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A COMPARATIVE STUDY OF SOME PHYSICO-CHEMICAL PROPERTIES OF HUMAN SERUM ALBUMIN SAMPLES FROM DIFFERENT SOURCES—II

THE CHARACTERISTICS OF THE N–B TRANSITION AND THE BINDING BEHAVIOUR WITH REGARD TO WARFARIN AND DIAZEPAM

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Abstract—A comparative study of the N–B transition and the drug binding properties of human serum albumin samples from various sources has been carried out with the help of circular dichroism and equilibrium dialysis. It was found that when warfarin was used as a marker the midpoint pH and the cooperative nature of the N–B transition that occurs in the albumin around physiological pH varied with the albumin sample. The midpoint pH was found to be related to the cooperative nature of the albumin samples. A similar relationship has been found for allosteric proteins. However, when diazepam was used as a marker molecule for the N–B transition, variations in the midpoint pH and cooperative nature of the N–B transition disappeared. This is attributed to the strong allosteric effect of diazepam on binding. The affinity of warfarin for albumin depends strongly on the sample, but this is not the case with diazepam. The cooperative binding properties found for the albumin samples are compared with those found for the albumin in serum. After a discussion it is concluded that the cooperative binding properties of a particular albumin sample should be taken into account when that sample is used in binding studies.

Albumin is one of the most extensively studied proteins. Nevertheless, it is only recently that the complete amino acid sequence of human serum albumin has been elucidated [1, 2]. Neither its physiological function nor its physico-chemical properties are properly understood. One of the physico-chemical properties of human and bovine serum albumin, the so-called N–B transition, is the subject of an increasing number of investigations [3–14]. This conformational change occurs in the protein around physiological pH. Physiologically important ions such as Ca^{2+} and Cl^- make the equilibrium between the N and B forms very sensitive to small changes in the pH [10, 11]. Most drugs have one or two high affinity sites and several sites of relatively low affinity on the albumin† molecule. The high affinity binding sites of most drugs can be divided into at least two binding sites, the so-called warfarin site, or site I, and the diazepam site, or site II [15–19]. Recently a third and a fourth binding site have been found [19, 20] but most drugs bind to the warfarin or the diazepam binding site [19]. Both sites are sensitive to the N–B transition [11, 12] and, therefore, the binding of many drugs to albumin will be pH dependent. Since the N–B transition also occurs in albumin in serum [21], and since free blood levels of drugs and other substances therefore depend on the pH of the blood and the presence of physiologically important substances in the blood, the N–B transition will have a physiological function.

Results of studies *in vivo* and in serum on the properties of the N–B transition are rather complex to interpret and it is, therefore, easier to carry out such investigations in solutions of pure albumin. Preliminary experiments [12], however, showed that the affinity of warfarin for albumin differs considerably from one sample to another, especially when the albumin is in the N conformation. In view of this, the choice of the albumin sample to be used for research purposes will be very important, especially when results of such studies are to be extrapolated to serum or *in vivo*. Therefore, we started a study on the properties of the N–B transition in several albumin samples and examined the consequences of the binding of drugs to albumin. Warfarin and diazepam, being the best known representatives of the two major binding sites of albumin, were the drugs chosen for the binding studies. The results are presented in this paper.

MATERIALS AND METHODS

The origin and lot numbers of the albumin samples used in this study are summarized in Table 1. We also isolated albumin from human plasma ourselves using a modified version of the method of Hao [22, 23]. The albumin was deionized before use and

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† In the rest of the text albumin means human serum albumin, unless otherwise stated.

its concentration determined as described elsewhere [11]. In all experiments the albumin concentration was 6.0×10^{-5} M.

Sodium warfarin (Brocacef, Maarssen, The Netherlands) and diazepam (gift from Hoffman–La Roche, Mijdrecht, The Netherlands) were used without further purification. All other chemicals were of analytical grade (Merck, Darmstadt, West Germany or J. T. Baker, Deventer, The Netherlands).

The binding of warfarin and diazepam was studied by means of equilibrium dialysis. The equilibrium dialysis experiments and the determination of the free concentration of warfarin and diazepam were carried out as described previously [11, 12]. For these experiments low drug-to-protein ratios of about 0.2 and 2×10^{-3} were chosen for warfarin and diazepam, respectively. Since warfarin and diazepam have only one high affinity binding site on the albumin, the high affinity binding constant of warfarin and diazepam to albumin can easily be calculated from one low drug-to-protein ratio [11]. In the dialysis experiments the protein-free compartment contained a phosphate or borate buffer ($I = 0.1$). The free concentration of warfarin was measured at two pH values: 6.1 and 8.8.

