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N-Iodoacetyl-D-Glucosamine, an Inhibitor of Growth and Glycoside Uptake in *Escherichia coli*

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1. Synthesis of *N*-iodoacetyl-D-glucosamine and its *N*-iodo[1,2-$^{14}C_2$]acetyl form has been achieved from the tetra-*O*-acetyl amino sugar and iodoacetic acid in the presence of dicyclohexylcarbodi-imide followed by catalytic deacetylation. 2. *N*-Iodoacetylglucosamine (up to 0.1mM) linearly inhibits uptake (up to 1min) of methyl α-D-glucoside by *Escherichia coli* ML308 and K12. Uptake of methyl β-D-thiogalactoside and glycerol is also inhibited. 3. Growth of the organism (strain ML308) on glucose, succinate and glycerol is strongly inhibited by the iodoacetyl compound. The inhibition is relieved by *N*-acetylglucosamine. 4. The inhibitor has multiple effects, some of which are considered to be intracellular. 5. A separate transport pathway exists for *N*-acetylglucosamine by means of which the iodoacetyl analogue may enter the cell.

The identification of the molecular structures involved in specific uptake of metabolites across membranes of living cells is a matter of obvious biological importance. According to a widely held hypothesis, the active transport mechanism across a biological membrane consists of at least two components, namely a substrate-specific component or 'carrier' which facilitates the operation or initiation of selective changes across the membrane and an associated mechanism which makes metabolic energy available for the concentration process (see, e.g., Winkler & Wilson, 1966). The possibility cannot be disregarded that both functions may reside in one molecular complex. Evidence indicates, however, that other selective processes operate also on the efflux of metabolites from erythrocytes and bacteria, and a satisfactory molecular mechanism of transport will have to account for these facts also.

Investigations by Fox & Kennedy (1965), Carter, Fox & Kennedy (1968) and Kennedy & Scarborough (1967) have been concerned with the identification of a cell-wall membrane component (M protein) in *Escherichia coli* having two distinct binding sites for sugars. By an ingenious procedure of isotopic double-labelling, the M protein was found to differ quantitatively in cells having the constitutive β-galactosidase-transport system and those mutants where it was absent. It is thus a product of the *y* gene of the *lac* operon. It was shown that one of the sugar-binding sites involves a thiol group, which could be protected by binding of certain carbohydrate substrates, e.g. melibiose and thiodigalactose (β-D-galactosyl-1-thio-β-D-galactopyranose). Kundig, Ghosh & Roseman (1964) and Simoni *et al.* (1967) have investigated the bacterial phosphotransferase system (which is closely related to carbohydrate permeases) in *E. coli*, *Aerobacter aerogenes* and *Salmonella typhimurium*. Two enzymes (I and II) have been isolated and characterized. One is concerned with the phosphorylation of a low-molecular-weight protein (HPr) by phosphoenolpyruvate. The second enzyme, which is membrane-bound and possesses a high degree of substrate-specificity, catalyses the transfer of phosphate from phospho-HPr to a carbohydrate acceptor. This is consistent with the observation that sugars accumulate solely or primarily within cells as phosphate esters. Kolber & Stein (1966, 1967) have described protein components of membranes from *E. coli* involved in transport and associated with the *lac* operon. In *Staphylococcus aureus* phosphorylation of some sugars only occurs in the presence of further inducible proteins (factor III) (Simoni, Smith & Roseman, 1968).

The role of specific phosphotransferase systems in the uptake of glycosides has been investigated by Tanaka & Lin (1967) and Tanaka, Fraenkel & Lin (1967) in *E. coli* and *A. aerogenes*.

