JCI The Journal of Clinical Investigation

INCREASED ELECTROPHORETIC MOBILITY OF ALBUMIN IN THE SERUM OF RATS SUBJECTED TO CARBON-TETRACHLORIDE TREATMENT OR TO TOURNIQUET SHOCK

Ulrich Westphal, Stanley G. Priest

J Clin Invest. 1955;34(9):1462-1466. https://doi.org/10.1172/JCI103196.

Research Article

INCREASED ELECTROPHORETIC MOBILITY OF ALBUMIN IN THE SERUM OF RATS SUBJECTED TO CARBON-TETRACHLORIDE TREATMENT OR TO TOURNIQUET SHOCK

By ULRICH WESTPHAL and STANLEY G. PRIEST

(From the Protein and Steroid Section, Biochemistry Department, Army Medical Research Laboratory, Fort Knox, Kentucky)

(Submitted for publication February 8, 1955; accepted May 25, 1955)

It has been previously reported (1) that binding of small quantities of certain fatty acids to serum albumin causes a decrease of its azorubin-binding capacity (ABC). It was assumed then that the low ABC of serum albumin observed in clinical cases (2) or in experimental animals (3, 4) was produced by a firm binding, *in vivo*, of increased amounts of certain anions, possibly higher fatty acids.

If this explanation were correct, a negative increment in the net charge of the albumin molecule would be expected. The electrophoretic mobilities of albumin were therefore determined in sera of rats subjected to carbon-tetrachloride poisoning and to tourniquet shock since these conditions are known to lower the ABC of serum albumin (3). Studies on the influence of dialysis on the ABC of sera and other protein solutions were included as supporting data.

EXPERIMENTAL

Animal experiments. Male Sprague-Dawley rats were given i.p. injections of 0.5 ml. CCl_4 per Kg. body weight every second day for six days and twice this volume of a 1 + 1 mixture of CCl_4 and ethanol on the eighth day. The blood was obtained on the tenth day. Other details have been given previously (3). The average weight of the rats declined from 336 gm. to 271 gm. under the injections of CCl_4. Tourniquet shock was produced by applying rubber bands to both hind legs (3). The average weight of the animals at the time of sacrificing was 269 gm. The blood of all experimental animals was drawn from the heart and the serum prepared as described (3). The average non-protein nitrogen concentration in the sera listed in Table I was found to be 72 mg. per cent, indicating a severe state of shock. The various samples of serum for the normal, CCl_4-treated and tourniquet-shock rats were obtained from a total of 88, 163, and 148 animals, respectively.

Chemical procedures. Total protein and non-protein nitrogen were determined by a micro-Kjeldahl procedure as outlined previously (3).

Electrophoretic analysis. The sera were dialyzed against sodium phosphate buffer of pH 7.7, $\mu = 0.1$, for a total of 22 hr. at 2° C. in a mechanically stirred dialyzer (5) with six changes of buffer. The diluted serum solution was used for the measurement of the pH (glass electrode, room temperature) and resistivity. The electrophoretic analyses were done in a standard size electrophoresis apparatus [1] using the long analytical cell. The separations were mostly run for 120 minutes at + 0.4° C. and 24 mA. The conductivity was measured in a Shedlovsky cell equilibrated at + 0.4° C.

The evaluation of the electrophoretic patterns was done as in earlier studies (3), using the descending boundaries only. The procedure of Longsworth and MacInnes (6) was applied to determine the distance traveled by albumin. As a check of the accuracy of the electrophoretic analysis, the mobility of crystalline bovine serum albumin (Armour) was determined in a 1 per cent solution in glycine sodium hydroxide buffer, $\mu = 0.2$ at pH 8.4. The value obtained (-5.79×10^{-5} cm.^{2} sec.$^{-1}$ volt^{-1} at pH 8.35) was in agreement with the mobility (-5.8×10^{-5} cm.^{2} sec.$^{-1}$ volt^{-1} at pH 8.4) reported in the literature (7).

