Spontaneous in vivo isomerization of bovine serum albumin as a determinant of its normal catabolism.

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Research Article

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Spontaneous In Vivo Isomerization of Bovine Serum Albumin as a Determinant of Its Normal Catabolism

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ABSTRACT By combination of isoelectric focusing and immunoelectrophoresis of fresh bovine plasma it is shown that 10% of the albumin in plasma has isoionic points equal to the intramolecular SS-interchanged isomers of bovine serum albumin (BSA). It is also shown that (*a*) albumin with the isoionic point of SS-interchanged BSA is produced in the cow from radioiodinated BSA depleted from SS-interchanged albumin before injection and (*b*) purified radiolabeled SS-interchanged BSA can be converted in vivo to albumin with the native isoionic point. On this basis, it is proposed that SS-interchanged albumin in vivo is in postsynthetic equilibrium with the "native" albumin conformation.

The SS-interchanged isomers purified either from commercial BSA or from BSA submitted to SH-SS interchange was, after radioiodination with ^{125}I, compared metabolically with "native" albumin labeled with ^{131}I in the same calf. Both species of SS-interchanged albumins have fast initial turnover rates but obtain a normal rate of degradation after the reversion to native albumin. If the isomers formed in vivo have the same properties as the ones present in commercial BSA, at least 50% of the physiological degradation of albumin can be accounted for by the 6–7 times faster catabolic rates of these isomers.

INTRODUCTION

Denatured serum albumin is catabolized faster in vivo than native albumin. This has been shown for grossly denatured albumin (1), but relatively small chemical modifications also lead to increased turnover rates (2). In vitro incubation of rabbit albumin at near physiological conditions will produce metabolically denatured molecules (3). On basis of this finding we suggested that part of the normal albumin degradation in vivo proceeds through a stochastic formation of denatured albumin with decreased resistance to catabolism (3, 4).

The heterogeneity of immunologically pure serum albumin has been studied extensively during the last decade (5), and recently intramolecular SS-interchanged isomers of bovine serum albumin (BSA)[1] have been isolated (6–9). The SS-interchanged isomers are present in commercial BSA and can be produced *in vitro* at conditions not far from the physiological state (9). Chemically, the SS-interchange is a SH-SS interchange reaction which takes place within the protein molecule leading to a reorganization of the position of the SS-bridges (10). In vitro the SH-SS interchange is favored at physiological pH and temperature (11). Similar reactions are known to be involved in vivo in the coupling of Bence Jones proteins to serum proteins (12).

The present investigation compares the metabolic properties of purified SS-interchanged isomers with "native" BSA and examines whether a conversion from one to the other actually takes place in vivo. Isomers present in commercial BSA as well as the ones formed in vitro will be studied.

METHODS

BSA was obtained from Behringwerke (Marburg-Lahn, West Germany; OHRD batch no. A 769); immunochemical purity was 100%. The albumin was defatted by the charcoal method of Chen (13) at pH 2.75. Defatting by this procedure does not change the metabolic properties of albumin.[2] Pure BSA monomer was isolated by preparative gel filtration on Sephadex G-150 at pH 5.6, 0.2 M KCl

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[1] *Abbreviations used in this paper:* BSA, bovine serum albumin; CY 5.45, "cysteinaged" BSA, fraction pI 5.45; NA 5.28, commercial BSA, fraction pI 5.28; NA 5.45, commercial BSA, fraction pI 5.45; pI, isoionic point.

[2] Wallevik, K. In preparation.

TABLE I

Fractions of Monomer Defatted BSA used for Comparative Catabolic Studies in Calves

Albumin basic material	pI of fraction isolated	Abbreviations	Point of iodination	Reference BSA in catabolic studies
Commercial	5.28	NA 5.28	After purification*	Commercial monomer
Commercial	5.45	NA 5.45	After purification*	Commercial monomer
Cysteine-aged‡	5.45	CY 5.45	Before incubation	NA 5.28

* Iodinated within 24 h before injection.

‡ Cysteine-ageing: I = 0, pH 8.2, cysteine 5 mol/mol, 23°C atmospheric oxygen.

(14). The protein was iodinated with carrier-free ^{125}I or ^{131}I (The Radiochemical Centre, Amersham, England) by the monochloride method (15, 16). This procedure gave material with one atom of iodine per molecule of BSA, and a radioactivity of 0.5 μCi/mg of protein.

Analytical electrofocusing was carried out on a 110-ml column, while preparative experiments were done on a 440-ml column (LKB-Produkter, Bromma, Sweden) in sucrose density gradients (17). Ampholine (LKB), pH range 4–6, was used at a concentration of 1%. After the preparative electrofocusings, NaCl was added to the pooled isoionic BSA fractions to a concentration of 0.2 M. Sucrose and ampholytes were removed by gel filtration on Sephadex G-50 (0.2 M KCl, pH 6.0). The albumin solutions were then sterilized by filtration and, when necessary, concentrated in Ficoll 40% to a volume of about 15 ml.

The following preparations were used for metabolic studies in calves (Table I): *Native BSA pI 5.28 (NA 5.28) and pI 5.45 (NA 5.45)* were isolated by preparative electrofocusing of 200–300 mg defatted commercial BSA monomer and labeled within 24 h of injection. *SS-interchanged BSA produced by "cysteine-ageing"* (9): 250 mg ^{125}I-labeled BSA was dialyzed against and diluted in deionized, distilled water. Cysteine (BDH Chemicals Ltd., Poole, England) was added to a concentration of 5 mol/mol BSA and the pH was adjusted to 8.0. The solution was sterilized by filtration into a sterile, rubber-sealed glass bottle and placed in the dark at room temperature for 5 days. The incubation mixture was then dialyzed against H_2O and submitted to preparative isoelectric focusing.

