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UNEXPECTED DIFFERENCE BETWEEN HUMAN SERUM ALBUMIN AND HUMAN SERUM TOWARD L-TRYPTOPHAN BINDING

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Abstract—The binding of L-tryptophan to human serum albumin (HSA) Fr. V in Krebs–Ringer phosphate buffer and to HSA in serum was studied by equilibrium dialysis. At constant albumin concentration (in the *in vivo* range) and various L-tryptophan concentrations, binding was greater in serum, with an apparent association constant (k) equal to 4.88×10^4 l/mole and the number of binding sites (n) equal to 0.62. The corresponding kinetic parameters obtained at constant HSA Fr. V concentration and various L-tryptophan concentrations in Krebs–Ringer phosphate buffer were 7.61×10^3 l/mole and 0.28, respectively. The Scatchard plot obtained from experiments with various albumin concentrations and a constant L-tryptophan concentration had a negative slope in serum and a positive slope in Krebs–Ringer phosphate buffer.

Rheumatoid arthritis is a disease associated with marked alterations in serum protein levels. A decreased content of albumin and an increased glycoprotein content of both alpha- and beta-globulin fractions were noted in both human [1–3] and rat sera [4–6]. An increased level of bound and total L-tryptophan was also reported in arthritic human serum [7, 8], and this alteration has been considered an important index of the course of rheumatoid arthritis and its response to anti-rheumatoid drug therapy [9], although a similar change has not been observed in serum of adjuvant rat.* Since McMenamy has already established that L-tryptophan binds specifically to albumin in human serum [10], the simultaneous occurrence of increased total and bound L-tryptophan levels and the decreased level of albumin is surprising and may reflect a qualitative change in this protein which alters its binding properties. One possible explanation for this is that a decrease in molecular aggregation [11] may occur at reduced albumin concentration, thereby enhancing the albumin binding capacity. The association constant (k) and the number of bindings sites (n) for some acidic and basic drugs were found to be dependent upon albumin concentration [12–15]. Moreover, Bowmer and Lindup [16, 17] reported that the binding capacity of bovine serum albumin (BSA) toward L-tryptophan increases upon albumin dilution. The investigation of the effect of dilution of albumin upon L-tryptophan binding using human serum and human serum albumin (HSA) over ranges related to the *in vivo* situation constitutes the basis of this report.

MATERIALS AND METHODS

Materials. Human serum obtained from healthy volunteers was pooled and used without freezing. HSA Fr. V lot No. 47C-0442-2, α-globulin (bovine) Fr. IV lot No. 97C-0345, β-globulin (bovine) Fr. III lot No. 26C-01971, γ-globulin (bovine) Fr. II lot No. 78C-0398, and unlabeled L-tryptophan were obtained from the Sigma Chemical Co., St. Louis, MO. Omnifluor and [^{14}C]-L-tryptophan, with a specific activity of 54.7 Ci/mole, were obtained from the New England Nuclear Corp., Boston, MA, and Soluene was obtained from the Packard Chemical Co., Downers Grove, IL. The stainless steel dialysis block was made according to the design of M. Kraml and W. T. Robinson from Ayerst Research Laboratories, Montréal, Canada. The dialyzing membranes were Type C, part No. 105-1058 POIA. These were obtained from Technicon Instruments Corp., Tarrytown, NY.

Determinations. The determination of total L-tryptophan level, based on norharman formation, and the determination of free L-tryptophan level, using equilibrium dialysis, were performed according to Wood *et al.* [18]. The albumin concentration was determined with bromocresol green according to the method of Lehane *et al.* [19].

Binding studies. For the binding studies which involved a constant L-tryptophan concentration and varying concentrations of albumin, the serum or HSA (4%, w/v) solution in Krebs–Ringer phosphate buffer, pH 7.4 [20], was diluted with the same buffer, to which L-tryptophan was added to give a concentration of 12.4 μg/ml. For the alternate study which involved a constant albumin concentration and varying concentrations of L-tryptophan, 0.2 ml of solutions containing various concentrations of L-tryptophan in Krebs–Ringer buffer, or the buffer alone, were added to 5 ml of serum on HSA (4%, w/v). The pH of these solutions was measured on a Radiometer pHM61 laboratory pH meter. One set of HSA samples with constant albumin concentration and another set of samples with varying albumin concentration had their pH adjusted to 7.4, with small volumes of 0.1 N NaOH. The free L-trypto-

