Aglycosylantibody

EFFECTS OF EXOGLYCOSIDASE TREATMENTS ON AUTOCHTHONOUS ANTIBODY SURVIVAL TIME IN THE CIRCULATION*

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Rabbit anti-hapten antibodies were purified by affinity chromatography and characterized immunochemically for in vivo studies of their blood clearance rate and organ distribution after treatment with various glycosidases. Following sequential removal of sialic acid, galactose, and N-acetylgalactosamine with the appropriate cellulose-immobilized exoglycosidases, the antibody populations were recharacterized, radiolabeled, and introduced intravenously into the original animals. Using double radioiodine labels it was possible to demonstrate alterations in purified antibody survival times in the circulation and altered organ distribution after glycolytic cleavage. Removal of terminal sialic acid resulted in rapid blood clearance and enhanced localization of asialoantibody in the liver. Subsequent removal of penultimate galactose residues returned both antibody survival time in the circulation and organ distribution to near normal. Removal of subpenultimate N-acetylglucosamine moieties resulted in aglycosylantibody survival values which were intermediate between asialo- and asialoagalactoantibodies. Removal of the three saccharides also increased kidney localization. The results are evaluated based on current concepts of the biological roles of protein-linked carbohydrate and plasma glycoprotein survival time in the circulation.

The concept that the terminal sialic acid moiety is crucial to the continued circulation of many, if not most, core-type and mucin-type (1) glycoproteins has been developed by Ashwell and Morell (2–4). This hypothesis is based on the fact that neuraminidase treatment of ceruloplasmin (5), orosomucoid, fetuin, haptoglobin, α₂-macroglobulin, chorionic gonadotropin, follicle-stimulating hormone (6), and plasminogen (7) has resulted in rapid clearance of these asialoglycoproteins from the blood when compared with untreated controls. The loss of asialoglycoproteins from the circulation was subsequently shown to be due to their binding by hepatic (parenchymal) cell surface receptors which "recognize" exposed, penultimate galactosyl moieties (8, 9). The hepatic cell surface receptor was initially proposed to be an enzyme-UDP-Gal-glycoprotein glycosyltransferase (10), but the involvement of transferase activity in the purified receptor (11) has been questioned by Hudgin and Ashwell (12). Independent verification that glycoprotein clearance is mediated through oligosaccharide moieties was obtained in studies where proteins were conjugated to asialoglycoprotein and clearance rates evaluated. Both lysozyme and albumin, when coupled to, fetuin, were selectively absorbed by liver cells (13) after neuraminidase treatment. There is, however, no direct evidence that the process of asialoglycoprotein clearance by hepatic receptors occurs naturally in vivo.

Since very little is known about the mechanisms or rates of antibody clearance from the circulation, we felt an analysis of whether the experimentally inducible clearance process is applicable to humoral antibody could give some indication of (a) molecular events which control levels of circulating antibody, (b) possible mechanisms for modulating circulatory longevity of different classes of immunoglobulins, and (c) possible mechanisms for control of circulatory survival times of different types (immune versus hyperimmune) of antibody molecules.

MATERIALS AND METHODS

Enzyme Purification and Immobilization on Cellulose—Two secreted exoglycosidases from Diplococcus pneumoniae (β-galactosidase...
and N-acetylglucosaminidase) were purified by combined salt fractionation, molecular sieve, and ion exchange chromatography as described by Hughes and Jeanloz (14). Neuraminidase (type V from \textit{Clostridium perfringens}; Sigma Chemical Co., St. Louis, Mo.) was purified by affinity chromatography using cellulose-immobilized fe-

fluorometer (23, 29). Antibody population heterogeneity indices were derived from Sips analysis (30) of binding data plotted according to Scatchard (31). Radioactive antigen for equilibrium dialysis was \textit{e}-\textit{phosphyl-3,5-\textit{H}N\textit{p}h\textit{t}-lysine (1.1 Ci/mmol; New England Nuclear, Boston, Mass.). Antibody concentration was adjusted to 100 mg/ml using 25 \textit{m}Ci/mg, labeled[^{35}S]amino acid with 15 \textit{m}M - 15, and the immunoglobulin was stored at -70 \degree C until use.

