The Metabolism of the Amino Sugars

1. THE BREAKDOWN OF *N*-ACETYLGLUCOSAMINE BY STRAINS OF *STREPTOCOCCUS HAEMOLYTICUS* AND OTHER *STREPTOCOCCI*

By H. J. ROGERS

Biological Research Department, The Dental School and Hospital, Leeds University

(Received 18 January 1949)

The amino sugars are important constituents of many polysaccharides such as chondroitin sulphate, hyaluronic acid, chitin, heparin and salivary 'mucins', yet little is known about their metabolism. Lutwak-Mann (1941) studied the breakdown of glucosamine by mammalian tissues and found that testicle, kidney and brain-slice preparations oxidized the hexosamine and liberated varying amounts of ammonia. An acid substance was formed which was neither lactic nor acetic acid. Monoiodoacetate, fluorides, toluene, and merthiolate all inhibited both oxidation and deamination, whereas cyanides and glyceraldehyde were more effective against the former. This author included some preliminary results for the three bacterial strains *Escherichia coli*, *Streptococcus faecalis* and *Proteus vulgaris* and stated that the reactions differed from those carried out by animal tissues in occurring under anaerobic as well as under aerobic conditions, but that the end products formed appeared to be similar.

Very recently Rosenberg (1948) has confirmed that *Esch. coli* can deaminate glucosamine under either aerobic or anaerobic conditions in the presence of phosphate. In the absence of phosphate or in the presence of azide he claims that deamination will no longer proceed anaerobically, although dehydrogenation of the substrate still occurs.

In the present studies strains of *Strep. haemolyticus* have been examined in greater detail because these organisms might be expected to have an active amino sugar metabolism. For example, some strains form enzymes (hyaluronidases) which break down hyaluronic acid and are then able to use the liberated amino sugar (McClean, Rogers & Hale, unpublished observation), whilst others form capsules composed of this substance (Kendall, Heidelberger & Dawson, 1937; McClean, 1941). Streptococci of the *viridans* type have been included because it has been shown (Rogers, 1948) that the hexosamine-containing substances in incubated saliva are broken down and the constituents utilized by the developing flora. Streptococci of this latter type form a very high proportion of the flora and may be responsible for the phenomenon. The streptococci have a further advantage for studying the metabolism of substituted sugars in that the products they form from glucose

are few in number, lactic acid accounting for 75–90% of the sugar used (Hewitt, 1932; Smith & Sherman, 1941); this simplifies analytical procedures involved in comparisons with the fermentation of glucose.

METHODS

N-Acetylglucosamine was synthesized from glucosamine hydrochloride by the method of Zuckerkandl & Messner-Klebermass (1931) and estimated by the Morgan & Elson (1934) procedure. NH_3 was determined by the microdiffusion method of Conway (1947), and lactic acid by ceric sulphate oxidation (Winnick, 1942); interfering substances were first removed from the samples by the method of Friedemann, Cotonio & Shaffer (1927) before making the latter estimations. Glucosamine was determined by Elson & Morgan's (1933) method and reducing sugars by that of Somogyi (1937). Nitrogen was estimated by the micro-Kjeldahl technique and volatile acids by steam distillation of the sample in the presence of McIlvaine buffer at pH 2.60 (cf. Clark, 1928). At this pH no deacetylation of *N*-acetylglucosamine can be detected after 20 hr. heating at 98–100° (Rogers, 1946).

