flux value, in comparison to suspensions, as well as a continuing reduction in flux with time.

As a final test of this explanation, and to ensure that the surfactant used in the suspension formulations did not influence the results, experiments were performed using a saturated solution of benzocaine in water to which excess benzocaine (1 mg/ml) was added.

The results are plotted in Fig. 4 for the penetration profiles with and without excess drug in donor. It is evident that the slopes for the two curves are different. The flux for the donor with excess drug (100×10^{-3} mg hr^{-1} cm^{-2}) was very close to the values obtained for the suspension systems (Table I).

REFERENCES

(1) U. G. Dalvi and J. L. Zatz, *J. Soc. Cosmet. Chem.*, **32,** 87 (1981).

(2) F. Bottari, G. Di Colo, E. Nannipieri, M. F. Saettone, and M. F. Serafini, *J. Pharm. Sci.*, **66,** 926 (1977).

(3) G. Di Colo, V. Carelli, B. Giannaccini, M. F. Serafini, and F. Bottari, *ibid.*, **69,** 387 (1980).

(4) G. D. Tucker, R. N. Boyes, P. O. Bridenbough, and D. C. Moore, *Anesthesiology*, **33,** 287 (1970).

(5) E. Menczel and H. I. Maibach, *J. Invest. Dermatol.*, **54,** 386 (1970).

(6) E. Menczel and H. I. Maibach, *Acta Derm. Venereol.*, **52,** 38 (1972).

(7) S. K. Chandrasekaran, A. S. Michaels, P. S. Campbell, and J. E. Shaw, *AIChE J.*, **22,** 828 (1976).

ACKNOWLEDGMENTS

U. G. Dalvi is grateful for fellowship support provided by the Society of Cosmetic Chemists.

Degradation of Melphalan in Aqueous Solutions—Influence of Human Albumin Binding

HANS EHRSSON ˣ **and ULLA LÖNROTH**

Received April 28, 1981, from the *Karolinska Apoteket, S-104 01 Stockholm, Sweden.* Accepted for publication September 28, 1981.

Abstract □ The protein binding and degradation rate of melphalan in human albumin solutions and plasma have been investigated. In plasma, melphalan is bound 69.0 ± 3.4% (25°, melphalan concentration 25 μg/ml). The stability of melphalan when bound to albumin is about three times higher ($k = 3.07 \times 10^{-2} \pm 0.48 \times 10^{-2}$ hr^{-1}) than unbound in solution ($k = 1.14 \times 10^{-1} \pm 0.01$ hr^{-1}).

Keyphrases □ Melphalan—degradation studies in aqueous solutions, influence of human albumin binding □ Binding—human albumin to melphalan, degradation of melphalan in aqueous solutions □ Albumin, human—influence in binding with melphalan, degradation in aqueous solutions

Melphalan, an alkylating agent of the nitrogen mustard type, can be bound to plasma proteins as a result of a chemical reaction with the protein molecules as well as by a process of reversible adsorption (1). At least 60% of melphalan was bound to serum proteins (26°) as studied by an equilibrium dialysis technique (2). However, the quantitative determinations were performed using an unselective technique, and it is unclear to what extent the results are affected by a codetermination of degradation products of melphalan formed during the dialysis procedure. Melphalan (30%) was observed previously to be undialyzable in human plasma (4°), the quantitative determinations being performed by high-performance liquid chromatography (1). In the present study the reversible protein binding of melphalan in albumin solutions and human plasma has been determined by a modified ultrafiltration technique (3). Since the binding of melphalan to albumin might have a profound effect on its stability *in vivo* (4), the rate constants for the degradation of protein-bound and free melphalan have been evaluated.