**Glycosidase Treatment of Purified Antibodies—** Aliquots of purified antibodies from eight rabbits were pooled for each analysis and titration with Im-enzymes. Enzymatic hydrolysis was performed by incubating 25 or 50 mg of antibodies with 0.2 to 0.6 ml (packed volume) of Im enzyme at 37 \degree C for 30 min as delineated under "Results." The appropriate pH was obtained by adding \textit{m}l volume of 10X buffers as described by Hughes and Jeanloz (14). Reactions were terminated by centrifugation (2,000 \times g for 2 min at 4 \degree C) and removal of supernatants. Total nase acid was determined using the resorcinol-HCl technique (32, 33) and correcting for hexose backgrounds according to Spiro (34). Release of sialic acid by Im-neuraminidase treatment was monitored by the thioarbituric acid method (19). Liberated Gal and GlcNAc were measured by the Gal-oxidase-chromagen method (35) (Galacto-

**RESULTS**

Blood survival times were evaluated by injecting 5.0-mg doses of differently labeled untreated and glycosidase-treated antibodies into the autologous animal (i.e., 2.5 mg of \[^{125}I\textit{a}-\textit{Siama-anti-N,ph\textit{t}}\) from rabbit 1 and 2.5 mg of \[^{125}I\textit{a}-\textit{Siama-anti-N,ph\textit{t}}\) from rabbit 2). Radioactivity was determined simultaneously into antibody 1. Treated and untreated antibodies were injected simultaneously in the left marginal ear vein. Rabbits were killed at various time intervals. Blood (5.0 ml) was collected into heparinized tubes and analyzed for iodine ratios of \[^{125}I\] to \[^{131}I\].

1 The abbreviations used are: AcNeu, N-acetylneuraminic acid; BAc-cellulose, bromoacetyl-derivatized cellulose; Im, cellulose-immobilized; N,ph., dimethylphenyl; fl, fluorescein; aSia, aGal; aGlcNAc, N-acetylglucosaminidase; aGal, galactosidase; aGlcNAc, without N-acetylglucosamine.

**Radiolabeling and In Vivo Analyses—** Radiolabelings were performed at room temperature as follows: Im-lactoperoxidase (0.1 ml of packed volume, 490 \mu g) was rinsed in phosphate-buffered saline and 5.0 mg of antibody was added in a final volume of 1 ml iodide concentration was adjusted to 0.1 \mu m by the addition of 1.0 \mu M KI and 0.05 \mu m of NaI (39). Specific activities were determined after dialysis against two changes of buffer (8 liters each 24 hours for a total of 48 hours). Antibody precipitation was accomplished by adding \textit{m}l volume 10\% ZnSO\textsubscript{4} mixing, and then adding \textit{m}l volume 0.5 N NaOH. After mixing again, the antibody precipitate was pelleted (10,000 \times g for 10 min) and the supernatants analyzed for Gal or GlcNAc as described above. Quantitative protein precipitation was verified in control experiments with \[^{125}I\]labeled antibody.

Based on the results of enzyme titrations, individual stock preparations of aSia-antibody, aSia-Gal-antibody, and aSia-aGal-GlcNAc antibody were prepared for in vivo studies by sequentially treating 10.0-ml aliquots of purified antibodies with appropriate amounts of Im-enzyme (see "Results").

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(Fig. 50 to 60% of the total amount of sialic acid on the antibody treated sequentially with Im-enzymes and the released mono-
saccharides determined (Fig. 2). Im-neuraminidase liberated
cellulose or to reactive groups near the enzyme acticie site.

nearly 5-fold in activity after immobilization. This loss in Im-
tivity except for N-acetylglucosaminidase which decreased
activity to the pH conditions used for optimal coupling to BAc-
glucosaminidase activity might have been due to enzyme sensi-
tation by other enzymes assayed (Table I). Activities were re-
examined after immobilization on BAc-cellulose confirming
that the enzymes were coupled essentially without loss of ac-
divities (a) of the purified anti-hapten antibodies and
aglycosylantibodies indicated that glycosidase treatment(s)
did not affect the antigen-binding properties (Table II).

