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THE ANTI-RECOGNITION FUNCTION OF SIALIC ACIDS:

STUDIES WITH ERYTHROCYTES AND MACROPHAGES

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Abstract - Sialic acids have been found to mask antigens, receptors and other recognition sites on molecules and cells. This has been investigated best with hepatocyte and macrophage lectins which recognize ß-galactose and N-acetyl-ß-galactosamine residues on molecules and cells exposed by sialidase treatment. The lectin on macrophages leads to the binding and phagocytosis of erythrocytes after the enzymic removal of a critical amount of sialic acids. New techniques (colorimetry, high-performance liquid chromatography, gas-liquid chromatography|mass spectrometry) for the determination of the amount and nature of these sialic acids were developed. The interaction of partially desialylated rat erythrocytes with homologous peritoneal macrophages can be inhibited by ß-galactosyl glycosides in a degree depending on the structure of the oligosaccharides and glycoconjugates tested. Although homologous serum stimulates binding and phagocytosis, immunoglobulins and complement factors were found to play no role in this process. The main factor responsible for the masking effect of sialic acids on subterminal ß-galactosyl groups seems to be the carboxyl group of neuraminic acid, as reduction of this group to an alcohol largely abolishes this important function of sialic acids in the macrophage and hepatocyte systems studied. The anti-recognition function of sialic acids illuminates the pathophysiological role of serum sialidases in infectious and other diseases.

INTRODUCTION

Sialic acids have many functions in biological systems and according to our knowledge their most important role seems to be anti-recognition (1). The first and most exciting example of this function are sialidase-treated serum glycoproteins, which are bound and endocytozed by hepatocytes (2). In these molecules sialic acid masks ß-glycosidically linked galactose residues, which after exposure by sialidase, are recognized by a ß-galactose-specific lectin on the hepatocyte surface. A lectin with similar or perhaps identical properties was detected on the surface of liver macrophages (Kupffer cells) of rat (3,4) or rabbit (5), as well as on rat peritoneal macrophages (6,7). It recognizes ß-galactose and N-acetyl-ß-galactosamine residues of sialidase-treated erythrocytes resulting in binding and phagocytosis of these cells. These sugar residues are masked by sialic acids on normal erythrocytes circulating in the blood stream. Sialic acid is thus an important factor for the regulation of the life-time of erythrocytes (1). Loss of this sugar leads to rapid and irreversible removal of red blood cells from the circulation (8,9 and work from other laboratories cited in Ref. 1). Similarly, sialidase-treated lymphocytes (10) and thrombocytes (11) disappear quickly from the blood stream. On the other hand, inclusion of sialoglycoprotein from human erythrocyte membranes into liposomes inhibits phagocytosis of the liposomes by human polymorphonuclear leucocytes (12). Further, Sporothrix schenckii yeast cells with sialic acid on the surface are not phagocytozed by mouse peritoneal macrophages (13).

Anti-recognition is exerted by sialic acids also in other systems. For example, the reactivity of IgG with the Fc receptor of human T lymphocytes (14), the number of receptors for luteinizing hormone (LH) and human chorionic gonadotropin (hCG) in rat ovary (15), the amount of carrier sites for dopamine in nerve cell membranes (16), the accessibility of ß-adrenergic receptors in rat heart (17), the sensitivity of cells from human cell lines to diphtheria toxin and Pseudomonas aeruginosa exotoxin (18), or the complement-dependent cytotoxicity against autologous, invasive human bladder tumor cells (19) were increased by sialidase treatment. Removal of sialic acid from the endothelium of rabbit arteria was found to stimulate the adhesion of blood platelets (20).

It has been reviewed in Refs. 1 & 21 that sialic acids can mask antigenic sites of molecules and of normal or malignant cells. An additional example is asialylated rabbit IgG which becomes immunogenic in autologous hosts and thus shows a relationship to the rheumatoid factor (22). Sialic acids furthermore mask antigenic sites in the cornified layer of newborn rat epidermis (23) and on lymphocytes (24). The involvement of sialic acids in the blood group reactivity of human erythrocytes and the exposure of T-antigen by sialidase-treatment are well known (25).

Sialic acids, whose many other functions were discussed in detail in Refs. 1 & 21, are components of receptors for hormones, bacterial toxins and infectious agents (e.g. viruses and mycoplasmae). They influence the shape of cells because of their negative charge (the pK values of the different sialic acids are around pH 2) and the conformation of macromolecules. This has various metabolic (e.g. hindrance of the activity of proteases) and biological consequences (e.g. influence on the viscosity of some mucins, and the masking effects discussed above). In addition, sialic acids bind positively charged compounds with nutritive and other physiological effects and thus help to regulate the numerous functions of cells.

In the following the role of sialic acid and galactose residues of erythrocyte membranes for the regulation of the life-time of erythrocytes and for the interaction of erythrocytes and macrophages will be described. As such studies require knowledge about the quantity and nature of sialic acids in the cells investigated, at first new methods for the isolation and analysis of these sugars will be presented. Then, the pathophysiological implications of the phenomena observed, also in the light of the occurrence of sialidases in blood serum in disease, will be discussed.

STRUCTURE AND ANALYSIS OF SIALIC ACIDS

Sialic acids occur in the animal kingdom since the echinoderms (26). Already in these animals, best investigated with starfishes and sea urchins, several kinds of sialic acids are formed (1,26 and Fig. 1). The greatest diversity of sialic acids, however, is found in mammals with a maximum of 14 neuraminic acid derivatives in cow (1,26 and Fig. 1). So far 23 saturated, natural sialic acids have been detected, which vary in the nature of the N-substituents of neuraminic acid (acetyl or glycolyl) and in the number, position or combination of O-substituents (acetyl, lactyl, methyl, sulfate and phosphate residues). No other natural sugar is known to exist in so many modifications. Various unsaturated and anhydro neuraminic acid derivatives occurring as free sugars in fluids of man and animals (27) have also been

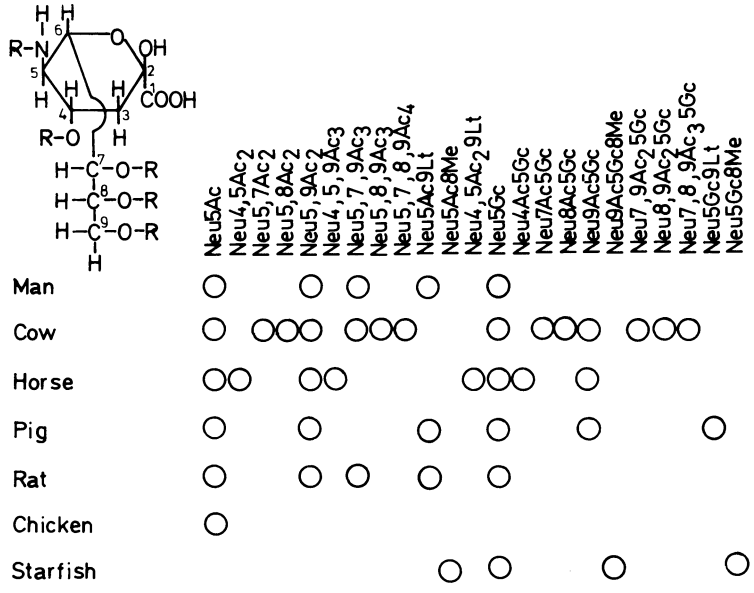

Fig. 1. Natural N,O-substituted sialic acids and examples of their occurrence. The sialic acid nomenclature corresponds to a proposal made at the 5th International Symposium on Glycoconjugates, held at Kiel-Damp in 1979 (see Ref. 1 & 26).
Neu, neuraminic acid. Substituents (R): Ac, acetyl; Gc, glycolyl; Lt, lactyl; Me, methyl. The numbers of abbreviations represent the position of substituents, for example: Neu7,9$Ac_2$5Gc means N-glycolyl-7,9-di-O-acetylneuraminic acid.