Cultures of capsulated and non-capsulated Lancefield Group A and Group C haemolytic streptococci and of a streptococcus of the *viridans* type were grown for 18 hr. in 500 ml. amounts of a medium consisting of 3% peptone (Evans Sons, Lescher and Webb Ltd.) and 0.2% glucose; for the haemolytic streptococci normal horse serum (5%) was included, and for the *viridans* organisms sodium glycerophosphate (2.5%) was added to prevent the pH falling lower than 6.5–6.8 (Rogers, 1945), as otherwise some inactivation of the enzyme systems occurred. After growth, the organisms were removed from the cultures by centrifuging, washed twice with $\text{m}/15$ phosphate, pH 7.2, and resuspended in the same buffer at such a concentration that a 1:100 dilution of the suspension in buffer caused a deflexion of 0.3 on the arbitrary scale of the Spekker photoelectric colorimeter (Adam Hilger and Watts Co. Ltd.); such suspensions were found to contain 1.0–1.2 mg. bacterial N/ml. Samples were taken from the original culture supernatants, washing fluids and final cell suspension for acid hydrolysis (2 hr. with 6*N*-HCl) and subsequent determination of glucosamine; similar measurements were also made on the uninoculated, incubated medium. Total N was estimated on the cell suspensions.

The rate at which the various cell suspensions utilized *N*-acetylglucosamine was measured by warming them to 37.5° and adding an equal volume of warm 0.02*M*-*N*-acetylglucosamine dissolved in water. Incubation of the mixtures in corked test tubes with an atmosphere of air at 37.5° was

continued until about 50% of the substrate had been used. The required period was judged by the amount of 0.1 N-NaOH used to keep the pH at 7.2 (cf. p. 102) using 0.001% phenol red as internal indicator. The reaction was stopped by the addition of 0.2 ml. of N-HCl. The organisms were immediately removed and the supernatants neutralized to pH 7.0. *N*-Acetylglucosamine was then determined by the usual procedure in the neutralized supernatant fluids.

RESULTS

Relation between rate of utilization of N-acetylglucosamine and synthesis of glucosamine-containing compounds

Table 1 illustrates the results. Strains 283 T, S 23 and E 14, which in young culture are capsulated, form considerable amounts of a glucosamine-containing substance, presumably hyaluronic acid; in 18 hr. cultures it is found wholly free in the culture supernatant. The amount of glucosamine associated with

rate at which the various haemolytic streptococci utilize *N*-acetylglucosamine varies from 0.5 to 7.0 mg./hr./mg. bacterial N. There is, however, no obvious relation between the ability of growing cultures to synthesize glucosamine and the rate at which washed suspensions utilize *N*-acetylglucosamine. There are insufficient results to justify calculation of correlation coefficients, nor is it thought that such an attempt would be profitable even with a more extended series until more is known of the conditions controlling synthesis. The results obtained suggest that the mechanism of synthesis of the capsular polysaccharides is unlikely to be by the direct incorporation of *N*-acetylglucosamine, initially present in the medium, since all the organisms utilize this substance rather rapidly. The rate of utilization by six out of the seven strains of the *viridans* types is very similar, and smaller than that for the haemolytic organisms.

Table 1. *Rate of utilization of N-acetylglucosamine by washed suspensions of streptococci, and glucosamine content of cultures of the organisms*

(Utilization experiments carried out at pH 7.2 and 37.5° in 0.033 M-phosphate buffer; cultures, 18 hr.)

Organisms			Rate of utilization by suspensions (mg./hr./mg. bacterial N) Exp. 1	Amount of glucosamine found in 18 hr. cultures (mg./mg. bacterial N)			
				Cells		Supernatants	
Group	Type	Strain		Exp. 1	Exp. 2	Exp. 1	Exp. 2
<i>Strep. haemolyticus</i>							
A	4	P 315	2.6	0.37	0.36	0	0
A	4	H 713	1.4	0.31	0.30	0	0
A	6	283 T	1.0	0.26	0.27	3.7	3.0
A	14	S 23	0.5	0.29	0.31	2.8	2.6
A	25	E 14	1.1	0.42	—	—	0.9
A	22	63 T	1.2	0.27	0.31	0	0
A	22	D 93	—	0.31	0.36	0	0
C	4	Pettman	0.9	0.34	0.36	0	0
C	20	Niel	7.0	0.30	0.25	0	0
C	—	5370	3.9	—	—	—	—
<i>Streptococci of viridans type</i>							
	1		0.5	0.31	—	—	0
	2		0.5	0.30	0.35	0	0
	3		0.75	0.30	0.30	0	0
	4		0.77	0.28	0.23	0	0
	5		0.82	0.30	—	0	0
	6		0.77	0.31	0.32	0	0
	7		—	—	0.26	0	0

