

EFFECT OF FREE FATTY ACIDS ON BINDING OF ANTI-INFLAMMATORY DRUGS BY BOVINE AND HUMAN SERUM ALBUMIN

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ABSTRACT

The effect of long chain fatty acids on the binding of anti-inflammatory drugs to bovine (BSA) and human serum albumin (HSA) was investigated with the hope that the results obtained would provide some informations about the forces involved in the binding process and the topography of the binding sites on the albumin molecule. The interaction between albumin molecule and oleic and palmitic acids has been investigated at pH 7.4 using Sorensen's phosphate buffer at 25°C by observing the competition, in binding to proteins, between each acid and either tiaprofenic acid or tenoxicam. By the equilibrium dialysis technique, the capacity of these albumin preparations to bind each tested drug was measured. Analysis of scatchard plots showed that the two drugs occupied two classes of binding sites in both BSA and HSA. The binding of the two drugs to BSA or HSA was inhibited by palmitate or oleate at a molar ratio of either 3.5 or 7. The inhibition affected either the number of binding sites or the association constant for the interaction of the site with the tested drug or both. Binding to either tiaprofenic acid or tenoxicam was markedly reduced, with a significant reduction in number of class 1 sites available. The inhibitory effect of oleate at a molar ratio of 7 on the binding properties of BSA for tiaprofenic acid and tenoxicam was nearly similar to that of 7 mol of palmitate. The binding of the two drugs to HSA containing 7, 3.5 mol of FFA or to HSA free from FFA was less than that observed upon using BSA preparations. These experiments suggest that FFA have a general inhibitory effect on the capacity of albumin to bind tiaprofenic acid or tenoxicam.

INTRODUCTION

Plasma free fatty acids and numerous drugs are transported in blood and primarily bound to plasma albumin^(1,2). The affinity of free fatty acids (FFA) for albumin is greater than that of most drugs. Thus, palmitate and oleate, the major FFA of mammalian plasma, occupy two classes of binding site on the human albumin molecule, which can be distinguished on the basis of the number of binding sites in each class (n) and the association constant of these sites for the fatty acid⁽³⁾. It was observed that an acute increase in plasma FFA of the rabbit was associated with a decrease in the plasma concentration of protein-bound thyroxine and an increase in the concentration of free thyroxine⁽⁴⁾. Supporting this conclusion was the observation of Tabachnik⁽⁵⁾ that addition of 1 or 3 moles of palmitate to defatted albumin reduced the protein's capacity to bind thyroxine *in vitro* and the finding of Hollander et al.⁽⁶⁾ that acute elevation of plasma FFA levels in man was with a 2- to 9-fold increase in percentage of free thyroxine in plasma. On the other hand, Braverman et al.⁽⁷⁾ and co-workers found little or no effect of variations in plasma FFA level upon the percentage of plasma free thyroxine, and Schatz et al.⁽⁸⁾, attributed the rise in plasma free thyroxine after heparin to an effect of the anticoagulant rather than FFA. Solomon et al.⁽⁹⁾ reported that the capacity of human serum albumin to bind phenylbutazone or warfarin was reduced in the presence of lauric, myristic or stearic acid.

Several studies pointed that the binding of hydroxyphenylazo-benzoate was sensitive to relatively small changes in FFA concentration⁽¹⁰⁾. This was due to FFA - induced weakening of drug binding to

albumin. Also, anilino-naphthalene sulfonate binding to albumin was altered by relatively small changes in FFA concentration⁽¹¹⁾. It was observed that octanoate binding to albumin was decreased by addition of FFA⁽¹²⁾. Some authors have reported that FFA reduced the ability of albumin to bind azorubin⁽¹³⁾, tryptophan⁽¹⁴⁾, skatole⁽¹⁵⁾, thyroxine⁽¹⁶⁾, triiodothyronine⁽¹⁷⁾, trinitrobenzenesulfonate⁽¹⁸⁾ and zomepirac⁽¹⁹⁾. It was reported that nonesterified fatty acid concentrations did not contribute to the pregnancy-associated decrease in theophylline binding⁽²⁰⁾, whereas Shum and Jusko⁽²¹⁾ concluded that obesity causes a moderate decrease in serum binding of theophylline which may be attributed to increase free fatty acids. Several authors⁽²²⁾ reported that the variations in free fraction of lidocaine and quinidine were strongly associated with variations in free fatty acids, but for propranolol, no significant correlation was observed. Also, several authors have reported that FFA reduced the ability of albumin to bind diazepam⁽²³⁾ in diabetes mellitus, warfarin and indomethacin in rheumatoid arthritis⁽²⁴⁾, and zomepirac⁽¹⁹⁾ in uremia. The effect of age on the *in vitro* binding of valproic acid to serum proteins was investigated in rats ranging in age from 14 days to 24 months; The influence of free fatty acids and total protein concentration on age-related change in binding was examined by Slattum et al⁽²⁵⁾. They found that changes in protein binding may contribute to age related changes in disposition of valproic acid in rats. These findings led us to suspect that physiological changes in FFA concentration might influence the binding of certain drugs to albumin. Thus, the questions have

