$$HOCH_2\cdot(CH_2)_n\cdot\underset{\displaystyle NH_2}{\underset{|}{C}}H\cdot COOH + DPN^+ \rightleftharpoons OCH\cdot(CH_2)_n\cdot\underset{\displaystyle NH_2}{\underset{|}{C}}H\cdot COOH + DPNH + H^+$$
(or TPN^+) (or TPNH)

where $n = 2$ or 3.

Presumably all the reactions described by this equation are catalyzed by a single enzyme, since an enzyme preparation that was more than 90% inactivated through heating at 60° C showed approximately the same relative activity with the respective substrates and nucleotides.

The fresh and heat-treated preparations also catalyze the reduction of DPN or TPN in the presence of a lower homologue of PHS, homoserine. In this case, with fresh preparations, TPN is reduced considerably faster than is DPN, whereas the reverse is true with heat-treated preparations (under similar test conditions). It is thus possible that the fresh preparations contain more than one enzyme capable of acting on homoserine. Accordingly, the relationship of the PHS- or HHS-dehydrogenating enzyme to the homoserine dehydrogenase[8] of yeast is uncertain. Serine, threonine, and acetaldehyde did not appear to be substrates for the enzyme preparations.

Very probably, the dehydrogenase that acts on PHS or HHS is involved in the growth response of certain *N. crassa* mutant strains to these ω-hydroxy-α-amino acids. PHS is utilized by mutants that respond alternatively to proline or to ornithine[9] and HHS by some mutants that respond to lysine[10]. The partial replacement of arginine by PHS in the diet of chicks[11] may well depend in part on a similar enzymic reaction. The growth-promoting activities of PHS and HHS are thus ascribed to the formation of the respective ω-semialdehydes. While it is thought likely that the ω-semialdehydes, but not the corresponding hydroxy compounds, are actual biosynthetic intermediates, the possibility that the hydroxy compounds have some "normal" metabolic function is not excluded. The key position of GSA in the glutamic family of *N. crassa* has been reported[12,13]. The previously considered possible role of α-aminoadipic δ-semialdehyde as an intermediate in lysine synthesis[14–17] is supported by the present finding that this semialdehyde is the product of an enzymic reaction whose substrate is known to satisfy a lysine requirement.

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The structure of the prosthetic group of bovine submaxillary gland mucoprotein

Bovine submaxillary gland mucoprotein (BSM) contains about 17% sialic acid and 9.2% N-acetylgalactosamine; in addition, N-acetylglucosamine, galactose, mannose, and fucose are present in very small amounts[1,2,3]. It was shown previously[4] that the reducing group of sialic acid is joined in a glycosidic linkage to the rest of the mucoprotein and that, at pH 1.0 and 80°, 78% sialic acid, and sialic acid only, is released from BSM. *Vibrio cholerae* neuraminidase[5] splits off up to 64%

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of the sialic acid of BSM[4]. The presence in BSM of equimolar quantities of sialic acid and galactosamine suggested that galactosamine was the partner of sialic acid in the glycoside bond[4]. This assumption was verified:

(1) 0.5 g BSM was treated with 20 ml 0.01 *N* $Ba(OH)_2$ at 80° for 15 min, the barium removed and the remaining solution dialysed against 10 volumes of water at 4°. Paper chromatography (*n*-butanol/pyridine/H_2O (6:4:3) as solvent) of the concentrated dialysate revealed a compound of low R_F (0.04) reacting with both Ehrlich's and orcinol reagents to give purple and reddish-purple colours; the R_F value was close to that of neuramin-lactose[5]. In addition free N-acetylneuraminic acid (NANA; R_F, 0.12) was traced by the same reagents, and a small amount of N-acetylgalactosamine (R_F, 0.31) by the Morgan-Elson reaction. At R_F 0.60 a chromogen was present, forming an intense but quickly fading purple colour with Ehrlich's reagent immediately in the cold, a reddish-purple colour with the orcinol reagent, and a faint brown colour with aniline hydrogen phthalate on heating. This chromogen coincided precisely in R_F value and colour reactions with a compound produced when N-acetylgalactosamine or N-acetylglucosamine were submitted to the same mild alkali treatment. Both compounds showed an absorption maximum at about 230 mμ. From these properties it seems rather certain that the chromogen is identical with the chromogen 1 (anhydro-acetylglucosamine) prepared from N-acetylglucosamine under similar conditions by KUHN AND KRÜGER[6] and that it has a double bond conjugated to the amide group.

(2) Treatment of the low R_F compound with influenza virus neuraminidase[5] (Lee strain) at 35° for 16 h resulted in its quantitative breakdown to about equal amounts of NANA and the chromogen, the control remaining unchanged.

(3) On heating the compound of low R_F in 0.6 *N* $Ba(OH)_2$ at 100° for 6 h, 2-carboxypyrrole known to derive from NANA was produced, along with a compound of high R_F (0.81) reacting in the cold instantaneously with Ehrlich's reagent to give a stable bright purple colour coinciding with a compound formed from N-acetylgalactosamine under same conditions. Both compounds were observed previously when BSM was treated accordingly[7].

These data would indicate that the prosthetic group of BSM can be detached by very gentle alkali treatment and that it is the disaccharide shown in Fig. 1. The enzymic results exclude a tetraose constitution. Alkali hydrolysis of the ester or N-glycosidic linkage *a* will result in the liberation of the disaccharide, its O-acetyl group being split off in the process. The reducing disaccharide thus formed is the glycoside of a β-hydroxyaldehyde. As such it behaves towards mild alkali as does 3-β-D-galactopyranosyl-N-acetylglucosamine[6]. Both glycosides break down by an elimination mechanism directly into the glycon and the same chromogen; both glycosides form only small amounts of unimpaired N-acetylhexosamine by a mechanism of alkaline hydrolysis[6]. This behaviour excludes linkage of NANA to C_4 or C_6 of N-acetylgalactosamine. The production of N-acetylglucosamine sometimes observed under the conditions of (1) may be accounted for by reverse aldolization of NANA[8].

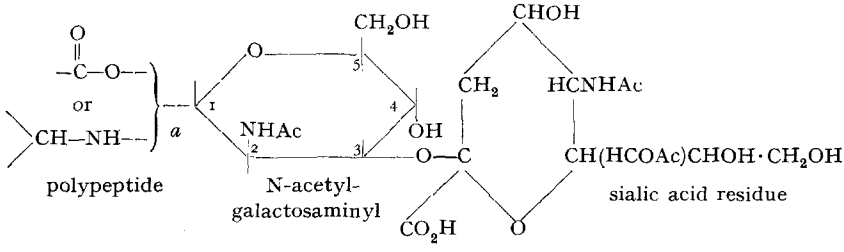

Fig. 1

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