Isoelectric focusing of plasma (18). 5 ml heparinized bovine plasma was defibrinated by addition of 10 mg protamine sulphate (Leo Pharmaceutical Co., Ballerup, Denmark). Preliminary experiments showed that electrofocusing of the dialyzed serum resulted in focusing of the albumin around pH 4.8, which is the pI region of BSA complexed with fatty acids (17). This could be avoided either by defatting or by gel filtration of the serum. *Defatting:* 5 ml of serum was dialyzed against 0.2 M KCl and defatted at pH 2.75 (13). The pH was then adjusted to 5.6. *Gel-filtration:* 5 ml of serum was diluted to 10 ml with 0.1 M phosphate buffer, pH 5.6, containing 0.1 M KCl and 0.02% sodium azide and gel-filtered on Sephadex G-200 in the buffer (column 2.5 × 100 cm) (19). The albumin peak was collected.

Before electrofocusing the samples were dialyzed against H_2O and precipitates were removed by centrifugation. Depending on the amount of radioactivity in the serum, about 70 mg albumin was electrofocused on the 110-ml column or the whole amount of protein on the 440-ml column. No precipitate was formed during the focusings when the serum was gel-filtered. Radioactivity and pH were measured on 1- to 3-ml fractions. The electrofocusings were executed between 2 days and 2 mo after sampling of the blood.

The distribution of ^{125}I activity among the isoionic fractions was calculated; the distribution of ^{131}I could only be measured in few of the plasma samples because of the short physical half-life. In the subsequent electrofocusings ^{131}I-labeled native albumin was added to the plasma as a point of reference.

Comparative catabolic studies in calves. These studies were performed according to conventional principles (20, 21, 3).

Three males of Dutch dairy breed, age 3–5 mo, weight 135–185 kg, were given inactive KI 2 days before and during the turnover study. The isomer labeled with 70–80 μCi ^{125}I was mixed with an equal amount of the ^{131}I-labeled reference albumin. After removal of a small sample, the mixture was injected into the jugular vein. 50 ml of blood was drained 15 min later; samples of 30 ml were obtained daily during the 1st wk, followed by 2–3 times a week for 1 mo. Radioactivity was measured on 10-ml plasma samples (Packard Auto-Gamma scintillation spectrometer, Packard Instrument Co., Inc., Downers Grove, Ill.).

Calculations of the catabolic data. The activity in the plasma samples is plotted semilogarithmically as percent of the sample taken 15 min after the injection, which is taken to represent the radioactivity present in the vascular compartment at time zero.

If the injected tracer albumin is undenatured, the fractional rate of degradation of albumin is given by the final slope of the plasma curve in its semilogarithmic presentation, while the distribution ratio of albumin between the intra- and extravascular compartments can be estimated from the intercept on the ordinate of the linear extrapolation of this slope to zero time (22, 23). From the initial curved part of the plasma curve the "distribution rate"[3] of the tracer in the "exchangeable albumin pool" can be derived by a simple mathematical resolution of the plasma curve (22, 23).

When the tracer albumin is a mixture of native (normally catabolized) and denatured (quickly catabolized) molecules the initial part of the plasma curve is the sum of the rates of degradation of the different molecular species and their distribution rates (2).

In the simultaneous turnover study it is assumed that there are identical distribution rates of the native ^{131}I-labeled reference albumin and the ^{125}I-albumin under study (see Discussion). The estimated distribution rate of the

[3] The "distribution rate" is the algebraic sum of the rate of escape (the transcapillary escape rate) and the rate of backflux into the vascular bed.

reference albumin can therefore be used to eliminate the distribution portion from the plasma curve of the albumin studied. This is done by using the calculated ratios between the plasma activities of the reference ^{131}I-albumin and the corresponding values on the extrapolation of its terminal decay slope to zero time. The plasma activities of the ^{125}I-albumin are then divided by these ratios.

The rate of degradation of the different molecular species in the albumin studied is finally estimated by resolution of the corrected decay curve into its monoexponential components by the classical mathematical analysis (24).

Rocket and fused rocket immunoelectrophoresis. These techniques were performed as described by Weeke (25) and by Svendsen (26). The fractions eluted from the electrofocusings were diluted 1:1 with Tris buffer, 0.1 M, pH 8.6, and 10 μl was placed in the corresponding well in the agar gel. Rabbit antiserum against total bovine serum was used (Dakopatts A/S, Copenhagen).

Miscellaneous. Polyacrylamide electrophoresis was performed vertically according to Raymond (27) as described elsewhere (3). Concentration of BSA was measured spectrophotometrically with an $E_{1\,cm}^{1\%}$ (280 nm) of 6.67. All chemicals used were reagent grade.

RESULTS

The method of isoelectric focusing is not only sensitive to small changes in the examined protein but involves the risk of introducing artefacts (17). The following precautions were taken: Unlabeled and ^{125}I-labeled defatted BSA show identical isoelectric focusing (Fig. 1A) (17). Incubation of the iodinated monomeric BSA under "cysteine-ageing" conditions (Fig. 1B) results in increase of the same isoionic fractions as for unlabeled BSA (9) and both the incubated and the unincubated albumin have corresponding ^{125}I specific activities in the focused protein fractions (Fig. 1).

The fractions marked in Fig. 1 were isolated from commercial and "cysteine-aged" BSA by preparative isoelectric focusing. Characteristics and abbreviations for the single fractions are given in Table I.

