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Partial resialylation of human asialotransferrin type 3 in the rat

(receptor-mediated endocytosis/glycosylation/Golgi apparatus/catabolism of asialoglycoproteins)

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ABSTRACT **After the injection of a small dose (1 μg/100 g of body weight) of ^{125}I-labeled human asialotransferrin type 3 in rats, the radioactivity became rapidly associated with the liver. However, during the ensuing 12 hr a significant fraction of the dose returned to the circulation as protein-bound ^{125}I. The protein released by the liver was indistinguishable by gel filtration from the original preparation and was precipitable by an antiserum to human transferrin. Nevertheless, it no longer bound to the immobilized Gal/GalN-specific lectin from rabbit liver. However, binding could be restored to a large extent by treatment with neuraminidase, indicating that the loss of binding was due to resialylation. Changes in the electrophoretic mobility of asialotransferrin released by the liver showed that resialylation was partial—i.e., it involved the attachment of two or three sialyl residues. From analysis by deconvolution of the plasma curve of partially resialylated asialotransferrin it was calculated that the liver "repaired" this way approximately one asialotransferrin molecule out of four. Plasma clearance of partially resialylated asialotransferrin was similar to that of nondesialylated transferrin.**

A minor portion (15–17%) of human transferrin phenotype C consists of molecules which, after desialylation, bind avidly (1) to the Gal/GalN-specific lectin (2) of the rat liver. We designated this fraction human asialotransferrin type 3 (HAsTf-3; ref. 3). Suspended rat hepatocytes internalize HAsTf-3 via the above lectin (4). However, only a small fraction of the intracellular HAsTf-3 undergoes catabolism, whereas the rest is released showing no signs of proteolytic digestion. Cell-associated radioactivity decreases considerably more slowly in the suspension than is the time required for ^{125}I-labeled HAsTf-3 (^{125}I-HAsTf-3) to emerge from the hepatocytes, implying that the ligand is being repeatedly endo- and exocytosed. We termed this movement of HAsTf-3 the diacytic pathway (4) in contradistinction to the well-known lysosomal pathway for other asialoglycoproteins (5). During diacytosis, HAsTf-3 is entrapped in a subcellular particle that is of a lesser equilibrium density than the vesicle that transports internalized asialoorosomucoid (6).

The unusual handling of HAsTf-3 by the hepatocyte raises the question of how the liver of the intact rat ultimately disposes of this asialoglycoprotein. Because our earlier experiments *in vivo* were too short to provide an answer (3), we have now conducted studies lasting up to 12 hr. These showed that rat liver processed small doses of HAsTf-3 slowly in (at least) two ways—namely, catabolism and partial resialylation. Here we report our findings relating to resialylation.

MATERIALS AND METHODS

Materials. Na^{125}I, Na^{131}I, $^{59}FeCl_3$, and *N*-acetyl-D-[6-^{3}H(N)]mannosamine (19 Ci/mmol; 1 Ci = 3.7 × 10^{10} becquerels) were obtained from New England Nuclear. Neuraminidase from *Vibrio cholerae* was from GIBCO and neuraminidase from *Diplococcus pneumoniae* was a gift from M. Lowe and G. Ashwell (National Institutes of Health, Bethesda, MD). Human transferrin and HAsTf-3, both in the diferric form, were prepared as described elsewhere (3). They were iodinated differentially with ^{131}I and ^{125}I by using ICl or chloramine T so that the average number of iodine atoms substituted per molecule of protein ranged between 0.5 and 1.5.

Animals. Sprague–Dawley rats (220–280 g) of either sex (Canadian Breeding Farm & Laboratories, St-Constant, PQ) were given free access to Purina chow and water. For injection and blood sampling the rats were placed in a cylindrical restrainer. No drugs were administered. Injections were in one of the tail veins by using 0.2–0.3 ml of 0.15 M NaCl as the vehicle. Small blood samples were collected from an incision on another tail vein. For large samples of blood the rats were placed under sodium pentobarbital anesthesia and exsanguinated from the heart. In each case, dry sodium heparin (Sigma) was used as an anticoagulant.

