Plasma Protein Binding of Drugs as a Function of Age in Adult Human Subjects

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Abstract □ The intent of this study was to determine what influence, if any, increasing age has on the binding of drugs by plasma proteins. Plasma from healthy subjects ranging in age from 21 to 94 years was used. The binding of phenytoin (diphenylhydantoin) (acid), penicillin G potassium (benzylpenicillin potassium), and phenobarbituric acid was determined by equilibrium dialysis of ^{14}C-labeled compounds. No differences were found in total protein concentration; however, albumin was reduced in subjects over 50 years of age. Plasma binding of each drug studied was not related to age; this finding suggests that age *per se* is not a factor in the binding of drugs by plasma proteins.

Keyphrases □ Plasma protein binding—drugs, effect of age, adult human subjects ■ Binding—drugs to plasma protein, effect of age, adult human subjects □ Age—effect on binding of drugs to plasma proteins, human subjects

Several years ago, Ehrnebo *et al.* (1) reported that human fetal and neonatal plasma has a lower binding capacity for drugs than plasma from young healthy adult subjects. The investigators attributed the lower binding capacity to lower total protein and albumin concentrations in fetal and newborn plasma. They also implied that qualitative differences in protein binding may have contributed to the decrease in binding capacity.

From a practical viewpoint, the decreased drug binding capacity in the newborn means that more free drug is available at a given total drug concentration in the plasma. Therefore, there exists the potential for an increase in drug activity and the incidence of undesirable effects.

Elderly patients exhibit an increased sensitivity to drugs (2–4), which in some cases has been shown to be related to higher and more prolonged blood drug levels brought about by a slower rate of drug metabolism and elimination in older patients (5–7). The objectives of the present study were to assess the influence of increasing age on the binding of drugs by plasma proteins and, thus, to determine whether or not altered drug binding could potentially contribute to the enhanced effect of drugs in geriatric patients.

Table I—Concentrations and Specific Activities of Drugs Used in Determining Drug Binding Capacity of Human Plasma

Drug	Concentration, μg/ml	Specific Activity, μCi/mg
Phenytoin (acid)		
Day 1	16.87	0.82
Day 2[a]	12.93	2.92
Phenobarbituric acid		
Day 1[b]	20.21	4.14
Penicillin G potassium		
Day 1[b]	13.78	2.43

[a] A fresh solution was prepared for the analysis of a second batch of human plasma. [b] The same solution was used with succeeding batches of human plasma on different days. The solution was stored at −10° when not in use.

Table II—Total Protein, Globulin, and Albumin Concentrations, A/G Ratio, and Other Laboratory Parameters of Plasma from Normal Subjects

	Subjects Less than 50 Years of Age (n = 14, Mean = 29 Years)	Subjects Greater than 50 Years of Age (n = 10, Mean = 63.5 Years)
Total protein, g/100 ml	7.1 ± 0.10[a]	7.0 ± 0.10
Globulin, g/100 ml	3.1 ± 0.08	3.5 ± 0.16[b]
Albumin, g/100 ml	4.0 ± 0.06	3.4 ± 0.09[c]
A/G ratio	1.3 ± 0.04	1.0 ± 0.06[c]
Blood urea nitrogen, mg/100 ml	14.5 ± 0.44	16.5 ± 1.16
Serum glutamic pyruvic transaminase, I.U.[d]	13.5 ± 1.03	11.7 ± 1.25
Serum glutamic oxaloacetic transaminase, I.U.	16.5 ± 1.21	15.0 ± 1.68
Alkaline phosphatase, I.U.	54.0 ± 2.84	58.0 ± 4.30

[a] Mean and standard error. [b] Significant difference at $p < 0.05$. [c] Significant difference at $p < 0.01$. [d] I.U. = international units.

EXPERIMENTAL

Plasma Samples—Blood was obtained by venipuncture from 25 healthy subjects ranging in age from 21–94 years. The subjects had not received any drug for at least 1 week prior to the time blood was withdrawn. All blood samples were heparinized and centrifuged, and the red cell-free plasma was stored at −10°. Prior to analysis, the plasma was thawed at 4° and then allowed to reach room temperature. Ehrnebo *et al.* (1) showed that freezing and thawing plasma samples do not affect the drug binding capacity of plasma.

