

α -Glucosidase-Albumin Conjugates: Effect of Chronic Administration in Mice¹

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ABSTRACT

Enzyme albumin conjugates have been proposed as a means of increasing the efficacy of enzyme use *in vivo* and decreasing immune response to the enzyme. Particulate drug carriers, however, have a pronounced tendency to localize in the mononuclear phagocyte (reticuloendothelial) system. We have examined in mice the effect on phagocytic index, tissue distribution and organ size of continued administration of conjugates of α -glucosidase with either homologous or heterologous albumin. Mice received 10×2 -mg injections of bovine serum albumin (BSA) or mouse serum albumin (MSA), either free, polymerized or conjugated with α -glucosidase. Experiments involving BSA had to be ter-

minated before the end of the experiment because of anaphylaxis, but these reactions were less severe to the polymerized albumin than to free albumin. Free BSA, BSA polymer and BSA-enzyme conjugates all caused a decrease in phagocytic index after six injections. Mice receiving MSA showed no evidence of anaphylaxis, but mice receiving six or more injections of free MSA, MSA polymer or MSA-enzyme conjugate had significantly decreased phagocytic indices as compared to controls. Phagocytic indices had returned to normal by 7 days after the final injection. Tissue distribution of ¹²⁵I-labeled albumin preparations was determined in either naive or chronically injected mice.

A variety of different drug carrier systems are being pursued actively in order to improve drug half-lives *in vivo*, to reduce immunological complications of drug administration and to improve the selective delivery of drugs to their desired site of action. Some of the drug carrier systems currently under development include drug-polymer conjugates, red blood cell ghosts, liposomes, albumin microspheres, DNA-drug complexes and enzyme albumin conjugates (reviewed by Gregoriadis, 1979; Poznansky and Juliano, 1984).

The principle site of uptake and localization of the two particulate drug carriers which have been extensively studied to date (liposomes and red blood cell ghosts) is the mononuclear phagocyte system, also called the RE system. It is likely that other particulate drug carriers will also rapidly localize in the RE system and this system is one of the major barriers to the *in vivo* targeting of drug carriers. The RE system is a major host defense system and in addition to its role in removing foreign particulate matter from circulation it is involved in a myriad of other functions including host defense against path-

ogenic micro-organisms, parasites and tumor cells, in host responses to endotoxin and hemorrhagic shock, in drug response and in response to circulating immune complexes (Saba, 1970; Altura, 1980).

The consequences of accumulation of large amounts of drug carriers in the RE system is little understood, but has been addressed recently for chronic administration of liposomes by Poste (1983) and by Allen *et al.* (1984).

Enzyme albumin conjugates have shown some promise for the treatment of lysosomal storage diseases following the lysosomotropic behavior of the complex (Poznansky, 1983). Using homologous albumin in a 10 to 1 molar ratio to the foreign protein (enzyme) the immunogenic property of the enzyme may be reduced greatly (reviewed in Poznansky, 1983). Attempts at targeting the enzyme complex using polyclonal antibodies (Poznansky and Bhardwaj, 1981), monoclonal antibodies (Poznansky *et al.*, 1982) and peptide hormones (Poznansky *et al.*, 1984) have met with considerable success. The interaction of these conjugates with the RE system is of concern as it detracts from the ability of the conjugate to seek out and find a particular target.

The purpose of this study was to examine in mice the effects of chronic administration of homologous and heterologous enzyme albumin conjugates on RE function, spleen and liver size, immunological response and tissue distribution. We compared

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ABBREVIATIONS: RE, reticuloendothelial; MSA, mouse serum albumin; BSA, bovine serum albumin; PNPG, *p*-nitrophenyl- α -D-glucosidase; PBS, phosphate-buffered saline; DSPC-CH, distearoylphosphatidylcholine-cholesterol; SUV, small unilamellar vesicles; RES, reticuloendothelial system.

the observed effects with the effects accompanying chronic administration of liposomes, another drug carrier system which has been widely studied and which shows significant ability to depress RE function with chronic administration (Poste, 1983; Allen *et al.*, 1984).