through 1.5 ml of buffer of pH 7.4. The plasma albumin concentration was determined by a colorimetric method using a spectrophotometer at 660 nm. The results are expressed as the mean \pm S.E.M. of three separate experiments.

The effect of the addition of albumin to the drug solution was studied. The capacity of human and bovine serum albumin to bind the two drugs under consideration is assessed by varying concentrations of drug (1.5×10^{-5} M) and albumin (from 0.1 to 1.0 M) in a constant volume of 1.5 ml. The binding capacity was measured from equilibrium dialysis in terms of free drug. The results are expressed as the mean \pm S.E.M. of three separate experiments. The association constant (K) for the interaction between each drug and albumin was calculated.

EXPERIMENTAL

Materials and Equipment

1. Bovine and human serum albumin (crystallized fraction V, molecular weight 66000) (Sigma Chemical Co., Louis, MO 63178 U.S.A.)
2. Cellulose dialysis sheets (Dipon Dialyzer, St. Louis, MO 63178 U.S.A.)
3. Thermometrically controlled shaking water bath (Chalovon, Labortechnik, D-3162, Westphalen/W, Germany)
4. UV spectrophotometer (UV 1201 Shimadzu Co., Japan)
5. Tetracaine (kindly supplied by Egyptian Pharmaceutical Industries Co. (E.P.I.C.) A.S.E.)
6. Tropicamide acid (kindly supplied by Hoechst Chem. S.A.E., Cairo, Egypt)
7. Citric acid and palmitic acid (El-Nar Pharmaceutical Chemicals (Advis), Egypt)

Methods

Binding of the two selected drugs to bovine and human serum albumin in presence of free fatty acids.

Cellulose dialysis sheets were boiled in deionized double distilled water before use, in order to eliminate the release of UV light absorbing material from the membrane during equilibrium dialysis experiments. Each internal cell (two sides opened by a pair of tubes) was filled with 5 ml of Hensen's phosphate buffer, pH 7.4, containing 5 mg of BSA (1.5×10^{-5} M). Outside the internal cell, it was placed 25 ml of the same buffer containing appropriate concentration of each drug (from 1 to 20×10^{-5} M). To determine that the dialysis membrane was freely permeable to each tested drug under study and to measure the quantity of drug which might be bound by

the membrane, albumin free solution from the internal cell is then evaporated. The equilibrium dialysis was carried out in two cells separated by cellulose dialysis sheet 5 ml of albumin solution (1.5×10^{-5} M) was placed in the protein of the tested free fatty acid, which is pipetted into the inner compartment and 25 ml of drug solution was placed into the outer cell. The inner cell was immersed into the same one to reach a balance for the surface levels of the two solutions on the same. The upper opening of the inner cell was covered with a fine perforated albumin free sheet to keep the volume of the inner solution constant during the equilibrium dialysis experiments (2).

The equilibrium dialysis units were dialyzed in a thermometrically controlled shaking water bath previously adjusted at $25 \pm 0.2^\circ\text{C}$ and the dialysis was done for 18 hours (equilibrium time). Each experiment was carried out at a fixed concentration of BSA (1.5×10^{-5} M) and different concentrations of each drug. At the end of 18 hours, the outer solution was spectrophotometrically analyzed and measured at 210 and 270 nm for tetracaine acid and tropicamide respectively. The tested free fatty acids were used at a ratio of 1:1 and 1:2 molar of BSA per molar of albumin.

Preliminary experiments showed that the two drugs under study passed freely through the cellulose dialysis membrane without significant binding, and that in the absence of albumin equal concentrations were established at 18 hours on both sides of the membrane. Palmitic acid and citric acid do not pass across the membrane. BSA concentrations in the outer solution were therefore zero at the end of equilibration. The concentration of free drug outside the dialysis sheet (DF) which equals that inside was determined spectrophotometrically. The number of moles of free drug, calculated from the volume of the system, was subtracted from the total number present, giving the total number of moles of drug bound by the albumin (DB). The molar ratio of bound drug to albumin (r) was also calculated. r/DF was then plotted against r from this curve, n (number of binding classes) and k (association constants) values for the binding sites available to each drug were calculated according to Scatchard plot (3). Each Scatchard curve relating r/DF vs r/DF was constructed on the basis of the average of duplicate determinations of each experimental point.

