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Dosage Form Design for Improvement of Bioavailability of Levodopa V: Absorption and Metabolism of Levodopa in Intestinal Segments of Dogs

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Received November 10, 1980, from the *Product Development Laboratories, Sankyo Company, Ltd., 1-2-58, Hiromachi, Shinagawa, Tokyo, Japan.* Accepted for publication March 10, 1981.

Abstract □ Plasma levels of levodopa, total dopamine, and residual amounts of levodopa and its metabolites at the administered site were analyzed following administration of single 100-mg doses of levodopa in solution into isolated segments of the duodenum, jejunum, and ileum of the dog. The largest area under the plasma concentration–time curve (*AUC*) of levodopa during the 1.0-hr study was obtained following administration in the duodenum, followed by the jejunum and ileum. In addition, the residual amounts of levodopa and its metabolites detected at the administration sites were: ileum, 23%; jejunum, 7%; and duodenum, <1%. The largest *AUC* of total dopamine was obtained following administration in the jejunum, followed by the ileum and duodenum. This order was consistent with the order of levodopa decarboxylase enzyme activity reported previously. Therefore, it can be concluded that the major absorption site of levodopa in the intestine resides in the upper small intestine. Levodopa in 10-, 50-, and 100-mg doses was administered into isolated duodenal segments. The *AUC* of levodopa increased nonlinearly with increasing dose. Negligible amounts of both levodopa and its metabolites were observed in the segment at 1.0 hr after administration, indicating that the duodenal absorption of levodopa was not saturable within the dose range tested.

Keyphrases □ Levodopa—absorption and metabolism, intestinal segments, dogs, bioavailability, dosage form design □ Dosage form design—bioavailability of levodopa, absorption and metabolism in dog intestinal segments □ Bioavailability—levodopa, dosage form design, absorption and metabolism in dog intestinal segments

It was previously reported (1) that the reduced bioavailability of levodopa following oral administration was primarily due to levodopa metabolism by levodopa decarboxylase in the intestinal tissue, with the most enzyme activity in the jejunum and the least in the duodenum. The present investigation attempted to validate these findings by a study of levodopa absorption from intestinal segments.

EXPERIMENTAL

Major Absorption Site of Levodopa in Intestine of Dogs—Nine healthy male mongrel dogs, 11.1–13.9 kg, were fasted for ~16 hr and anesthetized with 25-mg/kg iv doses of pentobarbital sodium. They were divided into three equal groups according to the segment to be ligated. After the dog was fixed on its back, a laparotomy was performed on each dog, and a 20-cm segment of the duodenum, the jejunum, or the ileum was ligated. A single 100-mg dose of levodopa was injected as a 1% solution[1] into the ligated segment of each dog. Blood samples were withdrawn with a heparinized syringe from the brachial or femoral vein.

At 0, 2, 5, 15, 30, and 60 min after dosing, blood samples were withdrawn and processed as described previously (2). The animals were killed by exsanguination at 1 hr after levodopa administration, and the ligated loop was washed with saline and then three times with 0.04 *N* $HClO_4$ solution. The irrigating solution was assayed for residual amounts of levodopa and its metabolites.

Influence of Levodopa Dose on Absorption and Metabolism of Levodopa in Duodenum of Dogs—Nine healthy male mongrel dogs, 10.2–13.5 kg, were fasted for ~16 hr and anesthetized as described previously. After the dog was fixed on its back, a laparotomy was performed and a 20-cm segment of the duodenum was ligated. Ten-milligram doses of levodopa in a 0.2% solution[1] were injected into the duodenal loops of the first three dogs, 50-mg doses in a 0.5% solution[1] were injected into the duodenal loops of the second three dogs, and 100-mg doses in a 1.0% solution[1] were injected into the duodenal loops of the third group.

Blood samples were withdrawn with a heparinized syringe from the hepatoportal vein at 0, 2, 5, 15, 30, and 60 min after administration. Blood samples and the irrigating solution obtained from the final wash of the ligated loops were processed as already described.

Assay of Levodopa and Its Metabolites in Plasma and in Administered Site—Levodopa and its metabolite in plasma were determined according to a reported method (3). Residual amounts of levodopa and its metabolites in the dosing site of the intestine were determined according to the method reported previously (1).