Measurement of the Release of ^{125}I-HAsTf-3 by the Liver into the Circulation. Rats (total, 43) received a small amount (0.9–1.1 μg/100 g of body weight) of ^{125}I-HAsTf-3 and the quantity of radioactivity administered was determined in each case by a gravimetric method (7). Groups of four to six animals were sacrificed at intervals ranging from 20 min to 12 hr for the measurement of protein-bound ^{125}I in the plasma volume. Plasma volumes were calculated from the isotopic dilution of a dose of ^{131}I-labeled human transferrin (20–30 μg) injected 5 min before the end of the experiment. Protein-bound radioactivity was measured as the difference between total and acid-soluble ^{125}I as described elsewhere (8). Radioactivities were assayed in a Packard model 5986 multichannel analyzer.

Studies of the Properties of ^{125}I-HAsTf-3 Reappearing in Plasma. Groups of rats (total, 20) received 0.9–1.1 μg of ^{125}I-HAsTf-3 per 100 g of body weight and were exsanguinated either 15 min or 3 hr later. Corresponding plasmas from two or three animals were pooled prior to removal of the bulk of the proteins by applying the first two steps (salt fractionation and CM-Sephadex chromatography) of a scheme (8) for the purification of plasma transferrin. The material thus obtained—referred to below as partially repurified ^{125}I-HAsTf-3—was tested for binding by the immobilized Gal/GalN-specific lectin, size dispersity, electrophoretic mobility, and reactivity with a monospecific antiserum to human transferrin. The lectin was isolated from rabbit liver (9) and coupled to Sepharose 4B (10). Lectin columns (2 ml) were operated as described (11) and loads (<1 μg of ^{125}I-HAsTf-3) were matched on the basis of their specific activities. Size distribution of ^{125}I-HAsTf-3 released into the plasma was compared with that of ^{131}I-labeled HAsTf-3 (^{131}I-HAsTf-3), which had not been injected, by gel filtration on

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Abbreviation: HAsTf-3, human asialotransferrin type 3.

Sephadex G-150 (Pharmacia) under conditions given elsewhere (4).

To determine whether the electrophoretic mobility of ^{125}I-HAsTf-3 released by the liver had been altered in any way, samples of partially repurified ^{125}I-HAsTf-3 were added to and electrophoresed (12) with a human transferrin standard that was comprised of all six molecular forms of the protein with respect to sialic acid content. The standard was established by blending fully and partially desialylated transferrin fractions with fully sialylated transferrins of the identical and mixed glycan types (11). After staining, the gel was sliced and the radioactivity that migrated with each of the standard bands was determined.

Immunoprecipitation of partially repurified ^{125}I-HAsTf-3 was carried out by incubation (1 hr at 37°C, then 3 hr at 4°C) with a monospecific rabbit antiserum to human transferrin, followed by incubation (18 hr at 4°C) with a sheep antiserum to the Fc fragment of rabbit IgG. Precipitates were washed three times with 0.15 M NaCl, digested with protease from *Streptomyces griseus* (Sigma), and assayed for radioactivity. In control experiments, normal rabbit serum was substituted for the antiserum to transferrin.

Estimation of the Total Amount of ^{125}I-HAsTf-3 Released by the Liver. The task presenting itself was to correct the measured appearance curve of ^{125}I-HAsTf-3 in the plasma for concomitant losses due to catabolism and transcapillary diffusion over the observation period (13). The measured appearance curve can be corrected to provide a measure of the true portion of resialylated molecules from a dose of ^{125}I-HAsTf-3 by increasing the amplitude of the curve by an amount that compensates for the losses. If the output of the system is known, the input into the system can be computed by deconvolution (14). The output (i.e., disappearance of human transferrin from the circulation of rat) was calculated from changes in the plasma concentration of protein-bound radioactivity as a function of time after the pulse injection of iodine-labeled human transferrin in rats. The input (i.e., the total amount of resialylated ^{125}I-HAsTf-3 as a function of time) was computed from the integrated version of equation **16** of Norwich (15). To do so, plasma points established for the disappearance of normal transferrin from plasma on the one hand and for the appearance of resialylated ^{125}I-HAsTf-3 in the circulation on the other hand were converted into continuous algebraic functions by exponential fitting. A two-term exponential function—whose coefficients totaled unity—provided a good fit for the former and a three-term function—whose sum of coefficients was zero—for the latter. The computer routine (16) that was used for fitting adjusted the coefficients and exponents by the procedure of Nelder and Mead (17) until the sum of squares of residuals reached a minimum. Fitted functions of these kinds need not have any interpretation in terms of a biological model. Furthermore, deconvolution does not demand a knowledge of the chemical nature of the input and output processes involved. The only assumption made here (and confirmed by the results) was that the speed of disappearance of resialylated ^{125}I-HAsTf-3 from the circulation of the rat is similar to that of control human transferrin.

