in the intact dog was dose dependent and that administration of 10-, 50-, and 100-mg doses of levodopa to the duodenum led to the same results shown in Fig. 3. Yet negligible amounts of levodopa remained in the duodenal segment, and plasma levodopa levels resulted from saturable metabolism of levodopa to dopamine by the levodopa decarboxylase enzyme system in the duodenal tissue. Calimlim *et al.* (10) noted that the levodopa metabolites produced in the stomach may be responsible for nausea and vomiting. Their study and the present results indicate that levodopa bioavailability might be improved with an enteric-coated preparation that releases levodopa in high concentration at the upper small intestine.

REFERENCES

(1) K. Sasahara, T. Nitanai, T. Habara, T. Kojima, Y., Kawahara, T. Morioka, and E. Nakajima, *J. Pharm. Sci.*, **70**, 730 (1981).
(2) K. Sasahara, T. Nitanai, T. Morioka, and E. Nakajima, *ibid.*, **69**, 261 (1980).
(3) K. Sasahara, T. Nitanai, T. Habara, A. Ninomiya, T. Morioka, and E. Nakajima, *Ann. Rep. Sankyo Res. Lab.*, **30**, 65 (1978).
(4) K. Sasahara, T. Nitanai, T. Habara, T. Morioka, and E. Nakajima, *J. Pharm. Sci.*, **69**, 1374 (1980).
(5) A. Leon and H. Spiegel, *J. Clin. Pharmacol.*, **12**, 263 (1972).
(6) D. N. Wade, P. T. Mearrich, D. J. Birkett, and J. L. Morris, *Aust. N. Z. M. Med.*, **4**, 138 (1974).
(7) P. T. Mearrick, D. N. Wade, D. J. Birkett, and J. L. Morris, *ibid.*, **4**, 144 (1974).
(8) R. P. Spencer, *Am. J. Clin. Nutr.*, **22**, 292 (1969).
(9) D. M. Matheus and L. Laster, *Gut*, **6**, 411 (1965).
(10) L. R. Calimlim, C. A. Dujoune, J. P. Morgan, I. Lasagne, and J. R. Boamcjome, *Eur. J. Clin. Invest.*, **1**, 313 (1971).

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Blood Collection Technique: No Effect on *In Vitro* Protein Binding of Prednisolone

MARIO L. ROCCI, JR., and WILLIAM J. JUSKO ˣ

Received November 28, 1980, from the *Clinical Pharmacokinetics Laboratory, Department of Pharmaceutics, School of Pharmacy, State University of New York at Buffalo*, and the *Millard Fillmore Hospital, Buffalo, NY 14209.* Accepted for publication March 25, 1981.

Abstract □ The effect of the blood collection vessel and systemic heparin administration on *in vitro* protein binding of prednisolone was examined in blood collected from human subjects. No differences in the fractional binding of prednisolone were found in plasma from plain glass culture tubes, heparinized culture tubes, and two types of red- and green-top commercial vacuum tubes. Thus, these blood collection techniques do not alter serum or plasma albumin and transcortin binding of prednisolone.

Keyphrases □ Binding—prednisolone, effect of blood collection techniques □ Tris(2-butoxyethyl)phosphate—leached substance altering protein binding of selected drugs □ Prednisolone—protein binding in blood collection techniques

Blood collection techniques can affect the binding of ligands to serum proteins (1–7). Heparin administration stimulates lipoprotein lipase release *in vivo* (8), and this enzyme can increase the serum concentrations of nonesterified fatty acids *in vivo* (8) and *in vitro* (9). These fatty acids displace drugs from their binding sites on albumin (10). Heparin also has been shown to alter the plasma protein binding of several drugs when used to prevent blood clotting *in vitro* (6). Tris(2-butoxyethyl)phosphate, a substance leached from some stoppers, also alters the protein binding of selected drugs (1–4). Weak bases that bind to α_1-acid glycoprotein are principally affected (2).