0 = no increase detected in glucosamine content of supernatant compared with the uninoculated medium.

the cells of both capsulated and non-capsulated organisms is remarkably constant. This suggests that the substance or substances which contain glucosamine and are closely associated with the cells are altogether different from the capsular substance and may possibly form some essential building material of the cell rather than part of an extracellular polysaccharide or of a metabolic intermediate. The

The reproducibility of the figures given for the rates of utilization of *N*-acetylglucosamine was examined by testing one strain (5370) on twelve separate occasions with freshly prepared suspensions. The average rate (given in Table 1) was 3.9 and average variation of the twelve results $\pm 19.5\%$. These figures include two extreme values of 4.9 and 2.5. Other strains were also examined in a smaller

number of experiments and the results showed a similar degree of variation.

The results incidently suggest that the loss of capsules in old cultures of the haemolytic streptococci is due to their solution in the culture media and not to the formation of enzymes which hydrolyse them. If the latter were the case the liberated hexosamine would have been utilized and none found in the acid-hydrolysed culture supernatants.

The end products from N-acetylglucosamine

The formation of an acid product and of NH₃ from N-acetylglucosamine was observed in earlier experiments; qualitative tests showed that lactic acid was present. The same end products were also formed under anaerobic conditions. Estimations were therefore made of these two substances and of volatile acids, since it seemed likely that acetic acid might arise as a result of deacetylation of the amino group. The techniques used for the preparation of the cell suspensions and for following the metabolism of the amino sugar were those described above. The incubation period was extended until no further acid was formed, the pH being maintained at 7.2 as before.

Table 2. End products from N-acetylglucosamine and from glucose by non-proliferating suspensions of haemolytic and viridans streptococci

(NAG = N-acetylglucosamine; G = glucose; 0 = none detected; — = not examined.)

Substrate...	Mol./mol. sugar					
	NH ₃		Lactic acid		Acetic acid	
	NAG	G	NAG	G	NAG	G
<i>Strep. haemolyticus</i>						
Niel	0.97	0	1.60	1.57	—	—
283T	0.97	—	1.61	1.70	1.04	0
23N	1.00	—	1.71	1.65	1.00	0
P315	0.99	0	1.70	1.68	1.06	0
5370	1.00	—	1.60	—	1.05	—
<i>Streptococci of viridans type</i>						
3	0.96	0	1.12	1.30	1.40	—
4	0.98	0	1.33	1.36	—	—
5	0.92	0	1.44	1.42	1.10	—
6	0.94	0	1.20	1.34	1.04	—

Table 2 shows a selection of the results obtained. All the organisms yielded approximately 1 mol. of ammonia, 1 equivalent of volatile acid and from 1.2 to 1.7 mol. of lactic acid/mol. sugar. One of the viridans strains was anomalous in forming about 1.4 equivalent of volatile acid and only 1.1 mol. of lactic acid. The amount of lactic acid formed from glucose was examined in parallel experiments; as can be seen in the Table, the results are closely similar to those obtained using N-acetylglucosamine, and agree with those already reported in the literature for the metabolism of glucose by streptococci (Smith & Sherman, 1941; Hewitt, 1932).

