Hepatic Catabolism of Serum Amyloid A during an Acute Phase Response and Chronic Inflammation (43086)

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Abstract. **Degradation of serum amyloid A (SAA) was studied in isolated perfused livers of mice treated with either a single injection of casein to induce an acute phase response or with 14 daily casein injections to maintain chronic inflammation. Littermates administered sterile saline served as controls. Radioiodinated SAA and apolipoprotein A-I, reconstituted with high-density lipoproteins *in vivo*, were studied in parallel. Degradation was monitored by appearance of acid-soluble radioactivity in the perfusate.**

Induction of an acute phase response reduced hepatic catabolism of SAA by 14% (from 8.6 ± 1.2% to 7.4 ± 1.1%/g liver in 3 hr, $P < 0.05$, $n = 16$). The acute phase response had no effect on apolipoprotein A-I degradation or bile production. Livers from animals receiving 14 daily injections of casein were 31% less active than control livers at degrading SAA (8.1 ± 1.6%/g/3 hr for treated group vs. 11.7 ± 2.3%/g/3 hr for control group, $P < 0.025$). Apolipoprotein A-I degradation was decreased but differences were not statistically significant and bile production was the same in both treatment groups. However, livers from treated animals were larger (mean weight 1.8 g) than those from controls (1.5 g) ($P < 0.05$), although amyloid fibrils were not detected by Congo red stain.

The size of the degradation products was analyzed by column chromatography. Elution profiles of perfusates from livers of chronically inflamed animals contained a peak corresponding to the molecular weight of amyloid A which was not present in perfusates from control liver.

We conclude that hepatic catabolism of SAA is decreased both early and late in an inflammatory response and intermediate degradation products corresponding in size to amyloid A are released into the circulation following prolonged inflammation.

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Serum amyloid A (SAA) is an acute phase reactant (1, 2) related to amyloid A (AA), the fibril protein of secondary amyloidosis (3–5). The mouse has been used extensively as a model in the study of secondary amyloidosis since both SAA synthesis and amyloid deposition can be readily induced by administration of casein or endotoxin (6). SAA is detectable in plasma within 3 hr of casein injection and reaches maximum levels approximately 18 hr postinjection (7). Daily injections of casein can lead to the deposition of AA in the spleen, liver, intestine, and kidney (8). Both indirect and direct evidence support the hypothesis that AA is derived from SAA by proteolytic cleavage (3–5, 9, 10).

The liver appears to be involved in both normal catabolism of SAA and in amyloid formation. We have shown that the isolated perfused mouse liver degrades SAA (11) and Fuks and Zucker-Franklin (12) have demonstrated degradation of SAA by isolated Kupffer cells. Furthermore, Kupffer cells from normal mice degraded SAA completely, whereas cells from animals receiving eight or more daily casein injections produced an intermediate product with the characteristics of AA (12).

In the current study we have used the isolated,

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perfused mouse liver to study the effect of the acute phase response and prolonged inflammation on catabolism of AA. Isolated liver perfusion has several advantages over isolated cells or whole animals: (i) the perfusate content can be controlled, (ii) the integrity of intracellular functions and hepatocyte polarity are maintained since the hepatic circulation remains intact, and (iii) extrahepatic metabolism is not a confounding factor (13). We have investigated the catabolism of SAA by livers from animals receiving 1 and 14 daily injections of casein. SAA is transported in plasma in association with the high-density lipoproteins (HDL). However, we have shown that SAA degradation by rat liver is independent of HDL catabolism (11). Therefore, HDL apoproteins were studied in parallel as a measure of nonspecific changes in degradation rate.

Materials and Methods

Preparation of Lipoproteins and Apolipoproteins. Plasma obtained from The Miriam Hospital Hematology Laboratory was screened for SAA and used for isolation of human SAA and apolipoproteins. SAA_5 (14) and apolipoprotein A-I (apo A-I) (17) were purified from apo HDL by methods previously described in detail.

Preparation of Tracer. Isolated apo A-I and SAA_5, one of the major forms of SAA (14) (also termed SAA_2 (15) and SAA_1 des arg (16) in other nomenclature systems), were radioiodinated using commercial lactoperoxidase reagents (Bio-Rad enzymobeads). Since SAA_5 is often contaminated with apo A-II, the radiolabeled SAA was absorbed with anti-apo A-II bound to protein A Sepharose CL-4B (Pharmacia P-L Biochemicals). All immunoprecipitable ^{125}I-apo A-II was removed by this procedure.

