

apy has been overestimated because physiological drug concentrations are generally lower than the concentrations used in *in vitro* studies. Goolkasian et al. (197), who studied the displacement of lidocaine, concluded from their results that a clinically significant displacement interaction of the drugs studied occurs only when bupivacaine and lidocaine are used in combination. McNamara et al. (343) reported earlier that clinical concentrations of bupivacaine, disopyramide, and quinidine increase the lidocaine concentrations. Mueller et al. (364), who studied the drug displacement between psychotropics, concluded that competition phenomena *in vivo* may occur for methaqualone and thioridazine. Further binding studies in plasma using several combinations of drug will probably give more information about the clinical relevance of these competition phenomena *in vivo*.

B. Binding of Basic and Neutral Drugs to Alpha-1-acid Glycoprotein *in Vitro*

The binding of drugs to AGP in plasma has been discussed in section IV A. A different approach to the study of drug-protein interaction can be followed by first isolating the binding protein from plasma, redissolving the isolated protein in an appropriate solvent (generally an aqueous buffer solution), and using this protein solution for binding experiments. Studies of this type performed with AGP or HSA will be referred to as isolated AGP or isolated HSA binding studies, in order to distinguish them from the binding studies in plasma. Sometimes a mixture of AGP and HSA was used. Results of studies of this type done on isolated AGP or HSA are collected in table 11. In columns 4 and 5 of table 11, the free fractions measured in solutions of isolated AGP or HSA at variable concentrations (F_{AGP} and F_{HSA} , respectively) are reported so that the contribution that each of these proteins makes to the total plasma binding can be estimated. In columns 6 to 9 of table 11, the number of binding sites (n_{AGP} and n_{HSA}) and the binding constants (K_{AGP} and K_{HSA}) of drugs for isolated AGP and HSA are given.

One of the main purposes of this table is to collect data scattered throughout the literature. For a given drug, the combined *in vivo* and *in vitro* data can give a picture of the importance of protein binding. Because of the vast amount of data available, it is not possible to discuss the various compounds. Only some general comments will be made.

In a comparison of the binding parameters of the same drug obtained in different studies, it should be noted that the AGP samples were obtained using different methods and that these can have different effects on the physical-chemical properties of AGP, as discussed in section II. It should also be pointed out that, since the AGP concentrations used to determine the free fraction were not the same, different values for the free fraction may result. From studies on binding in solutions of AGP, it follows

that often two classes of binding sites are present on AGP. Therefore the *in vitro* results cannot be compared indiscriminately with the results obtained in plasma reported in section IV A.

Other factors influencing the binding parameters are discussed below in section IV E.

C. Binding of Acidic Drugs to Alpha-1-acid Glycoprotein *in Vitro*

It is generally assumed that in plasma acidic drugs are mainly bound to HSA. Four recent studies (249, 462, 544, 545) have shown, however, that the association constants of some acidic drugs to AGP are high enough to indicate that binding to AGP will contribute significantly to the total plasma binding of these drugs, especially in diseases in which the concentration of AGP increases and/or of HSA decreases.

The parameters describing the binding of acidic drugs to isolated AGP are summarized in table 12. Israili and El-Attar (249) found that the binding to AGP increased with increasing concentration of AGP and decreasing concentration of the drugs (therapeutic range). The maximum binding of each drug to AGP (at 200 mg/100 ml) was, however, always lower than the binding to HSA (at 4.5 g/100 ml).

Urien et al. (545) studied several acidic drugs with or without a carboxylic group and found that clofibrate, fenofibrate, salicylic, and valproic acid do not bind to AGP, and that benoxaprofen, indomethacin, and itanoxone at a molar drug/AGP ratio of 0.04 (AGP concentration, 90 mg/100 ml) bind very poorly (table 12). In contrast, the percentages of bound warfarin, acenocoumarol, and phenylbutazone are noticeably higher. The acidic drugs which exhibit a high or intermediate affinity to AGP do not exhibit any carboxyl moiety and share a common specific binding site on HSA, called site I or the warfarin site. By contrast, all the drugs having a poor affinity or no affinity to AGP exhibit carboxyl groups and bind specifically to another HSA binding site, called site II or the diazepam site (527). Moreover, these results demonstrate the existence of only one binding site on AGP, which is the result found earlier for basic drugs. For these acidic drugs, Urien et al. (545) made some calculations to estimate the relative contribution of the drug bound to HSA and AGP in plasma. Acenocoumarol, phenylbutazone, and warfarin would then bind for 90%, 99.3%, and 94%, respectively, to HSA and for 9%, 0.3%, and 5%, respectively, to AGP, whereas the sum of the binding to HSA and AGP is close to the value observed in human plasma.