Carbon dioxide formation from N-acetylglucosamine was measured for two strains only; 0.85 and 0.9 mol. were formed from 1 mol. of substrate. In these two examples 87 and 90 % of the carbon were recovered. Thus the end products formed from N-acetylglucosamine appear to be closely similar to those from glucose with the addition of ammonia and acetic acid from the acetamido group of the former compound.

pH optimum for utilization of N-acetylglucosamine

The rates of utilization and deamination of N-acetylglucosamine by *Strep. haemolyticus* 5370 acting at different pH's were examined as follows. A suspension of the organism was prepared in the usual manner with the exception that the final suspending fluid was water and not phosphate buffer; providing such a suspension was used immediately no detectable inactivation took place though on storage the pH fell rather rapidly and the enzyme systems were inactivated. The suspension was mixed with equal volumes of 0.2 M-phosphate buffers at the appropriate pH values which were checked in the presence of the cells, using the glass electrode. The mixtures were warmed to 37.5° and sufficient 0.1 M-N-acetylglucosamine added to make the final concentration 0.01 M. It was found that the pH drifted in those tubes in which the reaction was proceeding at optimal rate, even in the presence of the stronger buffer. Phenol red was, therefore, added to these tubes, which were within the pH range of 7.0-7.5, and the acid formed neutralized with 0.1 N-NaOH. When approximately half the substrate had been used in the optimal pH range the reaction was stopped by the addition of N-HCl and analyses carried out as usual on neutralized supernatant fluids from the centrifuged suspensions.

Fig. 1 shows that the optimum pH for both the rates of deamination and utilization as assessed by

Fig. 1. Influence of pH on the metabolism of N-acetylglucosamine by *Strep. haemolyticus* strain 5370 acting in the presence of 0.1 M-phosphate at 37.5°; —●— % N-acetylglucosamine utilized; —×— % of theoretical NH₃ formed.

the usual methods is between 7.0 and 7.5. During these experiments attempts were made to use buffers other than phosphate, such, for example, as veronal (Michaelis, 1930) or acetate. The rates of both utilization and deamination, however, were about

20–30 % lower than in the presence of phosphate at the same pH. This reduction was due neither to depression by the buffers used nor to absence of K^+ ion, since the addition of phosphate but not of potassium chloride restored the rate of reaction. It is taken as preliminary evidence that phosphorylation cycles are involved in the metabolism of the hexosamine.

The optimum pH is similar to that of 7.4–7.8 recorded by Lutwak-Mann (1941) for the deamination of glucosamine by animal tissue preparations. The streptococci apparently remain active at a slightly lower pH than the tissues, which were inactive at pH 6.0.

The relative rates of utilization, deamination and lactic acid formation

Lutwak-Mann (1941) found that oxidation of glucosamine preceded deamination, and that utilization and deamination were not equivalent. If, in the metabolism of *N*-acetylglucosamine by streptococci, oxidation at carbon atom 1 or 2 precedes deamination, *N*-acetylglucosamine would disappear without the appearance of an equivalent amount of ammonia or lactic acid, for the oxidized molecule would give no colour in the usual estimation (cf. Morgan, 1936; White, 1940). To examine this possibility a suspension of *Strep. haemolyticus* 5370 was prepared in the usual manner and mixed with an equal volume of 0.02M-*N*-acetylglucosamine. Both the suspension and the solution were first warmed to 37.5°. Samples (2.0 ml.) were then taken at the times indicated in Fig. 2 and added to 0.2 ml. of *N*-hydrochloric acid, centrifuged immediately and the supernatant fluids neutralized. The pH of the mixture was maintained at 7.2 throughout the incubation by addition of 0.1N-sodium hydroxide. Phenol red was present as internal indicator.

Fig. 2 shows that within the experimental error there is no lag in the liberation of ammonia or in the formation of lactic acid; the small difference between the curves was not a constant feature of the reaction and did not amount to more than 10 % at any point. Thus it seems unlikely that changes involving carbon atoms one or two in the *N*-acetylglucosamine structure appreciably precede liberation of the amino group. In other experiments the rate of disappearance of *N*-acetylglucosamine was correlated with that of total glucosamine remaining after acid hydrolysis and with that of reducing sugar. Agreement was again within experimental error.