Clinical Chemistry—Total protein[1], serum glutamic oxaloacetic transaminase[1], serum glutamic pyruvic transaminase[1], alkaline phosphatase[1], globulin[2], and blood urea nitrogen[2] levels were determined using commercially available procedures. Plasma albumin concentration was calculated as the difference between the total protein and globulin concentrations.

Radioactive Compounds—5,5-Diphenylhydantoin-4-^{14}C as the acid[3] (specific activity, 5.21 mCi/mmole), phenobarbituric acid-2-^{14}C[3] (specific activity, 3.34 mCi/mmole), and potassium 6-phenyl-1-^{14}C-acetamidopenicillinate[4] (specific activity, 24.7 mCi/mmole) were obtained commercially. All radiochemical purities were reported by the suppliers to be greater than 98%.

Because of the high specific activities of these compounds, it was necessary to dilute them with their respective nonradioactive carriers. Both the labeled and nonlabeled compounds were diluted in pH 7.38 buffer (1) to a suitable concentration that was amenable

[1] Smith Kline Instruments Inc., Palo Alto, Calif.
[2] Dow Chemical, Midland, Mich.
[3] New England Nuclear Corp., Boston, Mass.
[4] Amersham-Searle, Arlington Heights, Ill.

Table III—Drug Binding, Total Protein Concentration, and Albumin Concentration of Plasma from Normal Subjects[a]

Drug	Subjects Less than 50 Years of Age				Subjects Greater than 50 Years of Age			
	Patients	Total Protein, g/100 ml	Albumin, g/100 ml	Percent Drug Bound	Patients	Total Protein, g/100 ml	Albumin, g/100 ml	Percent Drug Bound
Phenytoin (acid)	6	7.0 ± 0.08[b]	4.0 ± 0.07	82.4 ± 0.84	3	7.3 ± 0.18	3.4 ± 0.18	83.6 ± 0.35
Phenobarbituric acid	5	7.3 ± 0.23	4.1 ± 0.09	41.8 ± 1.33	3	6.8 ± 0.12	3.4 ± 0.22	41.9 ± 2.07
Penicillin G potassium	5	7.0 ± 0.16	3.9 ± 0.13	42.4 ± 3.00	4	6.9 ± 0.15	3.5 ± 0.15	45.1 ± 3.62

[a] With one exception (plasma from 21-year-old subject), plasma samples were subjected to a single drug binding experiment. Not included are data from a 94-year-old subject with a serum albumin of 1.3 g/100 ml, elevated serum glutamic oxaloacetic transaminase, and alkaline phosphatase levels. These data are referred to in the text. [b] Mean and standard error.

to the drug binding experiment. The concentrations and specific activities are listed in Table I.

Equilibrium Dialysis—Equilibrium dialysis was carried out in two-compartment Lucite cells[5]. The compartments on each side of the dialysis membrane measured 17.46 mm in diameter and 3.98 mm in depth. The volume of each compartment was approximately 0.87 ml. The viscose membrane[6] had a thickness of 0.203 mm with an average pore size of 4.8 mμ. This type of membrane retains components in aqueous solutions with molecular weights of 12,000 and higher. Prior to its use, the membrane was soaked overnight in deionized water and the excess water was removed before placing it into the apparatus.

A plasma sample, 0.87 ml, was placed into one compartment of the cell and the labeled drug dissolved in the buffer was placed into the other. The cell was sealed with tape and attached to an apparatus described in USP XVIII (8). This device raised and lowered the dialysis cell 30 times/min in a 37 ± 1° water bath for 6 hr. This time period[7] was the same as was used previously (1).

Assay of Drugs—Liquid scintillation counting of the radioactivity was used to determine the amount of labeled drug in the plasma and buffer solutions. Exactly 0.300 ml of each solution was removed, and 0.6 ml of a 25% aqueous tetramethylammonium hydroxide solution was added to solubilize the protein in the plasma aliquot. The solutions were allowed to digest overnight at room temperature; then 15 ml of a liquid scintillator solution[8] was added, shaken, and counted[9] for 10 min. The absolute disintegration rate was obtained by determining the quench effect by conventional internal standardization technique with hexadecane-^{14}C.