identified. The different saturated sialic acids are components of glycoconjugates and are distributed in animals and some microorganisms in a species- and tissue-specific manner. Only N-acetylneuraminic acid, the precursor of all other sialic acids (1,28), occurs in all organisms capable to synthesize the neuraminic acid molecule. N-Glycolylneuraminic acid is also widely distributed, followed by 9-O-acetylated sialic acids. Strikingly, the latter sialic acid frequently occurs in erythrocyte membranes (29) (Fig. 2). The reason for this may be one of the functions of O-acetyl groups in sialic acids, i.e. to hinder rapid hydrolysis of this

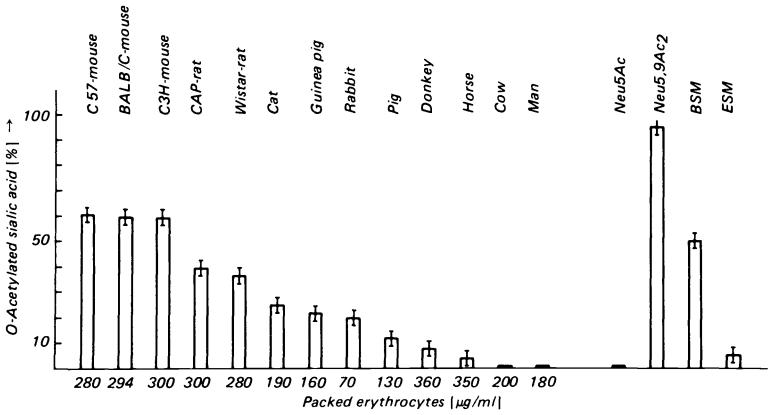

Fig. 2. Total amounts of sialic acids and the relative amounts of 9(8)-O-acetylated sialic acids in erythrocytes from various mammals using the periodate|acetylacetone method (29). The relative amounts of these O-acetylated sialic acids in glycoproteins of bovine (BSM) and equine submandibular glands (ESM) are also indicated

sugar by sialidases (1,28). Furthermore, Varki & Kornfeld observed an influence of O-acetylated sialic acid of erythrocytes on complement reactivity (30).

Since sialic acids in general and their O-acylated derivatives in particular are labile substances, special care must be taken in their isolation, especially when isolating them from cellular material in which their concentration usually is low (1,31). Hydrolysis of the glycosidic bonds of sialic acids by sialidase or mild acids, purification of sialic acids by ion-exchange and other chromatographic steps and eventually fractionation on cellulose is described in Refs. 1 & 31. When using sialidases one should be aware of the relative or absolute resistance of sialic acid residues towards these hydrolases. The hydrolysis rate is known to be reduced by the presence of N-glycolyl residues or of ester groups on the sialic acid side chain (1), or it may be hindered because of steric reasons, e.g. in cell membranes. Resistance of sialyl residues towards sialidase action has been found to be due to O-acetylation at C-4 or by linkage to the internal galactose in gangliosides (1).

Extensive purification of sialic acids is a prerequisite for most of the following analytical procedures (1,31). Underivatized sialic acids can quantitatively be determined or qualitatively be identified by different colorimetric methods, thin-layer chromatography (TLC) in one or two dimensions, high-performance liquid chromatography (HPLC) and NMR-spectroscopy (1,31, 32). HPLC analysis of sialic acids has recently been introduced (33). It revealed to be a valuable and sensitive tool for the rapid determination of sialic acids. For example, the analysis of sialic acids from horse erythrocytes is shown in Fig. 3. It is advantageous that sialic acids in most cases must not be completely purified before analysis by HPLC. This method is therefore especially useful in following enzymic reactions of sialic acids (34). Structural analysis of underivatized sialic acids is possible by combining HPLC with mass spectrometry (MS) (Shukla et al., unpublished): Sialic acids are eluted from a HPLC column (40x4.6 mm) filled with Aminex A-29 by a mixture (4:1) of aqueous 20 mM ammonium formate solution of pH 6 and acetonitrile at a flow rate of 0.5 ml|min and a pressure of 20 bar. About 3% of the effluent is directly transferred to the ion source of the mass spectrometer by a liquid inlet (Hewlett-Packard 5985 B with LC|MS interface). Simultaneously the eluting solvent is used as a reagent for chemical ionization. In some cases pure sialic acids were applied directly without column on-line to the mass spectrometer. Mass spectra of the different chemically ionized sialic acids were obtained allowing the identification of the nature and number of N- and O-acyl substituents of sialic acids. Fig. 4a gives the mass spectrum of N-acetylneuraminic acid, and in Table 1 the mass fragments of different sialic acids are shown. In Fig. 4b the very simple mass spectrum of 2-deoxy-2,3-didehydro-N-acetylneuraminic acid is illustrated revealing the molecular ion $[M+H]^+$ and one fragment with 18 (= H_2O) mass units less.

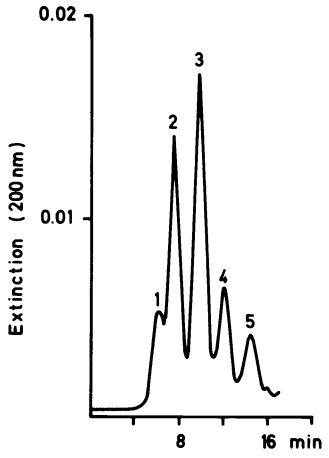

Fig. 3
Elution profile from anion-exchange HPLC (33) of a sialic acid mixture isolated by mild acid hydrolysis (31) from equine erythrocyte membranes.
1, N-acetylneuraminic acid;
2, N-glycolylneuraminic acid;
3, N-glycolyl-4-mono-O-acetylneuraminic acid;
4, N-glycolyl-4,9-di-O-acetylneuraminic acid;
5, presumably, N-glycolyl-4,7,9-tri-O-acetylneuraminic acid.