Materials and Methods

Materials

MSA was purchased from Cappel Laboratories (Malvern, PA). BSA, α -glucosidase, PNPG, 25% glutaraldehyde (Grade I), distearoylphosphatidylcholine, cholesterol and glycine were purchased from Sigma Chemical Co. (St. Louis, MO). [^{14}C]Inulin, 1.9 mCi/g, was purchased from New England Nuclear, (Boston, MA). [^{125}I]Na was purchased from Edmonton Radiopharmaceuticals (Edmonton, Alberta, Canada).

Methods

Preparation of albumin conjugates. α -Glucosidase-albumin conjugates were produced using primary amino groups as the reactive sites and the bifunctional dialdehyde, glutaraldehyde, to form a Schiff base linkage. The bond was stabilized by reduction with sodium cyanoborohydride.

α -Glucosidase (8 mg) in 0.8 ml of PBS, pH 6.8 and 50 mg of albumin in 15.0 ml of PBS were put in a glass vial and cooled to 4°C, 24 mg of PNPG in 3 ml of PBS was added and allowed to stir for 10 min at 4°C. Two hundred microliters of glutaraldehyde (25% w/v) was then added dropwise. The mixture was allowed to stir at 4°C for 3.5 hr and then dialyzed against PBS containing 1% glycine. The resultant conjugate was separated from free enzyme, albumin or small molecular weight conjugates by ultra-filtration using XM300 filters (MW cutoff of 300,000). The conjugate was then separated further by molecular sieve chromatography on Sepharose 4B. A fraction with an average MW of 800,000 (80% of eluting protein in the MW range of 6×10^5 to 10^6) was used for all experiments. Albumin was labeled with [^{125}I] using chloramine T yielding a specific activity of 0.5 mCi/mg. The [^{125}I]-labeled albumin was diluted with cold albumin (100-fold excess) before production of the conjugate, or before introduction of free [^{125}I]albumin. The stability of the [^{125}I]-label was determined in PBS (pH 7.4) at 4 and at 37°C. At 4°C less than 1% of the label is lost from either the free albumin or from the conjugate over a 72-hr period. At 37°C, less than 2% of the label is lost from either preparation over a 12-hr period. In the presence of fresh serum at 37°C, an increased rate of [^{125}I] release results, although greater than 95% of the label remains trichloroacetic acid-precipitable over a 6-hr period. Before injection, polymers were dialyzed against normal saline and then sterilized by filtration through 0.22 μ filters.

Animal experiments. ICR mice were obtained from the Animal Breeding Unit of the University of Alberta.

Mice were divided into four groups and each group received respectively, by tail vein, 10 injections of 2 mg each of free albumin, albumin polymer, α -glucosidase albumin conjugate or 0.2 ml of sterile saline, 0.9% NaCl, USP. In the conjugated form, 2 mg of total protein represents an amount of enzyme (320 μg) which is approximately 30 $\times$ more than the α -glucosidase deficit in patients with Type II glycogenosis (or a per weight basis). Larger doses would be prohibitive because of the cost of mouse serum albumin. Injections were in 0.2 ml of sterile saline and given on Mondays, Wednesdays and Fridays. In the first set of experiments mice received BSA and in the second set of experiments mice received MSA. Twenty-four hours after BSA or MSA injections, phagocytic indices were determined on groups of six mice after tail vein injection of 2 mg of carbon particles per mouse according to the technique of Allen *et al.* (1984). At this dose of carbon particles, phagocytosis plays the major role in early clearance (Donald and Tennent, 1975). Mouse body weight and liver and spleen weight were determined and tissue samples were saved for histological examination.

Twenty-four hours after the final (10th) injection in mice receiving MSA, test and control mice were injected with 2 mg each of [^{125}I]MSA,

[^{125}I]MSA polymer or [^{125}I]MSA enzyme conjugate. Each mouse received 700,000 cpm of [^{125}I]. Mice were sacrificed 0.5, 2, 6 or 18 hr after injection and [^{125}I] counts were determined in liver, spleen, lung, heart, kidney, thyroid and blood. Previous studies have shown little uptake of enzyme albumin conjugates into tissues other than those examined (Wong *et al.*, 1980; Poznansky *et al.*, 1984). Tissue samples were counted in a Beckman Gamma 8000 instrument. Tissue distributions of DSPC-CH, 2:1 small unilamellar liposomes containing 6×10^4 cpm of entrapped [^{14}C]inulin was determined according to the procedure of Allen and Everest (1983).

