morganii

Growth. Glutamine ($10^{-5}\text{--}10^{-3} \text{ M}$) did not affect the rate of growth of *Pr. morganii* under the conditions in which this substance was essential to streptococci, nor under the conditions employed in growing the organisms for the present metabolic experiments.

Action on glucose. The two strains rapidly produced acid from glucose anaerobically and their Q_{CO_2} in bicarbonate-containing media was equal to, or greater than, that of the streptococci. Added glutamine did not affect their Q_{CO_2} .

Action on glutamine and ammonium salts. *Pr. morganii* caused a rapid breakdown of glutamine both with and without glucose. Without glucose, NH_3 was formed in approximately equimolar quantities (Table 2), but with glucose, much less NH_3 was found. This appeared to be due to a secondary reaction.

The disappearance of NH_3 from glutamine was not confined to NH_3 from this source; NH_3 production from asparagine was also less in the presence of glucose than in its absence. The strain 2818 produced NH_3 anaerobically and in the absence of glutamine, at pH 7.6, at the rate of $2.9 \mu\text{mol./mg./hr.}$ from asparagine, and at $2.8 \mu\text{mol./mg./hr.}$ from aspartic acid; the reactions, if any, with pyrrolidone- α -carboxylic acid or with arginine gave $<0.2 \mu\text{mol.}$

Table 2. *Reaction of Pr. morganii with glutamine and ammonium salts*

Experimental arrangement as described in Table 1				
Strain of organism; dry wt. (mg.); duration of experiment (min.)	Substrates ($\mu\text{mol.}$)	Change ($\mu\text{mol.}$) in		
		Glutamine	NH_3	CO_2
'2818'; 3.1; 35	Glutamine, 14.1	-13.8	+14.0	+ 2
	Glucose, 200	0	0	+59.5
	Glucose, 200 and glutamine, 14.1	-12.4	+ 8.1	+58.5
	Glucose, 200 and glutamine, 5.6	- 5.3	0	+61
'2818'; 5.1; 35	Glucose, 200	0	0	+55
	Glucose, 200 and ammonium glutamate, 15	0	- 6.7	+54.5
	Glucose, 200 and ammonium chloride, 15	0	- 7.0	+55.5
	Ammonium glutamate, 15	0	+ 0.2	0
	Ammonium chloride, 15	0	+ 0.2	0
	Glutamine, 15.8	-14.8	+15.0	+ 0.5
Lab. strain 2; 3.8; 45	Glutamine, 15.8 and glucose	-14.5	+ 8.4	+53
	Ammonium glutamate, 14.8 and glucose	0	- 3.2	+51
	Ammonium chloride, 15.8 and glucose	0	- 7.1	+54
	Ammonium chloride, 15.8	0	- 0.2	+ 0.5
	Glucose, 200	0	0	+54

$\text{NH}_3/\text{mg./hr.}$ Ammonia added as NH_4Cl to *Pr. morganii*, in the presence of glucose, also disappeared at about the same rate as did that from glutamine (Table 2), though in the absence of glucose little change occurred in NH_3 . The disappearance of added NH_3 during reaction with glucose was frequently less in the presence of glutamic acid, but ammonium glutamate yielded no glutamine either in the presence or absence of glucose. It is understandable that little or no glutamine should accumulate as such (though the organisms presumably synthesize and assimilate it in growth), because its breakdown is rapid.

Pr. morganii thus differed from the staphylococci in its much more rapid glutaminase action, and in the fact that such action was unaffected by added glucose. The disappearance of NH_3 in the presence of glucose but without corresponding glutamine synthesis (a feature common to both these organisms and many others) was carried out more rapidly by *Pr. morganii*.

Extraction of a glutaminase from Pr. morganii. The cells were easily broken by ground glass and in the preparation used for the experiments of Table 3, 99% of the cells had been broken. The extract had about one-third of the activity manifested by the intact cells at pH 7.6; the debris had one-quarter the activity. The remainder was not necessarily lost as the pH of the test

system was not that which was later found to be optimal for glutamine breakdown. The extract was much more active at pH 5.

Autolysis also yielded an active solution, containing some 60 % of the activity of the cells from which it was prepared. Like the intact cells this yielded 1 mol. NH_3 /mol. glutamine decomposed (Table 3).

