DISCUSSION

The USP method (1) for the quantitative determination of spironolactone in tablets does not appear to be stability indicating since an almost completely decomposed sample indicated that 33.4% of the drug was still intact. The HPLC method showed a potency of 0% (Fig. 3B). The blue tetrazolium method, which indicated a potency of 4%, cannot be reliable because of the presence of a small amount of water in this system; water interferes with this reaction (see *Experimental* and Ref. 6). At lower concentrations (less than 5% of I), clearly identifiable peaks were obtained by the HPLC method. The quantitation of the results was not very accurate.

A high concentration of polyethylene glycol ointment base (above 100 mg in the reaction flask) strongly interfered with a blue tetrazolium assay. In the presence of polyethylene glycols and/or water, the HPLC method should be preferred. The HPLC method is also more sensitive (Table I). There was no interference from hydrochlorothiazide in the HPLC method (Table II and Fig. 1C). In the blue tetrazolium method, an equivalent amount of hydrochlorothiazide must be added to the reagent blank for more accurate results.

The HPLC method is sensitive, accurate, and precise (the standard deviation based on five readings was ±0.98%). The area of the peak was directly related to the concentration range tested (0.25–1.0 μg of I). In water, the peak was sharper than in alcohol (Figs. 1A and 1B). With the UV absorption technique, the standard solution in alcohol absorbed no light at 288 nm (maximum was at 239 nm) while the completely decomposed sample (with 0.1 *N* NaOH) showed maximum absorption at 288 nm. It may be possible to develop a stability-indicating assay based on two readings at two different wavelengths. The study was not pursued further.

Spironolactone decomposition, both in water and polyethylene glycol ointment base USP (5), appears to follow pseudo-first-order reaction kinetics (Figs. 2 and 4). The *K* values at 65° were estimated to be 0.0253/day in water *versus* 0.115/day in polyethylene glycol ointment base.

Whenever new prepacked HPLC columns are purchased, the flow rate or the percentage of alcohol may have to be changed to obtain the same retention time.

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COMMUNICATIONS

Plasma Protein Binding of Diazepam after a Single Dose of Sodium Oleate

Keyphrases □ Diazepam—plasma protein binding, effect of sodium oleate in rats □ Binding, plasma protein—diazepam, effect of sodium oleate in rats □ Sodium oleate—effect on plasma protein binding of diazepam in rats □ Sedatives—diazepam, plasma protein binding, effect of sodium oleate in rats

To the Editor:

Competitive displacement of drugs from plasma protein binding sites is well known, but most information has been derived from *in vitro* studies. Accordingly, little is known regarding the time course of such drug interactions. Specifically, we have been studying whether the decreased plasma protein binding of drugs after the administration of a displacing agent can be accounted for by the concentration of the displacing agent in the plasma. The unusually prolonged impairment of the plasma protein binding of diazepam after a single intravenous dose of sodium oleate is the subject of this communication.

In the first experiment, six male Sprague–Dawley rats were infused (0.6 ml/hr iv) for 1 hr with a buffered (phosphate) aqueous solution (pH 7.2) of sodium ^{3}H-oleate (7 mEq/liter). Blood samples were obtained during and for 14 days after intravenous infusion. The rats were randomly paired into three groups, and the blood was pooled for each pair to ensure adequate amounts of plasma. Three control rats were infused with saline (0.6 ml) for 1 hr, and blood was drawn periodically for 14 days.

Plasma oleate concentrations in treated rats were determined using the modified Dole extraction method (1, 2) in conjunction with liquid scintillation counting. Plasma binding of ^{14}C-diazepam (initial concentration of 400 ng/ml) was determined by equilibrium dialysis against an equal volume of phosphate buffer (0.1 *M*, pH 7.2) at 37° for 18 hr, followed by liquid scintillation counting of buffer and plasma.

The second experiment consisted of administering a bolus dose of sodium oleate (0.6 ml, 7 mEq/liter, premixed with 1 ml of blood) to two rats or of saline (0.6 ml) to two rats. Blood samples were taken immediately before, 5–10 min after, and 24 hr after the intravenous dose. Diazepam binding in plasma was determined by equilibrium dialysis as described.