Results

The first set of experiments involving mice receiving BSA polymers had to be terminated before the 10th injection because of the large numbers of mice dying from anaphylaxis. Severe anaphylactic reactions were noted in mice who received from three to six injections of free BSA. Milder transient reactions were noted in mice receiving polymerized BSA and some of these mice were able to receive up to nine injections of BSA enzyme polymer without severe anaphylactic reactions. Phagocytic indices after the 6th injection were 50% of control for mice receiving free BSA and 36% of control for mice receiving BSA enzyme polymer. After nine injections of BSA enzyme polymer phagocytic indices were 24% of control (results not shown). No increases in spleen or liver weight were observed.

Phagocytic indices for mice receiving 10 injections of 2 mg each of MSA are given in table 1. Phagocytic indices were slightly depressed 24 hr after three injections of either MSA, MSA polymer or MSA enzyme polymer and significantly depressed to approximately 30% of control values after 7 or 10 injections. One week after the final injection phagocytic indices had returned to normal. No alterations in spleen or liver weight were observed in these studies, unlike our observation in mice receiving chronic doses of liposomes (Allen *et al.* 1984; T. Allen, unpublished observations).

Tissue distributions of [^{125}I]-labeled albumin or albumin conjugates in mice receiving chronic albumin injections (test mice) and in sham-injected mice (control mice) are given in table 2. Tissue content is expressed as a percentage of radioactivity remaining *in vivo* for each respective time point. One-half hour postinjection, the earliest time point examined, there was already considerable radioactivity associated with liver. The amount localizing in liver was less for mice receiving free albumin (12 to 18%) than for mice receiving albumin polymers (20 to 27%) and had begun to drop by 18 hr postinjection. Uptake into liver in chronically injected mice was not significantly different than that in sham-injected mice. Very low uptake of label into spleen was observed, but uptake into spleen was 2 to 3 times higher in test mice than in control mice. Liver to spleen ratios were decreased an average of 55% in test mice as compared to control mice in mice receiving MSA, and an average of 30% in mice receiving MSA polymer or MSA enzyme conjugate. Decreased liver to spleen ratios have been reported by Allen *et al.* (1984) as being a sensitive measure of RE blockade. As can be seen from table 1, phagocytic indices were also reduced after the 10th injection.

Uptake of label into lung was around 3% and did not vary with any of the parameters of the experiment. Likewise, uptake into heart was invariably around 1% and uptake into kidney was 4 to 5%. Kidney levels were dropping by 18 hr postinjection, possibly reflecting the involvement of the kidneys in elimination of the labeled albumin from the body.

TABLE 1

Phagocytic indices in mice receiving chronic MSA polymer

ICR mice (six per group) were injected in the tail vein on Mondays, Wednesdays and Fridays for 10 injections with 2 mg each of MSA, cross-linked MSA polymer or cross-linked MSA containing α -glucosidase (MSA-enz polymer). Control mice received 10 injections of sterile saline. Twenty-four hours after each injection six mice from each group were injected with 2 mg of carbon per mouse and phagocytic indices were determined as detailed in Allen *et al.* (1984). Results are expressed as average phagocytic index $\pm$ S.D. Analysis of variance was performed on the results.

	Day (Injection No.)			
	6 (3)	15 (7)	22 (10)	29
Phagocytic index				
Control	0.025 $\pm$ 0.010	0.029 $\pm$ 0.011	0.026 $\pm$ 0.008	0.029 $\pm$ 0.008
MSA	0.011 $\pm$ 0.008	0.008 $\pm$ 0.004***	0.007 $\pm$ 0.005***	0.028 $\pm$ 0.005
MSA polymer	0.014 $\pm$ 0.008	0.009 $\pm$ 0.006***	0.009 $\pm$ 0.005***	0.034 $\pm$ 0.009
MSA-enz polymer	0.015 $\pm$ 0.010	0.008 $\pm$ 0.005***	0.009 $\pm$ 0.006***	0.029 $\pm$ 0.011