RESULTS

The binding data are presented as a series of plots of r/DF versus r (Figs. 1-6) and as terms of the n and K values calculated from these curves (Tables 1-6). A curve with one or more inflections is believed to represent binding by two or more sites, n_1 and n_2 and the slope of each curvature equals K_1 or K_2 (primary or secondary association constant), respectively.

In the scatchard plot for the binding of tenoxicam by BSA only (no FFA content) (Fig. 1a), the sharply ascending limb of the curve represents a high association constant value ($K = 6.50 \times 10^4$) with a low n values ($n = 1.8$); the nearly horizontal limb at r values > 14 represents a low association constant value ($K = 27$) with a large number of binding sites ($n > 20$). The bend of the curve ($r = 3.9$) indicates a class with intermediate

n (8.1) and K (1×10^3). The curve for binding of tenoxicam by BSA containing 7 moles of palmitate per mole of albumin (Fig. 1c) was displaced markedly downwards and to the left, reflecting reduced binding by the two classes of site. Class 1 was now undetectable. The value of n_2 was reduced from 36.7 to 22.3 with a significant reduction in K_2 (from 27 to 2).

Table (1) : Number of binding sites, n , and apparent association constants, K (litres per mole), for binding of tiaprofenic acid and tenoxicam by bovine serum albumin containing various concentrations of plamitic acid.

| Drug | Bovine serum albumin | | | | 3.5 moles of palmitate per mole of albumin | | | | 7 moles of palmitate per mole of albumin | | | |
|------------------|----------------------|-------|---------------------|-------|--------------------------------------------|-------|---------------------|-------|------------------------------------------|----------------|--------------------|-------|
| | K_1 | n_1 | K_1 | n_1 | K_1 | n_1 | K_1 | n_1 | K_1 | n_1 | K_1 | n_1 |
| Tenoxicam | 6.49×10^4 | 1.8 | 0.268×10^2 | 36.7 | 2.413×10^4 | 2.6 | 0.107×10^2 | 25.3 | Not-detectable | Not-detectable | 0.02×10^2 | 22.3 |
| Tiaprofenic acid | 4.35×10^6 | 2.3 | 1.669×10^3 | 33.2 | 2.238×10^5 | 1.3 | 1.225×10^2 | 28.4 | Not-detectable | Not-detectable | 0.39×10^2 | 18.6 |

Table (2) : Number of binding sites, n , and apparent association constants, K (litres per mole), for binding of tiaprofenic acid and tenoxicam by human serum albumin containing various concentrations of plamitic acid.

| Drug | Bovine serum albumin | | | | 3.5 moles of palmitate per mole of albumin | | | | 7 moles of palmitate per mole of albumin | | | |
|------------------|----------------------|-------|---------------------|-------|--------------------------------------------|-------|---------------------|-------|------------------------------------------|----------------|---------------------|-------|
| | K_1 | n_1 | K_1 | n_1 | K_1 | n_1 | K_1 | n_1 | K_1 | n_1 | K_1 | n_1 |
| Tenoxicam | 4.211×10^4 | 2.30 | 0.081×10^3 | 23.2 | 1.201×10^4 | 1.2 | 0.056×10^3 | 26.2 | Not-detectable | Not-detectable | 0.014×10^2 | 13.2 |
| Tiaprofenic acid | 1.239×10^5 | 2.33 | 3.392×10^2 | 19.01 | 3.511×10^4 | 2.2 | 1.602×10^2 | 16.4 | Not-detectable | Not-detectable | 0.013×10^3 | 19.5 |

Table (3) : Number of binding sites, n , and apparent association constants, K (litres per mole), for binding of tiaprofenic acid and tenoxicam by bovine serum albumin containing various concentrations of plamitic acid.

| Drug | Bovine serum albumin | | | | 3.5 moles of palmitate per mole of albumin | | | | 7 moles of palmitate per mole of albumin | | | |
|------------------|----------------------|-------|---------------------|-------|--------------------------------------------|-------|---------------------|-------|------------------------------------------|----------------|---------------------|-------|
| | K_1 | n_1 | K_1 | n_1 | K_1 | n_1 | K_1 | n_1 | K_1 | n_1 | K_1 | n_1 |
| Tenoxicam | 6.081×10^4 | 1.2 | 0.225×10^2 | 32.5 | 2.202×10^4 | 2.1 | 0.098×10^2 | 22.6 | Not-detectable | Not-detectable | 0.018×10^2 | 21.6 |
| Tiaprofenic acid | 4.155×10^6 | 2.01 | 1.439×10^3 | 29.6 | 2.151×10^5 | 1.04 | 1.113×10^2 | 25.6 | Not-detectable | Not-detectable | 0.31×10^3 | 16.4 |