We have previously shown that biologic screening is necessary to remove a form of ^{125}I-SAA which is easily dissociated from HDL and rapidly degraded (11). Therefore, labeled proteins (^{125}I-SAA and ^{131}I-apo A-I) were injected into mice through a jugular vein cannula, the animals were exsanguinated 30 min later and SAA and apo A-I incorporated into HDL ($1.08 <$ density < 1.21 g/ml) were recovered by ultracentrifugation. Gel filtration chromatography and agarose electrophoresis indicated that both tracers were HDL associated. Validation of tracer has been described in detail (11).

Induction of the Acute Phase Response. Swiss Webster mice (Charles River Laboratories) weighing 19–39 g and fed standard Purina Chow and water *ad libitum*, were used in all experiments. Mice were injected intraperitoneally with 0.50 ml of 10% casein (Sigma Chemical Co.) to induce an acute phase response (7). Development of an acute phase response was verified by analyzing for SAA in plasma HDL by polyacrylamide gel electrophoresis at acid pH (14, 18). Littermates were injected with an equal volume of sterile saline to serve as controls. Livers from a control animal and its experimental littermate were perfused simultaneously approximately 18 hr postinjection. It is important to note that paired comparisons were always made between littermates since there is considerable interlitter variation in SAA degradation rates.

Chronic inflammation was induced by 14–15 daily injections of casein. Congo red staining confirmed that amyloid had not deposited in either spleen or liver of these animals. Amyloid was demonstrable if injections were continued for 18–21 days. Littermates receiving an equal number of saline injections served as controls.

Liver Perfusion. A modification (11) of the method of Mortimore (19) was used for the perfusions. Livers were perfused at a rate of 2 ml/min/g liver with Eagle's basal medium (Gibco Laboratories) supplemented with 4% bovine serum albumin (Sigma Chemical Co.) and 20% washed human red blood cells. The pH of the perfusate was adjusted to 7.35 and was oxygenated with 95% O_2-5% CO_2. Temperature was maintained at 37°C. Liver viability was assessed by color, visual appearance, and volume of bile production. Tracer was added to the perfusate and samples were taken from the recirculating perfusion system at 8, 12, 20, 30, 60, 90, 120, and 180 min. The 8-min sample was used as an indicator of baseline concentrations assuming that mixing and equilibration with tissue spaces were complete. A second 8-min sample was placed in the perfusion chamber for the duration of the perfusion to test for degradation by the perfusion medium. Experiments were excluded when degradation by the medium exceeded 7%. Perfusate samples were radioassayed in a Packard Multi Prias 4 Gamma Counter. Protein-bound radioactivity was determined by trichloroacetic acid precipitation as described previously (11). Protein degradation was monitored by the appearance of acid-soluble radioactivity. Degradation by control and experimental livers was compared using a paired t test.

Column Chromatography. Tracer and perfusate samples were analyzed by gel filtration chromatography on a 150- × 1.2-cm column of Bio-Gel A-0.5 M equilibrated with 0.15 M NaCl and 0.05 M NH_4HCO_3. Low-density lipoprotein (LDL), HDL, SAA, monomeric apo A-II, and dinitrophenyl-lysine (DNP-lys) were used as molecular weight markers.

Statistical Analysis. Comparisons between control and experimental groups were made using paired t tests.

Results

Effects of Acute Phase Response on Degradation of SAA. SAA degradation by livers from mice undergoing an acute phase response was compared with that of normal littermates. SAA catabolism was decreased 14% (from 8.6 ± 1.2% to 7.4 ± 1.1%/g liver in 3 hr, $n = 16$, $P < 0.05$) in animals receiving a single

injection of casein (Fig. 1A). Apo A-I degradation measured simultaneously was not decreased by the acute phase response (Fig. 1B). Livers from control and acute phase mice degraded 2.0 ± 1.5 and 2.5 ± 0.9%/g (n = 7), respectively, of the labeled apo A-I during the 3-hr perfusion period. The ability of the liver to secrete bile was unchanged by the induction of an acute phase response (0.81 ± 0.40 μl/min vs 0.72 ± 0.26 μl/min in control livers) and the mean liver weights were 1.61 ± 0.44 g for control and 1.59 ± 0.43 g for acute phase animals.