Dialysis experiments. All dialyses were performed at 2° C. ± 0.5° C. The solutions of fatty acids in human albumin and the control samples were prepared as previously described (1). They were dialyzed in a mechanically stirred dialyzer (5) with buffer changes and for the time periods given in Table III. At pH 7.7, phosphate (0.02 μ)-sodium chloride (0.08 μ) buffer was used; at the higher pH values, glycine-sodium hydroxide-sodium chloride buffer of ionic strength 0.2 (8) was employed. The dialysis periods at pH 11.5 and 12.0 (Table III) were followed by a dialysis of about 16 hrs. against the phosphate-sodium chloride buffer at pH 7.7 with several changes of buffer. The final control and experimental solutions were adjusted to the same volume. This procedure assured an equal final ionic composition and approximately the same total dialysis times for all experiments of Table III. The determination of the ABC by a chromatographic procedure on anionotropic aluminum oxide and the calculation of the number of moles of fatty acid bound per mole albumin have been outlined previously (1).

For the dialysis of sera at neutral pH, distilled water, 0.6 per cent sodium chloride and the above phosphate-sodium chloride buffer were used. The dialyses at higher pH values were done against 0.1 μ glycine buffer (8), followed by phosphate-sodium chloride buffer, pH 7.7,

[1] Frank Pearson Associates, New York 12, N. Y.

TABLE I

Electrophoretic mobilities of albumin in rat sera
Average Values

Condition of rats	No. of determinations	Total protein in gm. %	−u Observed in 10^{-5} cm.^{2} sec.$^{-1}$ volt^{-1}	−u Corrected for 1.58% protein in electr. cell	−u Corrected for 1.76% protein in electr. cell
Normal	11	6.64	5.78	5.76	5.67
S.E. ±				0.032	0.033
F.L. ±				0.07*	0.06†
CCl_4 Treated	10	6.04	5.95	5.97	—
S.E. ±				0.057	
F.L. ±				0.13*	
Tourniquet Shock	5	5.90	5.78	—	5.88
S.E. ±					0.074
F.L. ±					0.16†

* Fiducial limits for probability level 0.95.
† Fiducial limits for probability level 0.90.

to complete equilibration. The dialysis times ranged between one and 3 days at pH values up to 9.5; at pH 11.5, the sera were dialyzed for 5½ hrs.

The various dialysates of the sera were acidified and extracted several times with petroleum ether. The dry residues of the petroleum-ether phase were combined with human serum albumin as described before (1). For the direct extractions of normal and low-ABC sera, alcohol-ether mixtures (3:1) were used at normal and elevated temperatures; the lipid material was also combined with albumin.

RESULTS AND DISCUSSION

The results of the electrophoretic analysis are given in Table I.[2] All essential requirements for accurate determinations of mobility, as outlined by Alberty (10), have been met in the present study. A correction was applied to the mobility values in order to account for the somewhat lower serum protein concentration observed in the injured rats (3, and Table I). The mobility value of a protein is influenced by the concentration of all proteins present, mainly through changes in viscosity. However, since mere correction for viscosity results in an overcorrection (10), the adjustment was made on the basis of experimental findings correlating the electrophoretic mobility with protein concentration. An interpolation of the data that Lippman and Banovitz (11) obtained for albumin in rat serum gave a dependency of the mobility (u) on the protein concentration (c), expressed in gm. per cent, $\Delta u/\Delta c = -0.46 \times 10^{-5}$. The same quotient was found in the present studies for albumin in rat serum (phosphate buffer, pH 7.7, $\mu = 0.1$) and was adopted for the calculations.

[2] Unabridged tables and a more detailed discussion have been given elsewhere (9).

TABLE II

Influence of pH on electrophoretic mobility of albumin in normal rat serum

Total protein concentration in serum: 6.33%
Total protein concentration in electrophoretic cell: 2.11%
Phosphate buffer, $\mu = 0.1$. Descending boundaries.

pH	−u in 10^{-5} cm.^{2} sec.$^{-1}$ volt^{-1}	$-\Delta u/\Delta pH \times 10^5$	$-\Delta u/\Delta pH \times 10^{5*}$
6.10	4.39		
7.68	5.58	0.75	0.75
8.48	6.00	0.53	0.50

* Values obtained by graphic interpolation of data given by Miller, Miller, and Eitelman (Figure 4 in [12]) for albumin in human plasma.

TABLE III

Influence of dialysis of albumin-fatty acid mixtures on ABC

Fatty acid	Dialysis pH	Dialysis Hours	Dialysis Changes of buffer	Decrease of ABC %	Corresponding to moles of fatty acid per mole albumin
	Before dialysis			44	2.3
Lauric	7.7	22	6	44	2.3
	11.5	5	5	32	1.5
	12.0	7	7	20	0.9
	Before dialysis			53	23
Caproic	7.7	22	6	10	1
	11.5	5	5	8	1

The corrections were made to the medium protein concentrations of the groups of sera to be compared with each other. In this way the extent of corrections was reduced to a minimum (for further details see [9]).