RESULTS

Major Absorption Site of Intestine of Levodopa in Dogs—The average plasma levels of levodopa and total dopamine[2] are shown in Fig. 1 following administration of single 100-mg doses of levodopa to the duodenum, jejunum, and ileum. The highest plasma levodopa levels were obtained after duodenal administration; peak concentrations of 9.5 ± 1.9 mg/liter were observed at 5 min. Plasma levodopa levels following administration to the jejunum reached the peak levels of 5.0 ± 0.8 mg/liter at 15 min. Plasma levodopa levels following administration to the ileum were not only lowest but also increased so slowly that they reached peak levels of only 3.2 ± 0.3 mg/liter at 30 min after administration.

The average *AUC* of levodopa up to 1.0 hr after administration is shown in Fig. 2. The *AUC* of levodopa after administration to the ileum was approximately one-half that observed after duodenal administration.

[1] The 0.2, 0.5, and 1% levodopa solutions were prepared by dissolving levodopa in the buffer solution containing 0.2% sodium bicarbonate and 0.5% polyvinyl acetate (~pH 8.0).

[2] Total dopamine = unconjugated dopamine + conjugated dopamine.

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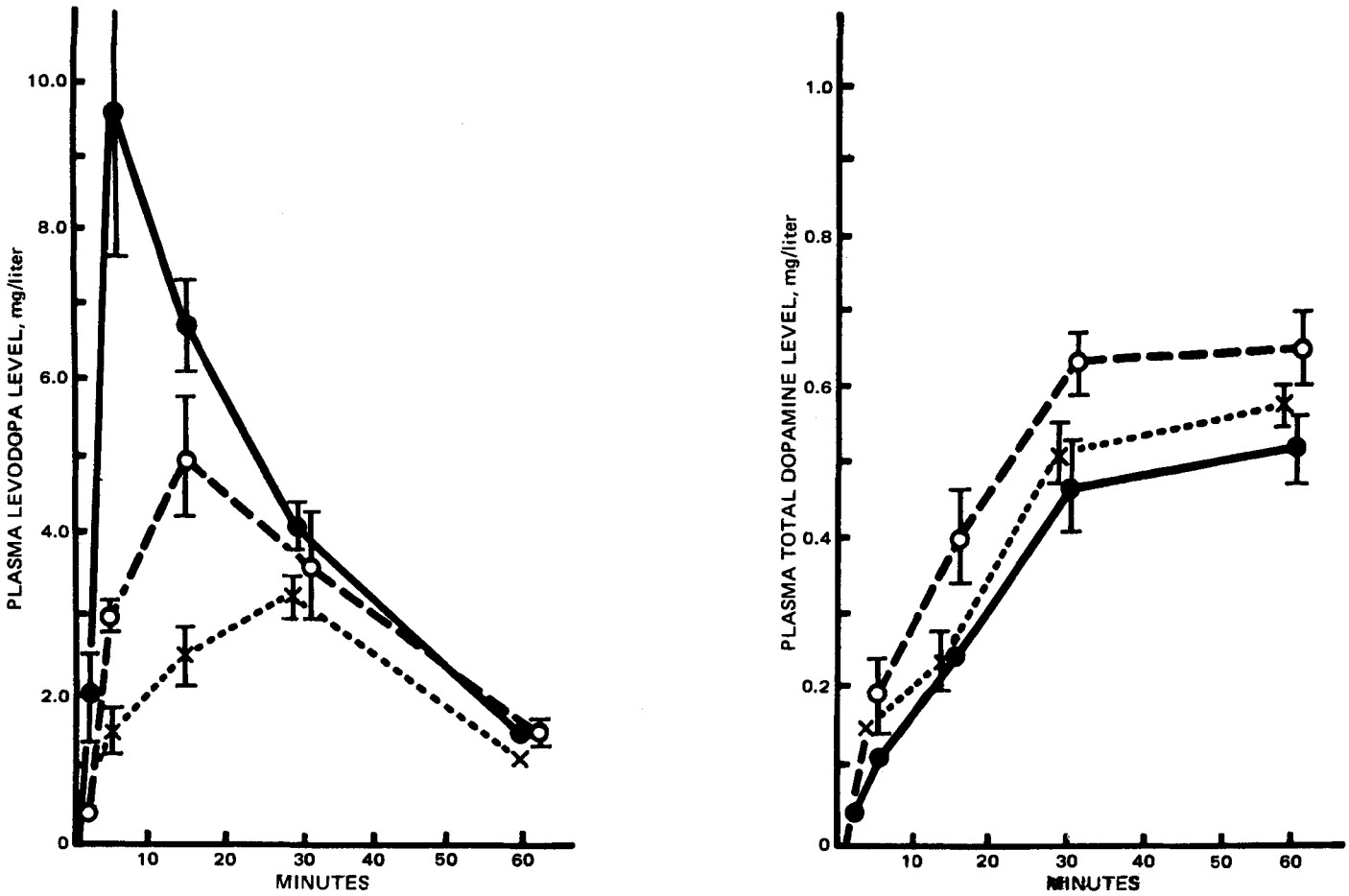