These results suggest that any intermediate step between the unmodified *N*-acetylglucosamine and the removal of the amino group is not one which is a major factor in determining the overall rate. There is no evidence for the accumulation of an amino compound. Lutwak-Mann's (1941) results for tissue-slice preparations are in striking contrast. For

example, kidney and testis liberated only 20–80 % of the theoretical amount of ammonia from glucosamine irrespective of the incubation time. It seems unlikely that results for the acetylated compound would have been different since the deamination of *N*-acetylglucosamine by the tissue slices was slower than that of glucosamine itself.

Fig. 2. Rates of utilization, deamination and lactate formation from *N*-acetylglucosamine using washed suspensions of *Strep. haemolyticus* strain 5370 acting at pH 7.2 in 0.033M-phosphate at 37.5°; —●— μmol . *N*-acetylglucosamine utilized; —×— μmol . NH_3 formed; —△— 0.5 μmol . lactate formed.

Inhibitors of the reaction

The influence of various known inhibitors of metabolism, on the rates of utilization and deamination of *N*-acetylglucosamine and on the rate of lactate formation was examined, using *Strep. haemolyticus* 5370 as test organism. Samples of the cell suspensions, prepared in the usual way, were warmed to 37.5° and mixed with sufficient of the inhibitors to give the concentrations indicated in Table 3 after the addition of substrate. An equal volume of warm 0.02M-*N*-acetylglucosamine was then added. The pH was maintained at 7.2 throughout the experiment by the addition of 0.1N-NaOH using phenol red as internal indicator. Incubation was continued until about 50% of the substrate had been used in the mixtures containing no inhibitor and the reaction was then stopped by the addition of *N*-HCl. Analyses were carried out on the neutralized supernatants as before.

All three stages of the reaction are inhibited by fluoride, copper ions, capryl alcohol, toluene, anisaldehyde, ketone fixatives, citrate and to a slight extent by dimedone (Table 3). Some of these substances appear to inhibit the rate of utilization rather less than they do the rate of either deamination or of lactate formation. The differences, however, are not very large and were not constant from experiment to experiment. Their significance is, therefore, doubtful. The inhibition by citrate was particularly interesting because it was later shown that glucose

also inhibited. Any possibility, however, that the mechanism of citrate action involved the four-carbon acid cycle, was excluded by the negative results obtained with fumarate, malate and malonate; moreover, citrate was effective under anaerobic conditions. The inhibition was completely reversed by the addition of 0.05 M-magnesium chloride and therefore seems to be due to removal of either Mg^{++} or Ca^{++} ions.

Table 3. Influence of inhibitors on utilization, deamination and lactate formation from N-acetylglucosamine by suspensions of *Strep. haemolyticus* strain 5370

Inhibitor	Concentration (M)	Inhibition (%)		
		Utilization	Deamination	Lactate formation
NaF	0.02	83	88.5	97.0
	0.01	83	81.8	87.0
	0.001	0	17.2	12.0
Citrate	0.04	50.5	70.0	53.8
	0.02	57.9	67.8	60.5
KCN	0.01	16.8	17.5	14.6
$NaHSO_3$	0.25	48.4	64.9	77
	0.12	27.9	43.2	52.8
	0.05	5.9	32.0	33.2
$NH_4OH.HCl$	0.01	42.2	63.2	72.0
	0.002	27.9	18.2	22.9
	0.001	17.3	0-10	5.0
Dimedone	0.02	0-10	21.6	—
	0.01	0-10	26.2	—
	0.005	0-10	17.3	—
Capryl alcohol	Saturated solution	22.2	40.4	37.9
*Toluene	Saturated solution (a)	24.3	43.6	20
	Saturated solution (b)	100	100	100
	Saturated solution (c)	100	100	100
Anisaldehyde	Saturated solution	87.0	93.0	—
Cu^{++} as $CuSO_4.5H_2O$	0.0015	22.2	17.3	39.0
	0.00015	0	4	6.0

* (a) = Toluene added to cell suspension and gently shaken immediately before experiment. (b) = Toluene and cell suspension incubated together for 24 hr. at normal temperature. (c) = As for (b) but incubation at 37°.