Since no information seemed to be available for the influence of pH on electrophoretic mobility of albumin in rat serum, these data were determined at three pH values. Table II shows that the quotients $\Delta u/\Delta pH$ obtained were almost identical with the values calculated by interpolation from results of Miller, Miller, and Eitelman (12) for albumin in human plasma. An application of the data of Table II to the mobility values of Table I showed that the hydrogen ion concentrations were sufficiently close to make corrections unnecessary (for the magnitude of such corrections see [9]).

In order to test the significance of differences between mobility values found in different sera, the reproducibility of the individual observations was checked. Duplicate determinations were made of the anodic mobilities of albumin in four (diluted) sera from normal rats and from rats treated with CCl_4. The results of the eight duplicate analyses showed that the deviations of the two duplicate observations from their medium values ranged from 0 to 0.8 per cent, the average deviation being 0.44 per cent, S.E. $= \pm 0.078$ per cent (F.L. 0.01 $= \pm 0.23$ per cent). This means that the reliability of the mobility values observed (Table I) is such that at a 0.99 probability level the statistical deviation from the "true" values is not greater than 0.025×10^{-5} cm.^{2} sec.$^{-1}$ volt^{-1}.

The data of Table I indicate that the albumin in the serum of CCl_4-treated rats had a greater anodic electrophoretic mobility than the albumin in normal serum. The difference averaged 0.21×10^{-5} cm.^{2} sec.$^{-1}$ volt^{-1} and was found to be statistically significant at a probability level of 0.95. The albumin mobility in the serum of rats subjected to tourniquet shock was also found to be greater than normal. The anodic mobility of the albumin in the sera of acidotic and x-irradiated rats (13) did not differ from the normal values (for details see [9]).

These results demonstrate that statistically significant elevations of the anodic mobility of albumin were found in all those sera that showed significant decreases in the ABC (sera from rats treated with carbon-tetrachloride or subjected to tourniquet shock), whereas normal albumin mobilities were observed in the sera with normal ABC (sera from acidotic and x-irradiated rats).

The difference between the anodic mobilities of albumin in the sera of normal and CCl_4-treated rats was found to be about 0.2×10^{-5} cm.^{2} sec.$^{-1}$ volt^{-1} (Table I). Since no data seemed to be available for the relation between electrophoretic mobility and net charge for rat serum albumin, the findings of Longsworth and Jacobsen (14) on the influence of net charge (z) on the mobility (u) of bovine serum albumin were utilized. The relationship was found to be a linear one, the slope of the curve being $\Delta u/\Delta z = 0.2 \times 10^{-5}$ (Figure 4 in [14]). On the assumption that the ratio $\Delta u/\Delta z$ for rat serum albumin is similar to that for bovine albumin it is concluded that the average albumin molecule in the serum of CCl_4-treated rats differs from that of normal rats by carrying about one more negative charge.

The possession of approximately one more negative charge on the albumin molecule of low-ABC sera is in agreement with the results obtained previously (1) on the combination of albumin with anions. After *in vitro* addition of the order of one to two moles of higher fatty acids per mole human serum albumin or albumin in rat serum, the ABC decreased by approximately the same degree as observed in CCl_4-treated and tourniquet-shock rats (3). On the basis of the extent of binding of various fatty acids by serum albumin (15) it is probable that the combination of the small amounts of fatty acids added to albumin to produce this decrease of the ABC (1) is essentially complete. The observation (Table I) of a higher negative net charge in the albumin of sera with lower ABC, therefore, corroborates the previous suggestion that a decrease in the ABC values is caused by a firm binding *in vivo* of increased amounts of certain anions, *e.g.*, fatty acids, to the albumin molecules.[8] The numerical value

[8] It should be mentioned that the serum from rats treated with CCl_4 was found to contain more non-esterified higher fatty acids firmly bound to albumin (1.30 moles, S.E. $= \pm 0.06$, per mole albumin, calculated as monocarboxylic acid) than that from normal rats (0.85 mole, S.E. $= \pm 0.04$). These analytical studies will be published elsewhere (see also G. L. Selden and U. F. Westphal, Concentration of non-esterified higher fatty acids in serum of carbon tetrachloride-treated and normal rats, and in rabbit and bovine serum. Army Medical Re-

of the difference in the net charge is in agreement with the assumption that one additional anion is firmly attached to the albumin molecule in the low-ABC sera. No greater accuracy, however, should be ascribed to this value than that of an order of magnitude.

