multiplying the ampul solution volume[9] times the respective solute concentration.

To gain a perspective on the relative amounts of amygdalin and the subject hydrolysis products in a given ampul, the amounts of amygdalin in the respective ampuls also were determined by the previously reported procedure (1).

The determination of 2-propanol was based on a GLC procedure with a porous polymer[10] column coupled to a flame-ionization detector. Aliquots of 0.50 ml from each ampul were each mixed with 0.50 ml of an internal standard solution[11] before being injected. The results derived were compared to a working curve obtained from standard solutions containing known amounts of 2-propanol and 2-butanol in water. The amount of 2-propanol in each ampul was calculated by multiplying its concentration times the ampul solution volume.

The amounts of amygdalin, amygdalinamide, amygdalin acid, and 2-propanol per ampul are listed in Table I.

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[9] Each ampul fill volume was determined by carefully scoring the liquid level in each ampul, emptying and drying the ampul, and refilling to the scored mark with water dispensed from a volumetric buret.

[10] Chromosorb 105.

[11] This internal standard solution was prepared by dissolving 400 mg of 2-butanol in 50 ml of water.

Tolbutamide Binding to Plasma Proteins of Young and Old Human Subjects

Keyphrases □ Tolbutamide—plasma protein binding in humans, effect of subject age □ Binding, plasma protein—tolbutamide in humans, effect of subject age □ Age—of subject, effect on plasma protein binding of tolbutamide in humans □ Antidiabetic agents—tolbutamide, plasma protein binding in humans, effect of subject age

To the Editor:

Some evidence supports the view that age may be an important determinant in drug pharmacokinetics and pharmacodynamics (1). Changes in plasma protein binding of some drugs were shown to account for the age-related differences in their pharmacokinetics. Diminished binding of meperidine, phenylbutazone, warfarin, and phenytoin was associated with decreased serum albumin concentrations in elderly subjects (2–5). Tolbutamide is an acidic drug that is highly bound primarily to the albumin fraction of plasma protein (6, 7).

Table I—Effect of Age on Protein and Albumin Concentrations and Unbound Fraction of Tolbutamide [a]

	Young Group (n = 24)	Old Group (n = 19)
Age, years	38.74 ± 10.76	72.05 ± 8.50
Weight, kg	79.37 ± 2.14	74.25 ± 1.91[b]
Serum protein concentration, g/100 ml	7.25 ± 0.11	7.22 ± 0.51
Serum albumin concentration, g/100 ml	5.25 ± 0.54	4.67 ± 0.65[b]
Unbound fraction of tolbutamide[c]	0.032 ± 0.006	0.040 ± 0.007[d]

[a] Mean ± *SD*. [b] Significantly different ($p < 0.01$; t test). [c] Total plasma tolbutamide concentration of 100 μg/ml. [d] Significantly different ($p < 0.001$; t test).

The present study was undertaken to investigate the effect of age on plasma protein binding of tolbutamide. Blood samples were collected from healthy, nonsmoking, drug-free adult males[1]. The subjects were divided into two groups. The young group (n = 24) ranged in age from 23 to 57 years, and the old group (n = 19) ranged in age from 61 to 87 years.

Protein binding studies were carried out in rigid, clear acrylic cells[2] separated into two 1-ml compartments by a cellophane membrane[3]. Plasma (0.9 ml) was placed into one compartment and then spiked with a 10-μl aliquot of ^{35}S-tolbutamide[4] (dissolved in absolute ethanol) to produce a total concentration of 100 μg/ml. A 0.9-ml volume of 0.067 M phosphate buffer (pH 7.4) was placed in the other compartment.

The cells were placed in a metabolic incubator shaker[5] set at 37° and 50 oscillations/min. At equilibrium (16 hr), 50-μl aliquots were sampled from both compartments and counted directly in a liquid scintillation counter[6]. The unbound fraction of tolbutamide was calculated by dividing the amount of radioactivity (counts per minute) in the buffer solution by that of the plasma sample.

While the albumin concentration was decreased ($p < 0.01$) by about 11% in the old group, there was no change in the total protein concentration (Table I). Similar results were observed previously (9, 10), and the lack of change in the total protein concentration was attributed to a rise in globulin concentration with age. The effect of these changes in protein composition on drug binding has not been predictable (11). Table I indicates that the unbound fraction of tolbutamide was increased ($p < 0.001$) by about 25% in the old group. This age-related difference in the plasma protein binding of the drug prompted a study of its pharmacokinetics in the same groups of subjects. Preliminary results indicate that the total plasma clearance and volume of distribution of tolbutamide increased significantly with age (12). These findings may provide a reasonable explanation for the high incidence of hypo-

[1] Volunteer participants in the Baltimore Longitudinal Study of Aging (8) at the Gerontology Research Center, National Institute on Aging, National Institutes of Health, Baltimore, Md.

