

TABLE 8

No.	Drug	Disease	AGP range (mg/100 ml)	<i>F</i> range	B/F	$\frac{^{14}C\text{-AGP}}{K_{app}} \times 10^{-3}$ [M <sup>-1</sup> ]	Ref.
XXVI	Lidocaine	Trauma, in four patients observed	194-298	0.22-0.12	$y = 0.029x - 1.83$ $r = 0.92$	1.15	155, fig. 4
			112-230	0.31-0.18	$y = 0.017x + 0.27$ $r = 0.78$	0.69	
			136-244	0.31-0.15	$y = 0.027x - 1.17$ $r = 0.96$	1.08	
			120-196	0.31-0.16	$y = 0.040x - 2.88$ $r = 0.99$	1.62	
XXVII	Mehtadone	Healthy people	28-120	0.18-0.07	$y = 0.054x + 5.21$ $r = 0.68$	2.18	436, fig. 1b, transformed
XXVIII	Mehtadone	Arthritis, hypoalbuminemia, healthy people	26-160	0.21-0.04	$y = 0.063x + 4.13$ $r = 0.70$	3.52	436, fig. 2, transformed
XXIX	Mehtadone	Cancer	41-251	0.25-0.06	$y = 0.036x + 1.25$ $r = 0.76$	1.89	7, fig. 1
XXX	Meperidine	Pregnancy (maternal/fetal plasma)	18.8-68.8	0.52-0.31	$y = 0.015x + 0.91$ $r = 0.60$	0.60	367, fig. 1
XXXI	Nortriptyline	Depressive patients	25-148	0.14-0.02	$y = 0.169x - 2.60$ $r = 0.49$	6.78	529, fig. 2, transformed
XXXII	Nortriptyline	Depressive patients	19-118 (concentration of S-variant of AGP)	0.12-0.02	$y = 0.316x - 1.77$ $r = 0.77$	12.6	580, fig. 4, transformed
→ Correlation with S-variant of AGP more evident than with total plasma AGP							
XXXIII	Perazine	Schizophrenic patients	45-124	0.044-0.029	$y = 0.091x + 19.1$ $r = 0.63$	3.66	465, fig. 3, transformed
XXXIV	Perazine	Psychiatric patients	67-163	0.08-0.03	$y = 0.122x + 9.08$ $r = 0.79$	4.87	84, fig. 2, transformed
XXXV	Perazine	Healthy people	53-140	0.06-0.03	$y = 0.086x + 14.8$ $r = 0.38$	3.43	84, fig. 2, transformed
XXXVI	Pranosin	Healthy people	64-284(?)	0.10-0.025	$y = 0.149x + 2.55$ $r = 0.97$	5.74	130, fig. 4B
XXXVII	Prednisolone (neutral drug)	Isolated AGP (II)	26-500	0.98-0.58	$y = 0.001x + 0.074$ $r = 0.91$	0.04	353, fig. 7
XXXVIII	Propranolol	Pregnancy (mother/newborn) oral contraceptives used	4-64	0.45-0.12	$y = 0.069x + 2.03$ $r = 0.68$	2.77	585, fig. 3
XXXIX	Propranolol	Elderly patients with acute illness	44-213	0.25-0.02	$y = 0.118x - 0.91$ $r = 0.85$	4.6	397, fig. 1
XL	Propranolol	Acute myocardial infarction	24-184	0.14-0.04	$y = 0.072x + 7.39$ $r = 0.66$	2.89	440, fig. 1
XLI	Propranolol	Healthy people	48-204(?)	0.12-0.05	$y = 0.064x + 5.40$ $r = 0.85$	2.56	450, fig. 4
XLII	Propranolol	Healthy people, renal failure, arthritis, Crohn's disease, cirrhosis	40-268	0.18-0.03	$y = 0.071x + 3.80$ $r = 0.77$	2.85	409, fig. 1, transformed
XLIII	Propranolol	Healthy people, elderly patients with acute illness	45-296	0.22-0.02	$y = 0.095x - 0.02$ $r = 0.86$	3.81	398, fig. 1
XLIV	Propranolol	Healthy people, elderly patients with acute illness	46-272	0.22-0.03	$y = 0.086x + 0.02$ $r = 0.88$	3.85	398, fig. 3
XLV	Propranolol	Smoking effect	43-100	0.20-0.09	$y = 0.081x + 0.92$ $r = 0.73$	3.26	46, fig. 1
XLVI	Propranolol	Obesity	36-122	0.15-0.07	$y = 0.058x + 3.67$ $r = 0.88$	2.32	48, fig. 1
XLVII	Propranolol	Cancer and its treatments	41-266	0.28-0.06	$y = 0.053x + 0.45$ $r = 0.93$	2.1	8, fig. 1
XLVIII	Propranolol	Healthy people	23-132	0.14-0.02	$y = 0.175x + 5.50$ $r = 0.67$	7	16, fig. 3

