139. *Methylglucosaminide: Its Structure, and the Kinetics of its Hydrolysis by Acids.*

By R. C. G. Moggridge and A. Neuberger.

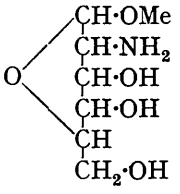
Consideration of the existing views regarding the structure of methylglucosaminide leads to the conclusion that they are inadequately founded. The betaine structure proposed for this compound by Irvine, McNicoll, and Hynd (J., 1911, **99**, 250; 1912, **101**, 1128) was based on (*a*) evolution of methylamine on distillation with alkali and (*b*) abnormal resistance to acid hydrolysis.

In this paper a satisfactory alternative explanation of (*a*) is given; it is further suggested that (*b*) is compatible with a normal glycosidic structure and is to be explained by the effect of the adjacent positively charged amino-group in repelling hydrions from its immediate vicinity.

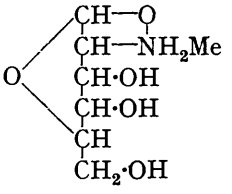
In order to test this hypothesis *N*-acetyl methylglucosaminide has been prepared, and a detailed kinetic study has been made of the comparative rates of acid hydrolysis of this compound and of methylglucosaminide.

The results are in full accord with the explanation suggested, and it is therefore concluded that methylglucosaminide has in fact a normal glycosidic structure.

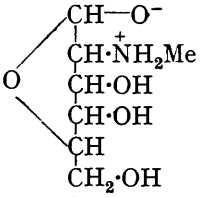
Methylglucosaminide hydrochloride was first prepared by Irvine, McNicoll, and Hynd (*loc. cit.*), who considered that its properties could not be fully explained in terms of the normal glycosidic structure (I), and suggested the presence of a betaine-ring (II). This suggestion was extended both to the structure of the free base, and to mucoproteins (Irvine and Hynd, J., 1913, **103**, 41). [The structure (II) would presumably now be written as a



(I.)



(II.)



(III.)

" zwitterion " (III).] This conclusion seems improbable, and it was thought that a further investigation would be of interest. The chief evidence advanced against the structure (I) was (*a*) the evolution of methylamine on treatment of the compound with alkali, and (*b*) the abnormal difficulty of hydrolysis of the compound by acids, so both these points were reinvestigated.

(*a*) On treatment of the compound with alkali the gases evolved did in fact contain small amounts of methylamine in addition to ammonia, but the conditions necessary for decomposition were extremely drastic, alkaline fusion for 4½ hours at 250° only liberating about half the theoretical amount of alkaline vapours. Less than 10% of the total gaseous nitrogen was in the form of methylamine. Since formaldehyde is produced by the action of alkali on sugars, and methylamine can be formed by the action of formaldehyde on ammonia at high temperature, it seems probable that under the conditions of the experiment methylamine might be formed from a glycoside of structure (I).

(*b*) Methylglucosaminide hydrochloride, as noted by Irvine and Hynd (*loc. cit.*), is hydrolysed much more slowly by acid than is any other known glycoside; but such behaviour can readily be explained by the presence of the charged amino-group in the α-position to the glycoside linkage. Coulomb forces will repel all positively charged ions, and thus decrease considerably the concentration of hydrions in the immediate vicinity of the amino-group, and this will decrease the rate of hydrolysis of the glycoside group, as that reaction is catalysed by hydrions. Analogous cases where reaction velocity is markedly influenced by the introduction of charge are (1) the reaction between thiosulphate and sodium or methyl bromoacetate (LaMer and Kammer, *J. Amer. Chem. Soc.*, 1931, **53**, 2832); (2) the reaction between silver ions and sodium or ethyl bromoacetate (Euler, *Ber.*, 1906, **39**, 2729); (3) the hydrolysis of esters of dicarboxylic acids (Ingold, J., 1935, 1482).