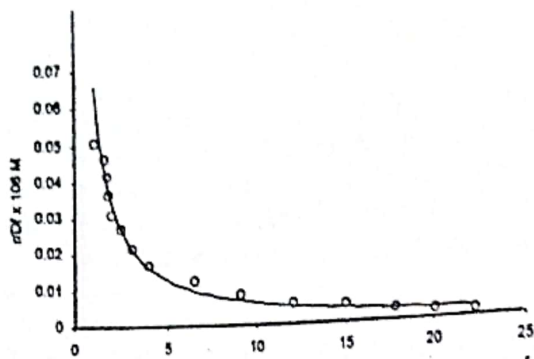


Fig. (1a) : Scatchard plot for binding of temoxicam by bovine serum albumin r = molar ratio of bound drug to albumin, D_f = concentration (mol/L) of free drug . The curve represents one dialysis equilibrium experiment. Each point on the curve is the average of three values.

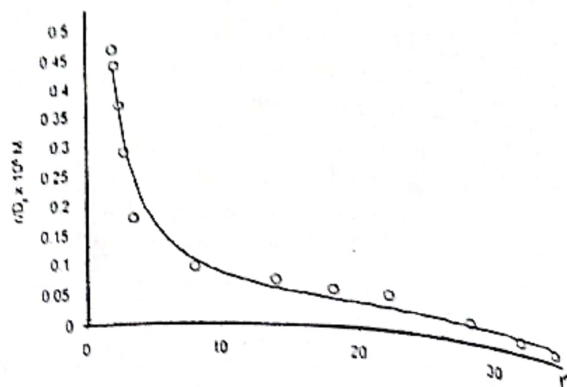


Fig. (2a) : Scatchard plot for binding of tiaprofenic acid by bovine serum albumin r = molar ratio of bound drug to albumin, D_f = concentration (mol/L) of free drug . The curve represents one dialysis equilibrium experiment. Each point on the curve is the average of three values.

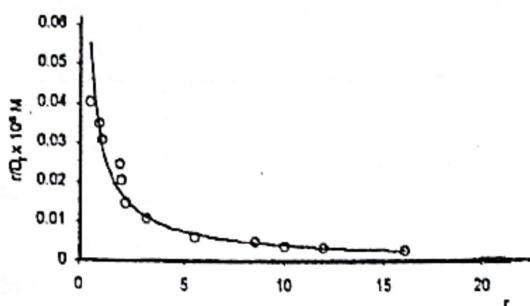


Fig. (1b) : Scatchard plot for binding of temoxicam by bovine serum albumin containing 3.5 moles of palmitate per mole of albumin, r = molar ratio of bound drug to albumin, D_f = concentration (mol/L) of free drug. The curve represents one dialysis equilibrium experiment. Each point on the curve is the average of three values.

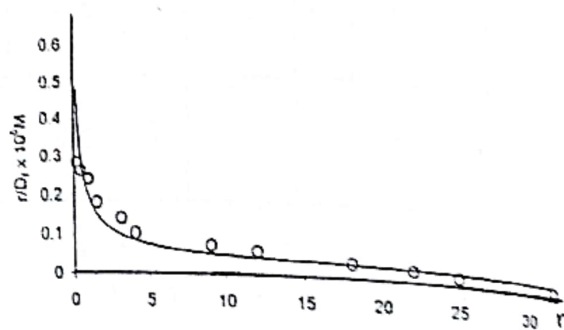


Fig. (2b) : Scatchard plot for binding of tiaprofenic acid by bovine serum albumin containing 3.5 moles of palmitate per mole of albumin, r = molar ratio of bound drug to albumin, D_f = concentration (mol/L) of free drug. The curve represents one dialysis equilibrium experiment. Each point on the curve is the average of three values.