The distribution among isoionic points of labeled albumin in the samples to be injected were determined by reelectrofocusing of trace amounts mixed into unlabeled commercial BSA or serum (Figs. 2 and 3).

A contamination of the injected fractions with protein of other isoionic points is caused by interconversion during the manipulations following the preparative focusing (9). For example, SS-interchanged albumin will partially reverse to the native pI value during the high pH conditions of the iodination (9). As NA 5.45 was iodinated after the purification (Table I) this explains why as much as 30% albumin pI 5.28 is present in the injected NA 5.45 sample (Fig. 4)

In the samples to be injected no polymers of BSA could be demonstrated by polyacrylamide electrophoresis.

Catabolic studies. The plasma curves of the ^{125}I-labeled fractions and the ^{131}I-labeled reference albumin,

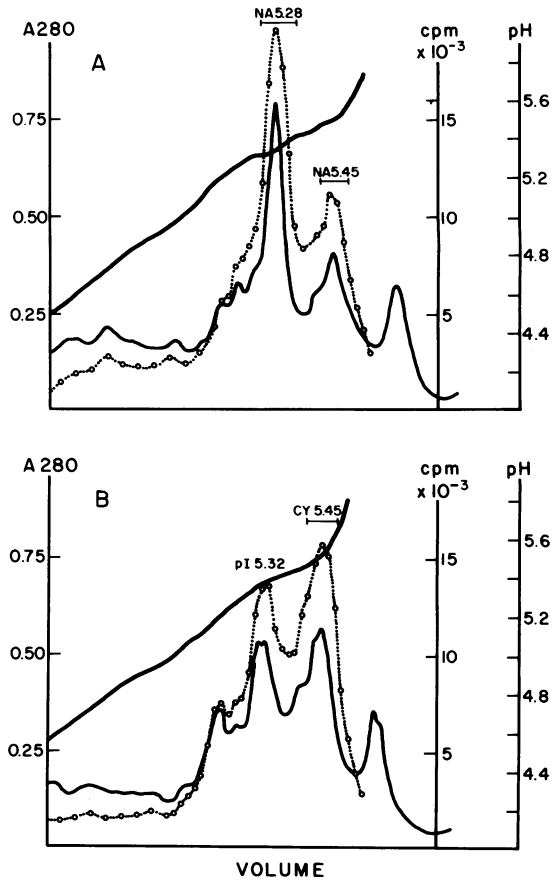

FIGURE 1 Isoelectric focusing of defatted ^{125}I-BSA immediately after iodination (A) and after incubation at pH 8.0, I = 0, cysteine 5 mol/mole BSA, 23°C for 5 days (B). The unbroken elution diagram is the directly recorded absorption at 280 nm of the effluent from the column. O···O: ^{125}I activity. The inclining line is the pH gradient. The fractions marked are the ones isolated by preparative isoelectric focusing and investigated catabolically in calves. Their notation is explained in Table I and in the text.

determined simultaneously in the same calves, are shown in Fig. 5. The NA 5.28 has a half-life similar to commercial BSA when compared in the same animal, indicating that the method of isoelectric focusing has no destructive influence on the catabolic properties of the albumin. NA 5.28 and the reference albumin have half-lives between 18 and 22 days, which is within the normal variation (28) (Table II). 38–42% of the injected NA 5.28 is situated intravascularly when the labeled protein after 4-6 days is in equilibrium between the intra- and extravascular compartments (Fig. 5).

An example of the basic results obtained from the turnover study of CY 5.45 is given in Table III. In the plasma obtained 15 min after injection, the ratios of ^{125}I and ^{131}I activities are the same as in the injected samples (Table III). As the reference albumins are shown