Table 3. *Ammonia formation from glutamine by extracts of Pr. morganii*

Preparation; equivalent dry wt. (mg.) of bacteria used	Buffering	NH_3 produced ($\mu\text{mol.}$) in 1 hr.	Glutamine lost ($\mu\text{mol.}$) in 1 hr.
Fresh cells; 8	Bicarbonate, pH 7.6	18.6	—
First extract of ground cells; 8.2	Bicarbonate, pH 7.6	4.0	—
Second extract of ground cells; 8.2	Bicarbonate, pH 7.6	2.6	—
Ground cell residue; 7.0	Bicarbonate, pH 7.6	3.6	—
First extract of ground cells; 8.2	Acetate, pH 4.5	22.0	—
Fresh cells; 2	Phosphate-citrate, pH 4.5	55.0	—
Autolysate; 2	Phosphate-citrate, pH 4.5	40.8	—
Residue from autolysis; 2.5	Phosphate-citrate, pH 4.5	25.3	—
Autolysate (different preparation); 2	Phosphate-citrate, pH 4.5	35.6	35.7
Autolysate (different preparation); 2	Phosphate-citrate, pH 4.5	35.6*	34.9*
Autolysate (different preparation); 2	Phosphate-citrate, pH 2.5	25.6	26.6

* Aerobic reaction.

Extracts were prepared from strain 2818 after 18 hr. growth. Reactions were performed anaerobically unless otherwise specified, and with initially 60 $\mu\text{mol.}$ glutamine per reaction mixture of 8 ml. Controls at the experimental pH values showed no spontaneous breakdown of glutamine.

Proteus vulgaris

Growth. In the casein hydrolysate medium employed in growing *Pr. vulgaris* for the present experiments, addition of glutamine (10^{-5} – 5×10^{-4} M) did not alter the mass of bacteria produced. The medium contained glutamic acid which was observed by Fildes & Gladstone (1939) to stimulate growth of a strain to almost the same extent as did glutamine when the substances were added to a medium of inorganic salts and lactate.

Action on glucose. The two strains produced acid from glucose. Carbon dioxide was evolved from bicarbonate-containing solutions at rates of 6–15 $\mu\text{mol./mg. dry wt./hr.}$ and these rates were increased by about 0.008 M ammonium salts or by glutamine to small and equal extents. Thus the strain B (Table 4), when evolving CO_2 at the rate of 15.8 $\mu\text{mol./mg./hr.}$, was stimulated 16 % by glutamine and 13 % by ammonium glutamate; in strain C, the rate of 16.1 $\mu\text{mol./mg./hr.}$ was increased 14 % by both compounds.

Action on glutamine and ammonium salts. Table 4 shows that, in marked distinction from the behaviour of *Pr. morganii*, *Pr. vulgaris* had little action on glutamine alone. Ammonia disappeared only slowly, without formation of glutamine, from solutions of ammonium glutamate added to suspensions of *Pr. vulgaris*, but this reaction and the decomposition of glutamine became considerable in the presence of glucose. Here again, metabolism of glucose was associated with a reaction leading to loss of the labile amide— NH_3 of glutamine,

at a rate of 1–2 $\mu\text{mol./mg. dry wt./hr.}$, but without accumulation of NH_3 . Acetyl phosphate did not induce breakdown of glutamine but led to loss of NH_3 ; the CO_2 produced (Table 4) during the experiments corresponded approximately to the compound's spontaneous hydrolysis (Lipmann & Tuttle, 1944).

The two strains were typical of *Pr. vulgaris* in exhibiting powerful urease activity.

Table 4. *Reaction of Pr. vulgaris with glutamine and ammonium salts*

Experimental arrangements as described in Table 1, except that pH was varied as indicated below.