The present work arises from a survey being made in this laboratory of biochemically active carbohydrate analogues (Kent, 1969). During these investigations a number of chemically reactive halo

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sugars were synthesized (see, e.g., Kent, Wood & Welch, 1964) in which the $-CO\cdot CH_2X$ group is present, where X is Cl, Br or I. Compounds of this class can react with a number of protein side groups and in particular they react with thiol groups at various rates, reflecting in part the diastereoisomeric character of reaction intermediates conferred by the conformation of the parent sugar structure. INAG* is a particularly interesting member, since the pyranose ring presents a conformation in a biochemical situation identical with or closely similar to that of glucose itself while the iodoacetamido side chain confers reactivity akin to the well-known behaviour of iodoacetamide. Reactive monosaccharide derivatives of this type may yield useful information about active sites in protein structures specific for the glucose conformation, especially where thiol, histidyl or other groups susceptible to alkylation are present. In particular this potential stereospecific alkylating agent offers promise as a possible direct approach to identifying sites involved in active transport of sugars in bacteria and other systems such as intestinal mucosa. Some studies have been made of stereospecific alkylation of proteins by using D(+) and L(−) isomers of 2-iodopropionic acid and their amides (Wallenfels & Eisele, 1968). In spite of the low order of conformational distinctiveness as compared with carbohydrate cyclic structures, marked differences were found in the rates of reaction of isomers in protein alkylation. Earlier Friedman & Rutenberg (1950), investigating the effect of *N*-iodoacetyl derivatives of L-tyrosine, L-phenylalanine, L-tryptophan and L-leucine on growth of transplanted sarcomas in mice, reported finding some activity in this respect not quantitatively related to the systematic toxicity of the compounds.

MATERIALS AND METHODS

Paper chromatography. This was performed by downward elution in solvent A (butan-1-ol–ethanol–water, 4:1:5, by vol.) on Whatman no. 1 paper, and in solvent B (butan-1-ol–ethanol–water, 13:8:4, by vol.) on Whatman 3MM paper.

N-Halogenoacyl-amino sugar derivatives were detected with alkaline acetylacetone and *p*-dimethylaminobenzaldehyde (Partridge, 1948), and free amino sugars were detected with $AgNO_3$ (Trevelyan, Procter & Harrison, 1950).

Optical rotation. This was determined on a Bendix Ericsson automatic polarimeter type 143A with the results displayed on a Sunvic potentiometric recorder (full-scale deflexion 10mV equivalent to angular rotation of 0.0115° at the sodium D line).

Tetra-O-acetyl-N-iodoacetyl-β-D-glucosamine (I). Tetra-*O*-acetyl-β-D-glucosamine (1.7g, 5mmol), prepared by the method of Bergman & Zervas (1931), and dicyclohexylcarbodi-imide (8.8g, 4mmol) were dissolved in a mixture of dry chloroform (12ml) and pyridine (3.4ml). After cooling to 2°C, iodoacetic acid (1.6g, 9mmol) in chloroform (10ml) was added. After 16h at 2°C, the filtered solution, washed successively with water, 2M-HCl and again with water, was dried over $MgSO_4$. Evaporation yielded the solid product (I), which, after recrystallization from ethanol, had m.p.169–170°C, yield 0.93g (37%), $[\alpha]_D^{25}$ +7.2° (*c*1.0 in chloroform), R_F0.83 (solvent A) (Found: C, 37.5; H, 4.0; I, 25.6; N, 3.0; $C_{16}H_{22}INO_{10}$ requires C, 37.3; H, 4.3; I, 24.6; N, 2.7%).

INAG (II). The above tetra-acetate (I) (0.47g) in dry methanol (40ml) was treated at 0°C with magnesium methoxide (0.1g) in dry methanol (10ml). After 1h the filtered solution was repeatedly passed down a column (2.1cm × 25cm) of Amberlite MB3 (75ml; mixed bed) prewashed with methanol. The solution and washings were evaporated to give the iodoacetate (II). After recrystallization from methanol, the product (yield 0.18g) had m.p. 177–178°C, $[\alpha]_D^{22}$ +39.4° → 23.9° (*c*1 in water), R_F0.26 (solvent A) and 0.44 (solvent B) (Found: C 28.1, H, 4.2; I, 37.0; N, 4.2; $C_8H_{14}INO_6$ requires C, 27.7; H, 4.1; I, 36.7; N, 4.0%). The i.r. spectrum showed strong bands at 3.0, 6.8, 7.3, 8.9 and 9.8μm. The n.m.r. spectrum (100 MHz) in D_2O showed initially a doublet at τ 4.8 with a splitting of 3Hz indicative of the α-configuration. The compound gave a mauve colour in the Morgan–Elson test.