Figure 1—*Average plasma levels of levodopa (± SE) and total dopamine (± SE) following administration of single 100-mg dose of levodopa to dog duodenum (●), jejunum (○), and ileum (×).*

However, plasma levels of total dopamine increased slowly and reached the peak levels at 30–60 min after administration, irrespective of the administration site. The highest plasma levels of total dopamine were observed following administration to the jejunum, followed by the ileum and duodenum. This order did not correspond with the order of plasma levodopa levels.

The residual amounts of levodopa and its metabolites recovered from the segment are shown in Table I. These results showed that the residual amounts of levodopa and its metabolites were proportional to the order of plasma levodopa levels. Virtually all drug was absorbed from the duodenal segments, and negligible amounts of metabolites of levodopa were detected. Therefore, it was concluded that the major absorption site of levodopa is the duodenum.

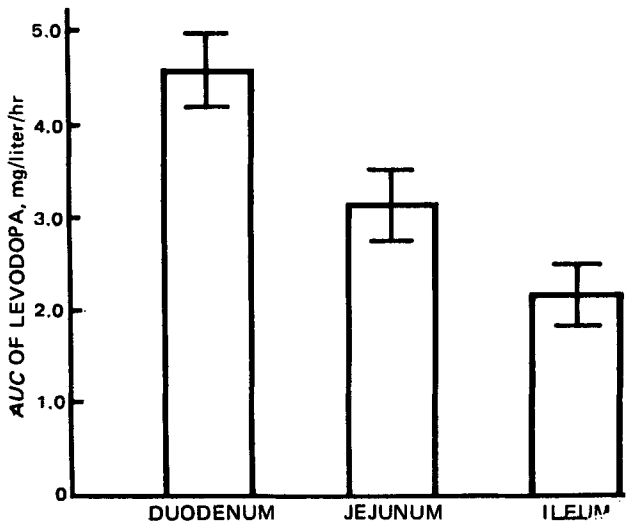

Figure 2—*Average* AUC *of levodopa (± SE) up to 1.0 hr after administration of single 100-mg doses of levodopa to dog duodenum, jejunum, and ileum.*

Influence of Levodopa Dose on Absorption and Metabolism of Levodopa in Duodenum in Dogs—The average plasma levels of levodopa and total dopamine following administration of single 10-, 50-, and 100-mg doses of levodopa to the duodenum of three dogs are shown in Fig. 3. As the administered dose increased, plasma levodopa levels in the hepatoportal vein appeared to be disproportionally high. In addition, the absorption rate from the duodenum was rapid since the peak time of plasma levodopa levels was observed at 2 min after dosing. However, total dopamine revealed an inverse situation; *i.e.*, as the dose increased, the relative plasma levels of total dopamine were progressively less. The relationship between the levodopa dose administered and the observed *AUC* of levodopa and the observed *AUC* of total dopamine up to 1.0 hr after dosing are shown in Fig. 4. These findings and the fact that levodopa obeys linear kinetics in the body after drug absorption (4) may indicate that metabolism of levodopa to dopamine by levodopa decarboxylase in the dog duodenum proceeds by a capacity-limited process.