Strain of organism; dry wt. (mg.)	Duration of experiment (min.)	pH	Substrates ($\mu\text{mol.}$)	Change ($\mu\text{mol.}$) in		
				Glutamine	NH_3	CO_2
B; 3.2	60	4.5*	Glutamine, 18.7	0	0	+ 0.7
	60	7.6	Glutamine, 18.7	0	+ 0.8	+ 1.1
	60	7.6	Glutamine, 18.7 and glucose, 200	– 5.9	0	+ 42.2
C; 7.2	60	4.5*	Glutamine, 18.7	– 1.0	0	+ 0.6
	60	7.6	Glutamine, 18.7	+ 0.2	+ 1.2	+ 1.0
	60	7.6	Glutamine, 18.7 and glucose, 200	– 5.1	0	+ 45.0
B; 6.35	100	7.6	Ammonium glutamate, 45.5	0	– 3.1	+ 0.5
	30	7.6	Ammonium glutamate, 45.5 and glucose, 200	0	– 6.5	+ 55.3
	30	7.6	Glutamine 21, and glucose, 200	– 5.4	+ 0.2	+ 57.8
	100	7.6	Glutamine, 21	0	+ 0.3	+ 0.6
	30	7.6	Urea, 25	0	+ 33.6	—
C; 6.95	100	7.6	Ammonium glutamate, 27	0	– 3.3	+ 0.3
	35	7.6	Ammonium glutamate, 27 and glucose, 200	0	– 6.3	+ 50.1
	100	7.6	Ammonium glutamate, 27 and acetyl phosphate, 50	0	– 4.3	+ 10.9
	100	7.6	Glutamine, 15.7	– 0.8	+ 0.5	+ 0.7
	100	7.6	Glutamine, 15.7, and acetyl phosphate, 50	– 0.3	+ 0.5	+ 12.2
	30	7.6	Glutamine, 15.7 and glucose, 200	– 7.8	+ 0.3	+ 58

* Acetate buffer used instead of bicarbonate.

Yeasts

The formation of NH_3 from glutamine by yeast extracts was reported by Geddes & Hunter (1928) and Grassman & Mayr (1933), without experimental details and in studies incidental to those of yeast asparaginase. Archibald (1945) found no glutaminase activity and stated that this was also the finding of Schwab (1936) and Nielsen (1941). The observations given below indicate well-marked glutaminase action, but show that the presence or absence of glucose greatly affects the reaction of yeasts to glutamine. Also, glutamine can be broken down without accumulation of NH_3 .

Growth. The effect of glutamine in concentrations between 10^{-5} M and 3×10^{-4} M on growth of the strains of *Saccharomyces cerevisiae* and *S. ellipsoideus* at 25 and 35° was examined in media consisting of ammonium salts, inorganic salts and glucose, and in the mixture of amino-acids used by Gladstone (1939). In the different experiments, with varying inocula, visible growth first appeared at 1–4 days, but in no case was a significant acceleration by glutamine observed.

Fermentation. The production of CO_2 from glucose by baker's yeast was observed by Smythe (1939) to be accelerated by glutamine, an effect which he concluded was mediated through NH_3 formed from the glutamine. The present observations are in agreement with this. Stimulation of CO_2 production at pH 7.5 by glutamine was smaller (4–8 %) than by ammonium glutamate (12–20 %). The yeasts were grown for periods between 30 and 72 hr. and were washed in saline, but were not prepared by the methods described by Smythe to obtain maximal response to NH_3 .

Action on glutamine and ammonium salts. Reaction mixtures with and without glucose were examined for anaerobic production or disappearance of ammonia and glutamine (Table 5). The values show that rarely, if ever, did a simple conversion of glutamine to glutamic acid and NH_3 , or the reverse reaction, take place. Decomposition of glutamine was slow, except in the presence of glucose. With glucose it reached values of 1 $\mu\text{mol./mg./hr.}$, and thus approximated to the rate of glutamine decomposition by streptococci. Unlike the reaction with streptococci, however, little NH_3 accumulated as a result of the loss of the labile amide group of glutamine in the presence of yeasts. Added NH_3 also disappeared either at a slightly greater (*S. ellipsoideus*) or much greater rate (*S. cerevisiae*) than that of the reaction with glutamine. Any NH_3 formed from glutamine would, therefore, be expected to undergo further reaction and not to accumulate. The two yeasts differed, not in their rates of reaction with ammonium salts, but in their rates of glutamine decomposition, which was lower in *S. cerevisiae*. A small accumulation of synthesized glutamine was observed in some cases (Table 5).