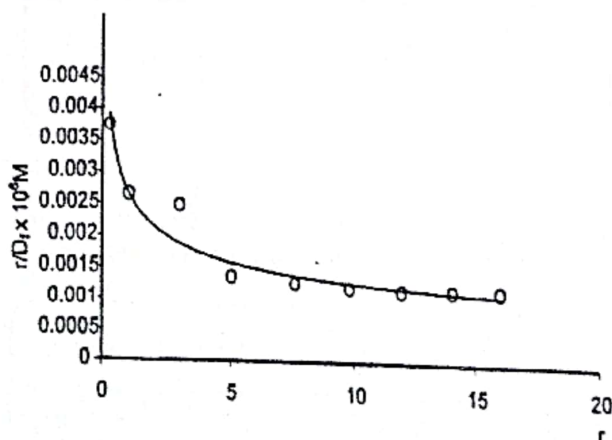


Fig. (1c) : Scatchard plot for binding of temoxicam by bovine serum albumin containing 7 moles of palmitate per mole of BSA . r = molar ratio of bound drug to albumin, D_f = concentration (mol/L) of free drug. The curve represents one dialysis equilibrium experiment. Each point on the curve is the average of three values.

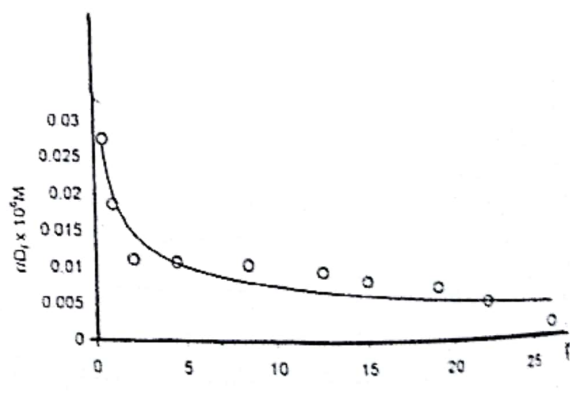


Fig. (2c) : Scatchard plot for binding of tiaprofenic acid by bovine serum albumin containing 7 moles of palmitate per mole of BSA . r = molar ratio of bound drug to albumin, D_f = concentration (mol/L) of free drug. The curve represents one dialysis equilibrium experiment. Each point on the curve is the average of three values.

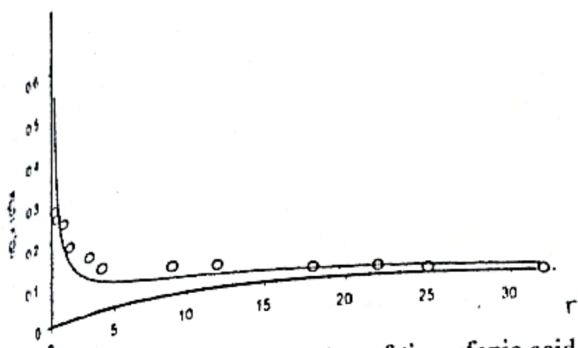


Fig. (3 a) : Scatchard plot for binding of tiaprofenic acid by bovine serum albumin containing 3.5 moles of oleic acid per mole of albumin. r = molar ratio of bound drug to albumin, D_f = concentration (mol/L) of free drug. The curve represents one dialysis equilibrium experiment. Each point on the curve is the average of three values.

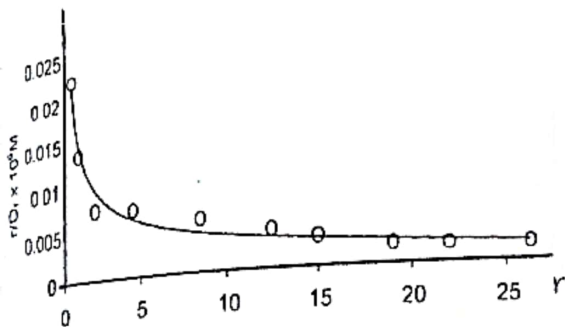


Fig. (3 b) : Scatchard plot for binding of tiaprofenic acid by bovine serum albumin containing 7 moles of oleic acid per mole of BSA. r = molar ratio of bound drug to albumin, D_f = concentration (mol/L) of free drug. The curve represents one dialysis equilibrium experiment. Each point on the curve is the average of three values.

The effect of oleate at a molar ratio of 7 was more or less nearly similar to that of palmitate (Table 3). The effect of palmitate at a ratio of 3.5 was detectable (Fig. 1b) but less marked than that seen at a ratio 7 where K_1 and K_2 were moderately reduced. HSA containing either 3.5 or 7 moles of palmitate per mole of protein or that free of palmitate exhibited differences in binding constants for tenoxicam and that binding was less than that observed with BSA preparations (Table 2).