Effect of Chronic Inflammation on SAA Degradation. Mice received daily injections of casein for 14 days and paired comparisons were made with littermates receiving daily saline injections. No amyloid deposits were detected by Congo red staining in the treated animals. Longer protocols (18–21 days) led to development of amyloidosis in these animals; however, we experienced difficulty in perfusing amyloid-laden livers. Therefore, experiments were conducted after only 14 days of casein injection. Repeated casein injection led to a 31% reduction in SAA catabolism, from 11.7 ± 2.3%/g/3 hr to 8.1 ± 1.6%/g/3 hr (n = 8, P < 0.025, Fig. 2).

Repeated administration of casein reduced apo A-I degradation from 2.6 ± 0.61 to 1.4 ± 0.4%/g (n = 8) but the decrease was not statistically significant (Fig. 2). Mice treated with casein had larger livers, 1.8 g vs 1.5 g for control littermates, P < 0.025. Bile production was not altered by chronic inflammation, 0.73 ± 0.28 vs 0.72 ± 0.26 μl/min in control livers.

Size of the Degradation Products. The medium perfusing the experimental (14 casein injections) and control livers was applied to a Bio-Gel A-0.5 M column to determine the size of the degradation products. The starting material introduced into the perfusion is also shown for comparison in Figure 3. Three major peaks were present in the control perfusate. These included the void volume (corresponding to the LDL marker), ^{125}I-SAA bound to HDL and free ^{125}I (corresponding to DNP-lys peak). The medium perfusing the experimental livers contained an additional peak of radioactivity

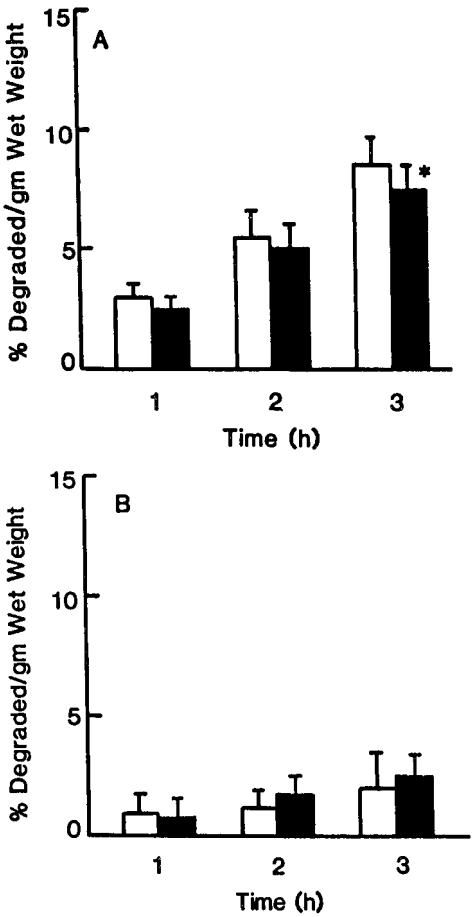

Figure 1. Effect of acute phase response on degradation of ^{125}I-SAA (A) and ^{131}I-apo A-I panel (B) by isolated, perfused mouse liver. Mice received 0.50 ml of sterile saline (□) or 10% casein (■) 18 hr before sacrifice. Bars represent mean ± SE. (A) n = 16. (B) n = 8. *P < 0.05, difference between saline and casein curves.

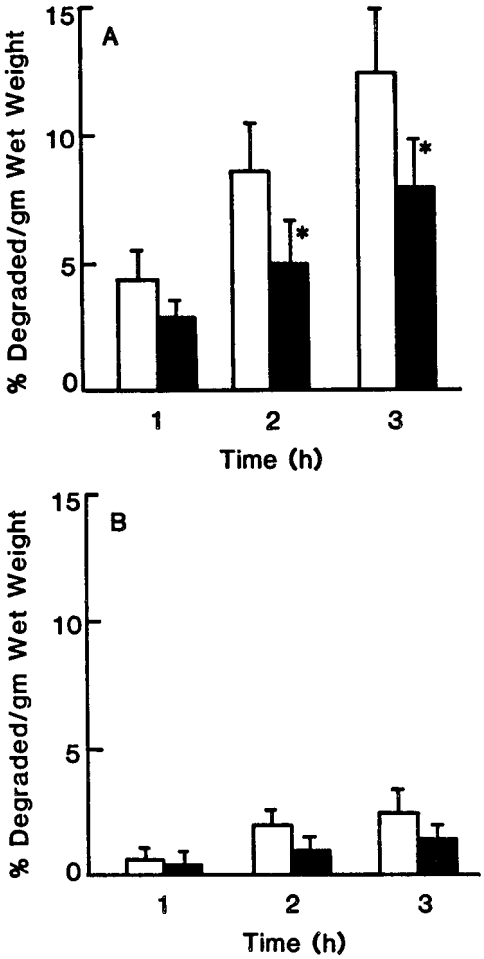

Figure 2. Effect of chronic inflammation on degradation of ^{125}I-SAA (A) and ^{131}I-apo A-I (B) by isolated, perfused mouse liver. Mice received 14 daily injections of sterile saline (□) or 10% casein (■) (n = 8). Bars represent mean ± SE. *P < 0.05, difference between saline and casein curves.