Calculation of Binding Capacity—Equation 1 (1) was used to determine the percentage of bound drug:

$$\% \text{ bound} = \frac{[C_{plasma} - C_{buffer}] \times 100}{C_{plasma}} \qquad \text{(Eq. 1)}$$

where C_{plasma} = concentration of drug in the plasma compartment after dialysis, and C_{buffer} = concentration of drug in the buffer compartment after dialysis.

RESULTS AND DISCUSSION

With the exception of elevated serum glutamic oxaloacetic transaminase and alkaline phosphatase levels in a 94-year-old subject, results of the clinical laboratory studies reveal no abnormal or significant findings (Table II) and thus confirm that the subjects were healthy and free of complicating disease. Data for this subject are not included in the statistical analysis but are discussed in reference to the relationship of drug binding capacity to plasma albumin levels.

In Table II, mean values are given for total protein, globulin (G), and albumin (A) concentrations along with the A/G ratio for subjects of different ages. These data show that plasma total protein is not altered by increasing age, while plasma albumin is significantly reduced in patients over 50 years of age with a corresponding increase in plasma globulin. The calculated A/G ratio is decreased from 1.3 for patients under 50 years of age to 1.0 for patients 50 years and over. These results are consistent with data reported by Libow (9). In this latter study, a total protein concentration of 7.2 g/100 ml was reported for healthy subjects 18–36 years of age and a value of 6.9 g/100 ml was given for healthy subjects 65–92 years of age. This difference was not statistically significant. Serum albumin, however, was significantly reduced in the older subjects. In the young group, the albumin concentration was 3.8 g/100 ml; in older subjects, the value was 3.3 g/100 ml.

The plasma binding values reported here are lower than those reported previously (1); however, the significance or explanation of this difference is difficult to judge since the specifications for the membrane used in the earlier investigation were not given. It has been pointed out that it is difficult to interpret the results of binding studies in which a single drug concentration was used, as was the case in this study. However, Shah *et al.* (10) showed that the percentage of phenytoin (diphenylhydantoin) bound to plasma protein is constant for a wide range of concentrations (1–40 μg/ml). Nevertheless, these issues, although of interest certainly to future studies in this area, do not affect the interpretation of the current results since the interest here was to establish the influence of age on the *relative*, not necessarily the *absolute*, drug binding capacity of plasma.

In Table III, the binding of each drug is compared for subjects under 50 years of age and those over 50 years of age. This comparison was made because after age 50 there was a significant reduction in serum albumin levels. Despite a reduction in plasma albumin concentration, no changes were noted in plasma binding capacity. These data suggest that age *per se* does not influence the binding of drugs to plasma proteins. More specifically, they indicate that increasing age is not associated with: (*a*) a defect in drug binding proteins or (*b*) the production of an endogenous substance which competes with a drug for binding sites on the protein molecule. With a reduction in serum albumin and no change in drug binding, the amount of drug bound for a given amount of albumin is increased with age.

While the data from the normal subjects in this study show no change in plasma binding capacity, this finding does not rule out the potential for a reduction in binding capacity in elderly patients, as with any other patients whose plasma albumin and/or total protein may be lower than that encountered in this study. For example, Ehrnebo *et al.* (1) reported a reduction in drug binding capacity in the newborn associated with a significant reduction in both plasma albumin and total protein. Andreasen (11) found a reduced binding capacity associated with lower concentrations of albumin in surgical patients with acute renal failure.

Indeed, in the present study, in a single subject of 94 years of age (not included in Table III) with a plasma albumin level of 1.3 g/100 ml, the percentage of phenobarbital bound to plasma protein was reduced to 12.1%, a figure far below the mean value for all patients regardless of age. In two other 90-year-old subjects with plasma albumin levels above 3.0 g/100 ml, plasma protein binding of phenytoin and phenobarbital was the same as that found for younger subjects.

The results of a recent study (12) are relevant to this issue. The incidence of unwanted central nervous system depression with diazepam was significantly greater in patients with a plasma albumin level of less than 3.0 g/100 ml than in patients with a value of greater than 4.0 g/100 ml. Interestingly, age was not a factor.