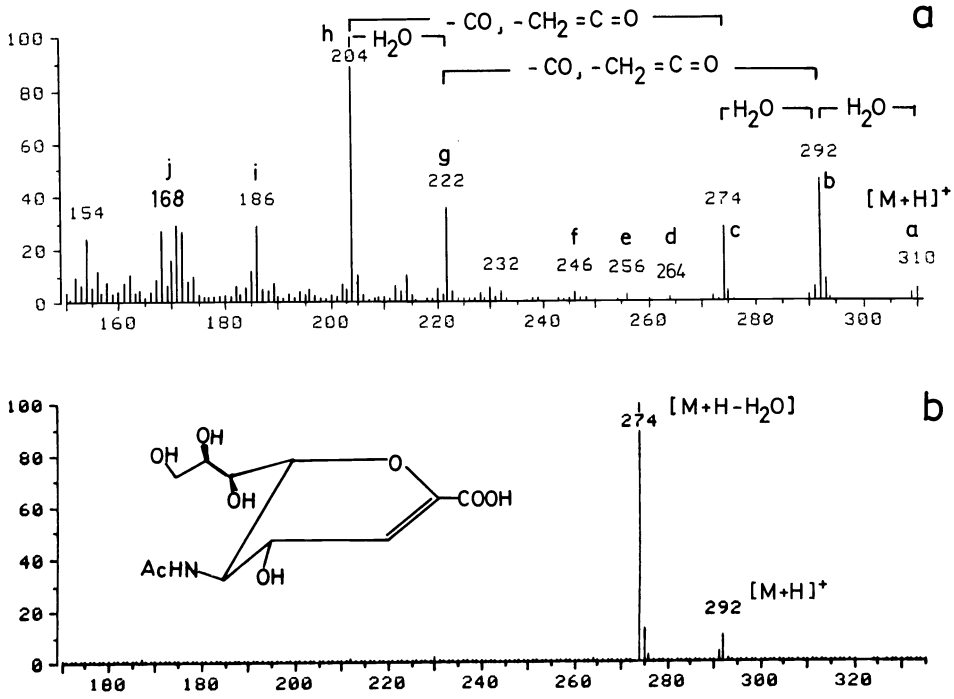

Fig. 4.
a) Chemical ionization mass spectrum of underivatized N-acetylneuraminic acid by HPLC-MS.
b) Chemical ionization mass spectrum of underivatized 2-deoxy-2,3-didehydro-N-acetylneuraminic acid (Neu2en5Ac) by HPLC-MS.

An efficient method for the analysis of sialic acids is gas-liquid chromatography (GLC), either alone or in combination with electron impact (EI) mass spectrometry (35). This method using packed columns enabled the elucidation of the structures of many sialic acids as methyl ester, trimethylsilyl (TMS) ether derivatives during the past ten years. A further analytical improvement represents the use of capillary columns for GLC analysis of sialic acids as TMS ester, per-O-TMS ether and partially N-TMS derivatives obtained by the reaction of the lyophilized material with N-methyl-N-trimethylsilyl-trifluoroacetamide (MSTFA) (36). Fig.5 shows a capillary gas-liquid chromatogram of the sialic acids from rat erythrocytes on OV-101.

TABLE 1. Mass fragmentation pattern of underivatized sialic acids analyzed by HPLC-MS. For sialic acid abbreviations see Figs. 1 & 4b.

	j	i	h	g	f	e	d	c	b	a
Neu5Ac	168	186	204	222	246	256	264	274	292	310
Neu2en5Ac	-	-	-	-	-	-	-	-	274	292
Neu5Gc	184	202	220	238	262	272	280	290	308	326
$Neu5,9Ac_2$	210	228	246	264	-	-	306	316	334	352
$Neu5,7,9Ac_3$	-	-	288	306	-	-	348	358	376	394
$Neu5,7,8,9Ac_4$	-	-	330	348	-	-	390	400	418	436

Using isobutane as reagent gas chemical ionization (CI) mass spectra were obtained at 270 eV from the GLC peaks, which indicate the molecular weight and the nature and position of the N- and O-substituents on the pyranose ring or the side chain of sialic acids (Schröder et al., unpublished). These spectra, which have not been described before, are equally well suited for the structural analysis of sialic acids as are the EI spectra (35), although the fragmentation patterns are different from those of the EI spectra (Fig. 6). An advantage of the CI mass spectra is the regular appearance of the molecular ion $[M+H]^+$ in the case of the N-TMS derivatives, which represents the base peak. Molecular ions are not regularly obtained in the EI spectra.

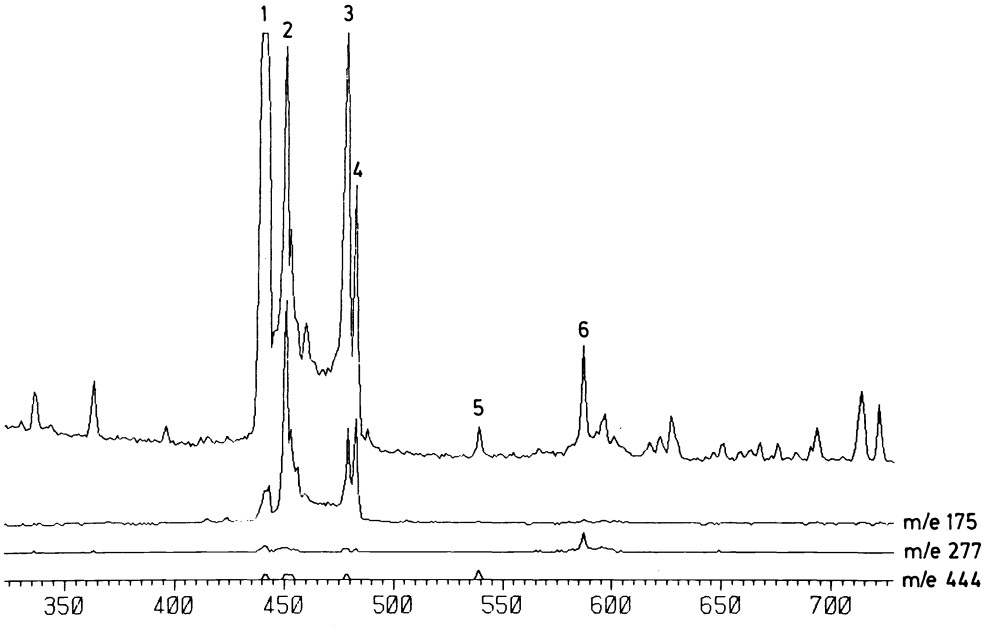

Fig. 5. Capillary gas-liquid chromatogram and mass chromatograms (EI; 35,36) of sialic acids from rat erythrocytes. Sialic acids were enzymically (Arthrobacter ureafaciens) released from the cell membranes. For GLC a fused silica capillary column OV-101, 25 m, was used. For sialic acid nomenclature see Fig. 1.
Peak identification:
1, Neu5Ac, per-O-TMS-
2, $Neu5,9Ac_2$, per-O-TMS- and $Neu5,7,9Ac_3$, per-O-TMS-
3, Neu5Ac, per-O-TMS-, N-TMS-
4, $Neu5,9Ac_2$, per-O-TMS-, N-TMS- and $Neu5,7,9Ac_3$, per-O-TMS-, N-TMS-
5, Neu5Gc, per-O-TMS-
6, Neu5Ac9Lt, per-O-TMS-