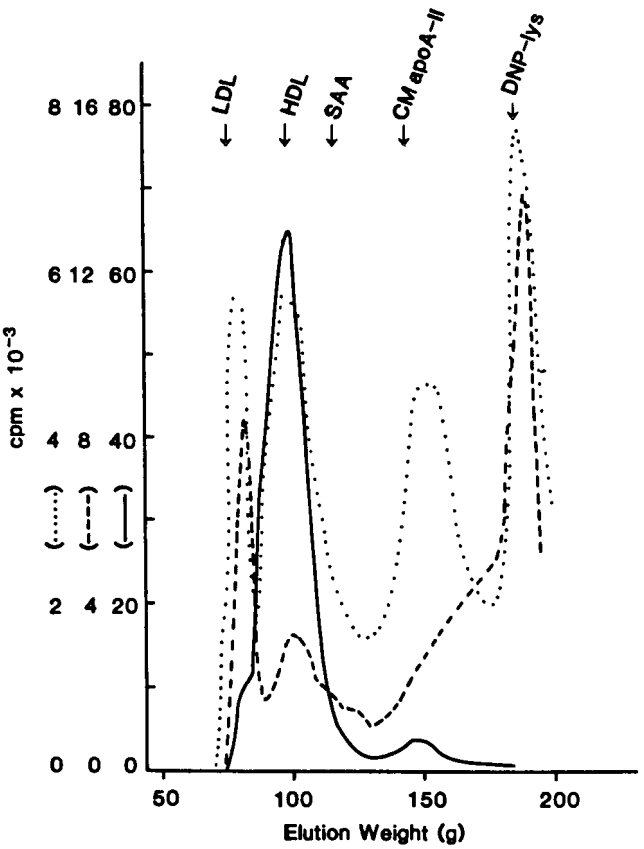

Figure 3. Gel chromatography elution profiles of ^{125}I-SAA starting material (——) and perfusates of livers from control (– – –) and chronically inflamed (· · · · ·) mice. Aliquots of perfusate were applied to a 1.2- × 150-cm column of Bio-Gel A-0.5 *M* and eluted with 0.15 *M* NaCl, 0.05 *M* NH_4HCO_3. Elution weights of molecular weight markers, LDL, HDL, M_r 150,000, SAA, M_r 12,000, monomeric carboxymethylated apo A-II (CM-apo A-II, M_r 8000), and dinitrophenyl lysine (DNP-lysine) are indicated.

which eluted in the size range of amyloid A (M_r 8500 corresponding to CM-apo A-II) (20). The area under this peak represented 26% of the total recovered radioactivity compared with 19% in the profile from control livers and 7% in the starting material. The control chromatogram contained a shoulder on the salt (DNP-lys) peak which overlapped into the amyloid A size range. This peak probably represents degradation products with a range of molecular weights smaller than amyloid A. The elution weight for glucagon, M_r 3550, for example, is 163 g compared with 143 g for CM-apo A-II (M_r 8000) and 185 for DNP-lys. The control perfusate may also have contained lipid-free ^{125}I-SAA which eluted as a shoulder on the HDL-bound SAA peak. HDL-bound ^{125}I-SAA comprised 93%, 18%, and 32% of the recovered radioactivity in the starting material and control and experimental perfusates, respectively, and free iodine accounted for 0%, 39%, and 24% of the recovered radioactivity.

Discussion

The liver is a major organ in SAA metabolism. In addition to synthesizing SAA (21), the liver degrades SAA (11) and is a site of amyloid deposition (22). We have estimated that the liver accounts for at least 16% of SAA catabolism in studies with partially hepatectomized rats (11). Previously, our laboratory used the isolated, perfused rat liver to characterize SAA degradation in normal animals (11). We found that SAA is catabolized independently of the HDL on which it is carried, that binding to lipoproteins protects SAA from degradation, and that degradation occurs at least in part through a specific cell-associated mechanism. In the current study, we sought to determine the effect of the acute phase response and chronic inflammation on the rate of SAA degradation by isolated perfused mouse liver.