Studies with Tritiated *N*-Acetyl-D-Mannosamine. The compound was lyophilized and redissolved in 0.15 M NaCl before use. Each of three rats received a mixture of HAsTf-3 (30 μg) and the precursor (25 μCi) by slow (10 min) intravenous injection. After 2.5 hr, the animals were exsanguinated and their pooled plasmas processed as described for the partial repurification of ^{125}I-HAsTf-3. The preparation was then labeled with ^{59}Fe and chromatographed on DEAE-cellulose for the isolation of the two allomers of rat transferrin (18). The peaks were pooled, concentrated, and freed of ^{59}Fe. Aliquots were taken for the estimation of total ^{3}H incorporated, ^{3}H releasable by neuraminidase, and ^{3}H precipitable by an antiserum to human transferrin by using the double antibody technique already mentioned.

RESULTS

In agreement with our previous observations (3), 94–95% of the injected ^{125}I-HAsTf-3 became associated with the liver in 5 min. Subsequently, hepatic protein-bound ^{125}I decreased continuously to 6–9% of the dose in 12 hr, this decrease being considerably slower than that one seen after injection of asialofetuin or asialoorosomucoid (19). A portion of the ^{125}I-HAsTf-3 lost from the liver could be accounted for by hepatic degradation. However, more interestingly, another portion of the dose was released by the liver into the circulation. The time course of this process was such that ^{125}I-HAsTf-3 in the plasma reached a broad plateau between 3 and 6 hr after injection (Fig. 1). The protein released in 3 hr was partially repurified from the plasmas of nine rats and filtered through Sephadex G-150 together with ^{131}I-HAsTf-3 that had not been injected. Elution profiles of both proteins were indistinguishable. Furthermore, 94–98% of the ^{125}I activity was precipitable with antitransferrin by using the double antibody technique outlined (two experiments). These observations indicated that the released radioactive protein was whole HAsTf-3 rather than its proteolytic fragments.

The ^{125}I-HAsTf-3 that was returned to the circulation was further analyzed by chromatography on the immobilized Gal/GalN-specific lectin from rabbit liver. As seen in Table 1, dramatic changes were found. At 15 min, when approximately 4% of the dose was present in the plasma volume, binding was decreased to 9–10%. We think this value represents remnants of the dose mixed with some ^{125}I-HAsTf-3 already released. By 3 hr, dose level in the plasma rose to 12–14%, of which virtually none bound. Incubation with neuraminidase restored binding. The extent to which this occurred depended on the time of incubation. The explanation is that—unlike the common form of human transferrin—the parent molecule to HAsTf-3 contains one sialyl residue that is cleaved very slowly by neuraminidases (unpublished observation).

Sialic acid contributes to the electrophoretic mobility of human transferrin at alkaline pH and, because some transferrin molecules contain five sialyl residues (3), six electrophoretic bands of the protein can be distinguished with respect to sialic acid content (11). We produced two such transferrin standards—one in the diferric and one in the apo form (Fig. 2)—to establish the number of sialyl residues attached by the rat liver to ^{125}I-HAsTf-3. ^{125}I-HAsTf-3 was partially repurified from 3-hr

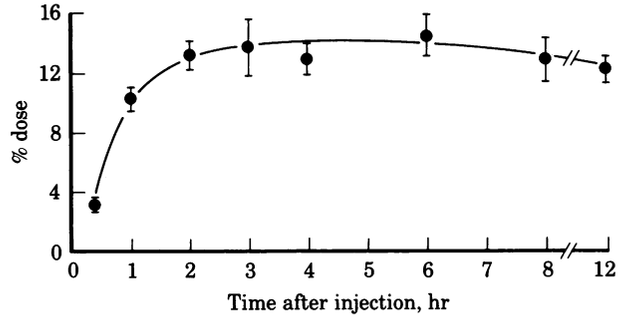

FIG. 1. Protein-bound radioactivity in the plasma volumes of 43 rats at different times after the injection of ^{125}I-HAsTf-3 (0.9–1.1 μg/100 g of body weight). Each value is the mean ± SEM from a group of four to six animals. Plasma volume was measured by isotopic dilution of a dose of ^{131}I-labeled human transferrin given 5 min before termination of the experiment.