Differences between untreated and aglycosylantibodies are not

Plasma Survival Times—The in vivo fates of untreated and
aglycosylantibodies was followed simultaneously by injection of
5.0 mg of 125I-untreated antibody with 5.0 mg of 125I-
glycosylantibody (119; 119 – 1). Rabbits were bled periodi-
cally and the blood analyzed for percentage of recoverable
radioactivity/ml. Figs. 3 to 6 show the survival times of
autochthonous aglycosylantibodies compared with their un-
treated counterparts. The average clearance rate for untreated
homologous antibodies was 0.05%/hour and the recovery of total
label was 0.3 to 0.5%/ml of blood (Fig. 3). aSia-antibodies were
cleared at a rate of 0.2%/hour (Fig. 4) while aSia-aGal-antibodies
were cleared at near normal rates (Fig. 5). The aSia-aGal
GlcNAc-antibodies showed a significant increase (0.1%/hour)
in blood clearance rates (Fig. 6).

TABLE I

| Enzyme                        | Substrate                         |
|-------------------------------|-----------------------------------|
| Beta-Galactosidase            | O-Nitrophenyl-β-D-galactopyranoside|
| Im-β-D-galactosidase          | 110*                             |
| N-Acetyl-β-glucosaminidase    | 75.5*                             |
| Im-acetylglucosaminidase      | 3.3*                              |
| Neuraminidase                 | 0.1*                              |
| Im-neuraminidase              | 0.1*                              |
| Lactoperoxidase               | 0.1*                              |
| Im-lactoperoxidase            | 0.1*                              |
| BAc-cellulose                 | 0.1*                              |

Activities and specificities of enzymes used for antibody treatment

Units/mg of enzyme protein as described under “Materials and
Methods.”

* - not detectable.

Im-enzyme concentration expressed in milligrams of enzyme/ml of
packed volume was: Im-β-galactosidase, 4.9; Im-N-acetylglucosami-
idase, 3.5; Im-neuraminidase, 4.6; Im-lactoperoxidase, 4.9.

BAC-cellulose inactivated by bicarbonate-ethanolamine treatment
(see “Materials and Methods”).

molecule (40) this corresponded to cleavage of about 20% of
the total galactose. Untreated antibodies, aSia-antibodies and
aSia-aGal-antibodies were next incubated with Im-N-acetyl-
glycosaminidase. The results (Fig. 2C) indicated that Im-N-
acetyl-β-glucosaminidase failed to release detectable amounts of
GlcNAc from untreated antibody, but could release about 5%
of the total GlcNAc from aSia-aGal-antibodies assuming that
there are 10 to 11 mol of GlcNAc/mol IgG (41, 42). There was a
small, measurable amount of GlcNAc released from aSia-
antibodies suggesting that (a) Im-N-acetylglucosaminidase
had some endoglycolytic activity, (b) antibody molecules
possessed some penultimate GlcNAc, or (c) the Im-neuramini-
dase contained small amounts of contaminating β-galactosi-
dase activity which was not detected by our assays (Table I).

Average intrinsic association constants (K_a) and heteroge-
neity indices (a) of the purified anti-hapten antibodies and
aglycosylantibodies indicated that glycosidase treatment(s)
did not affect the antigen-binding properties (Table II).