Circular dichroism (CD) experiments were performed as described previously [10, 11]. The CD signal of warfarin–albumin complexes with a drug to-protein ratio of 0.4 was measured in the pH range 5.5–9.5 at 310 nm in buffer ($I = 0.1$) and in 0.1 M NaCl. In the former case we used phosphate buffers ($I = 0.1$) below pH 8 and borate buffers ($I = 0.1$) above pH 8. In the experiments with 0.1 M NaCl the pH was adjusted with 0.1 N NaOH. The CD signal of the diazepam–albumin complexes with a drug-to-protein ratio of 2×10^{-3} was measured at 330 nm under conditions similar to those where warfarin was used as marker. The N–B transition, which is made visible when the observed molar ellipticity of the drug–albumin complex is plotted vs pH, is characterized by the pH_{50} and the Hill coefficient (HC). The midpoint pH of the pH dependence of the molar ellipticity, where it is assumed that half of the albumin is in the B form, stands for the pH_{50}, whereas the HC is the slope of the log $[f/(1-f)]$ vs pH curve at pH_{50}, where f is the fraction of albumin in the B conformation [10].

RESULTS

The pH_{50} and the Hill coefficient of the N–B transition of the various albumin samples are measured in buffer and 0.1 M chloride (pH_{50}^{buffer}, HC^{buffer} and $pH_{50}^{chloride}$, $HC^{chloride}$, respectively). In columns 4 and 5 of Table 1 pH_{50}^{buffer} and HC^{buffer} of the various warfarin–albumin complexes are summarized. As can be seen from this table, the pH_{50}^{buffer} is not the same for each sample. The N–B transition of the samples 01, 02, 03, 04, 11, 71 and 72 is characterized by a $pH_{50}^{buffer} = 7.4$, whereas the pH_{50}^{buffer} of the N–B

Table 1. The characteristics of the N–B transition of various albumin samples when warfarin is used as a marker, and the affinity of warfarin for albumin in the N and B conformations*

Origin of the albumin sample	Lot number	Code	pH_{50}^{buffer}	HC^{buffer}	$pH_{50}^{chloride}$	$HC^{chloride}$	$K_N^W \times 10^{-5}$ (M^{-1})	$K_B^W \times 10^{-5}$ (M^{-1})
Sigma Chemical Company, St. Louis, MO, U.S.A.; crystallized and lyophilized	39C-8085	01	7.4	0.9	7.3	1.3	5.5	
idem	76C-8145	02	7.4	0.9	7.3	1.3	5.6	
idem	65C-8320	03	7.4	0.9	7.3	1.3	5.7	
idem	126C-8070	04	7.4	0.9	7.3	1.3	5.9	
idem	18C-0518	05	7.8	1.1			2.4	
idem	18C-0519	07	7.9	1.1	7.7	1.4	2.7	14.1
Sigma Chemical Company, St. Louis, MO, U.S.A.; fraction V	47C-04422	11	7.4	0.8	7.3	1.2	6.0	
idem	49C-04851	13	8.2	1.3			2.0	13.3
Kabivitrum A.B., Stockholm, Sweden; highly purified	78862	21	8.6	1.8			2.0	13.2
idem	58801	22	7.5	0.9			3.3	22.1
Kabivitrum A.B., Stockholm, Sweden; infusion solution	77775	31			7.4	1.0	2.5	14.8
Preparation isolated by us	160181	41			7.5	1.5		
idem	230181	42			7.6	1.4	2.8	15.0
Biotest-Serum-Institut GmbH, Frankfurt am Main, West Germany; infusion solution	307100	71	7.4	0.8	7.3	1.2	6.0	30.0
idem	310071	72	7.4	0.8	7.3	1.0	1.7	7.4
idem, without the addition of stabilizers		73	7.9	1.3	7.6	1.0	2.7	15.9