The degree of inhibition by bisulphite varied from batch to batch of organisms. Whereas for one batch of cells 0.02 M-sodium hydrogen sulphite inhibited deamination by 50-60 %, with another the inhibition by a strength of 0.05 M was only 30-35 %. The relative efficiency of the three fixatives, bisulphite, hydroxylamine and dimedone, is also of interest and the results might most easily be interpreted by supposing that primarily they inhibit endocellular

reactions by combining with aldehyde or ketone prosthetic groups, rather than with metabolic intermediates free in the fluid. The comparative ineffectiveness of dimedone would then be explicable because cells might be expected to be less permeable to the large molecule of this substance.

The influence of glucose on the reaction

Concentrations of glucose from 0.05 to 0.2 M inhibit deamination of glucosamine by 50-70 % using tissue preparations (Lutwak-Mann, 1941). The influence of glucose upon the utilization and deamination of N-acetylglucosamine by streptococci was examined as follows. Cell suspensions of two haemolytic streptococci (Niel and 5370) were prepared and samples (1.0 ml.) distributed in tubes. Water was added to give a volume of 1.8 ml. after the addition of M-glucose (final glucose concentrations 0.2, 0.1, 0.05 and 0.02 M). The diluted cell suspensions were warmed to 37°, and the glucose solution added, followed immediately by N-acetylglucosamine to 0.01 M. The pH was maintained during subsequent incubation by the addition of 0.1 N-NaOH, with phenol red as indicator. Reaction was stopped by N-HCl when 50 % of the full amount of acid had been produced in a tube without glucose and N-acetylglucosamine and NH_3 were determined.

Table 4 shows that concentrations of glucose from 0.05 to 0.2 M inhibit both the utilization and the deamination of N-acetylglucosamine. The degree of

Table 4. The inhibition of utilization and deamination of N-acetylglucosamine by glucose, using washed suspensions of two strains of *Strep. haemolyticus*

Glucose concentration (M)	Inhibition (%)			
	Utilization		Deamination	
	Strain Niel	Strain 5370	Strain Niel	Strain 5370
0.2	79.0	—	83.8	93.5
0.1	79.5	75.9	67.5	71.2
0.05	54.0	49.6	50.2	62.2
0.02	29.6	4	23.6	5.5

inhibition is similar to that recorded by Lutwak-Mann (1941) for tissues acting upon glucosamine. One of the interesting results obtained with tissues was that the end products formed from glucose by testicular preparations also inhibited the deamination of the amino sugar. Streptococci, however, were not inhibited by the neutralized products formed from glucose; lactate itself was equally ineffective.

Relative rates of acid formation from glucose and N-acetylglucosamine

The rates, in two strains of *Strep. haemolyticus*, were measured by the amounts of 0.1 N-NaOH necessary to maintain a pH of 7.2. The technique of preparing the cell suspensions and of conducting the

reactions was as described for earlier experiments. The concentration of both substrates at the beginning of the experiment was 0.01 M. Fig. 3 shows that the rate of acid formation from glucose is very much faster than from *N*-acetylglucosamine. There is again no evidence of lag using *N*-acetylglucosamine.

Fig. 3. Relative rates of acid formation from *N*-acetylglucosamine and glucose by washed suspensions of two strains of *Strep. haemolyticus* (Niel and H 713) acting at pH 7.2 in 0.033 M-phosphate at 37.5°; —●— acid formation from glucose; —○— acid formation from *N*-acetylglucosamine.

The initial rates of the reaction were measured for nine strains of *Strep. haemolyticus* and seven of the *viridans* organisms. The rate of acid formation from glucose was always found to be faster than from the amino sugar, the ratios of the initial rates being from 2 to 20 for the former group of organisms and from 2 to 9 for the latter.