[^{14}C]*INAG.* This was prepared from compound (I) (210mg) by the above method by using iodo[1,2-$^{14}C_2$]-acetic acid (0.1mCi). The crystalline labelled product (25.2mg) had a specific radioactivity of 0.1285μCi/μmol.

Micro-organisms. E. coli ML308 ($i^-z^+y^+x^+$), constitutive for the transport system and for β-galactosidase (EC 3.2.1.23), was used principally in these investigations. *E. coli* K12, grown like strain ML308, was used in preliminary investigations. Organisms were grown overnight at 37°C in shaking culture. The minimal medium used was medium 63 (Sistrom, 1958) containing KH_2PO_4 (13.6g), $(NH_4)_2SO_4$ (2g), $MgSO_4,7H_2O$ (0.2g), $FeSO_4,7H_2O$ (0.5mg) and NaCl (11.7g) in 1litre of solution (adjusted to pH7 with KOH). Sufficient cells to give an extinction of 20–30 Klett units were transferred to 30ml of minimal medium (including carbon source where appropriate) and grown in exponential phase to approx. 200 Klett units (no. 42 filter) (100 Klett units were equivalent to 0.22mg dry wt./ml of cells or 0.6μl of cell water; Winkler & Wilson, 1966).

Transport of sugar derivatives. This was measured by Millipore filtration of cells after exposure to labelled sugar (Winkler & Wilson, 1966). Cells centrifuged at 4°C were resuspended in minimal medium containing chloramphenicol (0.01%) to give an extinction of 300 Klett units. Typically, cell suspension (1.4ml) was mixed with 0.5ml of inhibitor and kept at 25°C for a measured time (usually 20min). Labelled marker sugar (e.g. [U-^{14}C]MG, 0.1ml, 10mM; 7.5μCi/ml) was added and samples (0.5ml) were removed after 15s, 1min and 20min for filtration and measurement of radioactivity. Uptake experiments were

* Abbreviations: INAG, *N*-iodoacetyl-α-D-glucosamine; [^{14}C]INAG, *N*-iodo[1,2-$^{14}C_2$]acetyl-α-D-glucosamine; MG, methyl α-D-glucoside; [U-^{14}C]MG, methyl α-D-[U-^{14}C]glucoside; TMGal, methyl 1-thio-β-D-galactopyranoside; [U-^{14}C]TMGal, methyl 1-thio-β-D-[U-^{14}C]-galactopyranoside.

performed at a final cell concentration of 0.44mg cell dry wt./ml.

Assay of radioactivity. Radioactivity of whole cells and metabolite analogues was measured with a Nuclear-Chicago model 6860 scintillation counter. The scintillation fluid consisted of 2,5-diphenyloxazole (4g) and 1,4-bis-(5-phenyloxazol-2-yl)benzene (0.1g) in toluene (1 litre). Before use this was diluted with 580ml of ethanol. In later experiments modified conditions were employed as described in the succeeding paper (White & Kent, 1970).

RESULTS

In the present study, uptake refers to the specific processes involved in the transfer of isotopically labelled material from the exterior to the internal cellular environment.

Reaction of INAG with N-*acetyl*-L-*cysteine.* The rates of reaction of the iodoacetyl sugar (final concn. 0.5mM) with *N*-acetylcysteine (0.5mM) at pH values of 7 and 8 (phosphate buffer) were compared with those of iodoacetamide (0.5mM) under identical conditions (24°C). The disappearance of thiol was measured by the method of Ellman (1959). At pH8 the time for half-reaction of iodoacetamide was 42min and for the iodoacetyl sugar 120min, whereas at pH7 the value for iodoacetamide was 135min and the iodoacetyl sugar reacted very slowly.

Effect of INAG on the uptake of MG in E. coli *ML*308 *and K*12, *pre-grown on glucose* (0.2%). In a series of experiments with strain K12 it was shown that INAG (0.05mM) was very much more effective than 0.05mM-, 1mM- or 10mM-*N*-acetylglucosamine in inhibiting the uptake of MG. In this concentration range, iodoacetamide has a comparatively small effect on the uptake, e.g. in the presence of this inhibitor (0.5mM) uptake of MG was diminished to 86% of the control (Table 1). Investigation of the extent of inhibition of uptake shown by INAG at various concentrations gave a biphasic relationship (Fig. 1) both for strain K12 and for strain ML308. With the latter organism the inhibitor-free control at 1min had an internal concentration equivalent to 5.4mM-MG (i.e. 12.24mmol/g cell dry wt.).

