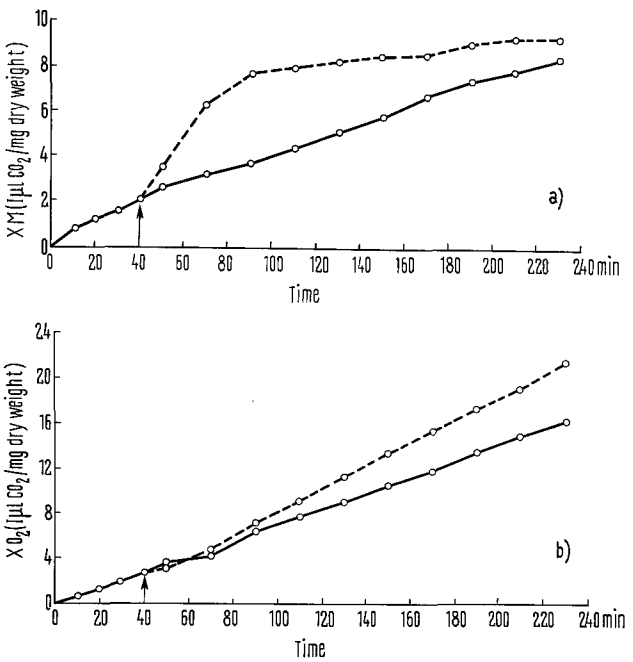

Fig. 1. Time course of (a) aerobic glycolysis and (b) respiration of rabbit-brain slices. ↑, addition of adenosine 3′,5′-monophosphate (AMP), final concentration 1 m*M*; ○—○, control; ○- - -○, presence of AMP.

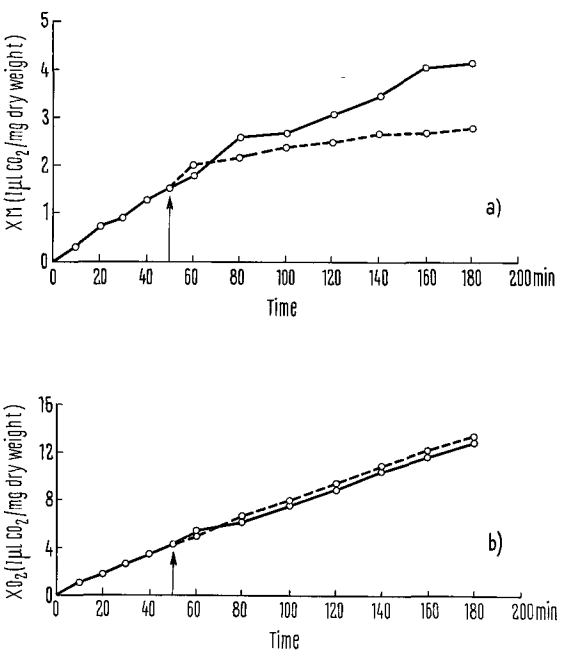

Fig. 2. Time course of (a) aerobic glycolysis and (b) respiration of rabbit-brain slices in the presence of 0.1 m*M* AMP. For symbols see Figure 1.

KUO, and GREENGARD[10]. They found that brain protein kinase was still stimulated by a concentration of 0.0005 m*M* AMP per litre incubation solution.

Zusammenfassung. Der Einfluss von Adenosin-3′,5′-monophosphat (AMP) auf den Glukosestoffwechsel von Grosshirnrindenschnitten des Kaninchens wurde untersucht. 0.1 bis 1 m*M* AMP pro Liter Incubationslösung stimulieren die Atmung der Schnitte. Die aerobe Glykolyse der Schnitte wird durch 0,5 bis 1 m*M* AMP anfänglich stark gesteigert und nach ca. 30 min völlig gehemmt. 0.1 m*M* AMP hemmt die Glykolyse lediglich.

