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.

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

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Received February 18, 1981, from the *Department of Pharmaceutics, School of Pharmacy, University of Washington, Seattle, WA 98195.* Accepted for publication March 23, 1981.

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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Table I—Sodium Chloride Equivalents and Freezing-Point Depressions [a]

Chemical	Concentration of Solution, sodium chloride Equivalents 0.5%	1%	2%	3%	5%	At Isosmotic Concentration	
Amikacin base	0.06	0.05	0.05	0.05	0.05	—	—
	0.016°	0.031°	0.062°	0.091°	0.153°	—	—
Benzquinamide hydrochloride	0.14	0.14	0.13	0.12	—	—	—
	0.041°	0.079°	0.150°	0.216°	—	—	—
Bretylium tosylate	0.16	0.14	0.13	0.12	0.11	—	—
	0.043°	0.081°	0.148°	0.208°	0.327°	—	—
Calcium gluceptate	0.12	0.12	0.11	0.10	0.10	—	—
	0.037°	0.068°	0.124°	0.178°	0.275°	—	—
Carbazochrome salicylate	0.38	0.36	0.36	—	—	0.35	2.57% [b]
	0.106°	0.210°	0.410°	—	—	0.52°	2.57%
Cefamandole nafate [c]	0.16	0.14	0.12	0.11	0.10	—	—
	0.045°	0.079°	0.137°	0.187°	0.290°	—	—
Cefazolin sodium	0.14	0.13	0.12	0.11	0.11	—	—
	0.042°	0.074°	0.132°	0.190°	0.303°	—	—
Cefoxitin sodium [c]	0.18	0.16	0.15	0.14	0.13	—	—
	0.050°	0.092°	0.166°	0.238°	0.384°	—	—
Cephapirin sodium	0.14	0.13	0.13	0.13	0.12	0.11	7.80%
	0.038°	0.075°	0.149°	0.222°	0.361°	0.52°	7.80%
Dobutamine hydrochloride	0.20	0.18	0.16	—	—	—	—
	0.053°	0.101°	0.188°	—	—	—	—
Dopamine hydrochloride	0.30	0.30	0.29	0.29	—	0.29	3.11%
	0.085°	0.170°	0.335°	0.502°	—	0.52°	3.11%
Etidocaine hydrochloride	0.18	0.18	0.18	0.18	0.18	0.18	5.08%
	0.051°	0.102°	0.204°	0.306°	0.510°	0.52°	5.08%
Isoetharine hydrochloride	0.24	0.23	0.22	0.21	—	0.21	4.27%
	0.068°	0.132°	0.250°	0.368°	—	0.52°	4.27%
Ketamine hydrochloride	0.21	0.21	0.21	0.21	—	0.21	4.29%
	0.061°	0.122°	0.244°	0.366°	—	0.52°	4.29%
Leucovorin calcium	0.06	0.05	0.05	0.04	0.04	—	—
	0.013°	0.026°	0.052°	0.077°	0.126°	—	—
Metoclopramide hydrochloride	0.16	0.15	0.13	0.12	0.11	—	—
	0.045°	0.084°	0.155°	0.216°	0.315°	—	—
Metrizamide	0.04	0.04	0.03	0.03	—	—	—
	0.010°	0.020°	0.040°	0.060°	—	—	—
Nalbuphine hydrochloride	0.16	0.15	0.14	—	—	—	—
	0.045°	0.085°	0.158°	—	—	—	—
Oxymorphone hydrochloride	0.16	0.16	0.15	0.14	0.13	—	—
	0.044°	0.088°	0.168°	0.244°	0.382°	—	—
Piperacillin sodium [c]	0.11	0.11	0.11	0.10	—	—	—
	0.032°	0.063°	0.123°	0.175°	—	—	—
Potassium thiocyanate	0.61	0.59	—	—	—	0.59	1.52%
	0.180°	0.341°	—	—	—	0.52°	1.52%
Propantheline bromide [c]	0.11	0.11	—	—	—	—	—
	0.032°	0.064°	—	—	—	—	—
Propranolol hydrochloride [c]	0.20	0.20	0.20	—	—	—	—
	0.060°	0.122°	0.230°	—	—	—	—
Sodium nitroprusside	0.30	0.29	0.28	0.28	—	0.27	3.30%
	0.086°	0.167°	0.322°	0.475°	—	0.52°	3.30%
Sodium thiocyanate	0.71	0.71	—	—	—	0.71	1.27%
	0.205°	0.410°	—	—	—	0.52°	1.27%
Terbutaline sulfate	0.14	0.14	0.14	0.14	0.13	0.13	6.75%
	0.042°	0.082°	0.161°	0.238°	0.390°	0.52°	6.75%
Ticarcillin disodium	0.20	0.20	0.20	0.19	—	0.19	4.62%
	0.056°	0.113°	0.226°	0.339°	—	0.52°	4.62%
Timolol maleate	0.14	0.13	0.12	—	—	—	—
	0.038°	0.077°	0.146°	—	—	—	—
Tobramycin	0.08	0.07	0.07	0.07	0.06	—	—
	0.019°	0.038°	0.075°	0.112°	0.187°	—	—

[a] Top value is sodium chloride equivalent, and bottom value is freezing-point depression. [b] Isosmotic concentration, % w/v. [c] Solution foams readily.

hemolytic investigations. Results are presented in tables to supplement the previous data.

EXPERIMENTAL

Cryoscopic Measurements—The method used to measure the freezing points of the solutions was the same as that already reported; all freezing-point data were obtained with a cryoscopic osmometer (4).

The freezing-point measurements were corrected for the amount of disengaged ice; −0.52° was used as the comparative freezing point for aqueous 0.9% reagent grade sodium chloride solution, which is isotonic and isosmotic with blood and tears. The materials studied were of the official grade of purity or better, and the grade of purity of the donated specialty preparations complied with the manufacturer's specifications.