Fig. 1. A, sodium dodecyl sulfate polyacrylamide gel electrophore-
tograms of purified antibodies. Numbers correspond to rabbit
identification numbers. Rabbits 1 to 4 produced anti-N-ph antibodies
while rabbits 5 to 8 produced anti-fluorescein antibodies. Molecular
weight calibration standards were: left, bovine serum albumin (BSA),
67,000; right, immunoglobulin M, 900,000; rabbit serum albumin
(RSA) 66,000, and ovalbumin (OVALB) 40,000. Antibody (100 to 150
µg) was applied to a 4% stacking gel (4 x 13 cm) cast over a 10%
resolving gel (13 x 13 cm). Electrophoresis was carried out for 5 hours
at 12.5 mA. B, immunoelectrophoretographs of whole rabbit serum
bodies from rabbits with numbers corresponding to A. All troughs
 contained goat anti-whole rabbit serum antiserum.

Fig. 2A). From
these data it was possible to estimate that 0.45
mol of AcNeu/mol of IgG was released by the immobilized
enzyme. Subsequent treatment of Im-neuraminidase treated
(aSia-antibodies) and untreated antibodies with Im-β-galac-
tosidase resulted in release of measurable amounts of galac-
tose from aSia-antibodies but not from untreated antibodies
(Fig. 2B). Assuming that there were 3 to 4 mol of galactose/IgG

Average packed volume was: Im-P-galactosidase, 4.9; Im-N-acetylglucosami-
idase, 3.9; Im-neuraminidase, 4.6; Im-lactoperoxidase, 4.9.

12.5 mA. B, immunoelectrophoretograms of whole rabbit serum
bodies from rabbits with numbers corresponding to A. All troughs
 contained goat anti-whole rabbit serum antiserum.
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FIG. 2. Immobilized-glycosidase treatments of purified rabbit antibodies. Figures correspond to the amount of saccharide released by: A, treatment of 50-mg aliquots of untreated antibodies with Im-neuraminidase (AcNeu released, 0). Optimal AcNeu release corresponded to 60% of the total AcNeu in 50 mg of antibody as estimated by comparing released AcNeu by the Warren method (19) with total AcNeu by the resorcinol-HCl method (32). B, treatment of 25-mg aliquots of previously untreated antibodies (●) and aSia-antibodies (▲) with Im-β-galactosidase. Supernatant (released) galactose values in the former case fell within the lower limits of assay sensitivity. C, treatment of 25-mg aliquots of aSia-antibodies (▲) and aSia-aGal-antibodies (■) with Im-N-acetylglucosaminidase. Exposure of previously untreated antibodies to Im-N-acetylglucosaminidase resulted in no measurable supernatant GlcNAc.

**Organ Distribution of Aglycosylantibodies**—In order to determine the organ distribution of the various antibody populations after measuring clearance from the circulation, rabbits were sacrificed and their organs removed, weighed, minced into approximately 5 mm² pieces, and monitored for total radiolabel content. The data summarized in Table III shows that aSia-antibodies were localized primarily in the liver while aSia-aGal-antibodies possessed essentially normal organ distribution compared with untreated antibodies injected simultaneously. The aSia-aGal-aGlcNAc-antibodies were found to a large degree in the kidney with some localization in the liver (Table III). The specificity of organ localization was tested in blocking experiments by injecting two additional rabbits with 5 mg of unlabeled native aglycosylantibodies plus 5 mg of unlabeled aglycosylantibodies. After 120 min, animals were sacrificed and their liver and kidneys evaluated for percentage of total radiolabel injected. Table IV shows that in comparison with ¹¹¹I-labeled aglycosylantibodies alone, both liver and kidney radiolabel uptake could be specifically inhibited with unlabeled aglycosylantibodies.

**Table II**

| Rabbit | Purified antibody | Kᵦ | (a) |
|--------|------------------|----|----|
| 1      | anti-            | 5.7 x 10⁶ | 0.81 |
| 2      | anti-N-ph        | 7.8 x 10⁵ | 0.55 |
| 3      | aSia-anti-N-ph   | 7.6 x 10⁵ | 0.50 |
| 4      | anti-            | 3.0 x 10⁵ | 0.73 |
| 5      | anti-N-ph        | 2.6 x 10⁴ | 0.72 |
| 6      | anti-N-ph        | 4.0 x 10⁴ | 0.69 |
| 7      | anti-N-ph        | 4.1 x 10⁴ | 0.89 |
| 8      | anti-N-ph        | 4.7 x 10⁴ | 0.53 |
| 9      | anti-N-ph        | 7.3 x 10⁴ | 0.69 |
| 10     | aSia-anti-f1     | 6.9 x 10⁴ | 0.88 |
| 11     | anti-f1          | 6.7 x 10⁴ | 0.84 |
| 12     | aSia-aGal-f1     | 6.8 x 10⁴ | 0.84 |
| 13     | anti-f1          | 1.3 x 10⁵ | 0.56 |
| 14     | aSia-aGal-aGlcNAc-f1 | 1.0 x 10⁵ | 0.80 |