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phan concentration was determined by equilibrium dialysis, using a multiple cells block. One milliliter of serum or HSA solution was dialyzed against an equal volume of buffer containing 93 pmoles [^{14}C]-L-tryptophan. The dialysis was terminated after 16 hr of incubation in a metabolic shaker at 22°. Samples (0.1 ml) were withdrawn from both protein and buffer compartments, placed in scintillation vials containing 0.5 ml Soluene and 15 ml of a 4% (v/v) Omnifluor solution in toluene (scintillation grade), and counted in a Beckman LS 8000 liquid scintillation counter, with an efficiency for ^{14}C of 90 per cent. When the possible effect of adding globulins to 4% HSA solution was inspected, 1% of α-, β-, or γ-globulin (bovine) was added either separately or together to 4% HSA Fr. V in Krebs–Ringer phosphate buffer solution using a single concentration of L-tryptophan of 12 μg/ml. All experiments were performed in triplicate or quadruplicate.

Calculations. The results were analyzed by means of the Scatchard equation for the law of mass action, $r/d = nk - rk$, where r is the molar ratio of bound L-tryptophan to albumin, d is the molar concentration of free L-tryptophan at equilibrium, k is the association constant and n the number of binding sites [21]. The molecular weight of albumin was taken as 66,248 [22]. The slope, the intercept and the correlation coefficient were calculated by linear regression analysis since the resulting data were well fitted with a straight line.

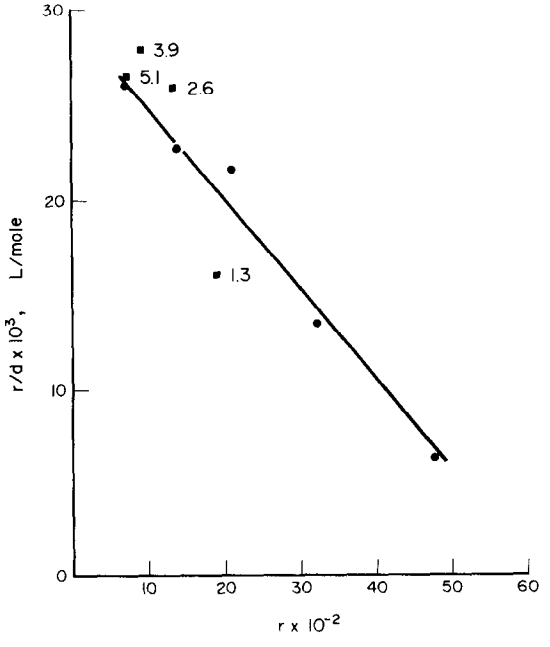

Fig. 1. Scatchard plot for the binding of L-tryptophan to human serum at 22°. Key: (■) L-tryptophan (29.1–247.8 μM) binding to 4.9% (w/v) albumin; (●) L-tryptophan (30.4 μM) binding to a range of albumin concentrations; the numerals are the albumin concentrations (%, w/v) at the points indicated. Each point is the mean of four experiments.

RESULTS AND DISCUSSION

The total L-tryptophan level in the pooled human serum was found to be 12.4 μg/ml. The albumin content was 5.1 g/100 ml. These values are within the normal ranges [7, 22]. Two studies were done with the human serum. In the first study, the albumin concentration was varied and the L-tryptophan concentration was kept constant. In the second study, the albumin concentration was kept constant and the L-tryptophan concentration was varied. The Scatchard plots of the data are shown in Fig. 1. Both slopes are negative and the data points are overlapping. The association constant, k, calculated by linear regression analysis for the experiment at constant albumin concentration, is equal to 4.88×10^4 l/mole, and the number of binding sites, n, is equal to 0.62 (correlation coefficient = 0.979). Three studies were done with HSA solutions in Krebs–Ringer phosphate buffer solution. The first study was done with variable albumin concentration and constant L-tryptophan concentration. It had already been shown that the L-tryptophan binding capacity of albumin increases with increasing pH [10, 23]. These observations prompted us to check the pH of our solutions.