The amounts of levodopa and its metabolites remaining at the ad-

Table I—Percentage of Residual Amounts to the Administered Dose at the Administered Site at 1.0 hr after Administration of Single 100-mg Dose of Levodopa to Dog Duodenum, Jejunum, and Ileum [a]

Site	Levodopa	Total Dopamine	Total[b]
Duodenum	0.8 ± 0.4[c]	0.1 ± 0.03	0.9 ± 0.4
Jejunum	6.6 ± 0.4	0.7 ± 0.1	7.2 ± 0.4
Ileum	21.8 ± 1.8	1.0 ± 0.1	23.1 ± 1.7

[a] 3,4-Dihydroxyphenylacetic acid and homovanillic acid were not detected. [b] Sum of levodopa, total dopamine, 3,4-dihydroxyphenylacetic acid, and homovanillic acid. [c] Average ± *SE*, $n = 3$.

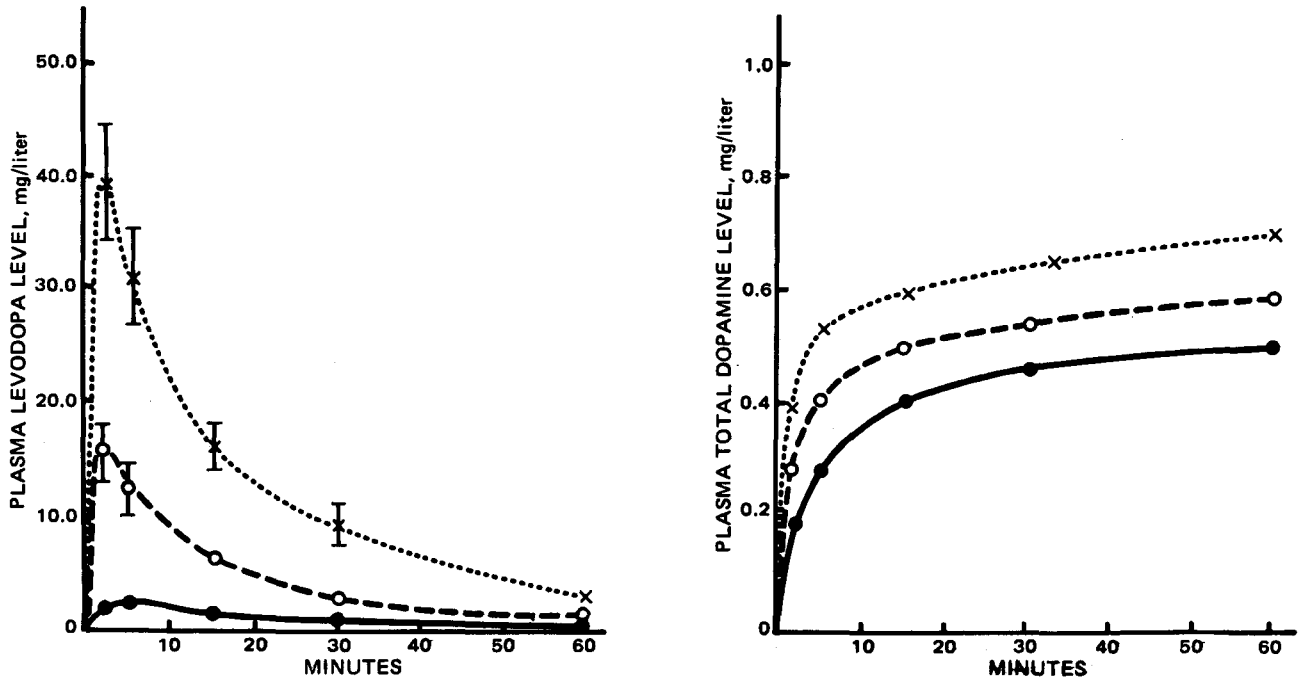

Figure 3—*Average plasma levels of levodopa (± SE) and total dopamine (± SE) following administration of single 10- (●), 50- (○), and 100-mg (×) doses of levodopa to dog duodenum.*

ministered site at 1.0 hr after administration were 1% of the administered dose, indicating that levodopa is completely absorbed within 1.0 hr after administration even at the largest dose studied.