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The Properties of Neuraminidase-Treated Crystalline Ceruloplasmin

Although the glycoprotein nature of ceruloplasmin has been described[1,2], little is known of the significance of the carbohydrate moiety for the function of this copper-containing protein. In a recent report by MORELL et al.[3], it has been shown that sialic-acid residues of ceruloplasmin protect the protein from being rapidly incorporated into the liver. In the present investigation the importance of sialic-acid residues of ceruloplasmin for its oxidase activity, physical properties and inhibition of viral hemagglutination has been studied.

Ceruloplasmin was obtained in a partially purified form from AB Kabi, Stockholm (retroplacental source) and recrystallized 3 times according to the method of DEUTSCH[4]. It had absorbance ratio ($A_{280}:A_{610}$) of 23.2 and appeared as one single protein band upon electrophoresis and ultracentrifugation. Liberation of sialic-acid residues was obtained by treatment of ceruloplasmin with neuraminidase either from *Cl. perfringens* (Type V enzyme from Sigma) or from *V. cholerae* (General Biochemicals). Sialic acid was determined by the thiobarbiturate method of WARREN[5]. The total sialic acid determined by hydrolysis in 0.1 *N* H_2SO_4 (Figure 1) amounted to 13 moles per mole ceruloplasmin (according to M.W. of 160,000[6]) while 9 moles sialic acid per mole cruloplasmin was removed enzymatically by treatment with neuraminidase. This value for sialic acid as well as the content of glucosamine[7] (23 moles), hexose[8] (26 moles) and fucose[9] (4 moles) in this preparation of crystalline ceruloplasmin are of the same order of magnitude as reported for ceruloplasmin

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from non-pregnant sera[1,2]. It appears that 30% of sialic acid is not easily removed by the action of neuraminidase. The report[10] that sialic acid bound to galacatose is readily removed by neuraminidase while the presence of N-acetyl-galactose introduces a resistance against enzymatic cleavage may be of importance in this connection.

A viral hemagglutination inhibitory action of crystalline ceruloplasmin was observed using 2 strains of virus according to the method of SALK[11]. Such an effect has previously been described with an impure preparation of ceruloplasmin[12]. Serial twofold dilutions of 2% ceruloplasmin solutions were made up in phosphate buffered saline (PBS), pH 7.35, in such a manner that each tube contained 0.25 ml. To each tube were added 0.25 ml of a suspension of indicator virus previously titrated to contain 4 HA (hemagglutination) units. The tubes were shaken and set aside for 10–15 min. $^1/_2$ ml of a 0.5% washed red-cell suspension was then added to each and the tubes were allowed to stand in the refrigerator overnight. Under these conditions it was found that 1 mg ceruloplasmin contains 320 hemagglutination inhibitor (HAI) units for Lee B virus and 8 HAI units for FR8 virus. Further it was observed that apoceruloplasmin prepared according to MORELL and SCHEINBERG[13] inhibited hemagglutination to the same extent. Treatment of ceruloplasmin and apoceruloplasmin with neuraminidase at 37 °C for 3 h resulted in the complete loss of inhibition. The inhibition of viral hemagglutination due to sialic-acid residues of ceruloplasmin is analogous to the effect of most glycoproteins with carbohydrate chains terminating in sialic-acid residues[14].

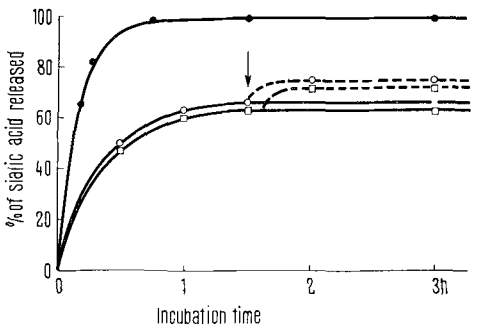

Fig. 1. Time course release of sialic acid from ceruloplasmin by acid and by neuraminidase. —●—, Hydrolysis in 0.1 N H_2SO_4 at 80 °C. -○-, Incubation with neuraminidase from *Cl. perfringens*: 1.25 μg enzyme-N, 400 μg ceruloplasmin, incubation volume 0.2 ml, pH 5.9. ↑, Effect of adding additional enzyme. -□-, Incubation with neuraminidase from *V. cholerae*: same conditions. ↑, Effect of adding additional enzyme.