*a Determined by equilibrium dialysis using ¹H]-N-ph-L-lysine.

*b Determined by quenching of fluorescence of fluorescein (fl) (disodium salt).

**Discussion**

The carbohydrate moiety of IgG constitutes 3 to 4% by weight of the total molecule and is known to consist of galactose, GlcNAc, AcNeu, GalNAc, mannose, and fucose (40). The exact sequence of these sugars on the antibody molecule is not known. However, the terminal oligosaccharide sequence, AcNeu-Gal-GlcNAc-, appears on an IgM myeloma protein (43) at a frequency equivalent to that found on many other glycoproteins (1) suggesting that this may be an important trisaccharide. The site of attachment of carbohydrate to the IgG of both rabbit and human origin is an asparagine residue in the Fc portion of the heavy chain. In rabbit IgG isolated from pooled sera, about 15% of the heavy chains
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contain an additional serine- or threonine-linked carbohydrate (44). These latter oligosaccharide residues generally contain GlcNAc, mannose, and galactose but no N-acetylneuraminic acid or fucose.

There are several roles which can be proposed for antibody-bound oligosaccharides. The carbohydrate portion of IgG is known to be involved in complement binding (24, 49). In addition, in a manner perhaps similar to parenchymal cell surface receptors which “recognize” exposed galactose moieties, macrophage and lymphocytes have receptors which may specifically recognize the carbohydrate sequences (perhaps, respectively, the Fd and Fc attached sugars) in the constant region of the IgG molecule (46, 47). Predictions have also been made for a role of carbohydrate in antibody secretion (48). Since serum albumin contains little or no carbohydrate, it appears that sugar moieties are not essential for secretion per se. It seems more likely that protein-linked oligosaccharides function in molecular recognition schemes and may confer important physicochemical characteristics to glycoproteins. For example, the carbohydrate portion of an Arctic fish “antifreeze” glycoprotein has been shown to be responsible for lowering the freezing temperature of serum (49-52). The presence of large amounts of carbohydrate on glycoproteins like fetuin could enhance solubility (53) or decrease partial specific volume (54).

The concept that plasma glycoprotein carbohydrate, specifically terminal AcNeu and penultimate galactose, plays a role in cell surface recognition has been under experimental investigation for several years (2-6, 8, 9, 13). The basic approach has been to remove terminal sialic acid residues with neuraminidase and to study the circulatory survival times of asialoglycoproteins. From the results of these studies, Ashwell and Morell concluded that the parenchymal cell of the liver has a surface receptor which recognizes exposed, AcNeu-penultimate, galactose moieties and binds the asialoglycoprotein for subsequent endocytosis and degradation (2-4). This mechanism is postulated to occur naturally as a means for maintenance of glycoprotein homeostasis.

**TABLE III**

Rabbit organ distribution of radiolabel after 120 min

| Rabbit | Antibody Type | Liver* | Kidneys | Lungs | Spleen | Brain |
|--------|---------------|--------|---------|-------|--------|-------|
| 1      | 131I-untreated | 0.045  | 0.068   | 0.357 | 0.078  | 0.005 |
| 1      | 125I-untreated | 0.038  | 0.045   | 0.034 | 0.069  | 0.004 |
| 2      | 131I-aSia     | 0.750  | 0.029   | 0.028 | 0.082  | 0.012 |
| 2      | 125I-untreated | 0.052  | 0.068   | 0.059 | 0.082  | 0.004 |
| 3      | 131I-aSia-aGal| 0.094  | 0.061   | 0.088 | 0.039  | 0.005 |
| 3      | 125I-untreated | 0.071  | 0.074   | 0.092 | 0.042  | 0.007 |
| 4      | 131I-aSia-aGal-aGlcNAc | 0.124 | 0.272  | 0.049 | 0.043  | 0.003 |
| 4      | 125I-untreated | 0.094  | 0.068   | 0.069 | 0.069  | 0.004 |