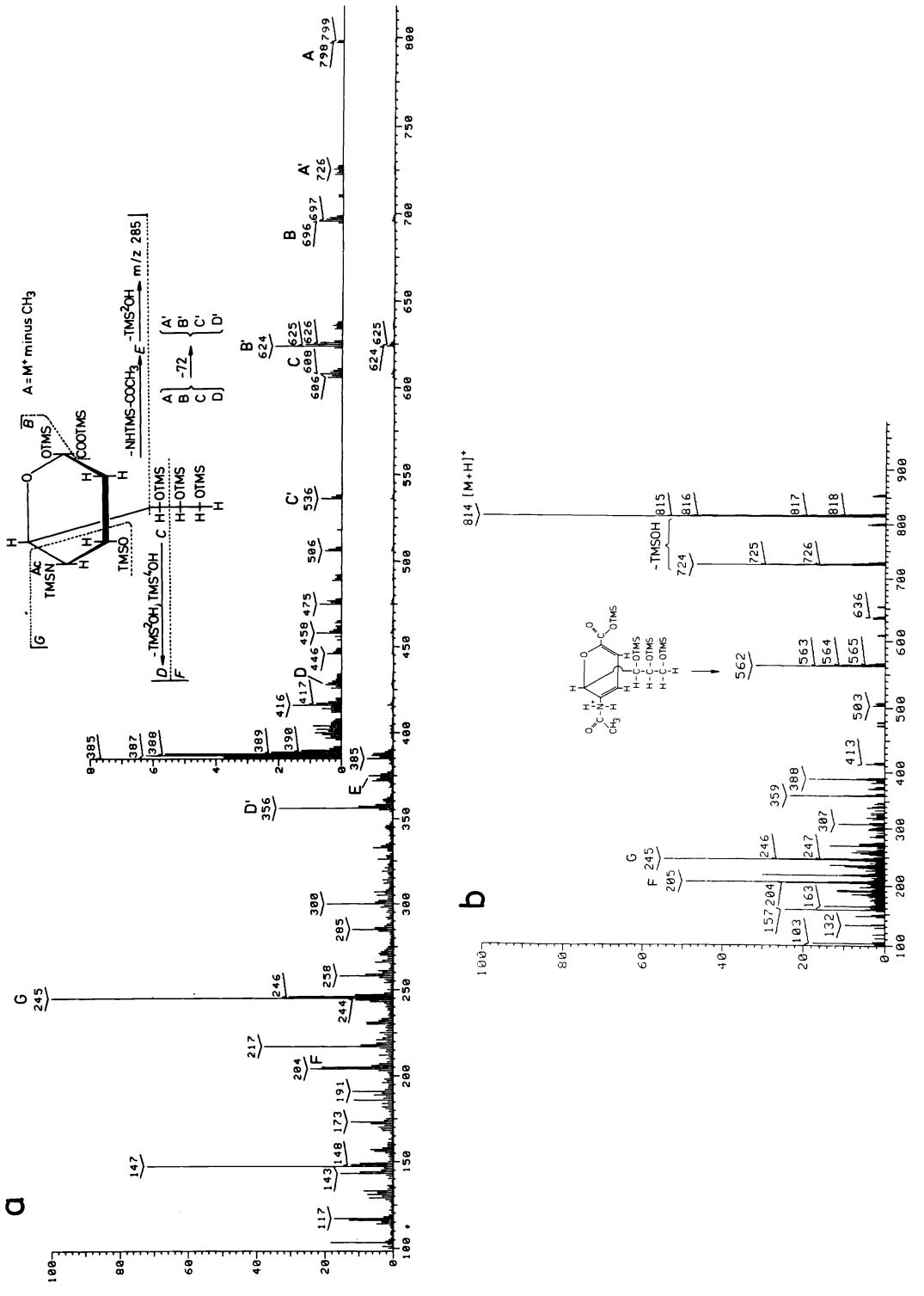

Fig. 6.
a) Electron ionization mass spectrum of N-acetylneuraminic acid, analyzed as trimethylsilyl ester, per-O-trimethylsilyl ether, N-trimethylsilyl derivative. The fragment nomenclature follows the known fragmentation pattern (35).
b) Chemical ionization mass spectrum of N-acetylneuraminic acid, analyzed as trimethylsilyl ester, per-O-trimethylsilyl ether, N-trimethylsilyl derivative, using isobutane as reagent gas.
The fragments F and G are the same as in the known fragmentation pattern (35) for EI mass spectra.

Since acid hydrolysis of sialic acids followed by purification is known to lead to the loss of 10-20% of these substances and of 30-50% of the O-acetyl groups of sialic acids (1,31), methods were developed for the determination of micro-quantities of sialic acids on intact cells or isolated erythrocyte membranes. In one of these procedures erythrocyte membranes are prepared on filters for colorimetric sialic acid analysis by the classical periodic acid|thiobarbituric acid or orcinol|ferric ion|HCl techniques (37). Alternatively, erythrocytes or their membranes are treated with 1 mM periodate resulting in one mole of formaldehyde per mole of sialic acid (29). The formaldehyde is determined by the reaction with acetylacetone and ammonium acetate leading to a fluorogen (F 410|510), the structure of which is shown in Fig. 7. As sialic acids O-acetylated at C-8 or C-9 are not oxidized under these conditions, they can quantitatively be measured by the increase of fluorescence after saponification of the ester groups by 0.1 M NaOH. The reactions leading to the production of formaldehyde in this sensitive sialic acid assay are shown in Fig. 7.