DISCUSSION

It was previously reported (5–7) that the shorter the transit time through the stomach, the higher the observed plasma levodopa levels. These reports suggested that the upper small intestine might play an important role in levodopa absorption *via* the oral route. Analogously, the major site of oral absorption of other amino acids has been shown to be the upper small intestine (8, 9). The isolated intestinal studies carried out in the present investigation showed that the major absorption site of levodopa was the duodenum and that complete absorption occurred in the dose range studied. The inverse relationship between the observed plasma levels of levodopa and total dopamine shown in Fig. 1 is consistent with the fact that activity of levodopa decarboxylase is the lowest in the duodenum and increases in the lower sections of the small intestine (1).

However, previous studies (4) reported that levodopa bioavailability

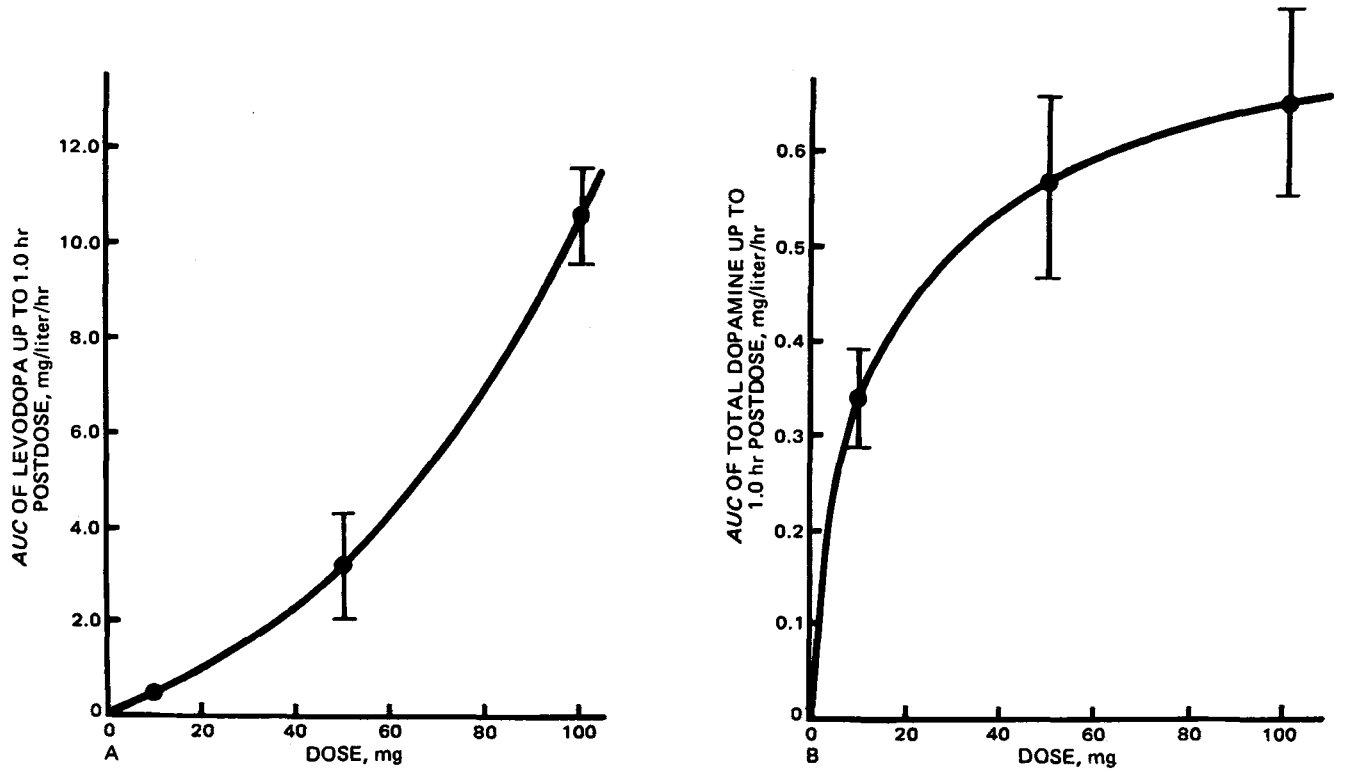

Figure 4—*Relationship between the levodopa dose administered and the observed* AUC *of levodopa (A) and the observed* AUC *of total dopamine (B) up to 1.0 hr after dosing.*

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.

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

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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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