Time-dependence of inhibitory action of INAG on MG uptake. In initial experiments, inhibition was observed when organisms were exposed to potential inhibitors for 20min before measurement of MG uptake. However, INAG appeared to exert no inhibitory action when given simultaneously with

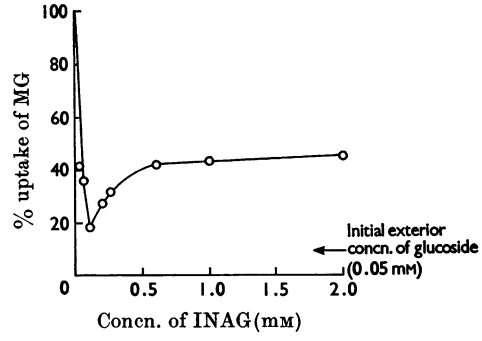

Fig. 1. Effect of INAG on uptake of MG by *E. coli* ML308. Organisms, pre-grown on glucose, were mixed with inhibitor in minimal medium at 25°C for 20min. Uptake (1min) was measured with 0.5mM-MG. Details are given in the text.

Table 1. *Uptake of MG by* E. coli *K*12 *in the presence of potential inhibitors*

Cells in minimal medium (+chloramphenicol) were preincubated for 20min with potential inhibitor and the uptake of MG was measured as intracellular concentration by exposure to [U-^{14}C]MG (initially 0.5mM) for 15s, 1min and 20min. Details are given in the Materials and Methods section. Results of duplicate experiments are shown in parentheses.

Inhibitor	Final concn. (mM)	Intracellular uptake of MG (mM)		
Time		15s	1min	20min
None		3.62 (3.00)	6.58 (5.50)	16.2 (11.7)
N-Acetylglucosamine	0.5	2.98	5.65	20.8
	1.0	1.65	4.25	11.6
Iodoacetamide	0.5	3.34	5.85	13.9
INAG	0.5	0.85 (0.68)	1.52 (1.08)	6.15 (6.15)
	0.25	0.65 (0.57)	1.37 (0.95)	4.85 (3.23)
	0.05	1.62	3.0	9.70
	0.025	3.02	5.0	15.8
	0.005	2.66	6.45	16.9
	1.0	1.07	2.19	6.15
	2.5	1.33	2.56	6.8

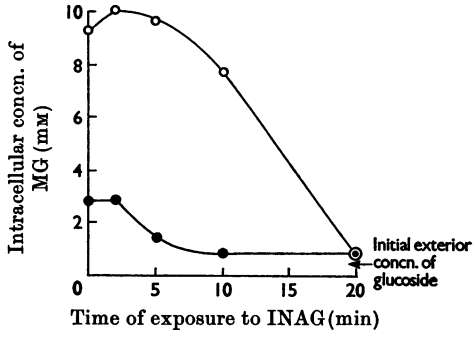

Fig. 2. Uptake (1min) of MG (0.5mM) by *E. coli* ML308 pre-exposed to INAG (0.05mM) for various times. ○, Glucose-grown organisms; ●, glycerol-grown organisms. Details are given in the text.

Table 2. *Effect of INAG on uptake of glycerol and glycosides by* E. coli ML308 *adapted to glycerol and galactose*

Adapted cells were exposed to INAG (0.05mM) for 20min and the uptake was measured for 1min. Details are given in the text.

	Intracellular uptake of ^{14}C-labelled analogue (mM)		
	Glycerol	TMGal	MG
Galactose-adapted cells			
Control		14.1	3.92
INAG		5.6	1.17
Glycerol-adapted cells			
Control	17.75		1.94
INAG	2.99		0.83

Table 3. *Binding of* [^{14}C]*INAG by* E. coli *in the presence of glucose and* N*-acetylglucosamine*

Cells, pre-grown in the appropriate medium, were centrifuged and resuspended in minimal medium containing chloramphenicol (100μg/ml) to give a final cell density of 250μg cell dry wt./ml. Mixtures of monosaccharide (final concn. 5mM) and [^{14}C]INAG (final concn. 0.05mM, 0.129μCi/μmol) were added in a total volume of 0.4ml. After 30min at 25°C samples (0.2ml) were filtered and washed and their radioactivities measured. In controls monosaccharide was omitted.