This study examined the effect of blood collection on the protein binding of prednisolone. This synthetic glucocorticoid is bound in serum by three proteins: transcortin, albumin, and α_1-acid glycoprotein, although the latter contributes minimally to the overall binding of this steroid *in vivo* (11). The limited capacity of transcortin for binding prednisolone results in a nonlinear relationship between prednisolone free fraction and serum concentration, with increases in the free fraction occurring at higher concentrations. This binding pattern contributes greatly to the nonlinear disposition of this compound in humans (12). In addition, the unbound drug appears to be the pharmacologically active moiety (11). Thus, accurate binding measurements are essential when examining prednisolone pharmacokinetics.

EXPERIMENTAL

Study Design—Four healthy adult males (nonsmokers), ages 24–37 years, were studied. A "scalp vein" infusion set[1] was inserted into an arm vein, and an intravenous drip of normal saline was infused at a rate of 0.5 ml/min to maintain the collection line open for 2 hr. At the end of this period, 10 ml of blood was collected into each of five tubes: (*a*) a plain glass culture tube, (*b*) a red-top commercial tube[2], (*c*) a green-top commercial tube with heparin[2], (*d*) another red-top commercial tube[3], and (*e*) a culture tube containing 0.2 ml of 1000-units/ml heparin injection[4]. Contact between the blood samples and stoppers from the two types of commercial tubes was assured through inversion of these tubes.

After initial blood collection, a dilute heparin solution in normal saline (20 units/ml) was connected to the infusion set to prevent clotting of the collection line. At 2, 2.5, 3, 3.5, and 4 hr, 5 ml of the heparin solution was flushed through the line to simulate heparin administration during intermittent blood sample collection. At 4 hr, blood was collected in various tubes as described.

All samples were maintained at 25° for 1 hr and then centrifuged; the plasma (or serum) was harvested and frozen (−20°) prior to analysis.

Analysis—Prednisolone containing trace quantities of [^{3}H]prednisolone[5] (specific activity 53 mCi/mmole) was added to each sample to produce concentrations of 100 ng/ml. The protein binding of prednisolone was assessed using equilibrium dialysis for 16 hr at 37° and radioactivity analysis as previously described (13).

Statistics—A two-way analysis of variance was performed (14) to differentiate between the effect of systemic heparin administration and the effect of the collection container on prednisolone protein binding. An interaction term was included so that the combined effects of these factors could be assessed.

[1] Pharmaseal Laboratories, Glendale, Calif.
[2] Vacutainer, Becton-Dickinson, Rutherford, N.J.
[3] Venoject, Kimble-Terumo, Elkton, Md.
[4] Lipo-Hepin, Riker Laboratories, Northridge, Calif.
[5] New England Nuclear, Boston, Mass.

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Table I—Mean (±*SD*) Percent *In Vitro* Binding of Prednisolone in Serum Samples Obtained in Various Collection Tubes before and after Systemic Heparin Administration

Tube	Before Heparin	After Heparin
Glass culture	84.6 (1.6)	84.7 (5.0)
Commercial[a] (red top)	83.3 (3.7)	86.5 (4.4)
Heparinized commercial[a] (green top)	83.6 (2.2)	84.6 (4.8)
Commercial[b] (red top)	79.9 (6.0)	84.2 (7.0)
Glass culture with heparin (200 units/0.2 ml)	86.6 (1.2)	87.2 (2.7)

[a] Vacutainer. [b] Venoject.

RESULTS AND DISCUSSION

The extent of prednisolone binding *in vitro* in the serum (or plasma) samples from the various collection tubes before and after systemic heparin administration is presented in Table I. There were no significant differences in the fractional binding of prednisolone in samples obtained from the various collection tubes ($F = 1.12$). In addition, heparin infusion had no significant effect on the fractional binding of prednisolone ($F = 2.84$). No dual interactions occurred between the collection vessel and heparin administration ($F = 0.67$).

Therefore, heparin administration has no effect on the *in vitro* binding of prednisolone at low steroid concentrations. A low concentration of prednisolone was chosen for these studies to reflect binding to transcortin, a protein that has not been assessed in relation to such potential binding displacement. Changes in the binding at this concentration would result in the greatest relative increase in the free fraction when compared to higher steroid concentrations where ~50–60% of prednisolone is bound (15). Elevation of free fatty acid levels by heparin increases the free fraction of drugs such as phenytoin, warfarin (10), and quinidine (5). The lipase responsible for the release of these fatty acids is active *in vitro* and can introduce a further artifact in the binding measurements *via in vitro* release of these displacing agents (9).