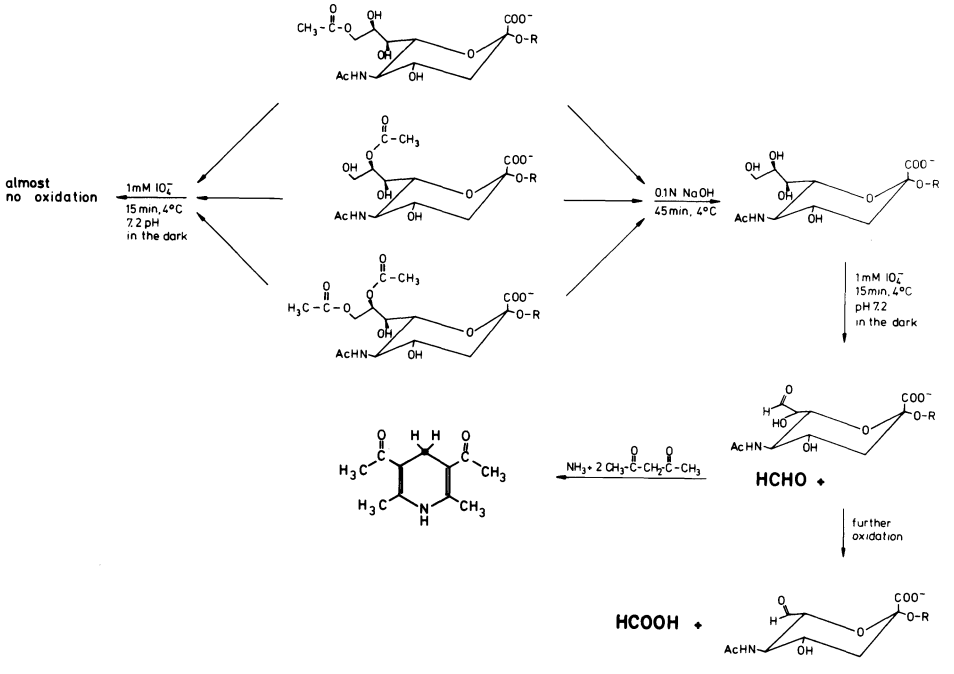

Fig. 7. Oxidation of the side chain of sialic acid with periodate and reaction of the formed formaldehyde with acetylacetone and ammonium acetate. The degree of O-acetylation at C-8 or C-9 can be measured by the increase of fluorescence after saponification of the ester group with 0.1 M NaOH (29).

INTERACTION OF ERYTHROCYTES WITH MACROPHAGES

Studies in vivo

Twenty years ago Perona et al. (38) observed a marked reduction of the life-time of human erythrocytes treated with sialidase. A similar observation was made by Jancik & Schauer (8) ten years ago with rabbit erythrocytes and later (9) also with human erythrocytes using pure Vibrio cholerae sialidase. Enzymic removal of about 60% of the sialic acids from human erythrocytes resulted in a reduction of the half life-time of these cells from normally 26 days to 2 h (Fig. 8). Sialidase-treated, 51-Cr-labelled erythrocytes were not lyzed in the blood stream but accumulated in spleen and liver of rabbits, as was shown by scintigraphy (5). Electron microscopy of these tissues showed that the enzyme-treated cells had been bound and phagocytozed by macrophages (5). Radio-labelling of sialidase-treated rabbit erythrocytes with galactose oxidase and borotritide followed by reinjection of these cells into the animals and autoradiography combined with electron microscopy of liver and spleen slices confirmed that only the enzyme-treated erythrocytes had been removed (unpublished).

Sequestration of sialidase-treated erythrocytes from the blood stream seems to be a general phenomenon for mammalian erythrocytes, which was also observed with cow (5) and with a variety of other mammals as reviewed in Refs. 1, 21 & 39. However, not only sialyl residues protect the circulation time of erythrocytes. In rabbit erythrocytes, terminal α-galactosyl residues have been found to have a similar role: after their removal by coffee bean α-galactosidase the cells undergo rapid lysis in homologous blood serum, mediated by complement factors (40).

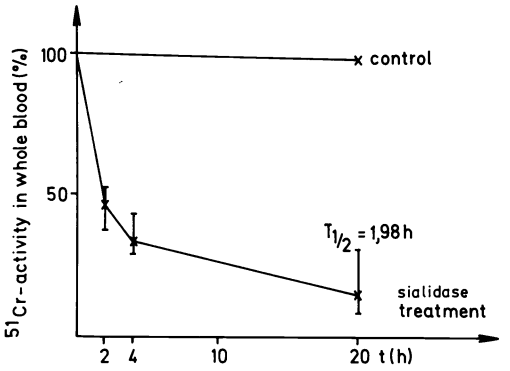

Fig. 8.
Disappearance of sialidase-treated and 51-Cr-labelled, homologous erythrocytes reinjected into the circulation of man (9).

Studies in vitro

Experiments with isolated macrophages (rat Kupffer cells or peritoneal macrophages) in culture enabled a detailed study of the mechanism involved in the sequestration of sialidase--treated erythrocytes by macrophages observed in vivo. One of the main aims was to find the amount of sialic acid which must be removed from the erythrocytes to induce binding and phagocytosis. Fig. 9 shows that the amount of rat erythrocytes bound and phagocytozed by rat peritoneal macrophages increased significantly after the removal of 10-20% and 30-40%, respectively, of sialic acid residues from erythrocytes (7). Values of 10-20% have been reported as being sufficient to eliminate erythrocytes from the circulation (e.g. 41). Hydrolysis of larger amounts of sialic acids led to an increase of the numbers of bound and engulfed erythrocytes (7) with a maximum after complete desialylation achieved by the use of sialidase from Arthrobacter ureafaciens. The amount of bound and endocytozed red cells was also proportional to the quantity of erythrocytes present in the incubation mixture (7), and exhibited a maximum after 30 and 45 min, respectively (42). These results were obtained by assays using rat peritoneal macrophages incubated with 51-Cr-labelled (6,7) or non-labelled (42) homologous erythrocytes followed by the determination of bound or phagocytozed erythrocytes either by radioactivity counting or by microscopy. Electron microscopy revealed strong deformations of erythrocytes interacting with peritoneal macrophages (Fig.10a). One macrophage is able to engulf between 1 and 10 erythrocytes; Fig. 10b shows a peritoneal macrophage with 3 ingested red blood cells.

The question arises which forces are responsible for the binding of partially desialylated erythrocytes by macrophages. As this interaction between the two cell types can occur in mere buffer, it is suggested that only the removal of sialyl residues from the erythrocyte surface causes macrophages to bind the red cell. A possible role of sialidase molecules themselves adhering to the erythrocyte membrane was excluded by the use of immobilized sialidase or the sialidase inhibitor 2-deoxy-2,3-didehydro-N-acetylneuraminic acid as well as by the additon of very low amounts of soluble sialidase (7,40). First insight into the nature of an erythrocyte membrane component demasked by sialidase treatment and involved in binding of erythrocytes to phagocytes came from studies with rat Kupffer cells by Kolb & Kolb-Bachofen (4), who

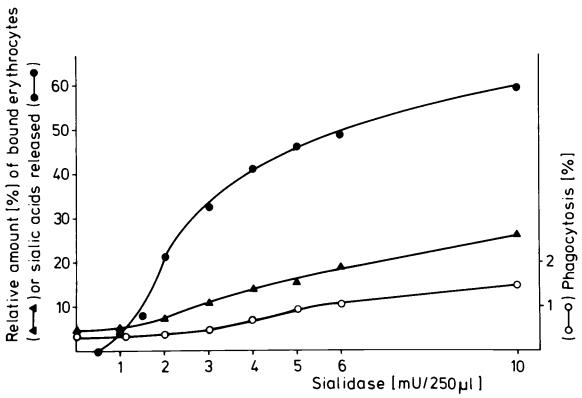

Fig. 9.
Binding (-▲-▲-, % of erythrocytes present in the incubation mixture) and phagocytosis (-○-○-) of sialidase-treated rat erythrocytes by homologous peritoneal macrophages in dependence on the percentage of sialic acids released by Vibrio cholerae sialidase (-●-●-) (7).