DISCUSSION

The end products formed by streptococci acting upon *N*-acetylglucosamine differ from those formed from glucose, by the additional presence of ammonia and volatile acid, but the amount of lactic acid is closely similar for the two substrates. The question is at what point or points in the fermentation is the acetamido group decomposed to ammonia and free acetic acid. One possible scheme for the breakdown of *N*-acetylglucosamine which would account for the end products obtained is deacetylation followed by hydrolysis of the amino group. The remaining glucose could then be fermented by the usual processes. The effect of inhibitors upon the reaction, however, does not favour this hypothesis, as the substances examined inhibited the formation of lactate and ammonia to about the same extent. Thus it seems that glycolysis and deamination are closely related. Otherwise we must assume that the sensitivity of the 'glucosamine deaminase' is exactly

the same as that of the various glycolytic enzymes, since inhibitors as widely apart in their mechanism of action as fluorides or citrate (which inhibit by removal of Mg^{++}), ketone and aldehyde fixatives such as bisulphite, iodoacetate (which effects enzymes depending upon thiol groups), and glucose (which presumably inhibits competitively) all influence deamination and lactate formation to about the same extent.

The way in which the glycolytic and deamination reactions are interrelated cannot be decided from the results so far obtained. The most likely hypothesis, however, on the present results and those obtained by Lutwak-Mann (1941) for mammalian tissue-slice preparations would seem to be that partial breakdown of the glucopyranose structure occurs before the amino group is liberated as ammonia. Although no evidence for the formation of intermediary amino compounds was obtained in the present work—the somewhat greater degree of inhibition of deamination as compared with utilization obtained using some inhibitors being too small and irregular to use as evidence—the observations of Lutwak-Mann (1941) that only 20–60% of the theoretical amounts of ammonia are liberated using testicular preparations could be explained in this way. No lactic acid is formed by this tissue, although the amino group remains free throughout the reaction. It appears that here some acid amino compound must be formed, and that the testicular preparations lack the enzyme systems, which can convert it to lactic acid. The deaminase in streptococci must be very active, assuming intermediate amino compounds are formed, since there is no lag between the disappearance of the hexosamine and the appearance of ammonia. Whatever reactions are pace-setting, they must leave sufficient of the *N*-acetylglucosamine molecule intact to give the colour reaction.

The other possible type of interrelation between glycolysis and deamination is that, as with glutamine (McIlwain, 1946), fermentation must be proceeding before deamination can take place. The inhibitory properties of glucose, however, do not support the idea. Using whole cells, however, permeability factors complicate the interpretation of results, and the problem would be better reserved for study with cell-free enzymes. The possibility that acid amino compounds are formed during the breakdown of hexosamines suggests that an amino-acid may be formed. Streptococci, however, have very limited potentialities in breaking down these substances. Hills (1940) examined washed suspensions of four strains of *Strep. haemolyticus*, Groups A and B, and one of *Strep. viridans*. He found that of the common amino-acids only arginine was deaminated rapidly. Clearly the involvement of arginine itself as an intermediate in the breakdown of *N*-acetylglucosamine is impossible from both structural con-

siderations and the nature of the end products; all the ammonia was liberated from the hexosamines, whereas two amino groups would remain fixed in ornithine if arginine were an intermediate. Nevertheless, the extremely poor array of deaminases present in the streptococci renders desirable a further examination of the relations between arginine dihydrolase and the deaminase active in the breakdown of the amino sugars.

SUMMARY

1. The rates at which suspensions of *Streptococcus haemolyticus* and *Strep. viridans* utilize *N*-acetylglucosamine show no obvious correlation with the amount of hexosamine synthesized by cultures.

2. The end products formed by streptococci from 1 mol. of *N*-acetylglucosamine are 1 mol. of ammonia, 1.2–1.7 mol. of lactate and 1 equivalent of volatile acid.