* pH_{50}^{buffer} stands for pH_{50} in phosphate or borate buffer, and $pH_{50}^{chloride}$ stands for pH_{50} in 0.1 M NaCl. HC^{buffer} and $HC^{chloride}$ stand for the Hill coefficient in phosphate or borate buffer and 0.1 M NaCl, respectively. K_N^W and K_B^W are the affinity constants of warfarin for albumin in the N and B conformations, respectively. The error in K_N^W and K_B^W was 5%. The accuracy is within about 0.05 for pH_{50} and 0.1 for HC.

transition of the other samples has a higher value. This means that at pH 7.4 the fraction of the albumin in the N conformation is 50% for samples 01, 02, 03, 04, 11, 71 and 72, whereas in the other samples the fraction in the N form is more than 50%. For instance about 75% of albumin 05 is in the N conformation at pH 7.4 and more than 90% of albumin 13. The samples with a $pH_{50}^{buffer} = 7.4$ have an HC^{buffer} of 0.8 or 0.9. The other samples have a somewhat larger HC^{buffer}.

The $pH_{50}^{chloride}$ and $HC^{chloride}$ of the various warfarin–albumin complexes are summarized in columns 6 and 7 of Table 1. It can be seen that for most samples $pH_{50}^{chloride}$ is somewhat lower than pH_{50}^{buffer}, whereas $HC^{chloride}$ is larger than HC^{buffer}. All samples with an N–B transition, characterized by $pH_{50}^{buffer} = 7.4$ and $HC^{buffer} = 0.8$–0.9, except sample 72, have an N–B transition that is characterized by $pH_{50}^{chloride} = 7.3$ and $HC^{chloride} = 1.2$–1.3.

Since warfarin binds only to the warfarin site on the albumin, it might be interesting to study the N–B transition of the various diazepam–albumin complexes, because diazepam binds specifically to the diazepam site. In Table 2 the pH_{50} and HC of the N–B transition of the diazepam–albumin complexes in buffer and in 0.1 M chloride are summarized. It can be seen from this table that neither pH_{50}^{buffer} and HC^{buffer} nor $pH_{50}^{chloride}$ and $HC^{chloride}$ are significantly different for the diazepam–albumin complexes, although there is a large variation in the pH_{50} and HC of the albumin samples with warfarin.

DISCUSSION

Janssen *et al.* [14] have shown that albumin can be regarded as an allosteric protein, if protons are considered as the ligand. This means that the fraction of the albumin in the N or in the B conformation is pH dependent. In the light of the two-state model of Monod *et al.* [24], i.e. the MWC model, the fraction of albumin in the N or in the B conformation as a function of the pH can be described by the following set of parameters: L, the allosteric constant of the $N \rightleftarrows B$ equilibrium, K_N^H, the dissociation constant, which describes the affinity of a proton for the N conformation, n, the number of proton binding sites and c, the ratio K_N^H/K_B^H, in which K_B^H represents the dissociation constant, which describes the affinity of a proton for the B conformation. Allosteric effector molecules affect the $N \rightleftarrows B$ equilibrium on binding to albumin, as is expressed in a change in the value of pH_{50} or HC. For instance, binding of diazepam to albumin results in a decrease in the pH_{50} and an increase in the HC [12]. A change in L will cause a change in the median of ligand (i.e. proton) binding (pH_m). This follows from [25]

$$pH_m = pK_N^H - \frac{1}{n}\log L. \qquad (1)$$

In our experiment, however, we measured pH_{50} instead of pH_m. Similarly a change in L will cause a change in pH_{50}, so changes in pH_{50} can be interpreted as changes in L.

The MWC model has been extensively tested on haemoglobin, where oxygen acts as the ligand. Many mutant haemoglobins are known which have a half

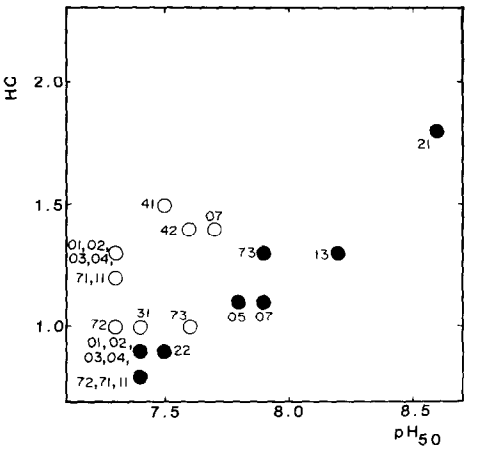

Fig. 1. The Hill coefficient (HC) of the various albumin samples as a function of pH_{50} in phosphate and borate buffer (●) and in 0.1 M chloride (○). The numbers in the figure correspond to the codes of the various albumin samples mentioned in Table 1.