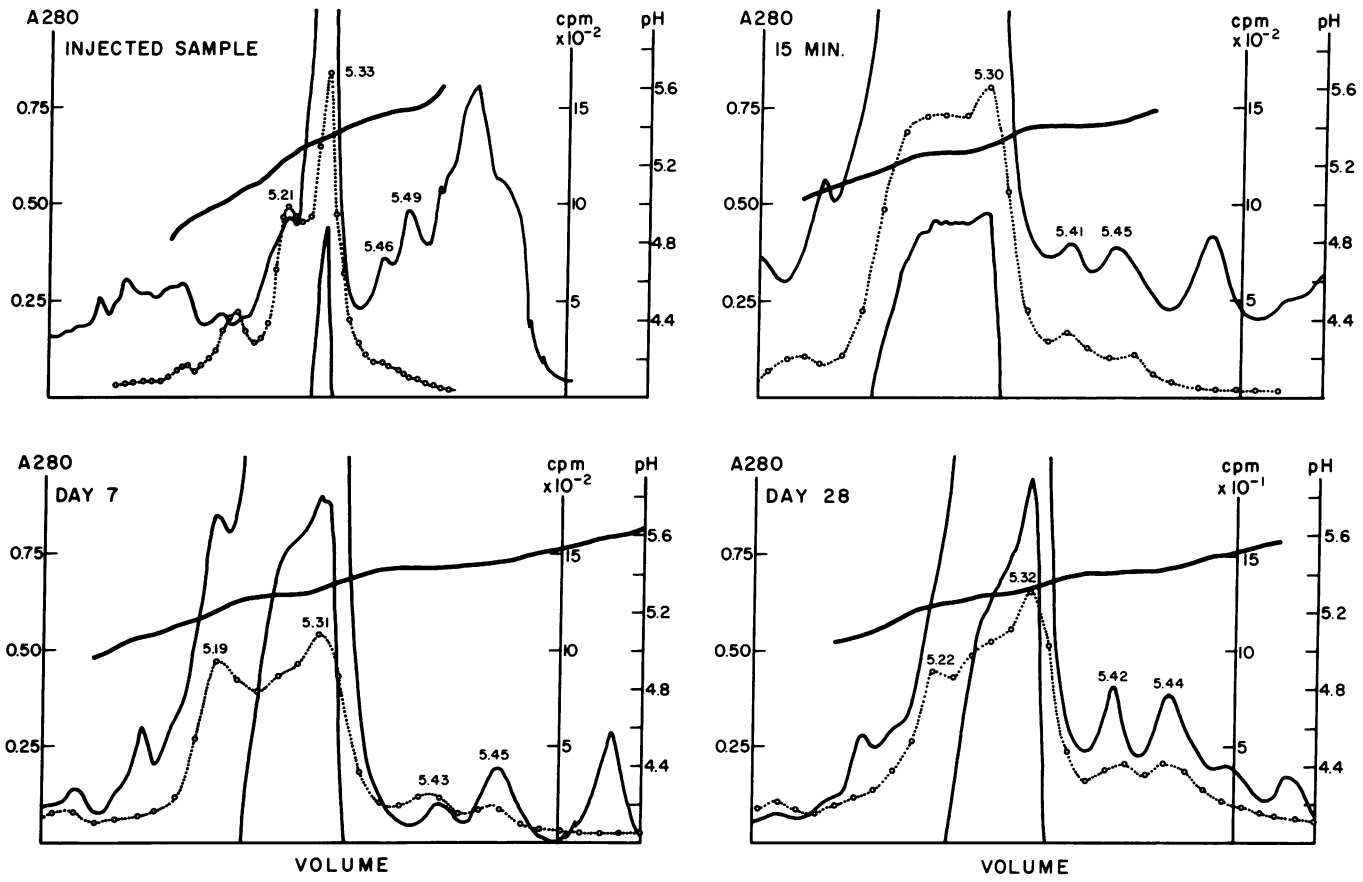

FIGURE 2 Isoelectric focusing of BSA crudely purified by gel filtration (Sephadex G-200) of serum from a calf injected with ^{125}I-labeled NA 5.28. Unbroken elution diagram: the directly recorded absorption at 280 nm of the effluent from the electrofocusing column. ○···○: ^{125}I activity. The inclining line is the pH gradient. The "injected sample" is mixed into freshly drawn serum and then treated as the other serum samples. The peaks which are not indicated by their isoionic points are other serum proteins present in the focused sample. The injected sample is electrofocused on a 110-ml, the subsequent ones on a 440-ml column.

to behave normally after injection, this indicates that none of the isomers contain grossly denatured molecules which are catabolized within the first 15 min after the injection (29).

The plasma curves of the isomers were corrected so as to have distribution properties in the "exchangeable albumin pool" equal to the reference albumin (see Methods) (Fig. 5). The justification of this correction will be discussed later. The corrected decay curves for the albumin isomers are not monoexponential, indicating that these albumins are metabolically inhomogeneous (2). However, after 1–2 wk of catabolism the slopes of the decay curves become linear, which makes resolution of the initial part of the curves into their monoexponential components possible (24). The resultant linear rate curves are inserted in Fig. 5. They are character-

TABLE II

Catabolic Half-Lives of ^{125}I-Labeled BSA Isomers Injected into Calves Together with a ^{131}I-Labeled Native Reference Albumin

^{125}I-labeled isomer injected	Half-life and apparent amount of the isomer to which it corresponds*						^{131}I-labeled reference
	I‡		II		III		
	$t_{\frac{1}{2}}$	%	$t_{\frac{1}{2}}$	%	$t_{\frac{1}{2}}$	%	$t_{\frac{1}{2}}$
NA 5.28	22	100					21
NA 5.45	19.8	74.5	2.9	25.5			19.8
CY 5.45	18.2	51.5	2.8	24.8	0.3	23.7	18.2

* Calculated from the intercepts on the ordinate of the extrapolations to zero time of the linear parts of the rate curves, with the intercept of the native reference albumin assigned a value of 100%.

‡ The Roman numerals refer to the linear parts of the plasma decay curves of Fig. 5 obtained by curve resolution.