* Complete organs were monitored for radioiodine. Average organ weights were: liver 103.1 ± 12 g; kidneys 17.7 ± 5 g; lungs 10.2 ± 1.2 g; spleen 1.7 ± 0.7 g; brain 5.3 ± 0.3 g. Expressed as: percent injected 131I per g wet tissue weight percent injected 125I per g wet tissue weight (values in parentheses indicate ratios.)
There are several potential difficulties in these types of studies, the majority of which have been overcome in the present study. One drawback has been the use of pooled glycoproteins from several animals. In such cases, observed clearance rates may be partially attributed to recognition of "foreigness" by mechanisms other than the galactose-specific parenchymal cell binding. This problem was overcome in the present study by using purified autochthonous antibodies. Secondly, in previous studies commercial enzymes were sometimes used without further purification or characterization. Commercial neuraminidase preparations have been shown to contain significant amounts of protease activity (15, 55-58), and protease "nicking" of neuraminidase-treated glycoproteins could have been easily overlooked with molecules such as fetuin which have no assayable function. By using neuraminidase purified by affinity chromatography and highly purified P-galactosidase and N-acetylglucosaminidase in immobilized form, we were able to sequentially cleave AcNeu, Gal, and GlcNAc, respectively, from purified antibodies without affecting antigen-binding characteristics (Fig. 2 and Table II) and without protein loss since Im-enzymes can be easily and completely removed by centrifugation after treatment. A third potential problem arises when radiolabeling aglycosylglycoproteins for in vivo tracing. Harsh oxidative methods for iodination such as the iodine monochloride technique could potentially modify glycoprotein structural integrity. Consequently, we used Im-lactoperoxidase, an enzyme which has been shown to be relatively gentle and efficient (59), to radiolabel antibody populations.

Under the described experimental conditions, aglycosylantibodies possessed different clearance rates compared with untreated antibodies. Our results are consistent with previous studies on the removal of aglycosylglycoproteins from the circulation. On introduction of purified, radiiodinated aglycosylantibodies into autochthonous animals, we found that loss of terminal sialic acid resulted in clearance of antibody from the circulation and increased localization in the liver (Table III, Fig. 4). This result is consistent with those found for murine IgG by Melcher (60). Clearance rates for rabbit aSia-antibodies were not as rapid as those reported for other asialoaglycoproteins (2). It could be that autochthonous glycoproteins are not cleared as rapidly as nonhomologous glycoproteins or that the antibody molecules with core-type oligosaccharides may be somewhat less susceptible to the hepatic clearance mechanism than mucin type glycoproteins like fetuin. Additionally, our results and those of other investigators (41, 42) indicate that antibody molecules contain relatively small amounts of AcNeu. Since there is evidence for "microheterogeneity" of sugar content within purified antibody populations (61) as well as quantitative differences occurring during the progression of the immune response (40), it is likely that antibody molecules contain different amounts of neuraminidase-susceptible sialic acid.

While Im-neuraminidase treatment of the purified antibodies resulted in enhanced clearance from the circulation, removal of penultimate galactose with Im-β-galactosidase resulted in blood survival times at near normal levels—consistent with the results of Ashwell et al. (3, 4). Removal of penultimate GlcNAc resulted in enhanced antibody clearance from the circulation compared with untreated antibody (Fig. 6). This enhanced clearance corresponded to increased localization of radiolabel in the kidneys (Table III). Elevated liver uptake of radiolabeled aSia-antibodies and kidney uptake of aSia-aglycoantibodies were specific processes which could be inhibited with the appropriate, unlabeled aglycosylantibody population (Table IV).