Hemolysis of Human Erythrocytes—Colorimetric hemoglobin determinations were made to indicate the degree of hemolysis for solutions that could be made isosmotic. The method (3–6) utilized a 45-min incubation period of erythrocytes in the isosmotic solution, followed by centrifugation of the erythrocytes and ghosts and determination of absorbance *versus* a standard at 540 nm in a colorimeter.

RESULTS AND DISCUSSION

Table I lists the sodium chloride equivalents and freezing-point depressions at various concentrations for the 29 substances investigated. To use these data, one should employ the sodium chloride equivalent that represents the concentration nearest to the desired final concentration of the medicinal substance used. Because of general interest in colligative properties of medicinal solutions, freezing-point depressions and sodium chloride equivalents are included for several substances that are not necessarily used as isotonic or isosmotic solutions. Sodium chloride equivalents and isosmotic concentrations are reported to the nearest 0.01.

Table II—Hemolysis of Erythrocytes in Isosmotic Solutions

Substances	Isosmotic Concentration, % w/v	Hemolysis, %	Approximate pH
Carbazochrome salicylate	2.57	82[a]	9.4
Cefazolin sodium	8.77	0	4.6
Cephapirin sodium	7.80	3[b]	6.7[c]
Dopamine hydrochloride	3.11	6	4.6
Etidocaine hydrochloride	5.08	100	4.3
Isoetharine hydrochloride	4.27	8	4.1
Ketamine hydrochloride	4.29	41	3.3
Potassium thiocyanate	1.52	0	6.3
Sodium nitroprusside	3.30	50[d]	9.1
Sodium thiocyanate	1.27	Trace	5.0
Terbutaline sulfate	6.75	11	4.1
Ticarcillin disodium	4.62	Trace	5.2

[a] Solution turned orange. [b] Solution turned yellow. [c] The pH was determined after addition of erythrocytes. [d] Solution turned red brown.

The percent of hemolysis found for the 12 isosmotic solutions studied, the isosmotic concentration used for each, and the solution's approximate pH before the addition of erythrocytes are listed in Table II. Any noticeable change in appearance of the erythrocytes or the solution was indicated. Of the 12 compounds studied, only four isosmotic solutions prevented hemolysis of human erythrocytes.

A compilation of the 305 substances whose isosmotic solutions were studied using the present hemolytic method shows that 144 failed to prevent hemolysis while 161 prevented hemolysis. Care must be taken not to equate isotonicity and isosmoticity without knowledge of the corresponding data whenever a biological membrane is utilized. This aspect was discussed previously (2, 4, 6–9).

Earlier studies (3–5) showed apparent aggregation of some substances in aqueous solution. In the present study, the cryoscopic graphs of cefamandole nafate and cefoxitin sodium (whose aqueous solutions foamed considerably) showed a slight discontinuity at 1%, suggesting that some aggregation may have taken place above that concentration; however, this phenomenon was not as distinct as with the previously reported drugs. Likewise, solutions of piperacillin sodium, propantheline bromide, and propranolol foamed considerably, suggesting that their surface tensions also were lowered; however, a plot of their cryoscopic graphs did not indicate aggregation. A sufficient number of compounds in the total study did tend to aggregate in solution, which suggests that this interfacial phenomenon may play a role in drug action by affecting biological activity. Associations taking place in the complex biological medium undoubtedly affect the thermodynamic activity of a given drug at the molecular level and, therefore, warrant increased study.

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Improved Liquid Chromatographic Assay for Serum Fluorouracil Concentrations in the Presence of Ftorafur

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Abstract ☐ An improved liquid chromatographic assay for serum ftorafur and fluorouracil is shown to be routine, sensitive, and reproducible using 200 μl of serum. Dilute ammonium acetate buffer at pH 10.2 is used for solubilization of the evaporated ethyl acetate extract for injection into the liquid chromatograph. A stability study indicated little or no *in vitro* formation of fluorouracil from ftorafur under the conditions described. Low serum fluorouracil levels were found after administration of therapeutic doses of ftorafur.

Keyphrases ☐ Liquid chromatographic assay—improved results for fluorouracil in presence of ftorafur ☐ Ftorafur—stability study, no *in vitro* formation of fluorouracil ☐ Anticancer agent—ftorafur potential for treatment of GI cancers

The recent development of an oral dosage form of the antineoplastic ftorafur [R_1S-*1*-(tetrahydro-2-furanyl)-5-fluorouracil] (I) has added new potential for the treatment of GI cancers on an outpatient basis. Ftorafur is generally considered to be a prodrug for fluorouracil (II), which, in turn, derives its cytotoxicity from its structural resemblance to uracil (IV). Inspection of the structure of I, II, and IV reveals the structural similarities between these molecules, and comparison of I and III indicates the unusual tetrahydrofuranyl ring of ftorafur.

BACKGROUND

During initial human studies with bolus ftorafur, investigators reported circulating levels of fluorouracil greater than those found after continuous intravenous infusion of fluorouracil (1, 2). In addition, there is some clinical similarity between bolus ftorafur and infusional fluorouracil in terms of patient toxicity (3). These findings suggested that the cytotoxic mechanism of ftorafur was probably due to the slow release of fluorouracil into the systemic circulation.

However, in a more recent study with bolus ftorafur, very low circulating levels of fluorouracil were reported and fluorouracil was not detected in the serum 8 hr after ftorafur administration (4). This report suggested that the high fluorouracil levels reported previously were the result of *in vitro* breakdown of ftorafur or perhaps its more labile dehydro metabolite (5). This metabolite is thought to differ from ftorafur by only a double bond located in an undetermined position in the furanyl ring

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