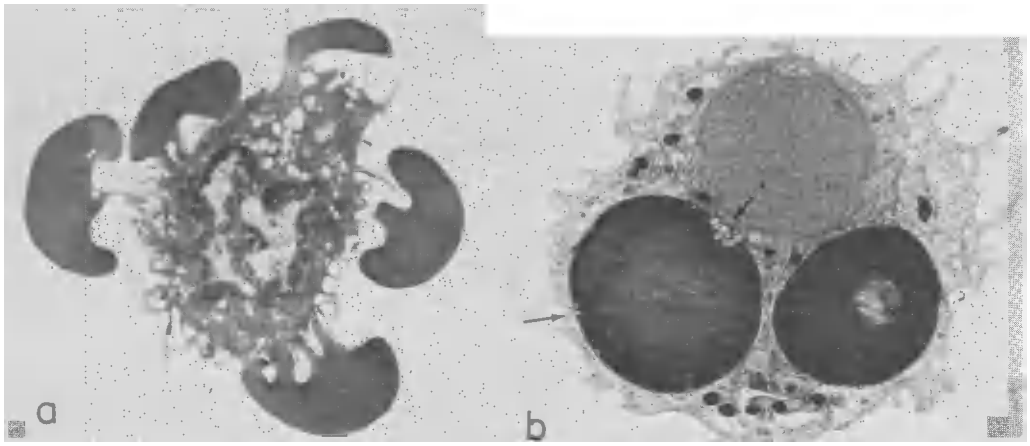

Fig. 10.
a) Binding of five sialidase-treated rat erythrocytes by a peritoneal macrophage with numerous microvilli. Electron micrograph, x 5000.
b) A peritoneal macrophage with three phagocytozed, sialidase-treated erythrocytes in different stages of decomposition. The arrows indicate vesicles considered to be lysosomes. Electron micrograph, x 6000.

could inhibit this interaction by galactose and N-acetylgalactosamine. The existence of a galactose-specific receptor on the Kupffer cell membrane was postulated. Although this receptor has yet not been isolated, further strong evidence for its existence is accumulating, mainly on the basis of inhibition studies. Table 2 shows galactose and a variety of complex carbohydrates with one or several terminal ß-galactosyl groups, all of them inhibiting both binding and phagocytosis of sialidase-treated rat erythrocytes by homologous, peritoneal macrophages. The inhibitory capacity of these compounds, however, is rather variable, free galactose exhibiting the weakest and human asialo-glycophorin as well as antifreeze-glycoprotein the strongest effects. Interestingly, branched oligosaccharides inhibit stronger than linear ones, a behaviour which is similar to that of the ß-galactose-specific lectin of human hepatocytes (43). Other similarities between the hepatocyte and macrophage receptors are described e.g. in Ref. 44. Mannose and N-acetylglucosamine (4) or mannan and chitobiose do not inhibit the interaction of sialidase-treated rat erythrocytes with peritoneal macrophages.
All these studies show that the macrophage receptor responsible for the binding of sialidase-treated erythrocytes recognizes ß-galactosyl groups in oligosaccharides and glycoconjugates and can thus be classified as a ß-galactose-specific lectin (1,4,7,44). The ß-galactosyl and perhaps N-acetylgalactosaminyl residues of erythrocytes interacting with the macrophage lectin are assumed to be demasked by sialidase-treatment on the basis of the following observations: a) sialic acids are known to be most frequently bound to galactose in glycoconjugates (1), b) sialidase-treated rat erythrocytes are agglutinated by galactose|N-acetylgalactosamine-specific plant lectins (7), c) T-antigen is exposed on human erythrocytes after sialidase treatment (25), and d) an increase of tritium incorporation into sialidase-treated rabbit erythrocyte membranes after galactose oxidase incubation has been observed (40). The macrophage lectin not only recognizes ß-galactosyl compounds in vitro, but also in vivo, as was shown by the inhibition of the clearance of sialidase-treated erythrocytes from the blood stream by repeated injections of lactose or asialo-fetuin into rabbits (45; Fig. 11).

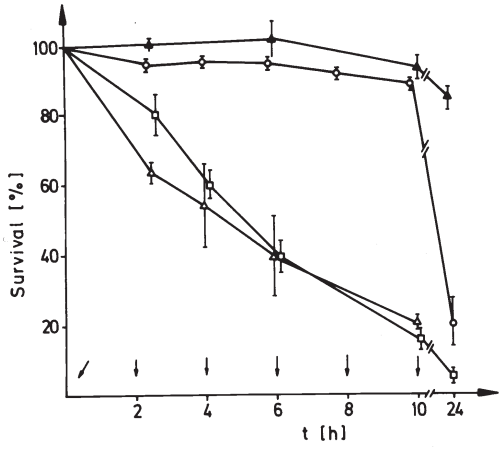

Fig. 11.
In vivo survival of sialidase-treated, 51-Cr-labelled, homologous rabbit erythrocytes under the influence of asialo-fetuin. -▲-▲-, control experiment; -□-□-, sialidase-treated erythrocytes without inhibitor; -△-△-, sialidase-treated erythrocytes in the presence of 10 µM native fetuin; -○-○-, sialidase-treated erythrocytes in the presence of 30 µM asialo-fetuin. The times of intravenous inhibitor injections are indicated by arrows.

Table 2. Inhibition by substances with terminal ß-galactosyl or sialyl-1-ol residues of binding of sialidase-treated rat erythrocytes by homologous peritoneal macrophages. Phagocytosis was inhibited at a similar degree. The values indicate concentrations leading to about 50% inhibition, with the exception of galactose, which inhibited by 21%. *The corresponding substances containing native, negatively charged sialic acid showed no significant inhibitory effect; **the oligosaccharides were a gift from J.-C. Michalski, Lille; ***mol. weight 2 600, contains 4 chains of Galß(1-3)GalNAc|molecule

Substances	µM
Galactose	30 000
Lactose	2 000
Sialyl-1-ol-α(2-3)lactitol*	2 000
GalNAcα(1-3)Galα(1-2)Fuc**	2 000
Lysozyme-phenylazo-ß-galactose	1 000
Galß(1-3)GlcNAcß(1-3)Galß(1-4)Glc** | α(1-4) Fuc	350
Galß(1-3)GalNAcß(1-4)Galß(1-4)Glcß(1-1)Cer	200
Galß(1-4)GlcNAcß(1-2)Manα(1-3) Manß(1-4)GlcNAc** Galß(1-4)GlcNAcß(1-2)Manα(1-6)	175
Albumin-galactose	100
Asialo-fetuin	10
Sialyl-1-ol-fetuin*	10
Sialyl-1-ol-glycophorin*	5
Galß(1-4)GlcNAcß(1-4) Galß(1-4)GlcNAcß(1-2)Manα(1-3) Manß(1-4)GlcNAc** Galß(1-4)GlcNAcß(1-2)Manα(1-6)	4
Asialo-glycophorin	1
Antifreeze-glycoprotein***	0.5

However, the lectin seems not to recognize α-galactosyl residues, as untreated rabbit (40) and rat (unpublished) erythrocytes bearing such sugar residues in terminal position of glycoconjugates on their surface survive in the circulation and are not bound to the macrophages studied. Thus, the mechanism of binding of sialidase-treated erythrocytes followed by engulfment, can be drawn as shown in Fig. 12.