TABLE 8—Continued

No.	Drug	Disease	AGP range (mg/100 ml)	F range	B/F	$n_{AGP}K_{AGP} \times 10^{-4} (M^{-1})$	Ref.
IL	Propranolol	Moderately obese male subjects	45-190	0.15-0.08	$y = 0.030x + 4.43$ $r = 0.73$	1.20	47, fig. 1
L	Propranolol	Arthritis, disseminated lupus, cancer, bacterial infection	90-410	0.11-0.02	$y = 0.185x + 0.83$ $r = 0.88$	5.4	107, fig. 4
I.I	Propranolol	Isolated AGP (!)	20-400	0.50-0.07	$y = 0.028x - 0.05$ $r = 0.97$	1.13	107, fig. 1
LII	Propranolol	Isolated AGP in presence of HSA (4 g/100 ml) (!)	20-400	0.17-0.02	$y = 0.096x + 3.69$ $r = 1.0$	3.84	107, fig. 1
— isolated AGP does not behave like AGP in serum; HSA potentiates binding to AGP; extent of potentiation depends on lipids associated with AGP and HSA							
LIII	Propranolol	Malnutrition	45-200	0.30-0.07	$y = 0.039x + 1.60$ $r = 0.71$	1.56	252, fig. 1, transformed
LIV	Quinidine	Healthy people	48-208(?)	0.39-0.15	$y = 0.017x + 1.71$ $r = 0.86$	0.67	377, fig. 3
LV	Quinidine	Traumatic injury	113-300	0.11-0.06	$y = 0.051x + 2.94$ $r = 0.88$	2.04	154, fig. 3
LVI	Triazolam	Patients on dialysis	72-205	0.15-0.06	$y = 0.051x + 3.21$ $r = 0.82$	2.05	290, fig. 1
LVII	Verapamil	Liver disease	14-58	0.23-0.07	$y = 0.174x + 8.72$ $r = 0.80$	7.09	187, fig. 3b
LVIII	Verapamil	Healthy people	40-130	0.11-0.06	$y = 0.046x + 6.85$ $r = 0.79$	1.85	328, fig. 1
LIX	Verapamil	Isolated AGP (!)	0-150	0.44-0.06	$y = 0.082x + 0.95$ $r = 0.99$	3.29	338, fig. 2
LX	Zimelidine	Depressed patients	38-106	0.13-0.05	$y = 0.113x + 3.11$ $r = 0.73$	4.5	101, fig. 3, transformed
LXI	Norzimelidine	Depressed patients	39-107	0.32-0.23	$y = 0.012x + 1.75$ $r = 0.69$	0.46	101, fig. 3, transformed

\* F, free fraction; B, bound fraction; B/F, binding ratio (under this heading the relation is given between B/F and the AGP concentration, as expressed by equation 6); y, B/F; x, AGP concentration in mg/100 ml; r, correlation coefficient;  $n_{AGP}$ , number of binding sites on AGP;  $K_{AGP}$ , association constant of drug for AGP; the value of  $n_{AGP}K_{AGP}$  is obtained from equation 6 and given in  $M^{-1}$ , assuming a molecular weight of 40,000.  
 † !, study done with isolated AGP (in vitro) instead of plasma/serum (in vivo); ?, very high value reported for AGP in serum of normals.