[5] Fabricated at the Smith Kline & French Laboratories.
[6] Catalog No. EDR3787-D22, A. H. Thomas Co., Philadelphia, Pa.
[7] In other studies in these laboratories with other drugs, no statistical difference in binding determined after 6 and 12 hr of dialysis was observed.
[8] Omnifluor–Cabosil–naphthalene–absolute ethanol–dioxane (9.6 g:50 g:100 g:100 ml:1000 ml).
[9] Packard Tri-Carb model 3003.

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Activity of Local Anesthetic Agents in Goldfish

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Abstract □ The activity of procaine hydrochloride, lidocaine hydrochloride, tetracaine hydrochloride, and dibucaine hydrochloride in producing overturn in goldfish was measured in pH 8.0 buffer. Calculation of the apparent minimum effective concentration of local anesthetic necessary to result in overturn of the goldfish showed that the activity of these agents increased in the following order: procaine hydrochloride < lidocaine hydrochloride < tetracaine hydrochloride < dibucaine hydrochloride. The effect of these agents on goldfish can be correlated with previous work on the minimum concentration necessary to block conduction in isolated nerve and muscle fibers. The activity of the local anesthetic agents could be explained, in part, by the relationship between the chloroform–pH 8 buffer partition coefficient and the minimum effective concentration in goldfish. Experimental results indicate that the unionized drug molecule is responsible for the observed effects.

Keyphrases □ Anesthetics, local—activity of procaine hydrochloride, lidocaine hydrochloride, tetracaine hydrochloride, and dibucaine hydrochloride in producing goldfish overturn, structure–activity relationships □ Structure–activity relationships—local anesthetics, goldfish overturn □ Goldfish—model for determining structure–activity relationships of local anesthetics

Recent reports (1–3) indicated that goldfish may serve as a biological test model in the assessment of structure–activity relationships of drugs. The goldfish was an adequate test system for discerning structure–toxicity relationships of substituted phenothiazine derivatives (1), and the goldfish was used as a model to study the effects of alkyl chain length on the alkyl *p*-aminobenzoate-induced narcosis in the fish (2). The use of this simple and inexpensive biological test system to study the correlation between pharmacological and toxicological activity with physical–chemical properties has many advantages (1). The present report concerns preliminary findings as to the relationships that may exist between structure and activity of local anesthetic agents in goldfish.

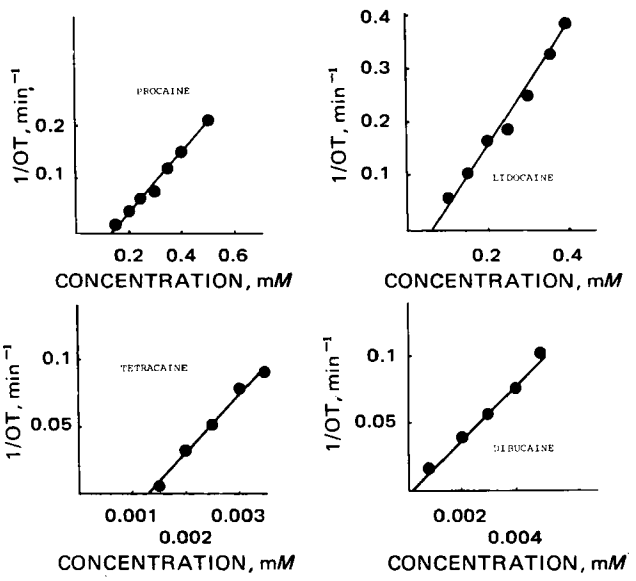

Figure 1—*Plot of the reciprocal of overturn time of local anesthetics in goldfish* versus *concentration of local anesthetic agent.*

EXPERIMENTAL

Goldfish, common variety (*Carassius auratus*), weighing 3–4 g were purchased locally. The overturn time (4) of individual goldfish in 100 ml of various concentrations of procaine hydrochloride[1], lidocaine hydrochloride[2], tetracaine hydrochloride[1], and dibucaine

[1] Amend Drug and Chemical Co., Livingston, N.J.

[2] Astra Pharmaceuticals, Worcester, Mass.