The rate of SAA degradation was decreased 14% by a single injection of casein. Bile production and appearance of the liver were normal. Furthermore, the degradation rate of apo A-I did not change. Thus, the decreased rate of SAA catabolism could not be explained by nonspecific changes in liver metabolism.

Administration of casein for 14 days resulted in a 31% decrease ($P < 0.05$) in SAA degradation after 3 hr. The livers were larger ($P < 0.05$) in the casein-treated animals. Fuks and Zucker-Franklin (12) have reported increased recoveries of Kupffer cells in animals receiving 8–25 injections of casein. Since our animals showed no evidence of amyloid formation, increased cell number may account for the enlarged livers. Bile production was maintained at control levels indicating that liver function was not severely compromised. However, the reduction in apo A-I degradation, although not statistically significant, suggests some nonspecific effects on liver metabolism during chronic inflammation. One explanation for the reduction of SAA degradation might be that endogenous SAA was saturating the enzymes/receptors responsible for degradation. However, we have shown in experiments with animals infused intravenously with radioactive tracers before perfusions that all residual blood is removed before the perfusate is connected for recirculation. Furthermore, we calculate a maximum concentration due to SAA synthesis during the perfusions of only 0.5 μg/ml compared with 120–150 μg/ml in serum at the peak of the acute phase response (23). The data are consistent with a specific decrease in SAA degradation during both short- and long-term inflammation. It would be of interest to compare degradation rates in amyloidotic livers. However, we have found that livers from mice with amyloidosis are very difficult to perfuse for 3 hr.

Analysis of the perfusates of livers from chronically inflamed animals revealed the presence of an intermediate degradation product which corresponded in size to amyloid A although AA deposits were not detected in the liver. This observation is consistent with the data of others showing production of AA by monocytes from humans with amyloidosis (24, 25) and by Kupffer cells

from amyloidotic and preamyloidotic mice (12). Benson *et al.* (7) have postulated that stimulants of the acute phase response may specifically or nonspecifically alter the function of the reticuloendothelial system resulting in incomplete hydrolysis of SAA. Others (12, 24, 25) have suggested that two enzymes or enzyme systems are involved. The first converts SAA to AA and the second degrades AA. Activities of both enzyme systems appear to be affected by inflammation since both AA and SAA accumulated in the cultures of affected cells.

One surprising result of our study was that the AA-sized intermediate was released into the circulation. AA has not been found in blood of either preamyloidotic or amyloidotic animals, and it is generally assumed that once AA is derived from SAA it is deposited locally. Studies of cells in culture indicate that SAA is converted to AA by elastases on the cell surface since the AA is recovered in the medium (12, 24, 25). The fate of AA *in vivo* is not known nor is the mechanism by which AA is deposited in fibrillar form.

It is also noteworthy that the AA-like material in the perfusate was not bound to the HDL despite the fact it was derived from HDL-bound SAA. In a previous study of SAA degradation by isolated, perfused rat liver, we concluded that SAA was catabolized independently of the HDL because SAA was degraded more rapidly than the HDL apoproteins A-I, A-II, and C-III and because excess normal HDL in the perfusate was less effective at inhibiting SAA degradation than SAA-rich HDL. Two observations in the current study are consistent with this hypothesis. First, the fact that the AA is not recovered with HDL suggests that the SAA is dissociated from the HDL before proteolysis. Second, apo A-I degradation rate is not affected by the acute phase response, indicating that SAA and apo A-I catabolism are under separate regulation.

SAA degradation by mouse liver is decreased within 24 hr of induction of an acute phase response and remains depressed for at least 14 days with chronic inflammation. An intermediate corresponding in size to amyloid A is produced by livers from chronically inflamed animals. This intermediate is released into the circulation and is no longer HDL bound.