Comparing the results of Urien et al. (545) and Israili and El-Attar (249), one can conclude that Urien et al. found a much higher affinity of phenylbutazone for AGP than Israili and El-Attar, presumably because Urien et al. used AGP samples from different origins, which led to different binding parameters as will be discussed later in section IV E.

TABLE II
Survey of binding parameters for the binding of basic and some neutral drugs with isolated AGP and HSA

No.	Drug	Origin of AGP	F_{AGP}	F_{HSA}	n_{AGP}	K_{AGP} [M^{-1}]	n_{HSA}	K_{HSA} [M^{-1}]	Ref.
I	Alfentanil	MiHes	AGP concentration dependent, 0.20 → 0.06 (50 → 200 mg/100 ml)	HSA concentration dependent, 0.97 → 0.65 (0.1 → 6 g/100 ml)					348
II	Alprenolol	Behringwerke, 57 mg/100 ml	0.45	0.60					75
		Behringwerke, 66 mg/100 ml							42, 43
III	Amitriptyline	Behringwerke	$F_{AGP + HSA} = 0.29$ (same range as in serum)			Two sites 0.97 and 1.94	6.16	3.7×10^7	83
		Behringwerke	0.165	0.146		3.4×10^5 and 1.3×10^6			82
		Gift from others	$F_{AGP + HSA} = 0.10$ $F_{AGP + HSA + LPS} = 0.054$ 0.334	0.365					413
IV	Aprindine	Behringwerke	Drug concentration dependent, 0.13-0.73	0.15-0.18		Two sites 1.1 and 2.9	1	9.8×10^6	524
		Own preparation using modification of method of Hsu and Wickerhauser (217)	$F_{AGP + HSA} = 0.06 - 0.16$ (same range as in serum)			In solution of mixture of HSA and AGP pH dependent pH 7.4 → 7.0 0.45 → 3.53	0.98	1×10^6 pH independent pH 7.4 → 7.0 $(6.3 \rightarrow 0.146) \times 10^6$ $(8.98 \rightarrow 4.37) \times 10^6$	140
V	Bupivacaine	Behringwerke	Concentration dependent, 0.14 → 0.69 (50 → 200 mg/100 ml)	0.27					404
		Not mentioned							410
		AGP (200 mg/100 ml) added to plasma (1)							410
VI	Carbamazepine (neutral drug)	Not mentioned	Concentration dependent, 0.71 → 0.90 (150 → 500 mg/100 ml)						339

Decrease from 0.16 to 0.14 after addition of isolated AGP to plasma

Agree with those calculated in plasma

0.50 (100 → 500 mg/100 ml)

Class	Drug	Concentration dependent	Concentration dependent, 0.60 → 0.88 (100 → 50 mg/100 ml)	Concentration dependent, 0.08-0.80	1.44 → 1.36, 0.66 → 0.52 (4 → 2 g/100 ml)	1.7 × 10 ⁴	7.5 → 7.33	4 × 10 ³	340
VII	Chlorpromazine	Sigma	Drug concentration dependent, 0.08-0.80	0.20	0.20	0.5 and 1	Two sites 9.4 × 10 ³	≥ 1	551
		Miles, defatted					5 × 10 ³ 3.4 × 10 ³		282
VIII	Ciclazindol	Behringwerke	0.076	0.41		0.83	3 × 10 ³ 3.45 × 10 ³		158 363
LX	Desmethylinprimine	Miles	0.25	0.38		1.3	4.7 × 10 ³ 6.3 × 10 ³	6.8	520 254, 265 482
X	Diazepam (neutral drug ⁽¹⁾)	Behringwerke	0.32			1			
		Own preparation and defatted by charcoal	0.81						282
		Not mentioned							5
		Not mentioned	Decreases after administration of AGP to HSA solution from 0.0155 → 0.0109	HSA concentration dependent, 0.013 → 0.024 (5 → 2.3 g/100 ml)					442
XI	Dipyridamole	Behringwerke					Two sites 1.55 × 10 ³ and 4 × 10 ³		167
		Own preparation				0.9 and 1	6.25 × 10 ³		514
		Own preparation				1	8 × 10 ³	$\alpha_{HSA}, K_{HSA} = 5.7 \times 10^4$	281
XII	Disopyramide	Sigma	Drug concentration dependent, 0.14-0.75			0.2	1.0 × 10 ³		313
		Not mentioned	Drug concentration dependent, 0.35-0.97	Drug concentration dependent, 0.81-0.98		0.256 and 0.506	Two sites 8.84 × 10 ³ and 2.43 × 10 ³		133
		Sigma	AGP concentration dependent, 0.15 → 0.76 (140 → 40 mg/100 ml)						315
		Sigma (600 mg/100 ml ⁽¹⁾)				0.02(?)	Stereoselective R(-) = 3.12 × 10 ³ S(+) = 5.9 × 10 ³ Racemic = 5.2 × 10 ³ 9.5 × 10 ³		317