Table 1. Binding of ^{125}I-HAsTf-3 to the immobilized Gal/GalN-specific lectin from rabbit liver after different times in the circulation

Experiment	Time, min	Assays, no.	% binding
A	0	6	95.5 ± 0.8
B	15	3	9.6 ± 2.7
C	180	3	1.2 ± 0.1
D	180	3	49.5 ± 0.3
E	180	2	60.9 ± 0.4
F	180	2	76.2 ± 0.6

Two groups of rats, each consisting of three animals, received ^{125}I-HAsTf-3 (0.9–1.1 μg/100 g of body weight). One group was exsanguinated after 15 min and the other after 3 hr. The corresponding plasmas were pooled and the ligand was partially repurified (see text) before testing its binding. Time refers to time in the circulation of rats. Values for binding are means ± SEM. Experiments A–D were repeated twice at weekly intervals. In experiment A, ^{125}I-HAsTf-3 was added to heparinized rat plasma *in vitro* and processed as above. In experiment D, partially repurified ligand from experiment C was incubated with 150–200 units (20) of *V. cholerae* neuraminidase at 37°C for 5–32 hr. The enzyme was removed chromatographically (3) prior to the binding assay. Experiment E is the same as experiment D except that incubation with *V. cholerae* neuraminidase was for 72 hr. In experiment F, material from experiment E was incubated for an additional 24 hr with 0.1 unit (21) of *D. pneumoniae* neuraminidase at 37°C and pH 6.0.

plasma samples of 15 animals and treated with ferric citrate complex or a chelating agent before mixing with the appropriate standard for electrophoresis. In all specimens examined, the

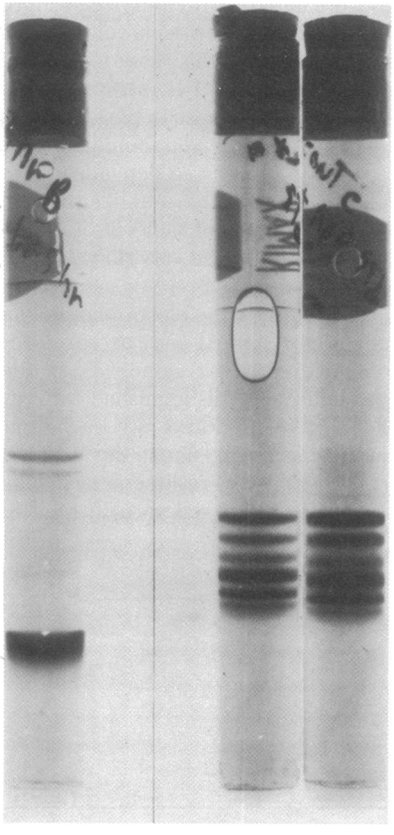

FIG. 2. Electrophoretograms of the proteins remaining in rat plasma after partial repurification of ^{125}I-HAsTf-3 (*Left*) and of the human transferrin standards in the diferric (*Center*) and apo (*Right*) forms. The alkaline (pH 8.1) 7.5% polyacrylamide gels were run for 75 min at 4 mA per gel. Migration was from top to bottom, with the anode at the bottom. Gels were stained with 0.04% Coomassie brilliant blue G 25 in 3.5% perchloric acid. The fastest moving band in the standards is the parent molecule to HAsTf-3 and the slowest is HAsTf-3.

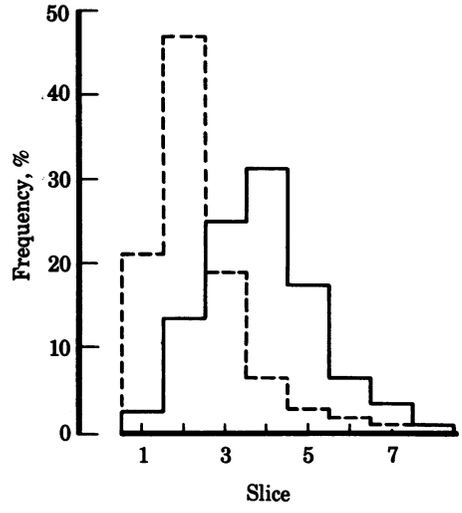

FIG. 3. Comparison of the electrophoretic mobility of ^{125}I-HAsTf-3 partially repurified from the plasma of a rat 3 hr after injection (solid line) with that of a sample of the same preparation added to rat plasma *in vitro* (dashed line). The specimens were mixed with diferric transferrin standard before electrophoresis in separate gels under conditions given in Fig. 2. The stained gels were sliced along the standard bands, radioactivity was counted, and frequency of ^{125}I in each band was calculated. Slice 1 corresponds to the slowest of the standard bands in Fig. 2; slices 7 and 8 are anodic to the fastest standard band that contained no stained protein.