As a first approximation it may be assumed that the velocity constant of the acid-catalysed reaction is proportional to the concentration of hydrogen ions. If k_0 is the constant in the absence of any charge effect, k_1 that of the charged methylglucosaminide ion, and C_0 and C_1 the concentration of hydrogen ions respectively in the bulk of the solution and in the immediate surroundings of the glycoside linkage, then

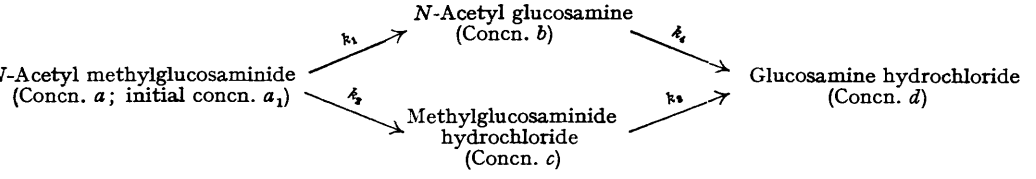
$$k_1 = C_1k_0/C_0 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

Applying the Boltzmann principle, we have, for univalent ions, with k_0 and k_1 extrapolated to zero ionic strength,

$$k_1 = k_0e^{-\varepsilon^2/DrkT} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$

where r is the distance of the glycoside linkage from the unit charge ε. It has already been pointed out (Ingold, J., 1931, 2179; Neuberger, *Proc. Roy. Soc.*, 1937, *A*, **158**, 68) that, owing to the difficulty of obtaining a correct value of the dielectric constant in the immediate surroundings of charged groups, the assumptions involved in the derivation of equation (2) are not correct if r is small, and, by analogy with the results obtained with the dissociation constants of dicarboxylic acids and amino-acids, it is to be expected that the value for r calculated from k_0 and k_1 will be too small.

If this interpretation of the abnormal behaviour of methylglucosaminide hydrochloride on acid hydrolysis is correct, it would be expected that an *N*-acyl methylglucosaminide, which does not carry a charge, should be hydrolysed at a rate comparable with that of ordinary glycosides. N-*Acetyl methylglucosaminide* was prepared to test this conclusion. Its hydrolysis is complicated by the fact that both acetyl and glycoside groups are hydrolysed by acid. We have the following scheme of reactions, all of which should be unimolecular:



In practice it is found that k_3 is negligibly small compared with the other constants. The reaction was followed by measuring (1) the free amino-nitrogen (V), representing $(c + d)$, by the Van Slyke method, and (2) the reducing power (R), representing $(b + d)$, and so the extent of the reaction k_1. Under the conditions used, the reducing powers of equimolecular quantities of glucosamine hydrochloride and *N*-acetyl glucosamine were equivalent to within 0·2%. For the reducing powers we have $dR/dt = k_1a$ and $da/dt = -(k_1 + k_2)a_1$, giving

$$a = a_1e^{-(k_1 + k_2)t} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (3)$$

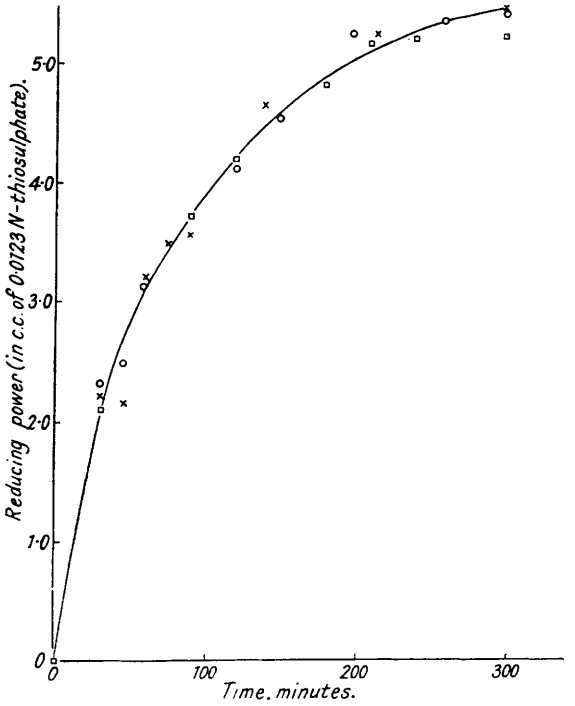
On substituting and integrating, we have

$$R = a_1k_1[1 - e^{-(k_1 + k_2)t}]/(k_1 + k_2) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (4)$$

This was used in the form

$$\frac{(k_1 + k_2)t}{2{\cdot}303} = \log_{10}\frac{a_1k_1}{k_1 + k_2} - \log_{10}\left(\frac{a_1k_1}{k_1 + k_2} - R\right) \quad . \quad . \quad . \quad . \quad (5)$$

An approximate value of $a_1k_1/(k_1 + k_2)$ was obtained from the fact that it represents the apparent end-point of the reaction; t was then plotted against the second logarithm in (5) for various values of $a_1k_1/(k_1 + k_2)$, that value giving the best straight line being adopted. The method was found to be sensitive to variations in $a_1k_1/(k_1 + k_2)$ of less than 1%.