The possibility was investigated that the amount of anions, *e.g.*, higher fatty acids, bound to albumin in the original serum would be altered by the dialysis. Table III shows that the decrease of ABC observed after dialysis of a human albumin-lauric acid mixture at pH 7.7 was the same as before, indicating that no change in the amount of bound lauric acid (1) took place. It is concluded that the amounts of anions firmly bound to albumin, *e.g.*, higher fatty acids, in the dialyzed sera used in the electrophoretic analysis (Table I) are the same as in the original sera. In accordance with the general experience that the firmness of binding between albumin and fatty acids decreases when their chain lengths become shorter (16), a substantial amount of caproic acid was found to be removed from albumin under the same conditions of dialysis (Table III).

The conclusions drawn from the dialysis experiments on albumin-lauric acid mixtures at pH 7.7, were confirmed by studies on sera. Normal sera and sera of low ABC were dialyzed at neutral pH in an attempt to reduce or eliminate the difference between the ABC values of the two types of sera. The difference remained unchanged. Equally unsuccessful was the endeavor to isolate material from normal and low-ABC sera at neutral pH by dialysis or extraction with organic solvents, to combine the material from the two types of sera with pure human albumin and thus demonstrate different influences on the ABC. In accordance with the dialysis experiments (Table III), these conditions appear to be too mild to affect the firm combinations of albumin and anions that are believed to influence the ABC of albumin in sera.

Dialysis of sera of normal and "tourniquet-shock rats" at pH 9.5 reduced the difference between the ABC values from an average of 27 per cent (3) to 7 to 8 per cent. Dialysis at pH 11.5 completely abolished the difference in the ABC of albumin between normal and "tourniquet-shock" sera. A corresponding result was obtained with the mixture of albumin and lauric acid: dialysis at pH 11.5 and 12.0 reduced the decrease of the ABC value from 44 per cent to 32 and 20 per cent, respectively (Table III). The explanation for these results is believed to be a removal of firmly bound anions from albumin by dialysis at the higher pH values. This interpretation is in accordance with the studies of Klotz, Walker, and Urquhart (17, 18) on the influence of pH on the binding of azosulfathiazole and methyl orange to bovine serum albumin.

It was observed in the present studies that the gradual disappearance of the differences between the ABC of normal and low-ABC solutions by dialysis at increased pH was accompanied by a general decline of the absolute ABC values to about three-fourths or two-thirds of the original. This general reduction is considered to be indicative of a partial denaturation of the albumin (19). However, under the conditions employed, the ABC was not completely destroyed; *e.g.*, dialysis at pH 12 for 5 hrs. reduced the ABC value of a normal human albumin solution to 73 per cent of the original. Subsequent addition of 2.3 moles of lauric acid per mole albumin caused a further decrease of the reduced ABC value by 47 per cent which is close to the 44 per cent observed in untreated albumin (Table III). In the light of these model studies it is concluded that the disappearance of the differences between the ABC of albumin in normal and low-ABC sera results from the removal of firmly bound anions, *e.g.*, higher fatty acids, by dialysis at the higher pH.

The results obtained on the electrophoretic mobilities of albumin in the sera of normal and injured rats have a direct bearing on the specific problem of the ABC. Besides, however, they appear to be of interest in view of the great number of data that were obtained in many laboratories on the electrophoretic mobility of albumin in normal and pathological human sera. A systematic analysis of such albumin mobility values, determined under strictly identical conditions, may assist in detecting abnormalities in the amounts of anions, *e.g.*, higher fatty acids, firmly bound to

search Laboratory Report No. 182, 10 March 1955 and also see G. L. Selden and U. Westphal, Non-esterified higher fatty acids in serum of CCl_4-treated and normal rats and other species. Proc. Soc. Exper. Biol. & Med., 1955, **89**, 159).

albumin and thus contribute to an elucidation of biochemical changes that occur in human blood under certain pathological conditions.

SUMMARY

1. The electrophoretic mobility of albumin in the serum of rats subjected to carbon-tetrachloride poisoning or tourniquet shock was found to be higher than that of the albumin in normal rat serum. The difference corresponded to an increment of approximately one negative charge per albumin molecule.