Tiaprofenic acid was likewise bound to two sites by BSA, with $K_1 = 4.35 \times 10^6$, $n_1 = 2.3$, $K_2 = 1.67 \times 10^3$ and $n_2 = 33.2$. As shown (Fig. 2c), BSA containing 7 moles of FFA per mole of albumin had a markedly reduced capacity to bind tiaprofenic acid where K_1 and n_1 with 7 moles of FFA had been reduced largely and became non-detectable. For class 2, K_2 and n_2 were significantly reduced from 1.67×10^3 to 0.39×10^2 and from 33.2 to 18.6 respectively. Thus, class 1 with BSA containing 7 moles of FFA was no longer detectable. Oleate at a ratio

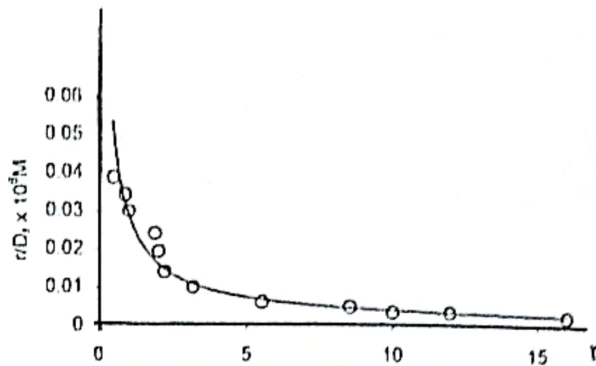


Fig. (4 a) : Scatchard plot for binding of temoxicam by bovine serum albumin containing 3.5 moles of oleic acid per mole of albumin. r = molar ratio of bound drug to albumin, D_f = concentration (mol/L) of free drug. The curve represents one dialysis equilibrium experiment. Each point on the curve is the average of three values.

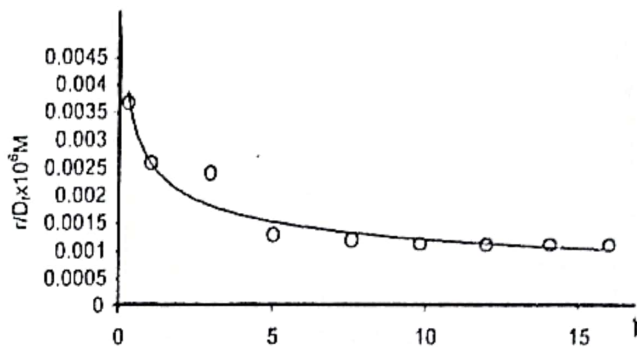


Fig. (4 b) : Scatchard plot for binding of temoxicam by bovine serum albumin containing 7 moles of oleic acid per mole of BSA. r = molar ratio of bound drug to albumin, D_f = concentration (mol/L) of free drug. The curve represents one dialysis equilibrium experiment. Each point on the curve is the average of three values.

of 7 moles per mole of BSA reduced K_1 , n_1 , K_2 and n_2 in a manner nearly similar to 7 moles of palmitate (Figs 3,4). Considerably less inhibitory were 3.5 mole of palmitate which reduced K_1 , n_1 , K_2 and n_2 to a considerable but less significant extent. Addition of 3.5 or 7 moles of palmitate to HSA produced more and largely inhibitory effects on the binding parameters of tiaprofenic acid and tenoxicam as compared to those observed with BSA (Figs. 5,6).

DISCUSSION

Tenoxicam and tiaprofenic acid are extensively bound by serum albumin. In every case the binding is reduced by palmitate or oleate. It should be mentioned that the values for n and K in the present dialysis-equilibrium studies are affected not only by FFA content of the albumin preparation, as the present data show, but also by temperature, species of albumin, ionic strength and pH of the buffer (9).

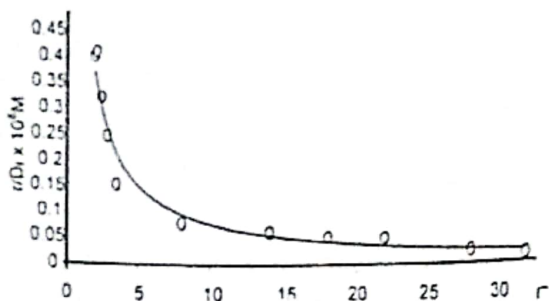


Fig. (5a) : Scatchard plot for binding of tiaprofenic acid by human serum albumin r = molar ratio of bound drug to albumin, D_f = concentration (mol/L) of free drug. The curve represents one dialysis equilibrium experiment. Each point on the curve is the average of three values.