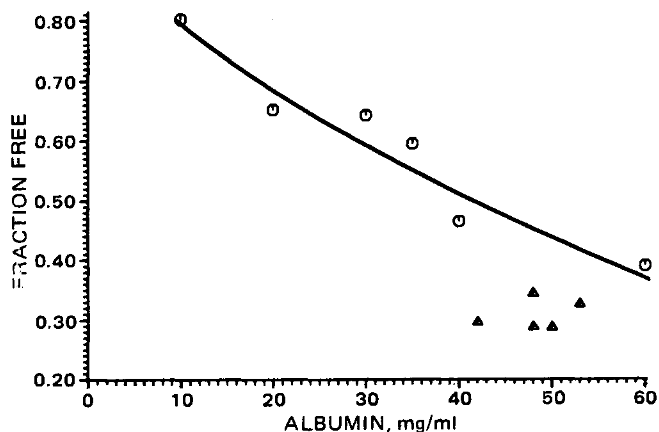

Figure 1—*Influence of albumin concentration on fraction free melphalan (25°; melphalan concentration: 25 μg/ml). The fraction free melphalan is a mean of four determinations. Key:* (○) *albumin solutions;* (▲) *plasma samples.*

EXPERIMENTAL

Degradation Studies—Melphalan[1], dissolved in 0.1 *M* HCl, was diluted 100 times with a solution of human albumin[2] in isotonic phosphate buffer pH 7.35 (NaCl concentration 0.095 *M*) or plasma[3] to a final melphalan concentration of 25 μg/ml. The mixture was incubated at 25.0 ± 0.1° and at appropriate times aliquots were analyzed by liquid chromatography.

Protein-Binding Studies—The protein binding was determined using a modified ultrafiltration technique (3). The studies were carried out at 25 ± 2° using albumin or plasma solutions containing 25 μg/ml of melphalan, unless otherwise stated. The degradation of melphalan in the ultrafiltrate was minimized by collecting it in albumin solutions containing the same concentration of albumin as the solution inside the dialysis tubing. No binding of melphalan to the dialysis membrane could be observed as studied by ultrafiltration of melphalan dissolved in isotonic phosphate buffer pH 7.35.

Quantitative Analysis—The quantitative determinations of melphalan were performed by liquid chromatography according to previous

[1] Sigma Chemical Co., St. Louis, Mo.

[2] Human albumin, essentially fatty acid free, Sigma Chemical Co. (A 1887), St. Louis, Mo.

[3] Blood was obtained from drug-free volunteers and collected in tubes containing heparin. The plasma was separated by centrifugation and stored at −20° until used.

0022-3549/82/0700-0826$01.00/0

© 1982, American Pharmaceutical Association

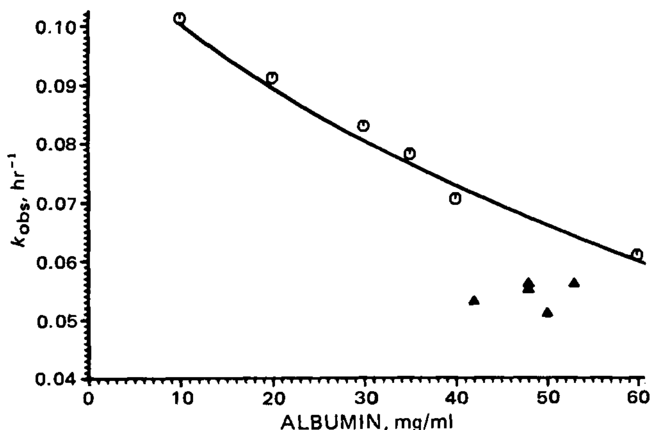

Figure 2—*Degradation rate of melphalan-influence of albumin concentration (25°; melphalan concentration: 25 µg/ml). Key: (○) albumin solutions; (▲) plasma samples.*

principles (1), using the same equipment as described previously (3). The results were evaluated using a standard curve prepared by adding known amounts of melphalan to albumin solutions and plasma.

RESULTS AND DISCUSSION

The protein binding of melphalan (25 µg/ml) was 69.0 ± 3.4% in plasma obtained from five donors, the mean albumin concentration being 48 mg/ml. No significant change in the degree of protein binding was observed in the concentration range 5–50 µg/ml of melphalan. The protein binding in albumin solutions was lower than in plasma containing comparable albumin concentrations (Fig. 1). The protein binding was 53.5 ± 1.7% at an albumin concentration of 40 mg/ml.

The influence of albumin concentration on the pseudo first-order rate constant for the degradation of melphalan is given in Fig. 2. The half-life for the degradation of melphalan is 9.88 ± 0.24 hr (n = 10, r = 0.998) at an albumin concentration of 40 mg/ml. The stability in plasma obtained from five donors is higher ($t_{1/2}$ = 12.80 ± 0.52 hr) than in albumin solutions containing comparable albumin concentrations.