*** $P < .001$.

TABLE 2

Tissue distribution in mice receiving MSA polymers

Tissue distribution in ICR mice (three per group) receiving 2 mg each of either MSA, cross-linked MSA (MSA polymer) or cross-linked MSA containing α -glucosidase (MSA-enz polymer) for 10 consecutive injections on Mondays, Wednesdays and Fridays. Control mice were injected with 0.2 ml of sterile saline. Twenty-four hours after the 10th injection all mice including controls received 2 mg of either [^{125} I]MSA, [^{125} I]MSA polymer or [^{125} I]MSA-enz polymer as appropriate. The results were corrected for blood content according to the technique of Hwang *et al.* (1980) and expressed as percentage of *in vivo* radioactivity $\pm$ S.D. Liver to spleen (L/S) ratio is the ratio of total counts in liver to total counts in spleen.

	MSA		MSA Polymer		MSA-Enz Polymer	
	Test	Control	Test	Control	Test	Control
0.5 Hr Postinjection						
Liver	18.0 $\pm$ 2.9	16.1 $\pm$ 2.6	23.6 $\pm$ 4.1	19.8 $\pm$ 1.4	23.4 $\pm$ 3.8	19.5 $\pm$ 0.7
Spleen	0.9 $\pm$ 0.2	0.3 $\pm$ 0.2	1.1 $\pm$ 0.1	1.0 $\pm$ 0.6	1.0 $\pm$ 0.2	0.6 $\pm$ 0.1
Lung	2.6 $\pm$ 0.9	3.3 $\pm$ 0.8	2.9 $\pm$ 0.6	3.0 $\pm$ 0.6	3.0 $\pm$ 1.0	2.5 $\pm$ 0.4
Heart	0.7 $\pm$ 0.2	1.0 $\pm$ 0.4	0.6 $\pm$ 0.3	0.8 $\pm$ 0.5	0.7 $\pm$ 0.2	0.6 $\pm$ 0.2
Kidney	5.6 $\pm$ 0.6	6.2 $\pm$ 0.8	3.9 $\pm$ 0.7	4.1 $\pm$ 0.7	3.8 $\pm$ 1.0	3.6 $\pm$ 0.5
Thyroid	1.1 $\pm$ 0.2	1.4 $\pm$ 0.6	0.7 $\pm$ 0.3	0.8 $\pm$ 0.1	0.7 $\pm$ 0.3	0.3 $\pm$ 0.1
Blood	71.0 $\pm$ 4.6	71.7 $\pm$ 2.0	67.2 $\pm$ 5.5	72.2 $\pm$ 3.1	67.3 $\pm$ 5.6	72.9 $\pm$ 1.8
L/S ratio	20.6 $\pm$ 3.7	58.2 $\pm$ 26.4	22.4 $\pm$ 1.3	24.2 $\pm$ 12.1	22.0 $\pm$ 6.8	33.3 $\pm$ 2.7
2 Hr Postinjection						
Liver	13.6 $\pm$ 1.0	12.4 $\pm$ 3.8	24.0 $\pm$ 2.3	22.4 $\pm$ 0.6	27.1 $\pm$ 1.5	23.4 $\pm$ 2.9
Spleen	0.8 $\pm$ 0.3	0.3 $\pm$ 0.1	1.7 $\pm$ 0.4	0.9 $\pm$ 0.1	1.9 $\pm$ 0.0	0.9 $\pm$ 0.2
Lung	2.4 $\pm$ 0.8	3.2 $\pm$ 0.3	2.9 $\pm$ 0.3	2.5 $\pm$ 0.5	2.4 $\pm$ 0.3	3.4 $\pm$ 1.0