saturation point with respect to oxygen binding and an HC which differ from normal haemoglobin [26]. The HC as a function of log L for a wide series of mutant and modified haemoglobins can be described by bell-shaped curves [26], as predicted by the MWC model [27]. From model calculations we found that curves which represent the HC as a function of the pH_{50} are also bell-shaped. In Fig. 1, therefore, we plotted the HC of the albumin samples vs the pH_{50}. The closed circles in Fig. 1 show the relation between pH_{50} and HC of the N–B transition in buffer. As can be seen, the HC^{buffer} increases with the pH_{50}. The curve obtained by joining the closed circles seems similar to one section of a bell-shaped curve. It should be noted that a decrease in L causes an increase in pH_{50}. Therefore, the curve in Fig. 1 resembles the part of one of the bell-shaped curves described by Rubin and Changeux [27], where the slope of the HC vs log L curve has a negative value. This means that the differences in the pH_{50} and HC of the N–B transition of the various albumin samples are due to the fact that the L values of the various samples are different, if it is assumed that n and c are the same for the albumin preparations. The question that now arises is why the L values are different for the various albumin samples. In principle there are two possible reasons. First the L value may be changed by the presence of allosteric effectors (different in nature and/or quantity). If this is the case it can be said that samples 05, 07, 13, 21 and 73 have bound allosteric effectors, which decrease L. Secondly, the albumin samples may differ intrinsically from each other; this may also induce different L values for the various preparations (see later). It is obvious, however, that intrinsic differences in the albumin samples can lead either to a change in L or to different n and/or c values. According to the MWC model a change in n or c will also change the pH_{50} and the HC. Consequently it is clear that the differences in the HC and pH_{50} of the various albumin samples may be due to differences in L, n, c or a combination of these parameters.

However, since the closed circles in Fig. 1 tend towards a bell-shaped curve, the most simple explanation is a change in L.

If the MWC model gives a reasonable explanation for the difference in the HC and the pH_{50} of the N–B transition in the albumin samples in buffer, then one should also find such a relationship in 0.1 M chloride. From Fig. 1 it is seen that a similar relationship was indeed found in 0.1 M chloride. The difference between the two curves in Fig. 1 is that the experimentally measured points in 0.1 M chloride, except for sample 73, show a shift to larger HC values and a somewhat smaller pH_{50}. In analogy with the model calculation of Rubin and Changeux [27] we found that an increase in the HC may be due to a decrease in the c value. Particularly in the part of the bell-shaped curve where the slope of the HC vs log L curve is negative, a small decrease in c results in a large increase in the HC. Because of the definition of c, a decrease in c can only be explained by an increase in pK_N^H or a decrease in pK_B^H. Since Beek *et al.* [28] found that the pK of some amino acid residues of the haemoglobin molecule increased when chloride was bound, the decrease in c is likely caused by an increase in pK_N^H. It is not known why sample 73 acts differently from the other samples in 0.1 M chloride.

To find further support for our statement that the observed differences in the N–B transition of the various albumin samples can be explained in terms of the MWC model, we use diazepam instead of warfarin as a label for the N–B transition. It is known that diazepam strongly affects the N–B transition as expressed by the pH_{50} and HC [12]. If we are correct in conducting that the observed differences in HC and pH_{50} of the N–B transition of the albumin samples are due to changes in the allosteric constant L, we can expect a strong effector such as diazepam to overrule these differences. From Table 2 it is seen that this was indeed the case when diazepam was bound to albumin. The albumin samples with the most marked differences in pH_{50} and HC, when warfarin was used as a label, did give nearly the same pH_{50} and HC when diazepam was bound, both in buffer and in 0.1 M chloride.

It should be noticed that the results found for the warfarin–albumin and diazepam–albumin complexes are probably not representative for the two binding sites of albumin. It is very likely that if a strong allosteric effector, which binds to the warfarin site, were used in the experiments instead of warfarin, the variations in pH_{50} and HC would be smoothed out too.