mean electrophoretic mobility‡ of released ^{125}I-HAsTf-3 was found to have increased corresponding to two or three sialyl residues by comparison with the original preparation (Fig. 3). After redesialylation, one specimen that was examined showed a corresponding reduction in electrophoretic mobility.

In view of the incomplete nature of resialylation of ^{125}I-HAsTf-3 by the rat liver, a theoretically interesting question is whether this kind of glycosylation extends over both or just one of the two transferrin glycans. We hoped that after incorporation of ^{3}H from precursor *N*-acetylmannosamine *in vivo* the released protein could be isolated from the plasma by immunoprecipitation, hydrolyzed, and its glycans determined after their separation on concanavalin A-Sepharose (23, 24). However, recovery of [^{3}H]HAsTf-3 was too low (50–80 dpm) to complete this task. By comparison, the specific activities of the rat transferrins were 1.3×10^5 to 1.8×10^5 dpm/mg, of which up to 40% could be released by incubation with neuraminidase. The necessity of keeping the HAsTf-3 dose very low (to prevent induction of catabolism) together with the protracted nature of the resialylation process seems to be the prime obstacle in accumulating analytically satisfactory quantities of ^{3}H in HAsTf-3 from a single precursor injection.

Reattachment by the liver of two or three sialyl residues markedly restored the biological properties of ^{125}I-HAsTf-3 as a plasma protein. This is concluded from the similarity of plasma disappearance curves obtained in groups of rats for ^{125}I-labeled control human transferrin and ^{125}I-HAsTf-3 partially repurified from the plasma of a first set of recipient animals (Fig. 4).

Because plasma proteins are known to be continuously lost

‡ It will be noticed in Fig. 3 that control (not injected) ^{125}I-HAsTf-3 did not electrophorese exactly in the position of unlabeled HAsTf-3—i.e., gel slice 1. The observed spread and somewhat increased overall electrophoretic mobility of the labeled protein were due to the lowered pK values of the phenolic OH groups of mono- and disubstituted tyrosyl residues (22). Nondesialylated transferrin undergoes a similar change on iodination. Altered ionization of iodotransferrins was taken into account in estimating the extent of resialylation.

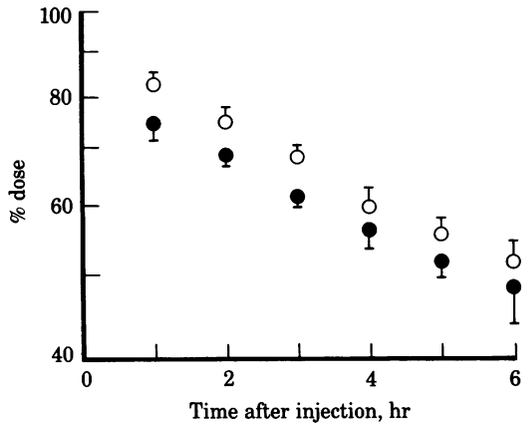

FIG. 4. Plasma clearance of ^{125}I-HAsTf-3 that had been released by the liver relative to that of ^{125}I-labeled control human transferrin. Two donor rats received ^{125}I-HAsTf-3 (1 μg/100 g of body weight). After 3 hr they were exsanguinated, the plasmas were pooled, and the ^{125}I-HAsTf-3 was partially repurified before injection in three rats (●). Another set of three rats (○) received ^{125}I-labeled control human transferrin. Values are mean ± SEM for protein-bound ^{125}I.