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1. Rosenthal CJ, Franklin EC. Variation with age and disease of an amyloid A protein-related serum component. J Clin Invest **55**:746–753, 1975.
2. McAdam KPWJ, Elin RJ, Sipe JD, Wolff SM. Changes in human serum amyloid A and C-reactive protein after etiocholanolone-induced inflammation. J Clin Invest **61**:390—394, 1978.
3. Franklin EC, Rosenthal CJ, Pras M. Studies on the amyloid A protein (AA protein) and a related serum component—purification—partial characterization and tissue origin and distribution in different types of amyloidosis. Adv Nephrol **5**:89–97, 1975.
4. Anders RF, Natvig JB, Michaelsen TE, Husby G. Isolation and characterization of amyloid-related serum protein SAA as a low molecular weight protein. Scand J Immunol **4**:397–401, 1975.
5. Linke RP, Sipe JD, Pollock PS, Ignaczak TF, Glenner GG. Isolation of a low-molecular-weight serum component antigenically related to an amyloid fibril protein of unknown origin. Proc Natl Acad Sci USA **72**:1473–1476, 1975.
6. McAdam KPWJ, Sipe JD. Murine model for human secondary amyloidosis: Genetic variability of the acute-phase serum protein SAA response to endotoxins and casein. J Exp Med **144**:1121–1127, 1976.
7. Benson MD, Scheinberg MA, Shirahama T, Cathcart ES, Skinner M. Kinetics of serum amyloid protein A in casein-induced murine amyloidosis. J Clin Invest **59**:412–417, 1977.
8. Skinner M, Shirahama T, Benson MD, Cohen AS. Murine amyloid protein AA in casein-induced experimental amyloidosis. Lab Invest **36**:420–427, 1977.
9. Husebekk A, Skogen B, Husby G, Marhaug G. Transformation of amyloid precursor SAA to protein AA and incorporation in amyloid fibrils in vivo. Scand J Immunol **21**:283–287, 1985.
10. Meek RL, Benditt EP. Amyloidogenesis. One serum amyloid A isotype is selectively removed from the circulation. J Exp Med **163**:499–510, 1986.
11. Bausserman LL, Saritelli AL, Van Zuiden P, Gollaher CJ, Herbert PN. Degradation of serum amyloid A by isolated perfused rat liver. J Biol Chem **262**:1583–1589, 1987.
12. Fuks A, Zucker-Franklin D. Impaired Kupffer cell function precedes development of secondary amyloidosis. J Exp Med **161**:1013–1028, 1985.
13. Styne PN, Vierling JM. Isolated perfusion of murine liver in situ: Technique and validation. Am J Physiol **249**:G719–G724, 1985.
14. Bausserman LL, Herbert PN, McAdam KPWJ. Heterogeneity of human serum amyloid A proteins. J Exp Med **142**:641–656, 1980.
15. Parmelee DC, Titani K, Ericsson LH, Eriksen N, Benditt EP, Walsh KA. Amino acid sequence of amyloid-related apoprotein (apo SAA_1) from human high-density lipoprotein. Biochemistry **21**:3298–3303, 1982.
16. Dwulet FE, Wallace DK, Benson MD. Amino acid structure of multiple forms of amyloid-related serum protein SAA from a single individual. Biochemistry **27**:1677–1682, 1988.
17. Herbert PN, Bausserman LL, Henderson LO, Heinen RJ, LaPiana MJ, Church EC, Shulman RS. In: Peeters H, Ed. The Lipoprotein Molecule. New York: Plenum Publishing Corp., pp35–56, 1978.
18. Jovin TM, Dante ML, Chrambach A. Multiphasic buffer systems output. Springfield, VA: National Technical Information Service, ppPB196085–196091, 1970.
19. Mortimer GE. Effect of insulin on release of glucose and urea by isolated rat liver. Am J Physiol **204**:699–704, 1963.
20. Levin M, Franklin EC, Frangione B, Pras M. The amino acid sequence of a major nonimmunoglobulin component of some amyloid fibrils. J Clin Invest **51**:2773–2776, 1972.
21. Hoffman JS, Benditt EP. Secretion of serum amyloid protein and assembly of serum amyloid protein-rich high density lipoprotein in primary mouse hepatocyte culture. J Biol Chem **257**:10518–10522, 1982.
22. Dunn TB. Amyloidosis in mice. In: Cotchin E, Roe FJ, Eds. Pathology of Laboratory Rats and Mice. Philadelphia: FA Davis Co., pp181–212, 1967.

23. Meek RL, Eriksen N, Benditt EP. Serum amyloid A in the mouse Sites of uptake and mRNA expression. Am J Pathol **135**:411–419, 1989.
24. Lavie G, Zucker-Franklin D, Franklin EC. Degradation of serum amyloid A protein by surface-associated enzymes of human blood monocytes. J Exp Med **148**:1020–1031, 1978.
25. Skogen B, Thorsteinsson L, Natvig JB. Degradation of protein SAA to an AA-like fragment by enzymes of monocytic origin. In: Glenner GG, Costa PP, deFreitas F, Eds. Amyloid and Amyloidosis. Amsterdam: Oxford-Princeton, pp465–470, 1980.