Hydrolysis of N-*acetyl methylglucosaminide with* N-HCl *at* 80°. *Theoretical end-point for complete hydrolysis,* 7·45 *c.c.*

For the Van Slyke estimation we have $V = c + d$, $dV/dt = k_2a + k_4b$; further, by equation (3) we have $db/dt = k_1a - k_4b = k_1a_1e^{-(k_1+k_2)t} - k_4b$, giving

$$b = -a_1k_1[e^{-(k_1+k_2)t} - e^{-k_4t}]/(k_1 + k_2 - k_4)$$

Hence $$dV/dt = k_2a_1e^{-(k_1+k_2)t} - \frac{a_1k_1k_4}{k_1 + k_2 - k_4}[e^{-(k_1+k_2)t} - e^{-k_4t}]$$

and on integration

$$V = a_1\{1 - [k_1e^{-k_4t} + (k_2 - k_4)e^{-(k_1+k_2)t}]/(k_1 + k_2 - k_4)\} \quad . \quad . \quad . \quad (6)$$

This expression was used to check the values of k_1, k_2, and k_4 found by other methods.

N-Acetyl methylglucosaminide was prepared from tetra-acetyl methylglucosaminide; this could not be obtained crystalline from *tetra-acetobromoglucosamine* (from penta-acetyl glucosamine) but was so obtained by direct acetylation of methylglucosaminide hydrochloride, and later from triacetyl methylglucosaminide hydrobromide, as described by Cutler, Haworth, and Peat (J., 1937, 1979), and on treatment with methyl-alcoholic hydrogen chloride it gave the desired *N*-acetyl methylglucosaminide. An alternative and extremely convenient method of preparation is from penta-acetyl glucosamine. The production of the same compound by these two methods is very hard to explain if the methyl group is associated with the nitrogen, as suggested by Irvine and Hynd (*loc. cit.*). If *O*-tetra-acetyl glucosamine hydrochloride (Bergmann and Zervas, *Ber.*, 1931, **64**, 975) is similarly treated with methyl-alcoholic hydrogen chloride, the product is not methyl-glucosaminide hydrochloride, but glucosamine hydrochloride.

Experimental.

Treatment of Methylglucosaminide with Alkali.—Methylglucosaminide hydrochloride, prepared by the method of Irvine, McNicoll, and Hynd (*loc. cit.*), was dissolved in 10N-sodium hydroxide and steam-distilled, but gave a negligible amount of alkaline vapours; 0·39 g. was therefore treated with alkali at 250° in a slow current of nitrogen, the gases being passed into hydrochloric acid. About half the calculated amount of alkaline vapours had come over in 4½ hours. The aqueous solution was concentrated to dryness in a vacuum, and the salts purified by one further distillation of the alkaline gases into acid. An almost colourless solid was obtained (Found: C, 3·1; H, 7·7; N, 25·4. Calc. for 82·5% of NH_4Cl + 17·5% of NH_3MeCl: C, 3·1; H, 7·7%; N, 25·2%).

Tetra-acetobromoglucosamine.—Penta-acetyl glucosamine—either the crystalline compound (Bergmann and Zervas, *loc. cit.*) or the dry syrup obtained by direct acetylation of glucosamine hydrobromide (10 g.) by means of acetic anhydride and sodium acetate—was dissolved with shaking at room temperature in a solution of hydrogen bromide (15 g.) in glacial acetic acid (30 g.), and left overnight. Chloroform was added, the solution extracted with bicarbonate solution, dried, concentrated to low bulk, and allowed to evaporate spontaneously. *Tetra-acetobromoglucosamine* (4·9 g.) was deposited as colourless needles, and was recrystallised from glacial acetic acid, $[\alpha]_D$ + 118·2°; it was stable on keeping, but on heating it charred without melting (Found: N, 3·35; Br, 19·7. $C_{14}H_{20}O_8NBr$ requires N, 3·4; Br, 19·5%). Attempts to replace bromine by methoxyl in methyl-alcoholic solution, with pyridine, silver oxide, and silver carbonate as condensing agents, led to uncrystallisable, bromine-free syrups.