Heart	0.7 $\pm$ 0.1	1.2 $\pm$ 0.6	0.9 $\pm$ 0.2	0.9 $\pm$ 0.3	0.8 $\pm$ 0.1	0.8 $\pm$ 0.2
Kidney	4.1 $\pm$ 0.2	4.6 $\pm$ 0.8	4.2 $\pm$ 0.1	4.2 $\pm$ 0.4	4.6 $\pm$ 0.4	4.2 $\pm$ 0.8
Thyroid	1.9 $\pm$ 0.6	3.3 $\pm$ 0.8	3.5 $\pm$ 0.6	2.6 $\pm$ 0.2	5.0 $\pm$ 0.8	3.0 $\pm$ 0.8
Blood	76.4 $\pm$ 1.3	75.0 $\pm$ 5.5	62.8 $\pm$ 2.8	66.5 $\pm$ 0.5	58.4 $\pm$ 1.8	64.4 $\pm$ 3.9
L/S ratio	17.7 $\pm$ 4.7	44.0 $\pm$ 10.9	14.9 $\pm$ 2.3	24.7 $\pm$ 4.7	14.3 $\pm$ 1.1	26.9 $\pm$ 1.2
6 Hr Postinjection						
Liver	14.3 $\pm$ 1.6	15.6 $\pm$ 3.6	20.6 $\pm$ 2.4	22.1 $\pm$ 1.5	26.3 $\pm$ 3.9	22.9 $\pm$ 2.0
Spleen	1.0 $\pm$ 0.1	0.6 $\pm$ 0.2	2.0 $\pm$ 0.1	1.3 $\pm$ 0.1	2.2 $\pm$ 0.4	1.3 $\pm$ 0.4
Lung	5.1 $\pm$ 1.1	4.4 $\pm$ 1.1	3.0 $\pm$ 0.5	3.4 $\pm$ 0.2	3.1 $\pm$ 0.4	8.0 $\pm$ 7.5
Heart	1.3 $\pm$ 0.7	1.2 $\pm$ 0.6	0.8 $\pm$ 0.1	1.1 $\pm$ 0.1	1.2 $\pm$ 0.2	1.3 $\pm$ 0.4
Kidney	4.6 $\pm$ 0.7	4.7 $\pm$ 1.0	4.2 $\pm$ 0.3	4.9 $\pm$ 0.1	5.5 $\pm$ 0.5	5.9 $\pm$ 0.9
Thyroid	8.8 $\pm$ 1.1	9.2 $\pm$ 0.6	23.9 $\pm$ 5.3	13.7 $\pm$ 4.0	24.1 $\pm$ 10.9	12.4 $\pm$ 16.7
Blood	65.0 $\pm$ 4.6	66.3 $\pm$ 2.3	45.6 $\pm$ 3.0	53.5 $\pm$ 5.1	37.6 $\pm$ 6.9	52.2 $\pm$ 8.4
L/S ratio	14.8 $\pm$ 0.3	28.5 $\pm$ 6.7	10.4 $\pm$ 1.2	16.7 $\pm$ 2.0	12.1 $\pm$ 2.9	18.5 $\pm$ 5.3
18 Hr Postinjection						
Liver	11.6 $\pm$ 0.9	12.8 $\pm$ 1.4	18.4 $\pm$ 2.3	11.1 $\pm$ 0.9	21.1 $\pm$ 3.7	13.4 $\pm$ 1.1
Spleen	1.0 $\pm$ 0.3	0.5 $\pm$ 0.1	1.1 $\pm$ 0.0	0.5 $\pm$ 0.2	1.1 $\pm$ 0.4	0.6 $\pm$ 0.2
Lung	3.1 $\pm$ 0.7	3.8 $\pm$ 0.5	1.8 $\pm$ 0.5	1.8 $\pm$ 0.5	1.6 $\pm$ 0.5	2.3 $\pm$ 0.4
Heart	0.9 $\pm$ 0.4	1.2 $\pm$ 0.2	0.8 $\pm$ 0.3	0.4 $\pm$ 0.0	0.5 $\pm$ 0.0	0.6 $\pm$ 0.2
Kidney	3.8 $\pm$ 0.4	4.0 $\pm$ 0.6	2.8 $\pm$ 0.4	1.9 $\pm$ 0.5	2.8 $\pm$ 0.5	2.4 $\pm$ 0.5
Thyroid	19.2 $\pm$ 3.8	17.4 $\pm$ 2.3	61.8 $\pm$ 6.8	71.3 $\pm$ 6.7	62.1 $\pm$ 7.7	65.6 $\pm$ 2.4
Blood	60.8 $\pm$ 4.1	60.5 $\pm$ 3.1	13.2 $\pm$ 3.2	14.1 $\pm$ 4.3	10.9 $\pm$ 3.1	14.9 $\pm$ 2.5
L/S ratio	15.8 $\pm$ 1.8	30.6 $\pm$ 9.6	17.0 $\pm$ 2.5	21.8 $\pm$ 6.3	20.0 $\pm$ 3.2	22.8 $\pm$ 7.0