[2] Fisher Scientific Co., Silver Spring, MD 20910.

[3] Union Carbide Corp., Chicago, IL 60638.

[4] Lot 1076/N19015 (specific activity 4.86 mCi/mmole), Amersham-Searle, Arlington Heights, IL 60005.

[5] Dubnoff, Precision Scientific Co., Chicago, IL 60647.

[6] Packard 2425, Packard Instrument Co., Downers Grove, IL 60515.

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glycemic reactions observed in older patients receiving tolbutamide (13, 14).

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Scatchard Plots with a Positive Slope and Role of Albumin Concentration

Keyphrases □ Scatchard plots—with positive slopes, various drugs, effect of albumin concentration □ Binding, protein—various drugs, Scatchard plots with positive slopes, effect of albumin concentration □ Albumin—effect on drug–protein binding, Scatchard plots with positive slopes

To the Editor:

A recent publication (1) reported Scatchard plots (2) with a positive slope for the binding of three narcotic analgesics and other basic drugs to human albumin. It was implied (1) that such Scatchard plots may be characteristic of organic bases and alkaloids. Positive Scatchard plots have been obtained for hydrocortisone (3), thiopental (4), phenytoin (5, 6), L-tryptophan (5, 6), and 2-(4′-hydroxybenzeneazo)benzoic acid[1] when the protein concentration was varied. Shen and Gibaldi (4) considered some aspects of this problem, but it was not mentioned in a recent review (7). Our own work and the reports listed in Table I suggest that this phenomenon is more common than hitherto supposed and requires explanation.

Two main experimental approaches are used to obtain drug binding data for Scatchard or similar analysis: A, vary the total ligand concentration and use one albumin concentration; and B, vary the albumin concentration and use a single total ligand concentration. Method A is more commonly used and was used in the study of narcotic analgesics (1). The apparent association constant, K, and the number of binding sites, n, derived from data obtained experimentally by Method A are tacitly assumed to be independent of protein concentration.

The positive Scatchard plots obtained for hydrocortisone, thiopental, phenytoin, L-tryptophan, and 2-(4′-hydroxybenzeneazo)benzoic acid indicate that n and/or K (*i.e.*, nK) decrease as the protein concentration increases. Method B was used for ultrafiltration or equilibrium dialysis with these five ligands, so the possibility arises that the results were an artifact of the method. However, this result is unlikely because identical Scatchard plots having the usual negative slopes were obtained experimentally by Methods A and B for both *o*-methyl red (6) and methyl orange[1], using experimental conditions similar to those for phenytoin and L-tryptophan. Previous workers (15, 16) found that nK for methyl orange may be dependent upon protein concentration with bovine albumin, but we have been unable to confirm this finding with human albumin.

Table I lists ligands for which there is an indication that nK may decrease when the protein concentration increases, although many of the data were not reported in a form suitable for Scatchard analysis. Several explanations for these findings are possible.

Commercial albumin preparations contain variable quantities of contaminants, *e.g.*, *N*-acetyl-L-tryptophan and other indoles (23) and fatty acids that may inhibit drug binding. At a constant drug concentration (Method B), the contaminant to ligand ratio increases as the albumin concentration increases and thereby decreases nK. Results with hydrocortisone (3) and 1-dodecanol (12) were attributed to contaminants. The sequential changes of buffer used in the dynamic dialysis technique may remove inhibitors of binding but seem unlikely to account for the results with the narcotic analgesics (1). Scholtan (20) found that drug binding in whole serum with pathologically lowered albumin concentration did not decrease as much as anticipated. Therefore, an increase in nK with lower albumin concentrations cannot be fully explained by dilution of endogenous inhibitory substances since no such dilution would have occurred in whole serum.

Cooperativity was implicated in several unusual ligand–protein interactions (4, 24, 25) and could explain Scatchard plots with positive slopes. However, these cooperative effects all were observed at one protein concentration or, at most, with only a small range of protein concentrations. If cooperativity is responsible, it should be manifest when the albumin concentration, as opposed to the ligand concentration, is varied. However, no such effects were apparent in studies where the ligand concentration was also varied.

[1] Unpublished results.

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