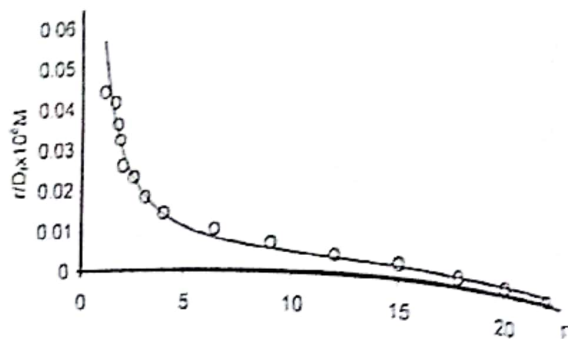


Fig. (6 a) : Scatchard plot for binding of temoxicam by human serum albumin r = molar ratio of bound drug to albumin, D_f = concentration (mol/L) of free drug. The curve represents one dialysis equilibrium experiment. Each point on the curve is the average of three values.

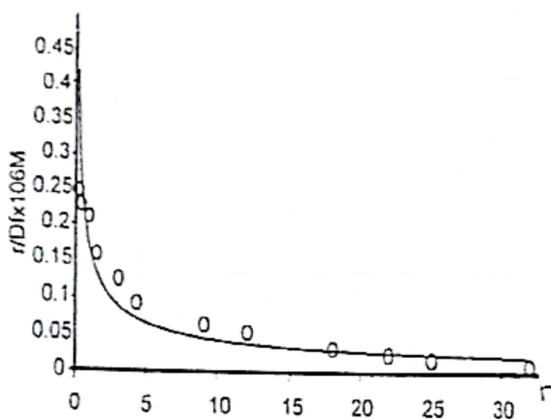


Fig. (5 b) : Scatchard plot for binding of tiaprofenic acid by human serum albumin containing 3.5 moles of palmitate per mole of albumin r = molar ratio of bound drug to albumin, D_f = concentration (mol/L) of free drug. The curve represents one dialysis equilibrium experiment. Each point on the curve is the average of three values.

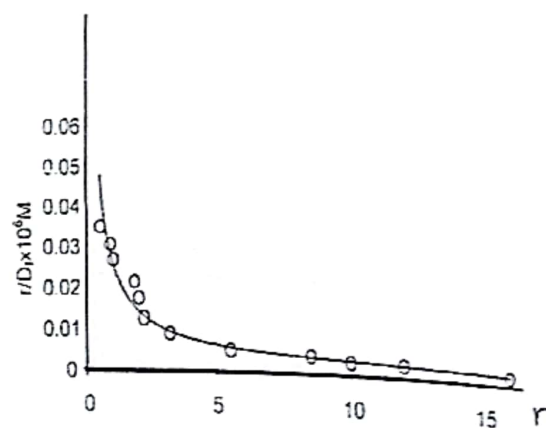


Fig. (6 b) : Scatchard plot for binding of temoxicam by human serum albumin containing 3.5 moles of palmitate per mole of albumin r = molar ratio of bound drug to albumin, D_f = concentration (mol/L) of free drug. The curve represents one dialysis equilibrium experiment. Each point on the curve is the average of three values.

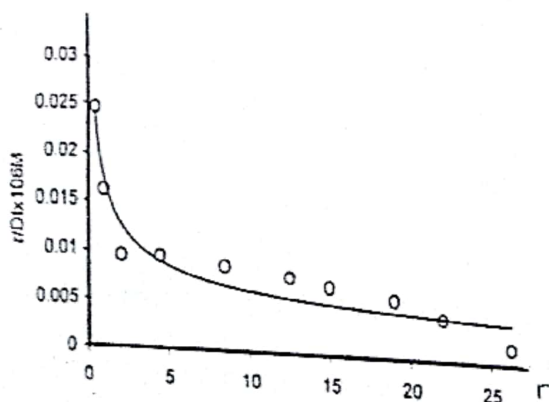


Fig. (5 c) : Scatchard plot for binding of tiaprofenic acid by human serum albumin containing 7 moles of palmitate per mole of BSA. r = molar ratio of bound drug to albumin, D_f = concentration (mol/L) of free drug. The curve represents one dialysis equilibrium experiment. Each point on the curve is the average of three values.

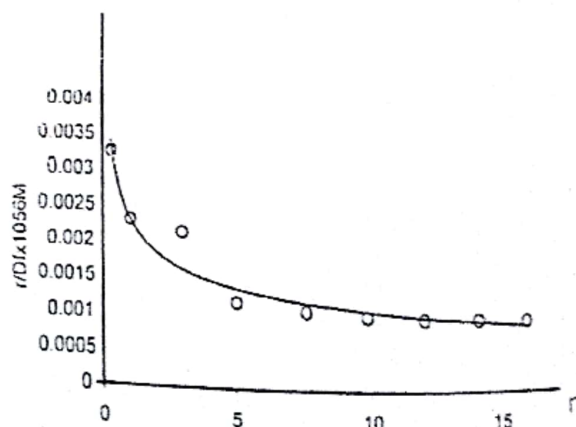


Fig. (6 c) : Scatchard plot for binding of temoxicam by human serum albumin containing 7 moles of palmitate per mole of BSA. r = molar ratio of bound drug to albumin, D_f = concentration (mol/L) of free drug. The curve represents one dialysis equilibrium experiment. Each point on the curve is the average of three values.