Tetra-acetyl Methylglucosaminide.—To methylglucosaminide hydrochloride (0·25 g.), suspended in pyridine (7 c.c.), acetic anhydride (7 c.c.) was added slowly at 0°. The clear solution was left in the ice-box overnight, poured into ice-water, and the product crystallised by extraction with chloroform and concentration. From alcohol it formed colourless needles, m. p. 160° (Found: N, 3·8. Calc.: N, 3·9%). A mixed m. p. showed identity with tetra-acetyl methylglucosaminide prepared as described by Cutler, Haworth, and Peat (*loc. cit.*).

N-*Acetyl Methylglucosaminide.*—(a) *From tetra-acetyl methylglucosaminide.* The tetra-acetyl compound (1·25 g.) was refluxed for 2 hours with dry methyl alcohol (30 c.c.) containing hydrogen chloride (2·2% by weight), and the solution then shaken with an excess of lead carbonate, filtered, and concentrated. N-*Acetyl methylglucosaminide* crystallised on standing (yield of once recrystallised material 45%). After three recrystallisations from alcohol it was obtained as colourless needles, m. p. 189°, $[\alpha]_D$ = + 105° (Found: N, 6·05. $C_9H_{17}O_6N$ requires N, 5·95%).

(b) *From penta-acetyl glucosamine.* Glucosamine hydrochloride (2·5 g.) was acetylated in the usual manner by treatment with acetic anhydride and sodium acetate; the dry syrupy product was refluxed with methyl-alcoholic hydrogen chloride (2·2% solution), and the product worked up as above. *N*-Acetyl methylglucosaminide (76% overall yield) crystallised at once; on recrystallisation it was obtained with m. p. 189°, and shown by mixed m. p. to be identical with the substance obtained as in (a).

Action of Methyl-alcoholic Hydrogen Chloride on O-Tetra-acetyl Glucosamine Hydrochloride.—This compound (3·0 g.) was refluxed with methyl-alcoholic hydrogen chloride (2·2% solution). After about ½ hour, glucosamine hydrochloride (1·5 g.) was deposited as colourless plates (Found: N, 6·55. Calc.: N, 6·5%).

Kinetic Measurements.—2-C.c. portions of a solution of the substance under investigation in hydrochloric acid of known strength were sealed into small test-tubes, and suspended in the vapour of a boiling liquid. The tubes were taken out at measured intervals of time, cooled in ice and their contents then estimated either in the Van Slyke apparatus, or by Hanes's modification of the Hagedorn–Jensen method (*Biochem. J.*, 1929, **23**, 99); in the latter case the acid was neutralised before the estimation. The amounts of thiosulphate needed were found to be strictly proportional to the amounts of substance present, being, in c.c. of N/100-solution per mg. of substance:

	No acid added.	N-HCl experiment.	2·5N-HCl experiment.
For glucosamine hydrochloride	2·60	2·70	3·14
For *N*-acetyl glucosamine	2·48	2·62	—

Constants were calculated by Guggenheim's method (*Phil. Mag.*, 1926, **2**, 540), applied to a smooth curve drawn through the experimental points. It is believed that most of the constants for methylglucosaminide hydrochloride are accurate to well within 10%. Blank experiments

showed that there was no appreciable destruction of glucosamine hydrochloride on heating with 2·5N-acid for 65 hours at 100°.

TABLE I.

Expt. No.	I.		II.		III.		IV.		V.		VI.	
	t.	*R.*	*t.*	*R.*	*t.*	*R.*	*t.*	*R.*	*t.*	*R.*	*t.*	*R.*
	1	0·24	1	0·43	24	0·82	2	0·17	0·5	0·78	2	0·70
	3	1·30	2	0·91	48	1·51	4	0·24	1·0	1·60	4	1·22
	5	1·93	3	1·17	72	2·43	6	0·28	1·5	2·37	6	2·00
	7	2·45	4	1·60	81	2·89	10	0·75	2·0	2·68	8·5	2·48
	8	2·70	5	1·93	95	3·02	24	1·26	2·5	3·50	10	2·68
	8·5	2·95	6	2·29	149	4·33	35	1·94	3	3·94	12	2·76
	9	2·94	8	2·71			52	2·26	4	4·40	22	3·95
	10	3·10	11	3·64			72	2·47	5	4·90	25	4·15
	11	3·40							6	5·33	29	4·59
Theoretical end-points		7·30		7·30		7·30		6·25		7·30		8·55