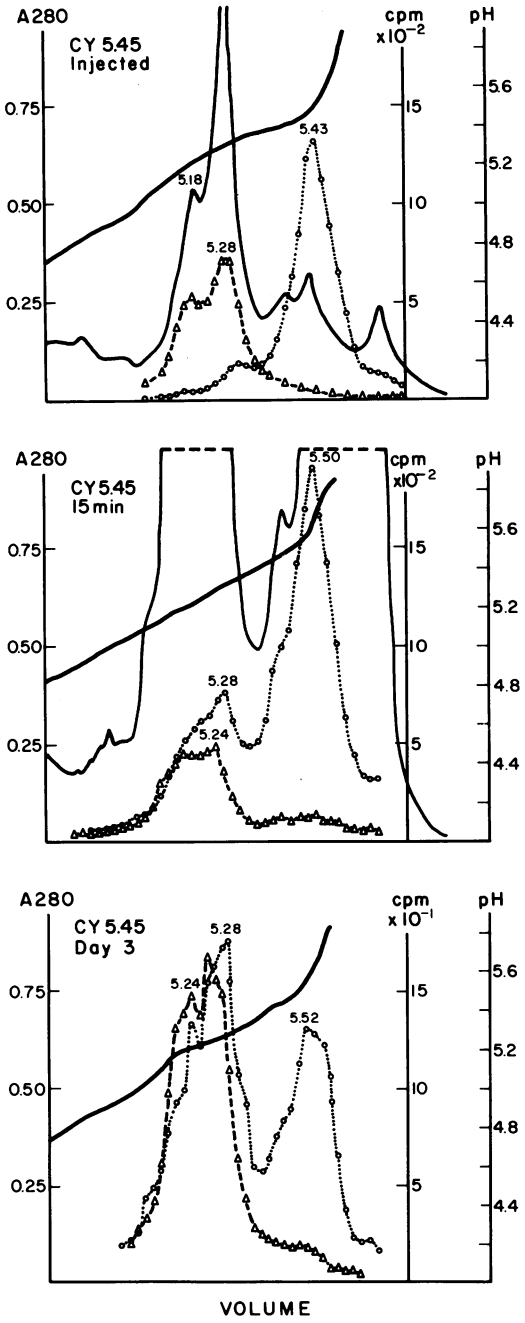

FIGURE 3 Isoelectric focusing of defatted serum from a calf injected with ^{125}I-labeled CY 5.45 and ^{131}I-NA 5.28. ○···○: ^{125}I activity; △—△: ^{131}I activity. Full drawn line: the directly recorded absorption at 280 nm of the effluent (only indicated when not disturbed by flocculation of other serum proteins on the column). The inclining line is the pH gradient. The "CY 5.45 injected" is mixed with unlabeled defatted BSA. In the serum obtained 15 min after injection the measured ^{131}I activity is the one present in the serum, in the subsequent samples ^{131}I-NA 5.28 is added to the serum.

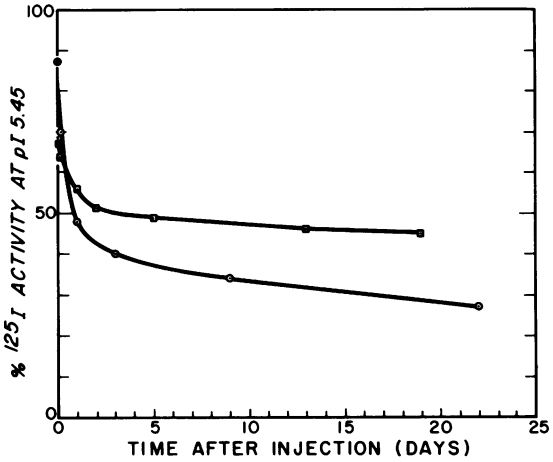

FIGURE 4 ^{125}I-Albumin with a pI value of 5.45 relative to the total amount of ^{125}I-albumin in the injected sample (closed symbols) and in the plasma samples (open symbols) taken from the catabolic studies of NA 5.45 (□—□) and CY 5.45 (○—○). The plotted values were obtained by calculating the distribution of ^{125}I activity among the isoionic albumin fractions in the electrofocusing diagrams of the injected and of defatted plasma samples from the catabolic studies of the two isomers (examples in Fig. 3). The values from the plasma are not corrected for the increase in fraction pI 5.45 caused by the defatting procedure (17).

ized by their slopes ($t_{\frac{1}{2}}$) and their normalized linear intercepts on the ordinate (Table II).

Metabolically, CY 5.45 and NA 5.45 have in common (*a*) that the slowly catabolized fraction has a turnover rate identical to native albumin and (*b*) that 25% of each of the isoionic fractions are catabolized with a $t_{\frac{1}{2}}$ close to 3 days. CY 5.45 contains in addition 25% of an albumin which is degraded faster with a $t_{\frac{1}{2}}$ in the vicinity of 0.3 days (Table II).

Isoelectric focusing of serum. Selected plasma samples from the catabolic studies were submitted to analytical electrofocusing (examples in Figs. 2 and 3). The recovery of ^{125}I activity in the pI 5.18–5.45 region was 78–82%. The resolution between fraction pI 5.28 and pI 5.39–5.45 was sufficient to calculate the distribution of radioactivity among the two pI regions with an accuracy of ±2%, when the same plasma sample was submitted to three independent gel-filtration and electrofocusing experiments (Table IV).[4]

When ^{125}I-labeled NA 5.28 mixed in freshly drawn unlabeled plasma is gel-filtered on Sephadex G-200 and electrofocused, 2–4% of the activity has isoionic points from 5.39 to 5.45 (Table IV), compared to 12% when plasma is taken 15 min after the injection or later (Table IV). The 12% figure is in agreement with the

[4] Defatting of serum before electrofocusing will overestimate the fraction pI 5.39–5.45 by 10%, which has to be subtracted (17).