Plasma binding of diazepam in saline-treated rats was essentially constant over 14 days (Fig. 1). In contrast, the free fraction of diazepam was elevated significantly in

Table I—Free Fraction of Diazepam in Rat Plasma Obtained before and after Intravenous Administration of Sodium Oleate or Saline

	Saline		Sodium Oleate	
Sample	Rat 1	Rat 2	Rat 1	Rat 2
Before dosing	0.100	0.112	0.106	0.121
5–10 min after dosing	0.099	0.112	0.176	0.194
24 hr after dosing	0.096	0.114	0.157	0.146

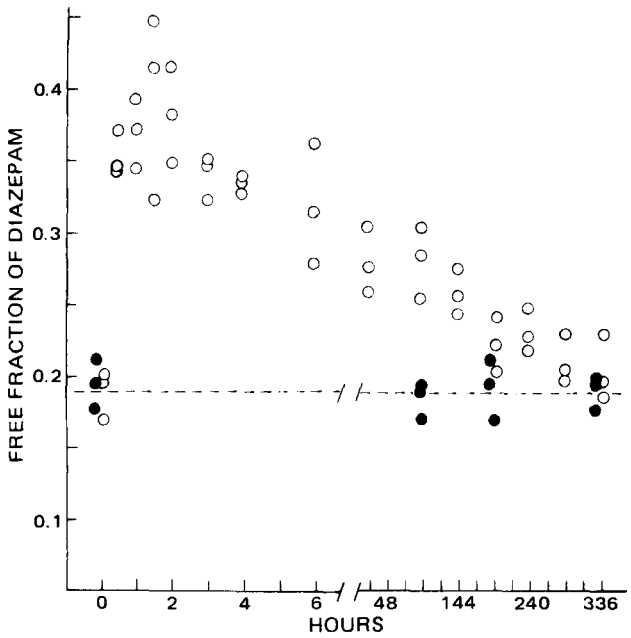

Figure 1—*Plasma protein binding of diazepam in rats after a 1-hr intravenous infusion of sodium oleate* (○) *or saline* (●).

oleate-treated rats. A maximum free fraction was observed 30–60 min after infusion. The unbound fraction of diazepam decreased continuously thereafter, but it was still higher than that observed in saline-treated rats after 7 days.

The maximum concentration of oleate in plasma (about 75 μmoles/liter) was observed at the termination of infusion. Thereafter, plasma concentrations of this fatty acid declined rapidly. Within 3 hr after administration, the plasma oleate concentration was less than 10 μmoles/liter. The reduction in plasma binding of diazepam appears to be unrelated to *in vivo* plasma oleate concentrations since *in vitro* addition of sodium oleate to plasma to produce concentrations comparable to those found 3 hr after *in vivo* administration had virtually no effect on diazepam binding. The same conclusions apply when sodium oleate is given as an intravenous bolus (Table I).

The prolonged impaired ability of plasma to bind diazepam after sodium oleate administration may be related to a slowly reversible change in the plasma proteins induced by the initially high concentrations of oleate or to the persistence of biotransformation products that can displace diazepam from binding sites. A more detailed report concerning the effects of sodium oleate on the plasma protein binding of phenytoin is in preparation.

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BOOKS

REVIEWS

The Alkaloids. Vol. 7. A Specialist Periodical Report. Edited by M. F. GRUNDON. The Chemical Society, Burlington House, London W1V OBN, England. 1977. x + 332 pp. 13.5 × 21.5 cm. Price $50.00. Available from Special Issues Sales, American Chemical Society, 1155 16th St., N.W., Washington, DC 20036.

M. F. Grundon is the senior reporter for the seventh volume of "The Alkaloids," which reviews the alkaloids literature published between July 1975 and June 1976. The reviews of Amaryllidaceae, *Erythrina*, imidazole, purine, and peptide alkaloids, which were not covered in Vol. 6, cover the period 1974–1976.

Each chapter effectively summarizes the important aspects of recent progress made on the particular types of alkaloids reviewed. Ample use of structures and schematics facilitates the clear presentations.

R. B. Herbert has again written an excellent chapter on biosynthesis, which includes recent work on secondary microbial metabolites as well as the various classes of alkaloids. It is of interest to note the increasing frequency of reporting the use of doubly-labeled precursors for biosynthetic work.

The major types of alkaloids are covered in the next 14 chapters, with the indole and isoquinoline alkaloids again representing the areas of most activity. The fast-growing use of ^{13}C-NMR spectroscopy for structure determination is becoming more evident. The final chapter (16) on miscellaneous alkaloids includes a section on unclassified alkaloids, which are presented in alphabetical order of plant or microbial source. The last section is noted to indicate the thorough reviewing characteristic of this series.

Volume 7 of this series continues the same concise format and style of the previous volumes. This reviewer especially likes the continued practice of placing the references on the page cited. For those who wish to keep abreast of the developments in the field of alkaloid chemistry, this volume is highly recommended despite the rather high cost.

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