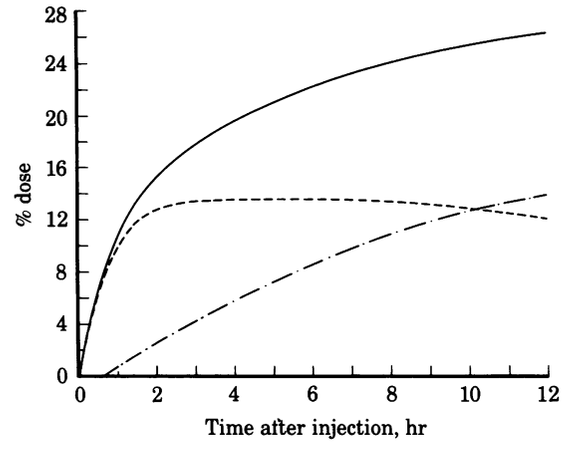

FIG. 5. Estimation by deconvolution of the total amount of ^{125}I-HAsTf-3 resialylated by the liver. ----, The plasma curve from Fig. 1 fitted by a sum of three exponential terms. ——, The total output of resialylated ^{125}I-HAsTf-3 by the liver. -·-·-, Resialylated ^{125}I-HAsTf-3 that had left the circulation as the result of diffusion and catabolism.

from the circulation by catabolism and diffusion into the extravascular space (13), the plasma curve in Fig. 1 cannot be taken as a true reflection of the fraction resialylated from a dose of ^{125}I-HAsTf-3. This value was therefore calculated by deconvolution. The results (Fig. 5) indicate that by the time protein-bound radioactivity in the liver had decreased to a negligible residue (6–9%), 25% of the injected ^{125}I-HAsTf-3 became resialylated.

DISCUSSION

From numerous investigations, transfer into and degradation by the lysosomes of a variety of asialoglycoproteins have emerged as a possible biological role for the Gal/GalN-specific hepatic lectin (5, 25). In addition, the lectin has been found to mediate, to a smaller extent, translocation to the bile of some asialoglycoproteins (26), particularly that of transcobalamins I and III (27). The present study shows that participation in the hepatic lectin pathway may also lead to the repair of an asialoglycoprotein by resialylation.

The restoration process exhibits three characteristic features: (*i*) resialylation of a dose of HAsTf-3 takes place slowly over many hours; (*ii*) only approximately one asialoglycoprotein molecule out of four is repaired; and (*iii*) not all available sites in HAsTf-3 become resialylated. The intracellular processing of HAsTf-3 has not yet been fully clarified, and therefore any attempt to explain these features must be regarded as tentative. It seems reasonable to assume that resialylation is a consequence of diacytosis. The latter term signifies that after binding by the Gal/GalN-specific lectin, HAsTf-3 is rapidly endocytosed but, instead of following the route for other asialoglycoproteins, it is transported back to the sinusoidal surface of the cell with a half-life of ≈4 min for recapture and a new diacytic cycle (unpublished work). It is not known at present whether diacytic vesicles are homed to a particular subcellular organelle on the endocytic leg of the cycle. If they were being homed directly to the Golgi, one would expect resialylation to affect a substantially larger fraction of the dose and in a much shorter time. In the absence of a corresponding finding it seems more likely that resialylation is corollary to the continuous movement of HAsTf-3 into and out of the hepatocyte (6). For example, it is conceivable that during their passage through the cell, some diacytic vesicles fuse with Golgi components on a random basis. If this were the case, one would have to assume that other diacytic vesicles fuse randomly with lysosomes to account for the rest of the dose eliminated by slow degradation. Alternatively, it is possible that ^{125}I-HAsTf-3 is a passive subcellular tracer of vesicles formed at the sinusoidal plasma membrane.

Because of the high metabolic activity of the hepatocyte, such vesicles are thought to be generated all the time and routed directly or indirectly to various subcellular organelles (28). ^{125}I-HAsTf-3, bound by the lectin in the plasma membrane, could be included with the vesicles and share the fate of their contents. Most of the ^{125}I-HAsTf-3 would then be contained at any one time in vesicles shuttling from and to the blood sinusoidal plasma membrane (28). However, frequent return from this pool to the plasma membrane would provide an opportunity for ^{125}I-HAsTf-3 to become associated also with endosomes of different destinies such as Golgi or lysosome. The remarkable dichotomy regarding the ultimate fate of ^{125}I-HAsTf-3 in the liver might thus be explained. The proposition that the asialoglycoprotein receptor is being internalized regardless of occupancy (29–31) supports the assumption made here about the passive routing of ^{125}I-HAsTf-3 in the hepatocyte.

Whatever nature's true intentions may be, the proportion of resialylated HAsTf-3 molecules is too high (25%) to be ignored. Further studies will have to show whether resialylation is unique to this ligand or is common to several asialoglycoproteins having similarly low carbohydrate contents as well as to highly glycosylated proteins in partially desialylated states. Not until this information is available will it be possible to assess the biological significance of hepatic lectin-mediated resialylation.