Yeast extracts and glutamine. An extract prepared by rubbing moist cells of *S. ellipsoideus* with powdered glass contained little free glutamine and NH_3 , and on incubation anaerobically with added glutamine at pH 4.5 or 7, it caused a slow breakdown of glutamine (–0.2 to –0.3 $\mu\text{mol./quantity of extract prepared from the equivalent of 1 mg. dry wt. of yeast/hr.}$). The rate was unaffected by glucose. The molar quantity of NH_3 produced was less than that of the glutamine lost.

On autolysis in the presence of fluoride, extracts with smaller glutaminase activity were obtained. These decomposed 0.1–0.2 $\mu\text{mol. glutamine/mg.-equivalent/hr.}$ with the formation of about an equimolar quantity of NH_3 . Such activity may account for the reports of Geddes & Hunter (1928) and Grassmann & Mayr (1933); it represents, however, only about one-tenth of the activity exhibited towards glutamine in the presence of glucose.

Table 5. *Reactions of yeasts with glutamine and ammonium glutamate*

Experimental arrangements as described in Table 1, except that pH was adjusted as indicated below, and the organisms were from cultures harvested at 48-52 hr. after inoculation.

Organism; dry wt. (mg.); duration of experiment (min.)	Buffering	Substrates ($\mu\text{mol.}$)	Change ($\mu\text{mol.}$) in		
			Glutamine	NH_3	CO_2
<i>S. ellipsoideus</i> ; 8.8; 45	Bicarbonate, pH 7.6	Glutamine, 15	0	+0.7	+1
	Bicarbonate, pH 7.6	Glutamine, 15 and glucose, 200	-5.8	0	+120
	Bicarbonate, pH 7.6	Ammonium glutamate, 15	+0.5	-0.3	+2
	Bicarbonate, pH 7.6	Ammonium glutamate, 15 and glucose, 200	0	-7.9	+135
<i>S. ellipsoideus</i> ; 7.5; 45	Phosphate-citrate, pH 6.5	Glutamine, 20	0	+0.3	+1
	Phosphate-citrate, pH 6.5	Glutamine, 20 and glucose, 200	-5.9	0	+64
	Phosphate-citrate, pH 4.5	Glutamine, 20	-2.0	0	+1
	Phosphate-citrate, pH 4.5	Glutamine, 20 and glucose, 200	-3.1	0	+88
<i>S. ellipsoideus</i> ; 8.7; 35	Phosphate-citrate, pH 4.5	Ammonium glutamate, 36	0	+0.5	—
	Phosphate-citrate, pH 4.5	Ammonium glutamate, 36 and glucose, 200	0	-6.9	+74
	Phosphate, pH 7	Ammonium glutamate, 36	0	-0.3	—
	Phosphate, pH 7	Ammonium glutamate, 36 and glucose, 200	0	-6.5	+74
	Phosphate, pH 7	Ammonium glutamate, 36 and glucose, 200 and fluoride, 250	0	+0.5	+0.1
	Phosphate, pH 7	Ammonium glutamate, 36 and glucose, 200 and fluoride, 50	0	-4.8	+44
	Phosphate, pH 7	Glutamine, 10.6	+0.2	+0.1	+1
<i>S. cerevisiae</i> ; 6.2; 65	Bicarbonate, pH 7.6	Glutamine, 10.6 and glucose, 200	-1.8	0	+63
	Bicarbonate, pH 7.6	dl-Ammonium glutamate, 18.5	+0.2	0	+0.6
	Bicarbonate, pH 7.6	dl-Ammonium glutamate, 18.5 and glucose, 200	0	-4.8	+65
	Phosphate-citrate, pH 6.5	Glutamine, 25	-2.4	+1.0	+0.3
<i>S. cerevisiae</i> ; 2.9; 60	Phosphate-citrate, pH 5.5	Glutamine, 25	-0.4	+0.2	+0.2
	Phosphate-citrate, pH 4.5	Glutamine, 25	+0.1	0	+0.1
	Phosphate-citrate, pH 4.5	Glutamine, 25 and glucose, 200	-3.4	0	+26.3