Comparison of the HC and pH_{50} of the N–B transition of the various albumin samples with those obtained in serum

It might be interesting to compare the results obtained with pure albumin samples with the result of studies on the behaviour of albumin in its natural surroundings: serum. Since in this study the N–B transition of the albumin samples is characterized by the HC and the pH_{50}, these values of the neutral to base transition of the various samples will be compared with the HC and pH_{50} of the N–B transition of albumin in serum. The pH_{50} and HC of the N–B transition of albumin in serum were measured by Giesen and Wilting [21]. They found that the N–B transition of albumin in serum can be described by $pH_{50} = 7.3$ and HC = 1.6. The pH_{50} of 7.3 in serum is the same as the $pH_{50}^{chloride}$ value of most albumin samples from Table 1. The HC of 1.6 in serum, however, is not the same as the $HC^{chloride}$ of 1.3, which was found for most albumin samples, which had a $pH_{50}^{chloride}$ of 7.3. Giesen and Wilting [21], however, showed that Ca^{2+} and Mg^{2+} ions, in physiological concentrations, increase the HC value by about 0.2. Consequently it is to be expected that the N–B transition of samples 01, 02, 03, 04, 11 and 71 will be similar to that of albumin in serum, with respect of pH_{50} and HC, whereas the N–B transition of samples 05, 07, 31, 41, 42 and 73 will not.

As we stated above, these differences are probably due to allosteric effectors bound to the albumin, or to intrinsic structural differences in the albumin samples. Several endogenous substances can act like allosteric effectors [30, 31]. The best known endogenous allosteric effectors are the non-esterified fatty acids and the demolition products of bilirubin. These substances do have a very large affinity for albumin, as a result of which they are very difficult to remove from albumin [29, 31–33]. Since we could not find a direct relation between the fatty acid content of the albumin samples and the pH_{50} or the HC of the N–B transition we think that the fatty acids alone cannot explain the differences found for

Table 2. The characteristics of the N–B transition of some albumin samples when diazepam is used as marker, and the affinity of diazepam for albumin in the N and B conformations*

Albumin sample	pH_{50}^{buffer}	HC^{buffer}	$pH_{50}^{chloride}$	$HC^{chloride}$	$K_N^R \times 10^{-5}$ (M^{-1})	$K_B^R \times 10^{-5}$ (M^{-1})
04	6.8	1.8			1.4	
07	6.9	1.9	6.0	2.8	1.5	2.3
71	6.9	1.9	6.0	2.7	1.4	2.2
72	6.8	2.0			1.3	2.4

* The numbers of the albumin samples refer to the codes mentioned in Table 1. pH_{50}^{buffer} stands for pH_{50} in phosphate or borate buffer, while $pH_{50}^{chloride}$ denotes pH_{50} in 0.1 M NaCl. HC^{buffer} and $HC^{chloride}$ represent the Hill coefficient in phosphate or borate buffer and 0.1 M NaCl, respectively. K_N^R and K_B^R stand for the affinity constants of diazepam for albumin in the N and B conformations, respectively. The error in K_N^R and K_B^R was less that 5%. The accuracy is within about 0.05 for pH_{50} and 0.1 for HC.

the various warfarin–albumin complexes. The presence of endogenous effectors (among which the fatty acids), which are probably bound to some of the albumin samples, is therefore a reasonable explanation for the differences in the pH_{50} and HC of the N–B transition of the various albumin samples. Very little is known about structural changes in the albumin molecule which can affect L. Mutant modifications of albumin have been discovered [34, 35], but we are unlikely to be dealing with these kinds of albumin, because the albumin samples, commercially available, have all been isolated from plasma pooled from many blood donors. Recently it was found that the amount of titratable histidines and carboxyl groups can vary from albumin sample to albumin sample [36]. The probable reason for this is that adventitious alterations in the albumin can arise as a result of the preparative procedures employed [37, 38]. It is not known whether these alterations affect L.