2. Elevated anodic mobilities were observed only for serum albumin of decreased azorubin-binding capacity (ABC), indicating the firm binding of an increased amount of anions to the albumin in sera of low ABC. The numerical value of the difference in net charge corresponded to a quantity of additionally bound anions that would decrease the ABC, according to model studies, to a similar degree as found for low-ABC albumin.

3. The difference between the ABC values of normal and low-ABC albumin or serum was reduced or eliminated by dialysis at pH 11.5.

REFERENCES

1. Westphal, U., Stets, J. F., and Priest, S. G., Influence of fatty acids and related anions on the azorubin-binding capacity of serum albumin. Arch. Biochem., 1953, **43**, 463.
2. Westphal, U., and Gedigk, P., Azorubin-binding capacity of normal and pathological sera. Proc. Soc. Exper. Biol. & Med., 1951, **76**, 838.
3. Westphal, U., DeArmond, R., Priest, S. G., and Stets, J. F., Azorubin-binding capacity and protein composition of. serum of rats subjected to tourniquet shock and to treatment with carbon tetrachloride. J. Clin. Invest., 1952, **31**, 1064.
4. Westphal, U., Priest, S. G., and Stets, J. F., Protein composition and azorubin-binding capacity of serum of rabbits subjected to tourniquet shock. Am. J. Physiol., 1953, **173**, 305.
5. Reiner, M., and Fenichel, R. L., Dialysis of protein solutions for electrophoresis. Science, 1948, **108**, 164.
6. Longsworth, L. G., and MacInnes, D. A., The interpretation of simple electrophoretic patterns. J. Am. Chem. Soc., 1940, **62**, 705.
7. Instruction Manual for Portable Tiselius Electrophoresis Apparatus, Model 38. P. 14. Norwalk, Connecticut, The Perkin-Elmer Corporation, 1951.
8. Miller, G. L., and Golder, R. H., Buffers of pH 2 to 12 for use in electrophoresis. Arch. Biochem., 1950, **29**, 420.
9. Westphal, U. F., and Priest, S. G., Electrophoretic mobility versus azorubin-binding capacity of albumin in the serum of normal and injured rats. Army Medical Research Laboratory Report No. 164, 20 January 1955. This report may be obtained on request.
10. Alberty, R. A., Electrochemical properties of the proteins and amino acids *in* Neurath, H., and Bailey, K., The Proteins. New York, Academic Press, Inc., 1953, Vol. 1, Part A, p. 461.
11. Lippman, R. W., and Banovitz, J., Influence of protein concentration upon electrophoretic mobility of serum proteins. J. Biol. Chem., 1952, **199**, 451.
12. Miller, G. L., Miller, E. E., and Eitelman, E. S., The pH-mobility relationships of components of human plasma. Arch. Biochem., 1950, **29**, 413.
13. Westphal, U., Priest, S. G., Stets, J. F., and Selden, G. L., Influence of whole body x-irradiation, cold exposure and experimental acidosis on protein composition and azorubin-binding capacity of rat serum. Am. J. Physiol., 1953, **175**, 424.
14. Longsworth, L. G., and Jacobsen, C. F., An electrophoretic study of the binding of salt ions by β-lactoglobulin and bovine serum albumin. J. Phys. & Coll. Chem., 1949, **53**, 126.
15. Boyer, P. D., Ballou, G. A., and Luck, J. M., The combination of fatty acids and related compounds with serum albumin. III. The nature and extent of the combination. J. Biol. Chem., 1947, **167**, 407.
16. Ballou, G. A., Boyer, P. D., and Luck, J. M., The electrophoretic mobility of human serum albumin as affected by lower fatty acid salts. J. Biol. Chem., 1945, **159**, 111.
17. Klotz, I. M., and Walker, F. M., The binding of organic ions by proteins. Charge and pH effects. J. Am. Chem. Soc., 1947, **69**, 1609.
18. Klotz, I. M., and Urquhart, J. M., The binding of organic ions by proteins. Buffer effects. J. Phys. & Coll. Chem., 1949, **53**, 100.
19. Klotz, I. M., Triwush, H., and Walker, F. M., The binding of organic ions by proteins. Competition phenomena and denaturation effects. J. Am. Chem. Soc., 1948, **70**, 2935.