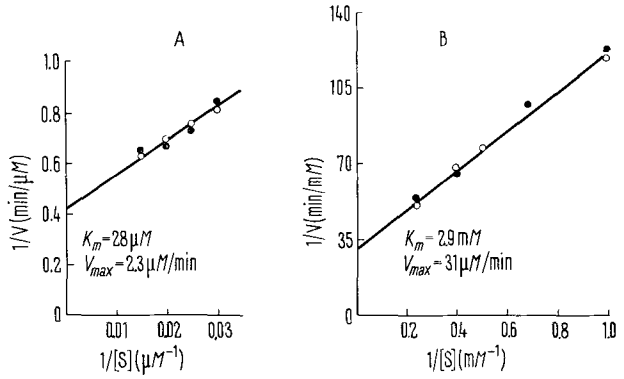

Fig. 2. Oxidase activity of ceruloplasmin and asialoceruloplasmin. (A) Determination of the 'low K_m site'. Reciprocal plots for activity against N,N-dimethyl-*p*-phenylenediamine. Conditions: 0.43 μM enzyme in 0.14 M acetate buffer pH 5.9, 0.5 μM desferrioxamine. Total volume 2.0 ml. Temperature 15 °C, 10 min incubation time. (B) Determination of the 'high K_m site'. Reciprocal plots for activity against 3-hydroxytyramine. Conditions: 2.5 μM enzyme in 0.14 M acetate buffer pH 5.9, 0.5 μM desferrioxamine. Total volume 1.0 ml. Temperature 38 °C, 10 min incubation time. —●—, Activity with ceruloplasmin. —○—, Activity with asialoceruloplasmin.

Oxidase activity of asialized ceruloplasmin has been determined with the neuraminidase-treated enzyme dialyzed for 18 h to removed sialic acid liberated. In kinetic experiments it has recently been shown that 2 different types of ceruloplasmin molecules with different K_m values exist[15,16]. As shown in Figure 2, removal of sialic-acid residues did not change the kinetic properties of oxidase activity. No difference of K_m or V_{max} of the 2 different types of ceruloplasmin molecules could be detected. The absorption spectrum of ceruloplasmin in UV and visible region and the absorbance ratio (A_{280}:A_{610}) was also unaffected after treatment with neuraminidase. However, the electrophoretic mobility at pH 5.9 was decreased and asialoceruloplasmin gave a slow-moving single band with oxidase activity.

The results indicate that the physical and enzymic properties of ceruloplasmin associated the copper-protein complex are independent of the sialic-acid residues. These observations have some counterparts seen with other protein molecules. After treatment of choline esterase with neuraminidase the electrophoretic mobility but not the enzymic activity was altered[17]. Removal of sialic acid from haptoglobin did not affect the formation of a haptoglobin-hemoglobin complex or its peroxidase activity[18]. Neuraminidase treatment of transferrin did not affect its antigenic properties and its iron-binding capacity was retained[19]. It therefore seems that sialic-acid residues occupying terminal position in the oligosaccaride chain of glycoproteins are not likely to influence the structure and function of the 'active sites' in these protein molecules.

Résumé. On sait que la plasmine céruléenne crystalline rétroplacentaire humaine ainsi qua la plasmine apocéruléenne empêchent l'hémaglutination virale. Cet effet pourrait être attribué aux résidus d'acide sialique, car l'action préventive disparaît après traitement de la plasmine céruléenne par la neuraminidase.

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