DISCUSSION

The changes which have been observed in glutamine and in ammonium glutamate, in the presence and absence of glucose, are summarized in Table 6. All the organisms are capable of reactions involving either the formation or the removal of NH_3 (or both reactions) at rates of at least 1–2 $\mu\text{mol./mg. dry wt./hr.}$ In straightforward hydrolysis of glutamine to glutamic acid and NH_3 , *Proteus morganii* is outstanding. Its reaction, at the rate of 6–8 $\mu\text{mol./mg./hr.}$, results

Table 6. *Summary of reactions of micro-organisms with glutamine and ammonium glutamate*

Organisms	Quotients* ($\mu\text{mol./mg. dry wt./hr.}$) with			
	Glutamine		Ammonium glutamate	
	Alone	With glucose	Alone	With glucose
β -Haemolytic streptococci	$\{ Q_{\text{NH}_3}$ 0 to +0.1	+1 to +3	<0.03	0 to -0.08
	$\{ Q_{\text{glut.}}$ 0 to -0.1	-1 to -3	<0.03	<0.03
Yeasts	$\{ Q_{\text{NH}_3}$ 0 to +0.03	0.02	0 to -0.03	-0.7 to -1.2
	$\{ Q_{\text{glut.}}$ <0.02	-0.3 to -1	+0.02 to +0.05	<0.02
Staphylococci	$\{ Q_{\text{NH}_3}$ +0.1 to +0.4	<0.02	0 to -0.02	-1 to -1.5
	$\{ Q_{\text{glut.}}$ -0.1 to -0.4	0 to -0.1	0 to +0.02	<0.02
<i>Pr. morganii</i>	$\{ Q_{\text{NH}_3}$ +6 to +8	+4	<0.02	-1.5 to -2.5
	$\{ Q_{\text{glut.}}$ -6 to -8	-6 to -8	<0.02	<0.02
<i>Pr. vulgaris</i>	$\{ Q_{\text{NH}_3}$ 0 to 0.15	0 to +0.1	-0.3	-1.6 to -2.1
	$\{ Q_{\text{glut.}}$ 0 to -0.1	-0.7 to -2.2	<0.03	<0.03

* Changes have been expressed as metabolic quotients for convenience in summarizing, but only in the cases of the haemolytic streptococci, staphylococci and *Pr. morganii* have some kinetic experiments been carried out; the remaining values are calculated from overall exchanges in experiments of the type of those of Tables 1–5. The experimental arrangements were similar throughout; reactions were carried out anaerobically at pH 7.6.

in decomposition in an hour of about its own weight of glutamine. Comparable reactions were not found in the other organisms, and *Pr. morganii* is seen to differ even from *Pr. vulgaris*. Rapid glutaminase action has also been observed in strains of *Clostridium welchii* (Krebs, 1948). It was easy to separate the glutaminase of *Pr. morganii* in cell-free form.

The glutaminase of *Pr. morganii* is characteristically distinct from the system in the exacting haemolytic streptococci, which decomposes glutamine to glutamic acid and NH_3 during glycolysis (McIlwain, 1946*a, b*). In this system extracts capable of hydrolysis of glutamine were not obtained (McIlwain *et al.* 1948), and it is possible that the true substrate of the streptococcal enzyme might not be glutamine, or that the reaction might be a phosphorylation rather than hydrolysis. Precise evidence is therefore required before the term 'glutaminase' can be applied to such a system, and before the relation between glutamine breakdown and glycolysis can be regarded as due only to permeability phenomena (cf. Gale, 1947). Breakdown of glutamine, associated with glycolysis, has now been demonstrated in yeasts and in *Pr. vulgaris*, so that its

occurrence is independent of assimilatory mechanisms peculiar to Gram-positive organisms.

One further feature of those haemolytic streptococci that are exacting towards glutamine in growth is their relative inertness towards ammonia, either added as such or produced during metabolism. Thus the ammonia formed from glutamine by these organisms, in the presence of glucose, was almost equivalent to the glutamine decomposed (McIlwain, 1946*a*), whereas in the case of other organisms it was much less (Table 6). This was true even with *Pr. morganii* which produced such NH_3 very rapidly. The equivalence, in streptococci, may be a reflexion of the relative lack in these organisms of synthetic reactions using NH_3 . The streptococci are nutritionally the most exacting of the organisms of Table 6, and require many preformed amino-acids, for their growth.

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