It is not possible to discuss all relationships in table 8 in detail. Instead, in table 9 the data relating to propranolol have been given. Propranolol was chosen because many studies have been done on this compound. Only those studies having  $r^2 \geq 0.5$  were taken from table 8. The F test of significance indicates a higher than 99% probability for these relationships. If one takes into account that these data originate from different sources and refer to different diseases, the correspondence in the slope of the various linear relationships is remarkable. According to equation 6, this slope represents  $n_{AGP}K_{AGP}$ , which should be constant as long as the molecular properties of the AGP have not changed. So despite the fact that heterogeneity can be expected due to the diseases (see section II D), it seems that this heterogeneity does not strongly influence the binding constants of propranolol.

The value of the constant term in the linear relationships in this column of table 9 represents the contribution of HSA and LIPO to B/F. This varies in the various cases described. According to equation 6, this is due to

variation in the concentration and number of binding sites of HSA and LIPO.

The linear relationships also allow us to calculate the contribution of AGP to the fraction bound. Examples are given in table 9. By substituting a given AGP concentration in the linear relationship (second column), the corresponding B/F, and therefore B value, can easily be calculated. This has been done for three values of the AGP concentration, namely, zero, 73, and 219 mg/100 ml. Note that the average value of the AGP concentration in normal conditions (see section III A) is 73 mg/100 ml. In comparing these B values, one should take the standard errors into account. It is then evident that the calculated values of B as predicted from the various studies are very consistent, as can be seen from the last three columns in table 9.

The linear relationships further permit one to calculate the contribution of AGP to the total binding in the following way. The value of  $(B/F)_{AGP}$  can be calculated for a given value of the AGP concentration. Dividing this by the value of the total B/F gives a number which

represents the fraction of the total drug bound that is accounted for by AGP. The last column in table 9 gives some numbers for an AGP concentration of 73 mg/100 ml. The results of the various studies show that AGP accounts for more than 50% of the binding.

In table 10 the binding parameters in plasma for AGP and HSA are summarized. These data were obtained from the fitting of Scatchard plots measured in plasma on the assumption of the presence on AGP and HSA of two classes of binding sites, one with a high affinity and a low capacity and the other one with a low affinity and a high capacity. From these parameters too it is possible to estimate the relative contribution of AGP and HSA to the total plasma binding. This method is a different way of approaching the problem discussed in this section. However, as only a very limited number of studies have been reported, a detailed comparison cannot be made.

Pike et al. (412, 414, 415) and Suzuki et al. (517) used plasma that was deficient in several plasma protein fractions in order to study the binding of acidic, neutral, and basic drugs in plasma. They found that the binding of basic drugs decreased considerably in AGP-deficient plasma. A decrease was observed for acidic and neutral drugs only in HSA-deficient plasma in accordance with the evidence presented above.

It is clear that the data presented in this section confirm that AGP makes an important contribution to the binding of many drugs.

Displacement studies have been performed in order to obtain information about the possibility of clinically relevant competition phenomena in vivo (158, 197, 337, 343, 362-364, 401, 460-465, 544, 581, 588). McElnay and D'Arcy (337) reported recently that the clinical importance of drug displacement during combined drug ther-