The results of the present study support the proposal of Ashwell and Morell that terminal sialic acid plays a key role in the survival of certain glycoproteins (2-6). Although direct proof for natural desialation of plasma glycoproteins occurring in vivo is lacking, there are reports of sialidase activity on mammalian cell surfaces (69). Consequently, it is possible to speculate that the half-life of certain glycoproteins in the circulation is based on both the probability that the glycoprotein will contact sialidase and the susceptibility of the glycoprotein's terminal sialic acid to cleavage. This "aging" process could have specific physiological significance. For example, one can speculate that glycoproteins such as high affinity antibody molecules of the secondary (hyperimmune) response could be "programmed" for long term survival by having additional sialic acid residues compared with low affinity molecules produced during the primary immune response. The synthesis of glycoproteins with different types of carbohydrate units by the same cell (1) and the occurrence within the same protein of unlike carbohydrate sequences and differing glycopeptide linkages makes simple interpretation difficult, but the possibility of "age-programming" by addition of sialic acid moieties adds a new dimension to the elegant model of Ashwell and Morell (2, 3) for liver clearance of plasma glycoproteins.

It is likely that the sugar components of glycoproteins function in a number of unrelated physiological processes. For example enhanced kidney uptake of aSia-aglycoantibodies (Fig. 6, Table III) may represent another mechanism for glycoprotein homeostasis. Studies of the mechanisms of hemoglobin turnover have shown that the glomerular epithelial foot possess properties which allow the kidney to act as a fine filter for plasma glycoproteins (63). Consequently, it is possible that certain aglycosylglycoproteins could form complexes (specific or nonspecific) with plasma components, and the complexes could then be trapped in the kidney's filtering network. Interestingly, complexes of asialo-IgG cryoglobulins occur in the kidneys of glomerulonephritic patients (64). While complete saccharide analyses were not performed on complexed cryoglobulin, it is possible that the molecules lack several sugar moieties (altered oligosaccharide structure could also confer different temperature-dependent solubility properties to the cryoglobulin). Alternatively, there may be kidney cell surface receptors which recognize unique oligosaccharide structures and specifically bind aSia-aglycoantibodies. These hypothesis should be amenable to study in autoimmune diseases where there are exaggerations of similarities.

| Rab- bit | Radiiodine tracer on antibody | Unlabeled inhibitor added | Percent radioactivity recovered | Liver | Kidneys |
|---------|-------------------------------|--------------------------|-------------------------------|-------|---------|
| 1       | 5 mg 121I-untreated           | None                     | 4.6                           | 0.83  |         |
| 2       | 5 mg 121I-aSia-antibody       | None                     | 77.1                          | 0.80  |         |
| 9       | 5 mg 121I-aSia-antibody       | 5 mg aSia-antibody       | 55.3                          | 0.87  |         |
| 4       | 5 mg 121I-aSia-agal-          | aGlcNAc-antibody         | None                          | 13.7  | 4.89    |
| 10      | 5 mg 121I-aSia-Gal-           | aGlcNAc-antibody         | None                          | 5.3   | 2.59    |

### TABLE IV

Specificity of organ uptake of radiolabeled aglycosylantibody

- **Percent radioactivity recovered**
- **Liver**
- **Kidneys**

- **Rabbit Radiiodine tracer on antibody Unlabeled inhibitor added**
  - 1 5 mg 121I-untreated None 4.6 0.83
  - 2 5 mg 121I-aSia-antibody None 77.1 0.80
  - 9 5 mg 121I-aSia-antibody 5 mg aSia-antibody 55.3 0.87
  - 4 5 mg 121I-aSia-agal- None 13.7 4.89
  - 10 5 mg 121I-aSia-Gal- aGlcNAc-antibody aGlcNAc-antibody 5.3 2.59
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(65) rather than heterogeneity of immunoglobulin-bound oligo-
saccharides.

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