Consequences for the binding properties of the albumin samples to drugs, i.e. warfarin and diazepam

To find out whether the albumin samples whose pH_{50} and HC differ from albumin in serum also differ in their drug binding to albumin in serum, free concentrations of warfarin and diazepam in pure albumin solutions at a constant drug-to-protein ratio were measured at two extreme pH values. In Table 1 the affinity constants (K_N^W and K_B^W) of warfarin for the N and B conformations, respectively, are summarized. The K_N^W and K_B^W in serum are 3.2×10^5 and $5.7 \times 10^5\ M^{-1}$, respectively. When the chloride effect is taken into account according to Wilting *et al.* [13] the values become 5.0×10^5 and $8.9 \times 10^5\ M^{-1}$, respectively. The albumin samples whose pH_{50} and HC differ from those of albumin in serum all have lower K_N^W values than were found in serum. Lima and Salzer (39) found that some albumin preparations were contaminated with α_1-acid glycoproteins, as a result of which ligands, especially diisopropamid, showed a variation in their free concentrations. In this study, contamination of the albumin samples with α_1-acid glycoproteins cannot be the reason for the lower K_N^W values of some albumin samples, because in serum warfarin binds exclusively to albumin [40]. Of the samples which have an N–B transition that is comparable to that of albumin in serum with respect to pH_{50} and HC, all, except samples 22 and 72, yield comparable values for K_N^W. How are we to explain the fact that some albumin preparations have lower K_N^W values than albumin in serum? Possible explanations are that when we are dealing with allosteric effectors, these substances may displace warfarin from its high affinity binding site or that when we are dealing with intrinsically different albumin samples, these intrinsic differences may alter the binding of warfarin to albumin. The K_B^W of all the albumin preparations is larger than the K_B^W of albumin in serum, if chloride is taken into account. So far we have no explanation for this.

In Table 2 the affinity constants (K_N^D and K_B^D) of diazepam for the N and B conformations, respectively, are summarized. As can be seen K_N^D as well as K_B^D are roughly the same for the various samples, although there were large variations in K_N^W and K_B^W of these samples. This means that the endogenous allosteric effectors or the intrinsic differences in the preparations do not affect the binding of diazepam to albumin. This means that the endogenous effectors do not have a displacing effect on site II or it means that the variations in L caused by intrinsic differences in the albumin samples are smoothed out by diazepam.

In summary, all albumin samples investigated were found to show the N–B transition around the physiological pH; however, there was a large variation in the HC and pH_{50} values. One of the important functions of albumin *in vivo* is to transport fatty acids and many other endogenous and exogenous substances which are hardly soluble in water. Most drugs at a therapeutic blood level are more or less tightly bound at the warfarin or at the diazepam binding site. The albumin molecule should therefore be able to adapt the local geometric structural properties of these sites easily in order to make it possible for molecules with completely different structural properties to bind to these sites. In terms of the function of the albumin molecule as an allosteric protein this could mean that the HC value of the N–B transition is very sensitive to the composition of the albumin solvent and to the nature of the ligand bound. Therefore the albumin molecule may be said to be a 'breathing' molecule. The 'breathing' can be said to 'cease' when the HC value is almost insensitive to the binding of allosteric effectors. Therefore, one should always take the properties of a particular albumin sample into account especially those discussed in this paper. If the allosteric properties of the albumin molecule are to be studied both fundamentally and with respect to the physiological function of albumin, one needs an albumin sample in which the HC value of the N–B transition is very sensitive to any alterations in the albumin surroundings. Therefore, a low HC in phosphate and borate buffers is preferable to a high HC, if weak allosteric effectors are used for the N–B transition. In any case, studies of this kind have to be carried out with the same batch of albumin, so that it is possible to compare the results. When the results of albumin binding studies are to be extrapolated to the binding of drugs to albumin in serum, the HC and pH_{50} values of the albumin sample should be as close as possible to the HC and pH_{50} values of the N–B transition of albumin in serum. The binding properties of the albumin samples should also be taken into account.

REFERENCES

1. P. Q. Behrens, A. M. Spiekerman and J. R. Brown, *Fedn Proc. Fedn Am. Socs exp. Biol.* **34**, 591 (1975).
2. A. Meloun, L. Moravek and V. Kostra, *FEBS Lett.* **58**, 134 (1975).
3. W. J. Leonard, Jr., K. K. Vijai and J. F. Foster, *J. biol. Chem.* **238**, 1984 (1963).
4. H. A. Saroff and M. S. Lewis, *J. phys. Chem.* **67**, 1211 (1963).
5. D. E. Goldsack and P. M. Wearn, *Can. J. Biochem.* **49**, 1267 (1971).
6. B. J. M. Harmsen, S. H. de Bruin, L. H. M. Janssen, J. F. Rodrigues de Miranda and G. A. J. van Os, *Biochemistry* **10**, 3217 (1971).