3. The pH optimum for the reaction is 7.2–7.5.

4. The rates of utilization, deamination, and lactate formation are equal.

5. Reagents inhibiting lactate formation also inhibit deamination and the disappearance of *N*-acetylglucosamine.

6. The most likely mechanism for the reaction appears to be the breakdown of the glucopyranose structure with the subsequent deamination of an intermediate amino compound.

The author acknowledges the very considerable technical assistance given by Mr A. Parkinson during this work, and thanks Dr Nuala Crowley for the strains of *Strep. haemolyticus*.

REFERENCES

- Clark, W. M. (1928). *The Determination of Hydrogen Ions*, 3rd ed. London: Baillière, Tindall and Cox.
- Conway, E. J. (1947). *Micro-diffusion Analysis and Volumetric Error*. London: Lockwood and Sons Ltd.
- Elson, L. A. & Morgan, W. T. J. (1933). *Biochem. J.* **27**, 1824.
- Friedemann, T. E., Cotonio, M. & Shaffer, P. A. (1927). *J. biol. Chem.* **73**, 335.
- Hewitt, L. F. (1932). *Biochem. J.* **26**, 208.
- Hills, G. M. (1940). *Biochem. J.* **34**, 1057.
- Kendall, F. E., Heidelberger, M. & Dawson, M. H. (1937). *J. biol. Chem.* **118**, 61.
- Lutwak-Mann, C. (1941). *Biochem. J.* **35**, 610.
- McClean, D. (1941). *J. Path. Bact.* **53**, 13.
- McIlwain, H. (1946). *Biochem. J.* **40**, 67.
- Michaelis, L. (1930). *J. biol. Chem.* **87**, 33.
- Morgan, W. T. J. (1936). *Biochem. J.* **30**, 909.
- Morgan, W. T. J. & Elson, L. A. (1934). *Biochem. J.* **28**, 988.
- Rogers, H. J. (1945). *Biochem. J.* **39**, 435.
- Rogers, H. J. (1946). *Biochem. J.* **40**, 782.
- Rogers, H. J. (1948). *Nature, Lond.*, **161**, 815.
- Rosenberg, A. J. (1948). *C.R. Acad. Sci., Paris*, **226**, 1751.
- Smith, P. A. & Sherman, J. M. (1941). *J. Bact.* **41**, 101.
- Somogyi, M. (1937). *J. biol. Chem.* **117**, 771.
- White, T. (1940). *J. Chem. Soc.* p. 428.
- Winnick, T. (1942). *J. biol. Chem.* **142**, 451.
- Zuckerkindl, F. & Messiner-Klebermass, L. (1931). *Biochem. Z.* **236**, 19.

The Kinetics and Specificities of Deamination of Nitrogenous Compounds by X-radiation

By W. M. DALE, J. V. DAVIES AND C. W. GILBERT
Christie Hospital and Holt Radium Institute, Manchester

(Received 31 January 1949)

In continuation of previous investigations on the specificity of radiation effects (Dale, 1942, 1947; Allsopp, 1944, 1948) we have found that deamination occurs when aqueous solutions of amino-acids are irradiated by X-rays (Dale & Davies, 1949), a fact which was independently observed by Stein & Weiss (1948). The specificity of this reaction and its peculiar kinetics described in this paper are of consequence with respect to the mode of the indirect action of radiation on solutions and probably for the radiobiology of cells. An attempt will be made to interpret the experimental results in terms of the primary formation of radicals from water by X-rays (Weiss,

1944). It is, however, possible that the current concept of radical formation may need modification in view of certain still unexplained facts, for example, the variation of the protective power of certain substances with their concentration (Dale, Davies & Meredith, 1949).

The approach to the problem of the relation between radiation effect and molecular structure of the solute, presented in this paper, is different from that used previously. Whereas then an overall picture of the radiosensitivity of a substance was indirectly obtained by measuring the competition between two solutes for the available radiation