Heparin affects the binding of warfarin, salicylic acid, and phenytoin when used as an anticoagulant *in vitro* (6). The lack of significant differences in prednisolone binding among heparinized and nonheparinized samples obtained prior to systemic heparin administration indicates that the *in vitro* presence of heparin is not of major concern when conducting prednisolone binding and disposition studies.

The leaching of tris(2-butoxyethyl)phosphate from the stoppers of one tube[2] increased the free fraction of basic drugs including propranolol (1), alprenolol (3), and quinidine (4). Prednisolone is a neutral molecule and thus is not affected by the presence of tris(2-butoxyethyl)phosphate. Therefore, the use of heparin and commercial tubes does not appear to pose a problem when examining the protein binding of prednisolone.

REFERENCES

(1) R. H. Cotham and D. Shand, *Clin. Pharmacol. Ther.*, **18**, 535 (1975).

(2) O. Borgå, K. M. Piafsky, and O. G. Nilsen, *ibid.*, **21**, 539 (1977).

(3) K. M. Piafsky and O. Borgå, *Lancet*, **2**, 963 (1976).

(4) D. Fremstad and K. Bergerud, *Acta Pharmacol. Toxicol.*, **39**, 570 (1976).

(5) K. M. Kessler, R. C. Leech, and J. F. Spann, *Clin. Pharmacol. Ther.*, **25**, 204 (1979).

(6) V. W. Wiegand, J. T. Slattery, K. L. Hintze, and G. Levy, *Life Sci.*, **25**, 471 (1979).

(7) M. Wood, D. G. Shand, and A. J. J. Wood, *Clin. Pharmacol. Ther.*, **25**, 103 (1979).

(8) J. R. E. Fraser, R. R. H. Lovell, and P. J. Nestel, *Clin. Sci.*, **20**, 351 (1961).

(9) K. M. Giacomini, S. E. Swezey, J. C. Giacomini, and T. F. Blaschke, *Life Sci.*, **27**, 771 (1980).

(10) R. Gugler, D. W. Shoeman, and D. L. Azarnoff, *Pharmacology*, **12**, 160 (1974).

(11) P. O. Ballard, *Monogr. Endocrinol.*, **12**, 25 (1975).

(12) W. J. Jusko and J. Q. Rose, *Ther. Drug Monit.*, **2**, 169 (1980).

(13) M. L. Rocci, Jr., N. F. Johnson, and W. J. Jusko, *J. Pharm. Sci.*, **69**, 977 (1980).

(14) J. L. Bruning and B. L. Kintz, "Computational Handbook of Statistics," Scott, Foresman, Glenview, Ill., 1977, pp. 48–54.

(15) G. P. Lewis, W. J. Jusko, C. W. Burke, and L. Graves, *Lancet*, **2**, 778 (1971).

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Sodium Chloride Equivalents, Cryoscopic Properties, and Hemolytic Effects of Certain Medicinals in Aqueous Solution IV: Supplemental Values

E. ROY HAMMARLUND

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Abstract □ A supplemental table of sodium chloride equivalents and freezing-point depressions at various concentrations for 29 substances in aqueous solution is presented. Also listed is the isosmotic concentration of each material that can form such a solution. The degree of hemolysis of human erythrocytes was determined in 12 different isosmotic solutions, and the data are presented to supplement the previously published values. While four isosmotic solutions prevented hemolysis, eight others did not.

Keyphrases □ Sodium chloride equivalents—data for 29 drugs □ Cryoscopic properties—data for 29 drugs □ Hemolytic effects—data for 29 drugs □ Medicinals—sodium chloride equivalents, cryoscopic properties, and hemolytic effects determined for 29 drugs

The sodium chloride equivalents and freezing-point depressions for 499 substances in aqueous solution were determined experimentally and reported previously (1–5). Furthermore, the degree of hemolysis of fresh human erythrocytes in certain aqueous isosmotic solutions was studied using the hemolytic method (3–6).

The current investigation studied some additional available substances not included in earlier cryoscopic and

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