TABLE 9  
Further analysis of propranolol binding data from table 8

No.*	$y = ax + b†$	F test of significance	$r^2$	$\frac{B}{[AGP]} - 0$	$\frac{R}{[AGP]} = 73$ mg/100 ml	$\frac{H}{[AGP]} = 219$ mg/100 ml	Fraction of bound drug, bound by AGP at $[AGP] = 73$ mg/100 ml
XXXIX	$y = 0.118 (0.007)x - 0.91 (0.86)$	1,102 = 285	0.74		0.88 (0.01)	0.96 (0.003)	1.12 (0.16)
XLI	$y = 0.064 (0.009)x + 5.40 (1.10)$	1,19 = 48	0.72	0.84 (0.03)	0.91 (0.01)	0.96 (0.005)	0.46 (0.09)
XLII	$y = 0.071 (0.007)x + 3.80 (0.80)$	1,78 = 111	0.59	0.79 (0.03)	0.90 (0.01)	0.95 (0.004)	0.58 (0.08)
XLIII	$y = 0.095 (0.007)x - 0.01 (0.086)$	1,55 = 185	0.77	0.00 (1.08)	0.87 (0.02)	0.95 (0.004)	1.00 (0.16)
XLIV	$y = 0.096 (0.008)x - 0.02 (0.81)$	1,40 = 138	0.78	0.00 (1.27)	0.87 (0.02)	0.95 (0.004)	1.00 (0.16)
XLVI	$y = 0.068 (0.010)x + 3.67 (0.93)$	1,9 = 31	0.78	0.79 (0.04)	0.89 (0.01)	0.94 (0.008)	0.54 (0.12)
XLVII	$y = 0.053 (0.004)x + 0.45 (0.56)$	1,21 = 137	0.87	0.31 (0.27)	0.81 (0.02)	0.92 (0.006)	0.90 (0.15)
L	$y = 0.135 (0.019)x + 0.63 (3.39)$	1,14 = 51	0.79	0.39 (1.28)	0.91 (0.03)	0.97 (0.006)	0.94 (0.35)
LI	$y = 0.028 (0.002)x + 0.05 (0.41)$	1,12 = 216	0.95	0.05 (0.37)	0.68 (0.05)	0.86 (0.012)	0.98 (0.21)
LII	$y = 0.096 (0.002)x + 3.59 (0.42)$	1,11 = 2,224	0.99	0.78 (0.03)	0.91 (0.00)	0.96 (0.001)	0.68 (0.03)
LIII	$y = 0.039 (0.006)x + 1.60 (0.68)$	1,38 = 38	0.50	0.61 (0.10)	0.82 (0.03)	0.81 (0.012)	0.64 (0.15)

\* The numbers in the first column refer to the compounds in table 8.

† In the second column,  $y$  represents  $B/F$  and  $x$  represents the AGP concentration in mg/100 ml. Numbers in parentheses in this and in other columns in this table represent the standard error in this parameter. The standard error in the value of  $B$ , denoted by  $S_B$ , follows from the relationship  $S_B = (1 + y)^{-2} \cdot S_y$ .

TABLE 10  
Survey of binding parameters for AGP and HSA in plasma

No.	Category	Drug	$n_{AGP}P_{AGP}^*$	$K_{AGP} [M^{-1}]†$	$n_{HSA}P_{HSA}$	$K_{HSA} [M^{-1}]$	Ref.
I	Anesthetic	Bupivacaine		pH 7.4 → 7.0 pH dependent $(1.56 \rightarrow 2.14) \times 10^{-6}$		pH 7.4 → 7.0 pH independent $(4.21 \rightarrow 5.03) \times 10^2$	140
II	Tricyclic antidepressant	Amitriptyline	$1.6 \times 10^{-4}$	$5.9 \times 10^4$	$4.1 \times 10^{-3}$	$7.3 \times 10^2$	83
III	Tricyclic antidepressant	Nortriptyline	$3.6 \times 10^{-4}$	$1.8 \times 10^4$	$1.8 \times 10^{-3}$	$1.4 \times 10^2$	83
IV	Tranquilizer	Thioridazine		$6.39 \times 10^7$			381
V	Beta-blocker	Alprenolol		$(3-5) \times 10^6$			236
VI	Beta-blocker	Oxprenolol	$8 \times 10^{-4}$	$1.3 \times 10^6$			44
VII	Beta-blocker	Propranolol					412 414
VIII	Beta-blocker	Propranolol					557
IX	Beta-blocker	Propranolol	$2.04 \times 10^{-4}$	$5.87 \times 10^4$			44
X	Antiarrhythmic	Quinidine					412
		Quinidine	$3.49 \times 10^{-6}$	$1.17 \times 10^5$	$3.14 \times 10^{-3}$	$1.38 \times 10^2$	164
XI	Antiepileptic	Carbamazepine	2.2	$2.4 \times 10^4$	9	$4.6 \times 10^2$	340

\*  $n_{AGP}P_{AGP}$  and  $n_{HSA}P_{HSA}$ , binding capacity to AGP and HSA, respectively, in plasma, using Scatchard plots.

†  $K_{AGP}$  and  $K_{HSA}$ , affinity constant to AGP and HSA, respectively, in plasma, calculated from Scatchard plots.