	^{14}C-labelling (% of that of monosaccharide-free control)	
	Glucose +[^{14}C]INAG	*N*-Acetylglucosamine +[^{14}C]INAG
Glucose-grown cells	85	4
N-Acetylglucosamine-grown cells	110	6

MG, and experiments were performed to elucidate the time-course of INAG inhibition. Cells (strain ML308) pre-grown in glucose (0.2%) or glycerol (0.4%) were incubated with INAG (0.05mM) for various times, and uptake (1min) of MG (0.5mM) was then measured. The results are given in Fig. 2.

Effect of INAG on TMGal and glycerol uptake. Washed organisms (0.35mg), pre-grown on galactose in minimal medium, were treated (for 20min at 25°C) with INAG (0.05mM). TMGal uptake (1min) was measured by using [U-^{14}C]TMGal (0.5mM, 0.36μCi). For comparison, MG uptake was also measured by using [U-^{14}C]MG (0.5mM, 0.25μCi). Organisms, pre-grown in glycerol, treated with INAG as in the preceding experiments, were exposed to [U-^{14}C]glycerol (0.5mM, 0.7μCi) for 1min (Table 2).

Binding of [^{14}C]*INAG by organisms in the presence of glucose and* N*-acetylglucosamine.* The binding of [^{14}C]INAG to cells was measured in the presence of glucose (5mM) and *N*-acetylglucosamine (5mM) after 30min. Results (Table 3) showed that, whereas glucose had little effect, *N*-acetylglucosamine severely diminished the amount of label present in the cells, irrespective of the pre-growth conditions. The glucose-grown control took up 31.6μmol of

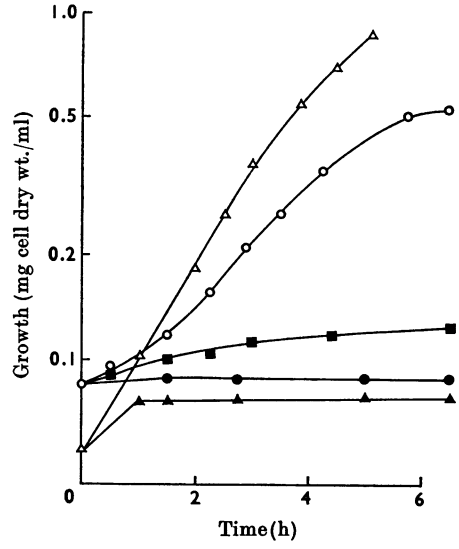

Fig. 3. Effect of INAG on growth of *E. coli* on various carbon sources. Cells, pre-grown in appropriate medium, were centrifuged and resuspended in an equal volume of fresh minimal medium+carbon source+INAG (0.05mM) or iodoacetamide (0.05mM). Controls were identical cultures from which inhibitor was omitted. Cells were grown at 37°C with shaking. ○, Succinate control; ●, succinate +INAG; ■, succinate+iodoacetamide; △, glycerol control; ▲, glycerol+INAG.

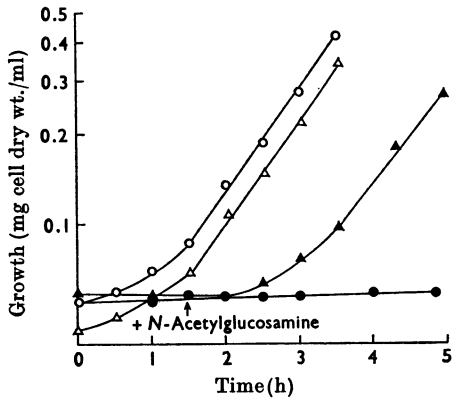

Fig. 4. Inhibition of growth of *E. coli* by INAG and its reversal by *N*-acetylglucosamine. ○, Glucose (10mM) present; ●, glucose (10mM)+INAG (0.05mM) present; △, *N*-acetylglucosamine (10mM) or *N*-acetylglucosamine (10mM)+INAG (0.05mM) present; ▲, glucose (10mM)+INAG (0.05mM) present, *N*-acetylglucosamine (10mM) added after 90min.