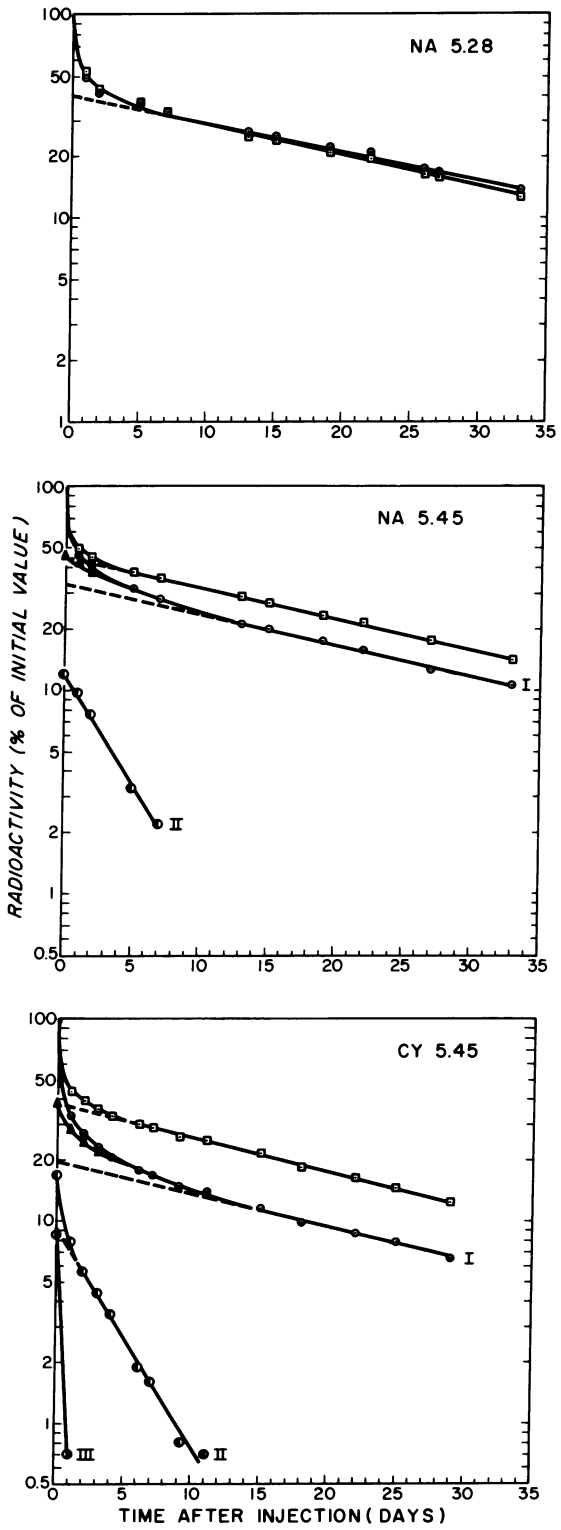

FIGURE 5 The plasma activity curves from the simultaneous turnover studies in calves of native albumin pI 5.28 (NA 5.28) and pI 5.45 (NA 5.45) and of "cysteine-aged" albumin pI 5.45 (CY 5.45). The experimental albumins are labeled with ^{125}I (○—○), the reference with ^{131}I (□—□). The plasma curves of the isomeric albumins are corrected for the rate of distribution of labeled albumin in the "exchangeable albumin pool" (see Methods), thus giving the rate curves for the degradation of the labeled isomers in plasma (△—△). The corrected catabolic decay curves are resolved into their monoexponential constituents (◐—◐). The estimated catabolic data are listed in Table II.

TABLE III

A Simultaneous Turnover Study of ^{125}I-CY 5.45 and ^{131}I-NA 5.28 in the Same Calf

Time after injection	^{125}I-CY 5.45*	^{131}I-NA 5.28*	$^{125}I/^{131}I$‡	dpm focused at pI 5.28§
	dpm			
0	4,462.1‖	188.3‖	23.70	6,901¶
15 min	53,089.2	2,241.9	23.68	16,458
days				
1	17,780.9	970.1	18.33	9,246
2	14,352.9	874.3	16.42	
3	12,321.9	791.0	15.58	7,393
4	11,052.2	741.5	14.91	
6	9,449.9	674.8	14.00	
7	9,008.2	648.5	13.89	
9	7,948.1	581.2	13.68	5,246
11	7,423.5	560.9	13.23	
15	6,085.1	480.2	12.67	
18	5,159.6	410.6	12.56	
22	4,612.0	368.3	12.52	3,367
25	4,225.3	333.8	12.66	
29	3,419.5	269.6	12.68	

* Corrected for physical decay.

‡ From day 15 the coefficient of variation of the ratio is 0.9%, which is equal to the influence of the counting inaccuracy on the ratio (29).

§ Calculated from the distribution of activity among the isoionic fractions (Fig. 4).

‖ The count rate at time zero is measured on an arbitrary sample of the injected mixture of isomers. The values are used only to calculate the $^{125}I/^{131}I$ ratio.

¶ The count rate in plasma at time zero is taken to be equal to the one obtained 15 min after injection, as the isotope ratio indicates that there is no measurable degradation of ^{125}I-labeled albumin within this period of time.

amount of protein obtained at pI 5.39–5.45 when gel-filtered, nondefatted bovine serum is submitted to electrofocusing (Table IV). That the protein focused at pI 5.45 is predominantly albumin is shown by fused rocket immunoelectrophoresis from the focusing of the bovine serum (Fig. 6). By quantitative immunoelectrophoresis the amount of albumin in serum with an isoionic point of 5.39–5.45 is estimated to be 15% of the total (Table IV).

The conversion of NA 5.28 to NA 5.45 can also be demonstrated in vitro when BSA with isoionic point

TABLE IV

Isomerization of NA 5.28 under Physiological Conditions Estimated by Isoelectric Focusing

In vivo: Serum from a catabolic study of ^{125}I-NA 5.28* in a calf‡

	% ^{125}I activity at pI:		
	5.18	5.28	5.39–5.45
Injected sample§	20±2	78±2	3±1
Time after injection:			
¼ h		89	11
240 h	34	54	12
720 h		89	11
Isoionic heterogeneity of albumin in serum‡			
	% BSA at pI:		
	5.18	5.28	5.39–5.45
Absorption at 280 nm‖	13±2	76±2	12±2
Quantitative immunoelectrophoresis	11	74	15

In vitro: NA 5.28¶ incubated at pH 7.4, 0.2 M KCl, 39°C, cysteine 5 mol/mole albumin

	% BSA focused at pI:		
	5.18	5.28	5.39–5.45
Before incubation	24	70	6
Incubation time:			
3 days	33	54	13
10 days	40	48	12

* NA 5.28 produced by preparative isoelectric focusing of defatted BSA.