Uptake of ^{125} I into the thyroid was initially low and gradually increased to as much as 70%. This increase was less dramatic for free albumin than for albumin polymers. Loss of label from free albumin or polymer was found to be low in the presence of serum at 37°C (less than 5% in 6 hr) and therefore the increase

in the percentage of label in the thyroid probably reflects breakdown and metabolism of the labeled compounds *in vivo* with release of free iodine which is rapidly taken up into the thyroid. MSA polymer and MSA enzyme conjugate appear to be cleared and catabolized more rapidly than free albumin.

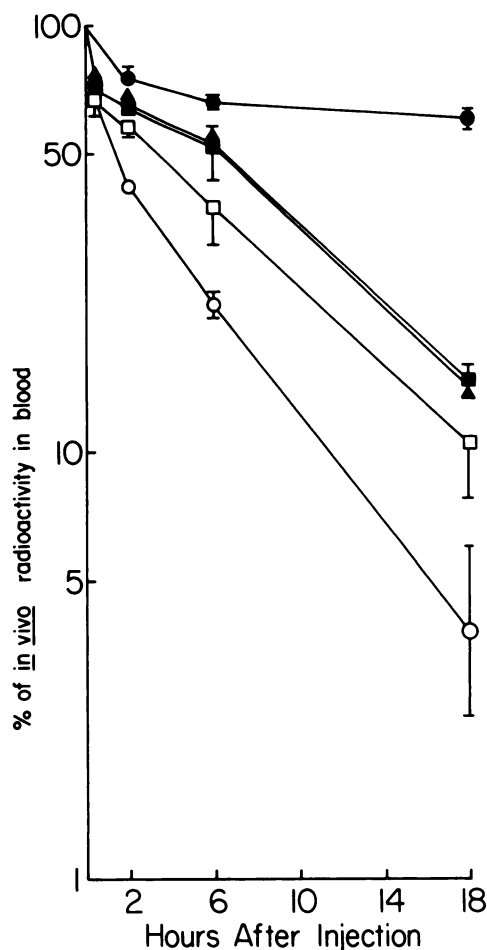


Fig. 1. Rate of elimination of [^{125}I]albumin or [^{14}C]inulin liposomes from blood of naive ICR mice (three per group) after tail-vein injection of 2 mg each of MSA (●), MSA polymer (▲), MSA enzyme polymer (■) or chronically injected ICR mice receiving 2 mg of MSA enzyme polymer (□). Another group of mice received DSPC-CH, 2:1, SUV (○) containing entrapped [^{14}C]inulin.

TABLE 3

Tissue distribution in mice receiving DSPC-CH, 2:1 Liposomes

Tissue distribution in ICR mice (three per group) of [^{14}C]inulin entrapped in DSPC-CH, 2:1, small unilamellar liposomes. Mice received 2 mg each of lipid by tail-vein injection, and tissues were sampled at 2, 6 and 18 hr after liposome administration. Tissues were corrected for blood content according to the technique of Hwang *et al.* (1980) and results were expressed as percentage of *in vivo* radioactivity $\pm$ S.D.