Hydrolysis of methylglucosaminide hydrochloride. The data are in Table I : t = time in hours; R = equivalent of reducing sugar in c.c. of 0·01233N-thiosulphate; 3·02 mg. of substance used in each reading in experiments I—V, and 4·12 mg. in experiment VI.

Expts. I & II (duplicate) : 2·5N-acid at 100°. $k = 2{\cdot}08 \times 10^{-5}$ (sec.$^{-1}$).
Expt. III : 2·5N-Acid at 80° (benzene). $k = 1{\cdot}585 \times 10^{-6}$.
,, IV : N-Acid at 100°. $k = 4{\cdot}05 \times 10^{-6}$.
,, V : 2·5N-Acid at 110·8° (fractionated toluene). $k = 7{\cdot}95 \times 10^{-5}$.
,, VI : N-Acid at 110·8°. $k = 1{\cdot}83 \times 10^{-5}$.

TABLE II.

Expt. No.	VII.		VIII.		IX.		X.		XI.		XII.		XIII.		
	t.	*R.*	*t.*	*R.*	*t.*	*R.*	*t.*	*R.*	*t.*	*R.*	*t.*	*V.*	*t.*	*V.*	*V'.*
	30	2·12	30	2·23	30	2·33	15	3·62	2	1·16	1	0·72	1	0·46	0·28
	90	3·71	45	2·17	45	2·50	19	3·78	4	1·94	2	1·16	2	0·82	0·59
	120	4·20	60	3·22	60	3·13	23	3·90	8	3·00	2·5	1·22	2·5	0·93	0·75
	180	4·83	75	3·50	120	4·13	39	4·35	25	4·20	3·0	1·31	3·0	1·01	0·87
	210	5·18	90	3·58	150	4·57	42	4·36	30	4·34	3·5	1·46	3·5	1·12	0·99
	240	5·22	140	4·79	200	5·27	45	4·50	48	4·48	4·5	1·59	4·5	1·28	1·16
	300	5·24	215	5·25	260	5·37	63	4·45	54	4·60			5·5	1·35	1·32
			300	5·47	300	5·43	65	4·45							
							67	4·53							
Theoretical end-points	7·45		7·45		7·45		7·45		7·45		1·94		1·61	1·61	

Hydrolysis of N-*acetyl methylglucosaminide.* 3·65 Mg. of substance per reading (R as before).

Expts. VII, VIII, and IX (triplicate; see fig.) : N-acid at 80° (benzene); t in minutes.
k_1 (glycoside hydrolysis) $= 1{\cdot}21 \times 10^{-4}$; k_2 (acetyl hydrolysis) $= 3{\cdot}55 \times 10^{-5}$.
Expts. X and XI (duplicate) : N-acid at 61·25° (chloroform); t in hours.

$$k_1 = 1{\cdot}25 \times 10^{-5}; k_2 = 8 \times 10^{-6}.$$

Van Slyke estimations. (i) On *N*-acetyl glucosamine (Expt. XII).—30·6 Mg. per reading; t in hours; V in mg. of amino-nitrogen; N-acid at 80°. $k_4 = 1{\cdot}3 \times 10^{-4}$.
(ii) On *N*-acetyl methylglucosaminide (Expt. XIII).—27 Mg. per reading; t in hours; V in mg. of amino-nitrogen; N-acid at 80°. V' = readings as calculated from equation (6) with $k_1 = 1{\cdot}2 \times 10^{-4}$, $k_2 = 3{\cdot}7 \times 10^{-5}$, $k_4 = 1{\cdot}3 \times 10^{-4}$. The agreement between V and V' is as good as could be expected.

DISCUSSION OF RESULTS.

Tables I and II show that methylglucosaminide hydrochloride is hydrolysed very much more slowly by acids than is N-acetyl methylglucosaminide, the glycoside linkage of the latter being, as is expected, split at a rate comparable with that of ordinary pyranosides. The figure shows the characteristic curve to be expected for the hydrolysis of N-acetyl methylglucosaminide, the rate of formation of reducing sugar being initially rapid, but

becoming slower as the acetyl group is hydrolysed off to give the relatively very stable methylglucosaminide hydrochloride.