‡ Before focusing, the albumin was partially purified from the serum by gel filtration on Sephadex G-200.

§ Injected ^{125}I-NA 5.28 mixed with fresh, not radioactive serum, then treated as in footnote ‡.

‖ Measured by planimetry of the direct UV-elution diagram from electrofocusing of five different gel-filtrated serum samples.

¶ NA 5.28 purified by preparative electrofocusing of fresh serum, pretreated as in footnote ‡.

5.28 prepared from defatted serum is incubated at pH 7.6, 0.2 M NaCl, 39°C, together with small amounts of cysteine (Table IV). Before incubation 6% of the albumin focuses at pI 5.45; at day 3 the amount has increased to and remains at 12%, indicating that an equilibrium between the two fractions has been obtained.

CY 5.45 and NA 5.45 labeled with ^{125}I also changes in vivo. Isoelectric focusing of the injected samples and of the plasma samples demonstrates that these isomers are reversed to albumin with a native isoionic point during the catabolic studies (Figs. 3 and 4). This conversion in vivo to albumin with a pI value of 5.28 cannot be accounted for solely on the basis of a much faster rate of catabolism of the ^{125}I-labeled 5.45 fractions compared to the impurities of ^{125}I-NA 5.28 already present in the samples at the time of injection, as there is an absolute increase of ^{125}I activity at pI 5.28 after the injection of both isomers (example in Table III).

During the catabolism of CY 5.45 the amount of ^{125}I activity which focuses at pI 5.45 changes from 88% of the activity in the injected sample to about 20% in the plasma obtained after 3 wk of catabolism (Fig. 4). In the case of NA 5.45 the corresponding figures are 68% in the injected sample decreasing to 35% of the radioactivity which remains in the plasma at day 19 (Fig. 4).

DISCUSSION

The present investigation shows that (*a*) 10% of the BSA has isoionic points in vivo of pI 5.39–5.45, (*b*) this isomeric albumin is produced in vivo from NA 5.28,[5] (*c*) the subfractions with isoionic points of 5.39–5.45, present in commercial albumin preparations or produced in vitro under SH-SS-interchanging conditions, are catabolized faster than NA 5.28, and (*d*) they are partially reversed in vivo to NA 5.28.

When samples of ^{125}I-NA 5.28 are injected they contain 3% albumin focused at pI 5.39–5.45. 15 min after

[5] The term "NA 5.28" is preferred for mercaptalbumin as it has been shown that NA 5.28 is heterogeneous (9).

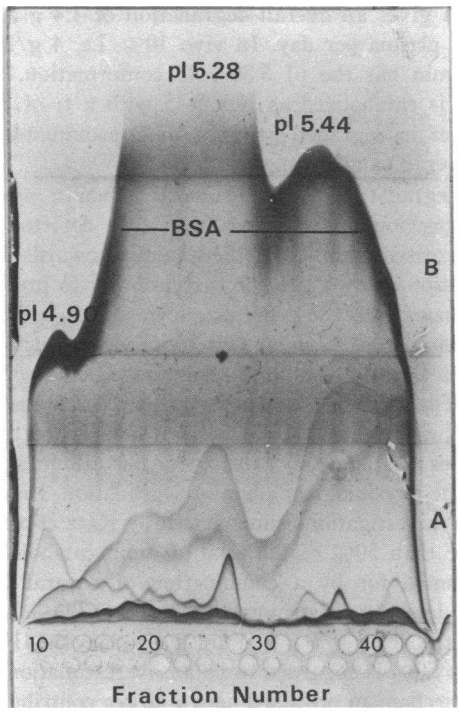

FIGURE 6 Immunochemical elution profile (fused rocket immunoelectrophoresis) of an isoelectric focusing of BSA isolated from freshly obtained bovine serum by gel filtration. The fractions eluted from the electrofocusing column (UV-elution diagram similar to the one shown in Fig. 2, "injected sample") were placed in their corresponding wells in the agar and precipitated with an antiserum against bovine serum present in the gels A and B by electrophoresis. The concentrations of antiserum in A and B were, respectively, 2.5 and 5%; still too much albumin is present in peak pI 5.18 and 5.28 to be precipitated. The traces of other serum proteins in the fractions are unidentified.

the injection these fractions increase 12%. A difference in the treatment of the samples cannot explain this finding, as the control sample of NA 5.28 was mixed into freshly drawn plasma and then handled identically to the plasma samples from the turnover study. Technical artefacts are improbable; isoelectric focusing has previously been shown to produce no more than a few percent BSA with isoionic points higher than NA 5.28 (9) and no increase in such a fraction was seen after storage of plasma up to 2 mo. It is thus unlikely that the 10% albumin focused at pI 5.39–5.45 is formed after collection of the blood.

The crucial question is whether the albumin with the isoionic points of 5.39 and 5.45 present in vivo is identical with the albumin with these isoionic points present in commercial BSA or obtained by the "cysteine-ageing" conditions.

By in vitro experiments it has been shown that both fraction pI 5.39 and 5.45 are SH-SS-interchanged conformations of BSA differing in the degree of intramolecular SS interchange (8, 9). Both fractions can be converted in vitro to albumin with the native isoionic point (8, 9), although this reversion is much slower than predicted from the forward reaction (8).

No other relevant in vitro modifications of BSA has been shown to increase the isoionic point of the albumin. Complex formation between BSA and organic compounds (17) and mild "oxidation" of BSA under "physiological" conditions (9) in vitro has been found to decrease the isoionic point of the reacted albumin compared to the native material.