	Tissue Distribution		
	2 hr	6 hr	18 hr
Liver	47.3 $\pm$ 0.8	64.6 $\pm$ 1.8	86.2 $\pm$ 4.8
Spleen	5.0 $\pm$ 1.2	5.3 $\pm$ 1.0	5.6 $\pm$ 1.0
Lung	1.5 $\pm$ 0.3	2.2 $\pm$ 0.1	0.4 $\pm$ 0.3
Heart	0.7 $\pm$ 0.2	0.8 $\pm$ 0.1	0.3 $\pm$ 0.1
Kidney	2.5 $\pm$ 0.6	3.8 $\pm$ 0.4	3.7 $\pm$ 0.5
Brain	0.7 $\pm$ 0.2	0.9 $\pm$ 0.1	0.2 $\pm$ 0.0
Blood	42.3 $\pm$ 1.6	22.6 $\pm$ 1.5	3.8 $\pm$ 2.8

MSA enzyme conjugate is also eliminated most rapidly from the body followed by MSA polymer, and free MSA is eliminated least rapidly (results not shown).

Blood levels of ^{125}I -labeled free MSA remained high throughout the 18-hr period of the experiment. Blood levels of MSA polymer and MSA enzyme conjugate dropped over the 18-hr period (fig. 1). Circulating levels of MSA enzyme conjugate decrease more rapidly than levels of MSA polymer. This is

probably due to their increased uptake into liver. Half-life of enzyme albumin conjugates in blood is approximately 6 hr.

For purposes of comparison, data on the blood levels of small unilamellar liposomes composed of DSPC-CH, 2:1 are included in figure 1 and tissue distribution of the entrapped aqueous space marker [^{14}C]inulin are reported in table 3. This particular composition was chosen because of their increased circulation times *in vivo* (Mauk and Gamble, 1979). Small unilamellar liposomes were chosen because they are only slightly larger in size than the enzyme albumin polymers.

As is readily apparent from a comparison of tables 2 and 3, SUV of DSPC-CH localized in liver and spleen to a significantly larger extent than do enzyme albumin conjugate. Liposomes are also removed from blood at a faster rate than albumin polymers (tables 2 and 3; fig. 1).

Discussion

A major consideration in the use of biological macromolecules or particulate materials such as erythrocyte ghosts or liposomes as carriers of either drugs or enzymes is their removal from circulation by the RES. In recognizing and removing these carriers from the circulation, the RES limits the circulatory half-lives of the drug/carrier complex and reduces its chances of finding a specific target. One possibility, that of RES blockade, has been suggested as a possible means of avoiding this rapid removal. In considering such blockade careful consideration must be given to the question of recovery of the RES and possible permanent alterations in function after repeated challenge. One of the objectives of this report is to examine the nature of the interaction of albumin and enzyme-albumin conjugates with the RES, to examine the effects of RES blockade and the subsequent recovery of the RES. In understanding the interaction between the enzyme-albumin conjugate and the RES it may be possible to modify the polymeric complexes to minimize their potential uptake by the RES and maximize the ability of the carrier to reach its target. Thus, it may be as important to consider targeting a carrier system "away" from the RES as it is important to target the carrier "to" a particular site.

α -Glucosidase deficiency (Type II glycogenosis or Pompe's disease) represents a glycogen storage disease occurring within lysosomes of cardiac, respiratory and hepatic tissue. Enzyme replacement therapy will be achieved only by delivering the specific enzyme to lysosomal fractions within those affected cells. Early attempts at enzyme replacement therapy (reviewed in Poznansky and Bhardwaj, 1981) failed at least partially because the enzyme was rapidly cleared from the circulation via the RES. The use of homologous albumin as a carrier (Poznansky, 1983) shows clearly reduced RES clearance and enhanced targetability of the enzyme-albumin complex (Poznansky *et al.*, 1984).

The experiments described here indicate that albumin polymers and albumin-enzyme conjugates do indeed interact with the RES, but in a reversible manner. However, MSA enzyme conjugates appear to be taken up less by liver and spleen and have longer circulation half-lives than do SUV of DSPC-CH, 2:1. This may in part be a function of the smaller size of the conjugates which are approximately half the diameter of SUV.

This study demonstrates that a nonliposomal particulate drug carrier causes reversible RE blockade in mice, but that this RE blockade is not as severe as that seen for some liposome

preparations (Allen *et al.*, 1984). More research into means of avoiding RE uptake and RE blockade is clearly needed if particulate drug carriers are to achieve optimum use *in vivo*.

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