INAG/g cell dry wt. and the *N*-acetylglucosamine-grown control took up 24.4μmol of INAG/g cell dry wt.

The inhibition by INAG of MG uptake was not influenced by pre-growth on glucose, casein or glycerol, nor was the inhibition diminished by the presence of glucose (100mM) in the preincubation period of 20min.

Influence of INAG on growth of E. coli *pre-grown on various carbon sources.* Experiments showed that INAG was a profound inhibitor of growth. Growth of cells on glucose (0.2%), casein or succinate (0.4%) was inhibited at concentrations of 0.05mM-INAG (Fig. 3). Growth was measured with a Klett photometer. There was some inoculum-dependence; a large inoculum of cells (0.5mg dry wt. of bacteria/ml) eventually overcame the inhibitory effects of 0.05mM-INAG.

Relief of INAG-inhibition of growth by N*-acetylglucosamine.* Minimal medium containing 10mM-glucose was inoculated with exponential-phase cells of *E. coli* ML308 and shaken at 37°C. INAG was added to a final concentration of 0.1mM. *N*-Acetylglucosamine (final concn. 10mM), added to some cultures at zero time and after 90min to others, clearly overcame the inhibitory action of INAG. Growth was measured with a nephelometer (Fig. 4).

DISCUSSION

Iodoacetamide, one of the most widely used metabolic inhibitors, owes its biochemical activity predominantly to its reactions with thiols, histidyl residues and certain amino groups. These effects have been extensively reviewed (Barron, 1951; Webb, 1965). The commonest type of inhibitory action appears to result from pH-dependent alkylation of thiol groups. Comparison of the rates of alkylation of *N*-acetyl-L-cysteine by iodoacetamide and INAG revealed that at pH8 and 25°C the former reacts about three times as fast as the latter. At pH7 iodoacetamide reacts more slowly and INAG is substantially unreactive. It appears likely therefore that, in the biochemical investigations that follow, INAG is chemically stable under the general conditions prevailing.

The effects of iodoacetamide and iodoacetate on transport phenomena in bacteria have been widely investigated. In *E. coli* iodoacetamide with sodium azide is a potent inhibitor of active transport, though sugars such as *o*-nitrophenyl β-galactoside can rapidly enter the cell (Winkler & Wilson, 1966). Experiments with strains K12 and ML308 showed that INAG powerfully inhibits the uptake of MG. In the concentration range 0–0.1mM there is a linear relationship with inhibition, resulting maximally in about 90% cessation of glucoside uptake. In no experiment was complete inhibition of uptake observed. At concentrations greater than 0.1mM the inhibitory effect was less marked and became constant at 35% above the initial external concentration (Fig. 1). This biphasic type of relationship suggests that INAG acts at more than one site and resembles the growth-inhibition curves found for penicillin (Eagle & Musselman, 1948). The inhibitory action of INAG on MG uptake is not instantaneous and it appears that penetration of cells by inhibitor must first occur. Investigation of the time-course indicates that the inhibitor requires to be in contact with micro-organisms for at least 4min before its effect is detectable. In general the maximal inhibiting effect was not observed before 20min. Experiments to determine whether INAG interacted with some membrane component dependent on pre-growth conditions showed that this was not the case.

Organisms pre-grown in glucose, glycerol, galactose and casein all took up MG to various extents, and uptake was similarly sensitive to INAG inhibition in each case. Further, the effect was not restricted to MG, and INAG also prevented uptake of TMGal and glycerol. It has to be concluded therefore that INAG acts in a relatively non-specific manner, possibly concerned with energetic steps of uptake rather than with selectivity. Lane & Macdonald (1968*a*,*b*) found that iodoacetate (up to 5mM) inhibited uptake of TMGal by *Staphylococcus aureus* HS1159, which in control and partly inhibited cells was all converted into the 6-phosphate. Cell-free extracts with added phosphoenolpyruvate showed undiminished phosphotransferase

activity in the presence of 5mM-iodoacetate, and it was therefore suggested that formation of phosphoenolpyruvate may be a rate-determining step in uptake.