As mentioned in the introduction SH-SS interchange reactions are chemically favored under physiological conditions and have actually been shown to take place in vivo between peptides and certain serum proteins (12). Furthermore, as albumin pI 5.39–5.45 is found to be produced from and probably is in equilibrium with NA 5.28 during in vitro "physiological" conditions it seems probable that the isomers formed after synthesis in vivo are SS-interchanged conformations. The proposed physiological SS interchange must also be intramolecular, as no dimer albumin is present in vivo (4). The isomers produced in vivo will probably be in closest agreement with the NA 5.45, which is isolated from albumin not submitted to the unphysiological low ionic strength of the "cysteine-ageing."

The differences in catabolism between native albumin pI 5.28 and the isomers are determined by simultaneous comparison of the differently labeled fractions in the same animal. This method has the advantage that the individual variation in catabolism among animals does not have to be taken into consideration (21, 29). If the mixture of the two differently labeled albumins has different plasma decay curves it can only be explained by a difference in behavior in (*a*) distribution rates between the intra- and extravascular compartments, (*b*) distribution ratio between the compartments, or (*c*) rates of catabolic degradation.

The main mechanisms of transcapillary protein escape from the plasma is filtration (30) and the transcapillary escape rates of the different serum proteins are determined by their hydrodynamic properties (31). Once a protein molecule has crossed the capillary wall it will return to the plasma predominantly via the lymph (32). As the back flux via the lymph is equal for the different serum proteins, this means that their distribution ratios will be determined chiefly by their transcapillary escape rates.

It has been shown that both albumin pI 5.39 and 5.45 are expanded conformations where CY 5.45 has a Stokes radius of 38.2 Å, which is 2.4 Å larger than NA 5.28 (9). This difference is, however, smaller than the difference in Stokes radius between transferrin (40 Å)

(33) and albumin, a difference, which has no detectable influence on the transcapillary escape rates of the two proteins.[6] The fast initial disappearance of the experimental albumins from the plasma compared to the reference thus cannot be explained by differences in transcapillary escape, but only by an initial fast degradation of both NA 5.45 and CY 5.45.

The finding that both NA 5.45 and CY 5.45 after injection (*a*) reverse to NA 5.28, (*b*) have initial fast turnover rates, and (*c*) have final turnover rates equal to the turnover of NA 5.28 can be depicted in the following reaction scheme:

$$\text{NA } 5.28 \rightleftarrows \text{SS-interchanged BSA} \rightarrow \text{degradation.}$$

The initial fast turnover rates measured for NA 5.45 and CY 5.45 should then indicate the catabolic rates of these conformational states before they obtain a normal catabolism by a conversion to NA 5.28.

10% of the albumin in vivo was found to have the isoionic points of the SS-interchanged isomers. If the conversion of the injected isomers to native albumin in vivo followed the simple reaction kinetics suggested in the reaction scheme, one would expect that both NA 5.45 and CY 5.45 would have reached this equilibrium point in plasma within the 4 wk of the catabolic study. As illustrated in Fig. 4 this is not the case, indicating that only a portion of the injected isomers pI 5.45 is subject to the SS interchange back to native albumin. However, this behavior of the isomers in vivo is in agreement with observations in vitro where the SS-interchanged isomers are found to reverse slowly and incompletely to native albumin (8, 9).

If the isomers formed in vivo have chemical and catabolic properties similar to the NA 5.45 injected, the proposed reaction scheme will represent a pathway for the normal in vivo degradation of albumin: the synthesized albumin molecules in vivo will be in equilibrium with isomeric, probably SS-interchanged conformations, which have considerably faster rates of catabolism. With these assumptions it is possible to make a crude quantitative estimate of the significance of the reactions from the scheme for the physiological albumin degradation.

In vivo the processes in the reaction scheme will normally be in a dynamic equilibrium so that the amount of protein in each conformational state is constant (steady-state conditions). In the steady state the rate of disappearance of albumin from plasma and from the whole body is equal. This means that the calculations based on the plasma parameters can be made representative for the degradation of the total albumin pool.

The concentration of albumin in plasma is 40 g/liter. With a mean $t_{\frac{1}{2}}$ of 20 days for native albumin in the cow, this gives an overall degradation of 1.4 g albumin per liter plasma per day. In vivo 10%, i.e. 4 g/liter, of the albumin has the pI 5.39–5.45 conformation. If this fraction is catabolized as NA 5.45 with a $t_{\frac{1}{2}}$ of 3 days, 0.8/g/liter is degraded per day, or approximately 50% of the overall catabolism.

The degradation of the isomeric albumin molecules can, as previously suggested (4, 3), be depicted either by a decreased stability of the albumin towards further denaturation or by a greater susceptibility to proteolysis (34), pinocytosis (35), or phagocytosis (36), all processes which have been shown to proceed much faster with even slightly denatured albumin molecules (34-37).

The demonstration of a postsynthetic isomerization of albumin with decreased catabolic resistance of the isomers gives a satisfactory account for the still unexplained part of the physiological albumin degradation. In spite of intensive investigations summarized by Katz et al. (38), not more than 50% of the total albumin catabolism can be accounted for by a combination of natural loss of albumin into the gastrointestinal tract (39), degradation in the liver (40) and in the kidneys (41), and even this 50% is regarded to be an overestimation (38). By the mechanism proposed here a larger contribution to the albumin degradation than the already estimated 50% can be explained by formation of small amounts of other isomers with very fast catabolic rates as, f.i., CY 5.45. Only 2% of the total albumin in plasma need have the fast catabolic rate of 0.3 days as actually measured initially for this fraction to account for another 50% of the overall degradation.

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