The rate constant for the degradation of melphalan in albumin solutions (k_{obs}) can be expressed by (3):

$$k_{obs} = k_1 + f_{free}(k_2 - k_1)$$

where k_1 and k_2 are pseudo first-order rate constants for the degradation of protein-bound and free melphalan, respectively, and f_{free} is the fraction of free melphalan in solution. A plot of k_{obs} *versus* f_{free} in albumin solutions and plasma is given in Fig. 3. The fraction free melphalan in the albumin solutions was changed by the use of different albumin concentrations (10–60 mg/ml) at a constant ligand concentration (25 µg/ml). Evaluation of the rate constants by linear regression analysis using the data obtained in albumin solutions gave $k_1 = 3.07 \times 10^{-2} \pm 0.48 \times 10^{-2}$ hr^{-1} and $k_2 = 1.15 \times 10^{-1} \pm 0.09 \times 10^{-1}$ hr^{-1} (r = 0.916, n = 24). The rate

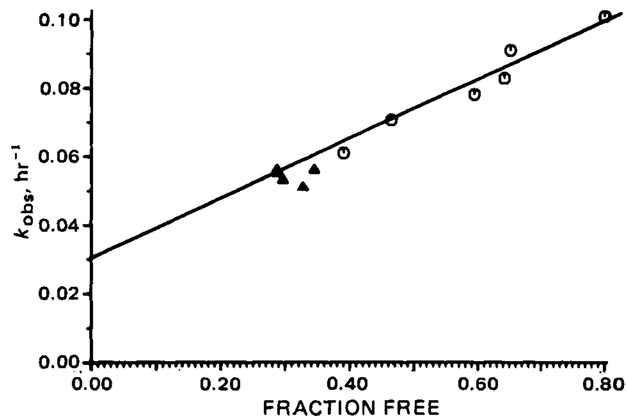

Figure 3—*Degradation rate* versus *fraction free melphalan (25°). Key: (○) albumin solutions; (▲) plasma samples.*

constant for free melphalan obtained from the plot is in good agreement with the value obtained from degradation studies in albumin-free isotonic phosphate buffer pH 7.35 ($k_{obs} = 1.14 \times 10^{-1} \pm 0.01 \times 10^{-1}$ hr^{-1}, n = 14, r = 0.999). Thus, the stability of melphalan bound to albumin is about three times higher than unbound in solution.

Since the degradation rate of nitrogen mustards is increased markedly with increasing dielectric constant of the solvent (5), it can be concluded that the nitrogen mustard group of melphalan when bound to albumin is in a chemical environment with solvating properties different from those in pure aqueous solution. It has been shown previously (3) that the nitrogen mustard drug, chlorambucil, was efficiently protected from degradation when bound to albumin, the rate constant for the bound form being ~100 times lower than for chlorambucil being free in solution. This indicates that melphalan is bound to albumin in a different way than chlorambucil. Furthermore, it can be observed (Fig. 3) that the data obtained for melphalan in plasma fall on the line obtained from studies in pure albumin solutions. The increased stability for melphalan observed in plasma must, therefore, be due to a more excessive binding to albumin or to other plasma ligand(s) which bind the nitrogen mustard group in a similar way as albumin.

REFERENCES

(1) S. Y. Chang, D. S. Alberts, D. Farquhar, L. R. Melnick, P. D. Walson, and S. E. Salmon, *J. Pharm. Sci.*, **67**, 682 (1978).

(2) M. Chirigos and J. A. R. Mead, *Anal. Biochem.*, **7**, 259 (1964).

(3) H. Ehrsson, U. Lönroth, I. Wallin, M. Ehrnebo, and S. O. Nilsson, *J. Pharm. Pharmacol.*, **33**, 313 (1981).

(4) D. S. Alberts, S. Y. Chang, H. S. G. Chen, T. E. Moon, T. L. Evans, R. L. Furner, K. Himmelstein, and J. F. Gross, *Clin Pharmacol. Ther.*, **26**, 73 (1979).

(5) W. R. Owen and P. J. Stewart, *J. Pharm. Sci.*, **68**, 992 (1979).