As the inhibitory action of INAG on MG uptake is unaffected by the presence of high concentrations of glucose (100mM), it was not possible to 'protect' the relevant sites by this means. With the aid of [^{14}C]INAG the inhibitor was found to become rapidly bound or combined with cells in the presence of glucose (Table 3). With added *N*-acetylglucosamine the binding of inhibitor is greatly decreased. This suggests that *N*-acetylglucosamine may enter the cell by a route different from that for MG and for which INAG competes.

In the initial transport experiments (Table 1) *N*-acetylglucosamine did not appear markedly to influence MG uptake as might be expected if a common transport site was involved. Further evidence for a separate pathway for *N*-acetylglucosamine uptake was obtained from growth studies. INAG, in concentrations less than 0.05mM, powerfully inhibits growth of organisms. Cells growing in succinate or glycerol appear to be somewhat more sensitive than those growing in glucose (Fig. 3) or casein. In the presence of *N*-acetylglucosamine, however, no inhibition of growth is observed, even in the presence of glucose (20mM), and if *N*-acetylglucosamine (10mM) is added to an inhibited culture growth is resumed (Fig. 4). The inhibition, further, is only exhibited while INAG is supplied to cells. An inhibited culture maintained for 90min in glucose and INAG (e.g. 0.05mM) resumes growth if centrifuged, washed (at room temperature) and resuspended in inhibitor-free glucose medium.

It therefore appears that INAG exerts multiple biochemical effects. The possibility exists that the inhibitor partially prevents uptake of marker sugars such as MG by competition for common selective sites. If it does so, it is in a reversible manner and does not appear to involve chemical reaction with the fixed structures of the membrane. The evidence points to the existence, in addition, of a separate selective site for *N*-acetylglucosamine by which the iodoacetyl analogue may gain entry into the cell. Within the cell the known pathway of amino sugar metabolism (White, 1968) includes a powerful deacetylase, which may act on the inhibitor (or a phosphorylated derivative of it) to release iodoacetate, which would then be available to inhibit energy-yielding reactions including those associated with transport. The greater sensitivity of glycerol-grown cells towards the inhibitor and the known ease of inhibition of glycerol phosphate dehydrogenase (Rapkine, 1938; Green, Needham & Dewan, 1937) is consistent with this view.

The biochemical action of INAG is unlikely to be due to its extracellular degradation to iodoacetate, nor are its overall properties entirely attributable to the latter substance. Some evidence indicates that, both as a transport and growth inhibitor, INAG is measurably more effective than iodoacetamide, and it is adduced therefore that the carbohydrate portion of the inhibitor molecule may facilitate its entry into the cell, where it may be susceptible to intracellular enzyme action.

Comparative experiments indicate that INAG behaves differently in animal cells. The substance is without marked effect on the uptake of MG by rabbit erythrocytes or in rat diaphragm. In hamster intestinal ring preparations, *N*-acetylglucosamine and INAG (1mM) had no effect on uptake of MG at 37°C, whereas iodoacetamide (1mM) resulted in 69% inhibition of the glucoside transport (P. W. Kent, unpublished work). These investigations underline features of fundamental biochemical difference in the metabolic role of *N*-acetylglucosamine in bacteria and in mammals. In bacteria uptake of the *N*-acetylglucosamine is accompanied by phosphorylation and, after deacetylation (White, 1968; White & Pasternak, 1967), leads to rapid metabolism for synthesis of structural components or, via deamination, to glycolysis. In animals the metabolic fate of *N*-acetylglucosamine is not known in detail. Deacetylation, though known in animals, is not a common metabolic route, and the enzymic pathway leading to the reutilization of this sugar remains to be established. There is evidence suggesting that the *N*-acetylglucosamine is not readily metabolized. There is also evidence that some of the control mechanisms for glycoprotein and mucopolysaccharide biosynthesis are in the enzymes involving the conversion of hexose into glucosamine 6-phosphate and its subsequent acylation (Phelps, Hardingham & Winterburn, 1970; Kent & Allen, 1968; Kent, 1970).

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