Values for the energies of activation can be calculated from the constants at different temperatures. For methylglucosaminide hydrochloride we have three independent readings; from Expts. I, II, and V, $E = 35{,}200$ cals.; from I, II and III, $E = 36{,}300$ cals.; from IV and VI, $E = 39{,}600$ cals. The accuracy in the particular case of Expt. IV is probably not more than 15% and this error is more than enough to account for the deviation in the last value of E. Probably the best value is about 36,000 cals.

For *N*-acetyl methylglucosaminide we have E (glycoside hydrolysis) = 28,400 cals., E (acetyl hydrolysis) = 19,200 cals. Most simple pyranosides have energies of activation of 28,000—34,000 cals.; these values then provide some confirmation, though of course no proof, for the assumption generally made that these glucosamine derivatives have a pyranoside ring structure. The energy of activation for the hydrolysis of methylglucosaminide is only a few thousand calories higher than for its uncharged analogues; so the main effect of the charge on the equation $k = PZe^{-E/RT}$ is to increase the PZ term.

All the constants refer to systems of high ionic strength; the collision between hydrions and the positively charged methylglucosaminide ions leads to a doubly charged complex, and the salt effect is therefore expected to be much larger for this reaction than for the hydrolysis of glycosides in general. The existence of a large salt effect is shown by the fact that at a given temperature the values of the hydrolysis constants are not proportional to the hydrion concentrations; a 2·5-fold increase of acid strength causes a nearly 5-fold increase of constant. For strict quantitative comparison the constants should be extrapolated to zero ionic strength; this, however, proved impossible owing to the stability of the glycoside linkage in methylglucosaminide hydrochloride, the hydrolysis being impracticably slow at acid concentrations of less than N, even at 110°. A further difficulty is that the configuration of methylglucosaminide hydrochloride is unknown, though from the work of Cutler, Haworth, and Peat (*loc.cit.*) it seems probable that the *N*-acetyl methylglucosaminide has been converted into the α-form by the use of methyl-alcoholic hydrogen chloride.

These two factors make a quantitative comparison of the hydrolysis constants of the charged and uncharged glycosides difficult; neither, however, would be expected to be large enough appreciably to affect the order of magnitude of the ratio of constants. If it is assumed that the variation of the constant with acid concentration is independent of the temperature, a constant of approximately $3{\cdot}5 \times 10^{-7}$ is found for methylglucosaminide in N-acid at 80°. Under these conditions the constants for *N*-acetyl methylglucosaminide, α- and β-methylglucosides (Moelwyn-Hughes, *Trans. Faraday Soc.*, 1929, **25**, 503) are approximately 350, 100, and 180 times higher respectively. Now at 80°, using the values of Wyman (*Physical Rev.*, 1930, **35**, 623) for the dielectric constant of water, equation (2) becomes $k_0/k_1 = 3{\cdot}39/r$, r being the distance (in A.) between the charge and the glycoside linkage. Substituting for k_0/k_1 the constant-ratios given above, the values of 1·3, 1·7, and 1·5 A. are obtained. These distances are certainly of the right order of magnitude.

It appears therefore that the conception of inhibition of acid catalysis by the charged amino-group provides an adequate explanation of the abnormal stability of methylglucosaminide towards acids. It can also explain (1) the fact that glucosamine alone, of all monosaccharides, is not converted into a glycoside by treatment with methyl-alcoholic hydrogen chloride; (2) the contrast between the behaviours of penta-acetyl glucosamine and *O*-tetra-acetyl glucosamine hydrochloride on treatment with methyl-alcoholic hydrogen chloride—the former gives *N*-acetyl methylglucosaminide, whereas the latter gives glucosamine hydrochloride, no glycoside formation having taken place.

The authors thank Professor C. R. Harington, F.R.S., for his help and advice. Acknowledgment is also made to the Beit Memorial Trustees for a Fellowship held by one them (A. N.).

UNIVERSITY COLLEGE HOSPITAL MEDICAL SCHOOL,
LONDON, W.C. 1.

[*Received, March 16th*, 1938.]