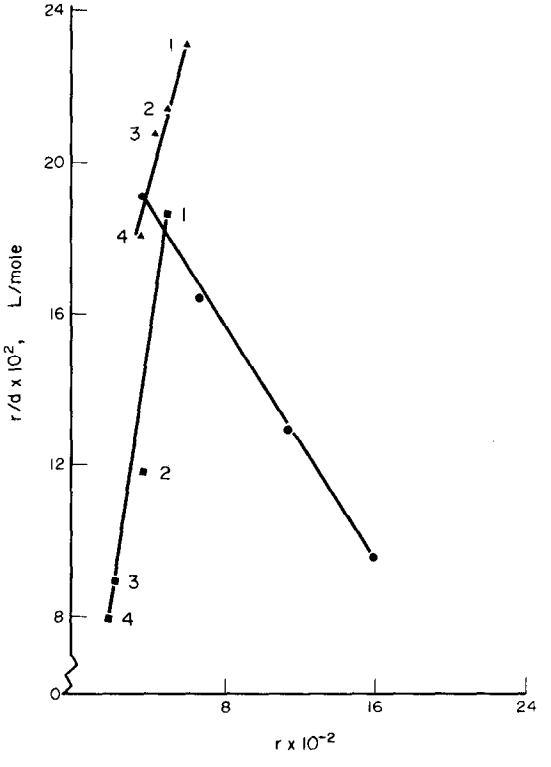

Fig. 2. Scatchard plot for the binding of L-tryptophan to HSA. Key: (●) L-tryptophan (29.1–214.3 μM) binding to 4% (w/v) HSA at pH 7.4 and 22°; (■) L-tryptophan (30.4 μM) binding to a range of concentrations (%, w/v) at the points indicated, pH equals 6.29 at 4%, 6.48 at 3%, 6.71 at 2% and 7.18 at 1% (w/v) HSA concentration; the numerals are the HSA concentrations (%, w/v) at the points indicated; (▲) L-tryptophan (30.4 μM) binding to a range of concentrations of HSA at pH 7.4. Each point is the mean of three experiments.

Significant changes were found with the HSA solution, going from pH 6.29 for the 4% solution to pH 7.18 for the 1% solution. Since these changes could induce some variation in the binding capacity of albumin, two more studies were done, one using constant albumin concentration and the other using variable albumin concentration with controlled pH at 7.4. The results of these studies are shown in Fig. 2. The Scatchard plot which was obtained from the experiment using a constant HSA concentration with varying concentrations of L-tryptophan at pH 7.4 has a negative slope from which the association constant can be determined to be equal to 7.61×10^3 l/mole and n, the number of sites, equal to 0.28 (correlation coefficient = 0.976). In the two experiments with varying albumin concentrations, the slopes are positive, and nearly identical. However, the absolute value of the data points shows, as expected, an improved binding capacity of the albumin at higher pH. The positive slopes are to be related to the similar results of Bowmer and Lindup [16] using varying concentrations of BSA in phosphate buffer, with a constant concentration of L-tryptophan. These authors suggested that the decreased binding capacity which occurs at a higher concentration is due to increased protein–protein interactions which mask the binding site. In human serum, no such phenomena occurs; both slopes are negative and the data points overlap. This contrasting behavior suggests that, in serum, no aggregation or polymerization of albumin does occur. The serum may contain some molecules (protein or endogenic ligand) which prevent the aggregation of albumin in the range of concentrations studied and leave the binding site available. The higher association constant and the larger number of binding sites support this hypothesis. The per cent L-tryptophan bound versus the albumin concentration is shown in Fig. 3. The per cent binding is much higher with human serum, and its decrease with decreasing albumin concentration is very slow at the *in vivo* concentration. From these results it appears that the reduced albumin concentration is not responsible for the increased L-tryptophan binding capacity depicted in arthritic human serum [7, 8]. The possibility that the increased globulin fraction is responsible for this increased binding capacity is very small, since McMenamy found that the L-tryptophan did not bind appreciably to any plasma fraction at pH 7.4 unless it contained albumin. Moreover, the addition of 1% of α-, β-, or γ-globulin (bovine), added either separately or together to 4% HSA Fr. V. in Krebs–Ringer phosphate buffer, did not change significantly the amount of bound L-tryptophan. The increased binding capacity could be due to a change in albumin conformation induced by a change in some ligand concentration. This is the hypothesis that is now under investigation in our laboratory.