Investigations using gold particles with diameters of 5, 17 and 50 nm and coated with asialo-fetuin or lactosylated bovine serum albumin have shown that Kupffer cells can engulf these particles in contrast to hepatocytes which can take up only the smallest particles (46). While the 5 and 17 nm beads were endocytozed by the macrophages via the coated pit|coated vesicle pathway, the 50 nm particles were not engulfed in this way. However, all particles accumulated in the lysosomal vacuoles.

Although the exposure of ß-galactosyl residues on the surface of particles and cells seems to be the only factor responsible for the initiation of uptake of these corpuscles by macrophages, the presence of homologus serum or plant lectins in subagglutinating concentrations was found to enhance both binding and phagocytosis of rat erythrocytes (6,7). Native serum stimulated binding and phagocytosis of sialidase-treated erythrocytes by a factor of two to five. Heat inactivation of serum (56°C, 30 min) reduced the stimulative effect by about the half. From these experiments it was concluded that immunoglobulins and|or complement factors as

well as the Fc receptor of macrophages may be involved in the mechanism of uptake of erythrocytes bound first by the ß-galactose-specific lectin (6). Careful experiments, however, using various immunoglobulins, anti-IgG, complement factors or inhibitors of the Fc receptor have not confirmed this assumption (7). Substances known to stimulate phagocytosis such as fibronectin, tuftsin or "substance P" were also excluded to play a role in the erythrocyte-macrophage system studied (7). It is not known at present whether this "serum-effect" is due to a specific compound present in serum or to an unspecific effect. When compared to serum the stimulative effect of peanut lectin (galactose-specific) or Erythrina crystagalli agglutinin (recognizing galactose and N-acetylgalactosamine) was much more pronounced (7; Fig. 13). An explanation for this phenomenon is not yet available. It may be speculated that the plant lectins cause alterations of the conformation or distribution of desialylated glycoconjugate molecules in the erythrocyte membrane which leads to the exposure of cryptic compounds triggering phagocytosis. Stimulation of phagocytosis by plant lectins has also been observed in other systems (e.g. 47).

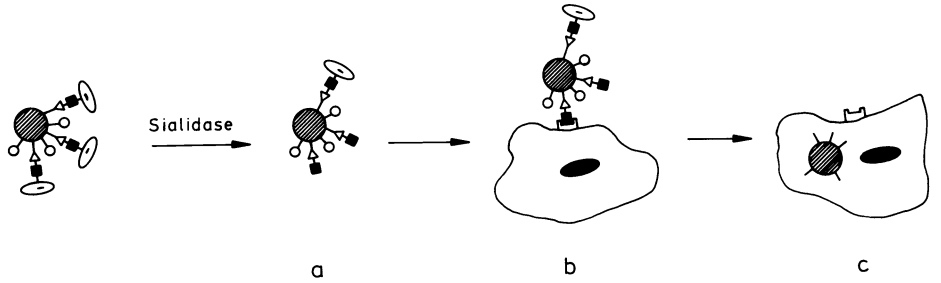

Fig. 12.
Mechanism of binding (b) and phagocytosis (c) of sialidase-treated erythrocytes (a) by macrophages.
⬭ , negatively charged sialic acid; ■ , galactose residue; ○ , other membrane components; ⌐¬ galactose-specific lectin on the macrophage surface

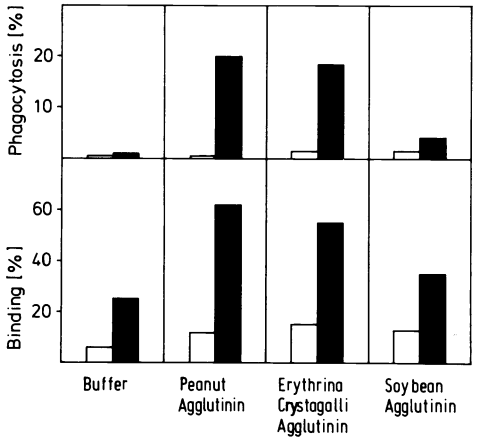

Fig. 13.
Effect of plant lectins on the binding and phagocytosis (% of erythrocytes present in the incubation mixture) of rat erythrocytes by homologous peritoneal macrophages (7).
▭ , Untreated erythrocytes;
■ , sialidase-treated cells.

Mode of masking by sialic acids

It is tempting to investigate in which way sialic acid residues can mask subterminal galactose or N-acetylgalactosamine residues of glycoconjugates and thus can protect the life-time of molecules and cells. One may think that this effect is caused by the bulkyness of the sialic acid molecule, a N-acylated polyhydroxyamino acid. Especially the carboxyl group is assumed to give the sialic acid residue a conformation suitable to shield the penultimate sugar effectively. Reduction of the carboxyl group of sialic acid to a primary alcohol indeed leads to a dramatic decrease of the masking effect of sialic acids. This was investigated with sialyllactitol containing carboxyl-reduced N-acetylneuraminic acid. The compounds inhibited binding and phagocytosis of sialidase-treated erythrocytes to a similar extent as did lactose (A.K. Shukla et al., unpublished) (Fig. 14 and Table 2). Sialyllactose and sialyllactitol were no inhibitors under the same conditions. Similarly, reduction of sialic acid carboxyl groups in fetuin or glycophorin gave these molecules an inhibitory effect, which was almost as strong as with the corresponding asialo-glycoproteins. The sialic acid residues of these compounds had been reduced by coupling to N-ethyl-N'-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) at pH 4.7 and treatment with sodium borohydride, according to a procedure described in Ref. 49 for uronic acid-containing polysaccharides. It was checked by several analytical procedures including TLC (Fig. 15), GLC and NMR-spectroscopy (J.F.G. Vliegenthart et al., unpublished), that 90-100% of the glycosidically bound sialic acid residues of the compounds tested were reduced in their carboxyl moiety.

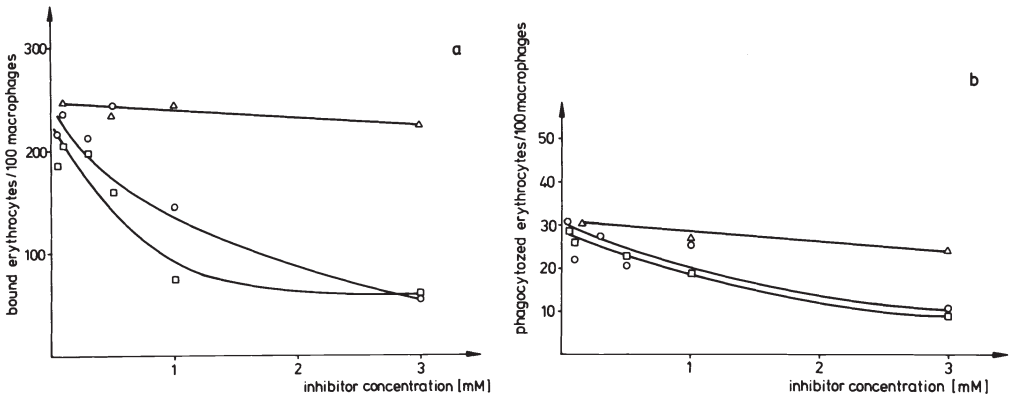

Fig. 14. Inhibition of binding (a) and phagocytosis (b) of sialidase-treated rat erythrocytes by homologous peritoneal macrophages by lactose (-□-□-) and sialyl--1-ol-α(2-3)lactitol (-○-○-). Sialyl-α(2-3)lactose (-△-△-) and sialyl-α(2-3)-lactitol had no significant inhibitory effect.