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1. Debanne, M. T., Chindemi, P. A. & Regoeczi, E. (1981) *J. Biol. Chem.* **256**, 4929–4933.
2. Stockert, R. J., Morell, A. G. & Scheinberg, I. H. (1974) *Science* **186**, 365–366.
3. Regoeczi, E., Taylor, P., Debanne, M. T., März, L. & Hatton, M. W. C. (1979) *Biochem. J.* **184**, 399–407.
4. Tolleshaug, H., Chindemi, P. A. & Regoeczi, E. (1981) *J. Biol. Chem.* **256**, 6526–6528.
5. Harford, J. & Ashwell, G. (1982) in *The Glycoconjugates*, ed. Horowitz, M. I. (Academic, New York), Vol. 4, in press.
6. Debanne, M. T. & Regoeczi, E. (1981) *J. Biol. Chem.* **256**, 11266–11272.
7. Regoeczi, E. (1975) *J. Nucl. Biol. Med.* **19**, 149–154.

8. Regoeczi, E., Hatton, M. W. C. & Wong, K.-L. (1974) *Can. J. Biochem.* **52,** 155–161.
9. Hudgin, R. L., Pricer, W. E., Jr., Ashwell, G., Stockert, R. J. & Morell, A. G. (1974) *J. Biol. Chem.* **249,** 5536–5543.
10. Porath, J., Aspberg, K., Drevin, H. & Axén, R. (1973) *J. Chromatogr.* **86,** 53–56.
11. Wong, K.-L., Debanne, M. T., Hatton, M. W. C. & Regoeczi, E. (1978) *Int. J. Pept. Protein Res.* **12,** 27–37.
12. Clarke, J. T. (1964) *Ann. N.Y. Acad. Sci.* **121,** 428–436.
13. Koj, A. & McFarlane, A. S. (1968) *Biochem. J.* **108,** 137–146.
14. Shipley, R. A. & Clarke, R. E. (1972) *Tracer Methods for in Vivo Kinetics* (Academic, New York), pp. 205–208.
15. Norwich, K. H. (1972) *Biochem. J.* **126,** 1124–1126.
16. Wong, K.-L., Charlwood, P. A., Hatton, M. W. C. & Regoeczi, E. (1974) *Clin. Sci. Mol. Med.* **46,** 763–774.
17. Nelder, J. A. & Mead, R. (1965) *Comput. J.* **7,** 308–313.
18. Gordon, A. H. & Louis, L. N. (1963) *Biochem. J.* **88,** 409–414.
19. Charlwood, P. A., Regoeczi, E. & Hatton, M. W. C. (1979) *Biochim. Biophys. Acta* **585,** 61–70.
20. Mohr, E. & Schramm, G. (1960) Z. *Naturforsch.* **15B,** 568–577.
21. Kawasaki, T. & Ashwell, G. (1976) *J. Biol. Chem.* **251,** 5292–5299.
22. Ramachandran, L. K. (1956) *Chem. Rev.* **56,** 199–218.
23. Krusius, T., Finne, J. & Rauvala, H. (1976) *FEBS Lett.* **71,** 117–120.
24. Hatton, M. W. C., März, L., Berry, L. R., Debanne, M. T. & Regoeczi, E. (1979) *Biochem. J.* **181,** 633–638.
25. Neufeld, E. F. & Ashwell, G. (1980) in *The Biochemistry of Glycoproteins and Proteoglycans,* ed. Lennarz, W. J. (Plenum, New York), pp. 241–266.
26. Thomas, P. & Summers, J. W. (1978) *Biochem. Biophys. Res. Commun.* **80,** 335–339.
27. Burger, R. L., Schneider, R. J., Mehlman, C. S. & Allen, R. H. (1975) *J. Biol. Chem.* **250,** 7707–7713.
28. Evans, W. H. (1980) *Biochim. Biophys. Acta* **604,** 27–64.
29. Kolset, S. O., Tolleshaug, H. & Berg, T. (1979) *Exp. Cell Res.* **122,** 159–167.
30. Tolleshaug, H. & Berg, T. (1979) *Biochem. Pharmacol.* **28,** 2919–2922.
31. Oka, J. & Weigel, P. (1980) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **39,** 2616.