At molar ratio 3.5 and 7, palmitate and oleate, which are the two principal components of the mixture of FFA in plasma, depress the binding of tenoxicam and tiaprofenic acid by BSA. This suggests that the inhibitory effect may be attributed to the long chain fatty acids. Each fatty acid was tested at several molar ratios, 0, 3.5 and 7 moles of FFA per mole of albumin to compare the inhibitory potency of each one. The n and K values for the binding of tenoxicam and tiaprofenic acid to HSA, and the effect of palmitate at ratios of 7 and 3.5 on these values, were generally largely affected when compared to the corresponding data obtained with BSA. This indicates that the structure of sites on these two types of albumin which bind these two drugs, and the effect of FFA on these sites, are more or less different to some extent.

Inspection of the scatchard curves in all figures and the calculated binding parameters, shows that the inhibitory effect of palmitate (or oleate) at molar ratio 7 is different in its magnitude from tenoxicam to tiaprofenic acid. FFA can reduce binding to other drugs by at least three different mechanisms:

1) Competition for the same binding site between FFA and the drug. This type of competition would cause a reduction in K without change in n for the drug. But the insolubility of FFA in aqueous buffer and tendency to micelle formation when saturation is approached may restrict the amount of fatty acid which can be displaced by another, more soluble drug competing for the same site, with consequent reduction in n as well as K values for the drug. 2) Increased electrostatic repulsion between albumin and anionic drugs. 3) Changes in the conformation of the albumin molecule caused by the binding of FFA⁽²⁸⁾. This type of effect could reduce either n or K values for any type of binding sites.

CONCLUSION

The present data show that palmitate at a molar ratio of 7 inhibits the binding by BSA of the two drugs tested, tenoxicam and tiaprofenic acid. For the two drugs, the inhibitory effect was confirmed with oleate in place of palmitate and with HSA in place of BSA. This suggests that at a molar ratio of 7, FFA may exert a general inhibitory effect on the binding of many drugs to several species of serum albumin. The data with BSA containing 3.5 as compared to 7 moles of palmitate suggest that the inhibitory effect may become significant only at serum FFA concentrations over 3.5 moles per mole of albumin. Elevation of this magnitude have been reported in patients with gram-negative septicemia⁽²⁹⁾. These conclusions need to be tested with additional drugs, additional concentrations of various FFA and albumins from additional species.

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تأثير الأحماض الدهنية طويلة السلسلة على إرتباط أدوية مضادات الإلتهابات بكل من زلال البقر والزلال الآدمى

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تضمن هذا البحث دراسة إرتباط عقارى التينوكسكام وحمض التايبروفينيك بكل من زلال البقر والزلال الآدمى وذلك فى وجود نسب مولارية مختلفة من الأحماض الدهنية طويلة السلسلة . وقد تم إجراء تجارب الإرتباط باستخدام طريقة الديليزة المتوازية عند درجة حرارة ٢٥ مئوية ومحلول منظم ذو أس أيديروجينى قدره ٧.٤ وذلك لدراسة مدى التنافس بين كل من العقارين وكل من حمض البالمتيك أو حمض الأوليك فى الإرتباط بالزلال.

وبتحليل منحنيات الإرتباط ، أظهرت النتائج أن كلا العقارين يشغلان طائفتين من مواقع الإرتباط على جزيء الزلال وأن هناك نقصان واضح فى درجة الإرتباط فى وجود حمض البالمتيك أو حمض الأوليك عند نسبة مولارية قدرها ٣.٥ أو ٧.

وأوضحت الدراسة أيضاً أن درجة إرتباط العقارين المذكورين بزلال البقر كان متشابهها إلى حد كبير فى وجود حمض البالمتيك أو حمض الأوليك وأن درجة إرتباط كل من العقارين بالزلال الآدمى كانت أقل من درجة إرتباطهما بزلال البقر وذلك فى وجود حمض البالمتيك .

وقد تمثل النقصان الواضح فى درجة إرتباط كل من العقارين بالزلال فى إنخفاض قيم معاملات الإرتباط وهى ثابت الإرتباط وعدد مواقع الإرتباط مما يؤكد أن الأحماض الدهنية طويلة السلسلة تؤدي إلى التقليل الواضح من سعة الزلال للإرتباط بكل من عقارى التينوكسكام وحمض التايبروفينيك.