The masking by sialic acid is not due to the anomeric configuration of the glycosidic bond of this sugar, as the galactose residue of sialyllactose or sialyllactosamine is biologically inactive, if sialic acid is bound to it in α- or ß-glycosidic linkage. In these unpublished studies sialyl-ß(2-6)lactosamine (48), a gift from H. Paulsen, Hamburg, was used. Shortening of the sialic acid side chains of intact rabbit erythrocytes by mild periodate oxidation followed by borohydride reduction only slightly reduced the life-time of these cells in the circulation, showing that the anti-recognition effect of sialic acids is not confined to this structural part of the sugar molecules (40).

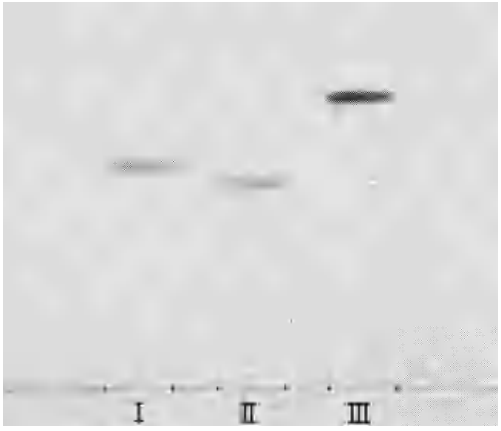

Fig. 15.
Thin-layer chromatography of sialyl-α-(2-3)lactose and reduced derivatives on silica gel thin-layer plates in ethanol|n-butanol|pyridine|water|acetic acid (100:10:10:30:3, v|v).
I, sialyl-α(2-3)lactose;
II, sialyl-α(2-3)lactitol;
III, sialyl-1-ol-α(2-3)lactitol (carboxyl-reduced sialyllactitol).

THE PATHOPHYSIOLOGICAL ROLE OF SIALIDASE

The observations regarding the anti-recognition by sialic acid in a variety of important biological systems ask for a strong regulation of the metabolism of this sugar. Although not yet much is known about the control of the anabolic side of this field, more insight into the modulation of the catabolic reactions e.g. by the study of substrate specificity, subcellular localization and genetic disorders of sialidases has been obtained (1,28,50). Knowledge about the occurrence of enhanced concentrations of sialidases in some infectious diseases, which was obtained in the past few years and will briefly be discussed in the following, shows that the phenomena of the uptake of serum glycoproteins by hepatocytes and blood cells by a variety of macrophages, may not only be of academic interest but also of pathophysiological importance. Physicians have reported unexplainable anemic states after traumatization or infections (personal communications and Refs. 5,9), which have been suspected to be due to a loss of erythrocyte sialic acids by elevated serum sialidase levels. This assumption was supported by the following observations: T-antigen was found to be exposed on the erythrocytes from an anemic patient with pneumococcal infection, which was believed to be due to the action of bacterial sialidase (51). In gas oedema of man (52) and rabbit (Fig. 16a; Sander-Wewer et al., unpublished) caused by Clostridium perfringens, high sialidase concentrations in blood

serum were determined. In this disease, which is frequently accompanied by severe anemia, desialylated glycoproteins were detected in serum by electrophoresis, too (Sander-Wewer et al., unpublished). In collaboration with R. Roos, Munich, we found high sialidase activities in both blood serum and brain liquor of a child suffering from pneumonia (Fig. 16b; unpublished). This patient had also developed a severe anemia. Enzyme activity decreased rapidly by antibiotic treatment (Fig. 16b). Furthermore, an increase of sialidase activity in cow serum accompanied by a decrease of the amount of erythrocytes in circulation during infection with Trypanosoma vivax was reported by Esievo et al. (53).

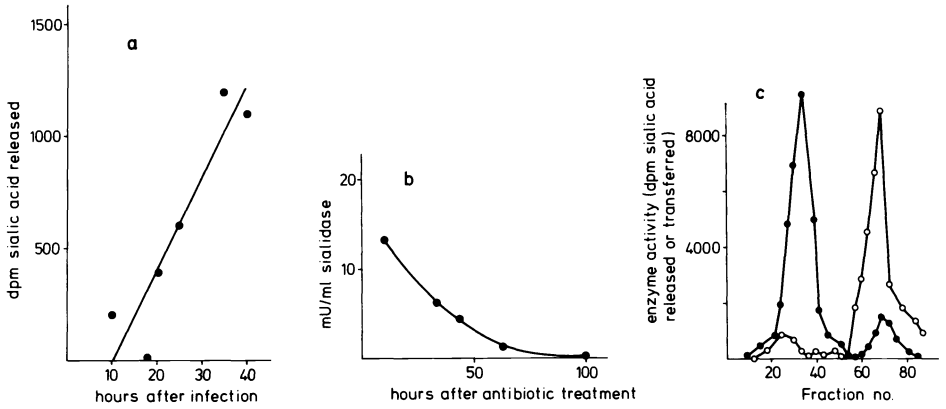

Fig. 16.
Occurrence of sialidase in blood serum under normal and pathological conditions.
a) Increase of serum sialidase activity in rabbit blood serum in the course of infection with Clostridium perfringens (Sander-Wewer et al., unpublished), determined with radioactive sialyllactose.
b) Elevated sialidase activity in the serum of a patient with pneumococcal infection. The disappearance of enzyme activity is due to treatment with antibiotics (R. Roos et al., unpublished).
c) Enrichment (300-fold) of sialidase (—●—●—) and sialyltransferase (—○—○—) from the serum of a healthy person by preparative polyacrylamide gel electrophoresis according to Ref. 55. Substrates for sialidase were radioactive sialyllactose and for sialyltransferase asialo-fetuin as acceptor and radioactive CMP-N-acetylneuraminic acid as donor. Sialidase concentration in this serum was about 5 μU|ml.

Normally,in the serum of healthy individuals only very low activities of sialidase can be detected (54), and values of 0.1-10 μU|ml were determined in about 300 persons (M. Schindelhauer, Dissertation, University Kiel, 1984). An increase of sialidase activity above this level may be harmful to the organism. It is expected that future research will reveal more examples of an increase of sialidase activity in patients and give more knowledge about a pathophysiological role of the galactose|N-acetylgalactosamine-specific lectins on hepatocytes and macrophages and perhaps also on other cells.

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