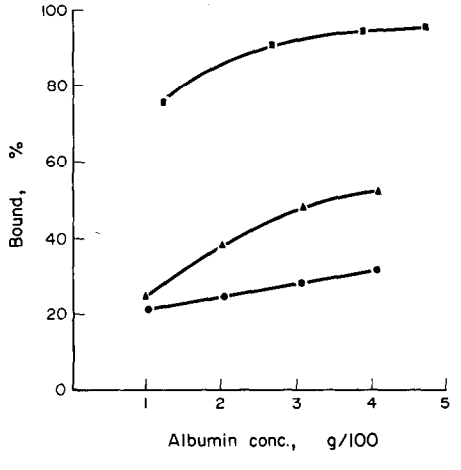

Fig. 3. Effect of the albumin concentration on the % binding of L-tryptophan (30.4 μM) . Key: (■) human serum; (●) HSA at pH 6.29 to 7.18; and (▲) HSA at pH 7.4. Each point is the mean of three or four experiments.

In conclusion, the binding of L-tryptophan to HSA may vary considerably depending on whether the binding occurs in serum or in Krebs–Ringer phosphate buffer. At a constant albumin concentration (in the *in vivo* range), and various L-tryptophan concentrations, binding is greater in serum, the constant of association being about six times larger, and the number of binding sites twice as large. Moreover, the Scatchard plot obtained from experiments with various albumin concentrations and a constant L-tryptophan concentration has a negative slope in serum and a positive slope in Krebs–Ringer phosphate buffer. These results imply that the albumin in serum is not aggregated. since dilution did not improve its binding capacity. Consequently, the reduced albumin concentration found in the sera of rheumatoid-arthritic patients may not be responsible for the observed increased binding of L-tryptophan.

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REFERENCES

1. P. Wilkinson, R. Jeremy, F. P. Brook and J. L. Hollander, *Ann. intern. Med.* **63**, 109 (1965).
2. R. W. Payne, *Ann. N. Y. Acad. Sci.* **94**, 284 (1961).
3. B. Decker, W. F. McGuckin, B. F. McKenzie and C. H. Slocumb, *Arthritis Rheum.* **3**, 49 (1960).
4. R. Hirschelmann, H. Bekemeir and A. Stelzner, *Z. Rheumatol.* **36**, 305 (1977).
5. H. E. Weimer, F. D. Wood and C. M. Pearson, *Can. J. Biochem.* **46**, 743 (1968).
6. J. S. Lowe, *Biochem. Pharmac.* **13**, 633 (1964).
7. M. Aylward and J. Maddock, *Rheumatol. Rehabil.* **13**, 62 (1974).
8. M. Aylward and J. Maddock, *J. Pharm. Pharmac.* **25**, 570 (1973).
9. M. Aylward, J. Maddock, R. Wheeldon and R. J. Parker, *Rheumatol. Rehabil.* **14**, 101 (1975).
10. R. H. McMenamy and J. L. Oncley, *J. biol. chem.* **233**, 1436 (1958).
11. D. C. Teller, *Nature, Lond.* **260**, 729 (1976).
12. R. Zini, P. d'Athis, A. Hoareau and J. P. Tillement, *Eur. J. clin. Pharmac.* **10**, 139 (1976).
13. D. Shen and M. Gibaldi, *J. pharm. Sci.* **63**, 1698 (1974).
14. J. P. Paubel and P. Niviere, *Chim. Ther.* **4**, 469 (1973).
15. J. P. Paubel and P. Niviere, *Eur. J. med. Chem.* **9**, 508 (1974).
16. C. J. Bowmer and W. L. Lindup, *Biochem. Pharmac.* **27**, 937 (1978).

17. C. J. Bowmer and W. L. Lindup, *J. pharm. Sci.* **67**, 1193 (1978).
18. K. Wood, C. Swade, J. Hardwood, E. Eccleston, M. Bishop and A. Coppen, *Clinica chim. Acta* **80**, 299 (1977).
19. D. P. Lehane, A. L. Levy and A. Erglis, *Ann. clin. Lab. Sci.* **8**, 122 (1978).
20. R. M. C. Dawson, D. C. Elliott, W. H. Elliott and K. M. Jones (Eds.), *Data for Biochemical Research*, p. 507. Clarendon Press, Oxford (1972).
21. G. Scatchard, *Ann. N. Y. Acad. Sci.* **51**, 660 (1949).
22. T. Peters, Jr., in *The Plasma Proteins* (Ed. F. W. Putnam), Vol. 1, p. 133. Academic Press, New York (1975).
23. J. N. McArthur, *Life Sci.* **18**, 947 (1976).