7. H. J. Nikkel and J. F. Foster, *Biochemistry* **10**, 4479 (1971).
8. K. Wallevik, *J. biol. Chem.* **248**, 2650 (1973).
9. V. R. Zurawski, Jr. and J. F. Foster, *Biochemistry* **13**, 3465 (1974).
10. J. Wilting, M. M. Weideman, A. C. J. Roomer and J. H. Perrin, *Biochim. biophys. Acta* **579**, 469 (1979).
11. J. Wilting, W. F. van der Giesen, L. H. M. Janssen, M. M. Weideman, M. Otagiri and J. H. Perrin, *J. biol. Chem.* **255**, 3032 (1980).
12. J. Wilting, B. J. 't Hart and J. J. Gier, *Biochim. biophys. Acta* **626**, 291 (1980).
13. J. Wilting, W. F. van der Giesen and L. H. M. Janssen, *Biochem. Pharmac.* **30**, 1025 (1981).
14. L. H. M. Janssen, M. T. van Wilgenburg and J. Wilting. *Biochim. biophys. Acta* **669**, 244 (1981).
15. G. Sudlow, D. H. Birkett and D. N. Wade, *Molec. Pharmac.* **12**, 1052 (1976).
16. R. Brodersen, T. Sjödin and I. Sjöholm, *J. biol. Chem.* **252**, 5067 (1977).
17. W. E. Müller and U. Wollert, *Pharmacology* **19**, 59 (1979).
18. K. J. Fehske, W. E. Müller and U. Wollert, *Biochim. biophys. Acta* **577**, 346 (1979).
19. I. Sjöholm, B. Ekman, A. Kober, I. Ljungstedt-Påhlman, B. Serving and T. Sjödin, *Molec. Pharmac.* **16**, 767 (1979).
20. I. Sjöholm, *Acta Pharm. Suec.* **17**, 76 (1980).
21. W. F. van der Giesen and J. Wilting, *Biochem. Pharmac.* (in press).
22. Y. Hao, *Vox Sang.* **36**, 313 (1979).
23. J. M. H. Kremer, *Vox Sang.* **42**, 223 (1982).
24. J. Monod, J. Wyman and J.-P. Changeux, *J. molec. Biol.* **12**, 88 (1965).
25. L. H. M. Janssen and S. H. de Bruin, *Int. J. Peptide Protein Res.* **5**, 27 (1973).
26. J. M. Baldwin, *Prog. Biophys. molec. Biol.* **29**, 227 (1975).
27. M. M. Rubin and J.-P. Changeux, *J. molec. Biol.* **21**, 265 (1966).
28. G. G. M. van Beek, E. R. P. Zuiderweg and S. H. de Bruin, *Eur. J. Biochem.* **99**, 379 (1979).
29. U. Kragh-Hansen, *Pharmac. Rev.* **33**, 17 (1981).
30. I. Sjöholm, A. Kober, I. Odar-Cederlöf and O. Borgå, *Biochem. Pharmac.* **25**, 1205 (1976).
31. A. A. Spector, *J. Lipid Res.* **16**, 165 (1975).
32. J. Jacobsen, *FEBS Lett.* **5**, 112 (1969).
33. R. F. Chen and V. J. Koester, *Analyt. Biochem.* **105**, 348 (1980).
34. Y. Abdo, J. Rousseaux and M. Dautrevaux, *FEBS Lett.* **131**, 286 (1981).
35. G. Wilding, B. S. Blumberg and E. S. Veseli, *Science* **195**, 991 (1977).
36. J. H. M. Dröge, L. H. M. Janssen and J. Wilting, *Biochem. Pharmac.* **31**, 3775 (1982).
37. M. Sogami and J. F. Foster, *J. biol. Chem.* **238**, PC 2245 (1963).
38. J. F. Foster, in *The Plasma Proteins*, (Ed. F. W. Putnam), Vol. 1, p. 179. Academic Press, New York (1960).
39. J. J. Lima and L. B. Salzer, *Biochem. Pharmac.* **30**, 2633 (1981).
40. K. M. Piafsky and O. Borgå, *Clin. Pharmac. Ther.* **22**, 545 (1977).