Keyphrases

Dexamethasone tablets—stability
Degradation rate—dexamethasone tablets
Half-life—dexamethasone tablets
Thick-layer chromatography—separation
TLC—analysis
UV spectrophotometry—analysis
Colorimetric analysis

Problem in the Estimation of Drugs in Biologic Tissue Recovery of Phenol Red from the GI Tract

By STUART FELDMAN and MILO GIBALDI

Recovery of phenol red from homogenates of rat intestinal tissue, using aqueous extraction procedures, was near quantitative. However, significantly poorer recovery was observed after incubation of phenol red with intact intestinal sacs. It appears likely that phenol red binds to tissue or mucosa when in intimate contact, and the bound material is relatively resistant to aqueous extraction. The relevance of this phenomenon to tissue assays is considered.

THE CLASSIC approach to the determination of drug concentration in tissue involves the extraction of the compounds from homogenates. The general principles of this approach have been set forth by Brodie *et al.* (1). Accordingly, experimental standards are based on the recoveries of known amounts of drug from tissue homogenates. The assumption is made that the extent of recovery of standard quantities from the homogenate is equivalent to the recovery from *in vivo* tissue after drug uptake. The authors have recently observed anomalies in the recovery of phenol red from the gastrointestinal tracts of intact rats, rat intestinal sacs, and tissue homogenates, which suggest that the usual assumption of equivalent recovery may lead to significant error.

EXPERIMENTAL

Materials—Phenol red, trichloroacetic acid, and sodium hydroxide were obtained from Fisher Scientific Company as certified reagent grade.

Methods—Male Sprague-Dawley descent rats (Blue Spruce Farms, Altamont, N. Y.) weighing 140–180 Gm. were fasted 24 hr. The animals were then sacrified by decapitation, and the stomach and small intestine were removed. The small intestine was divided into three segments of equal length.

Tissue Homogenates—The stomach and each intestinal segment were homogenized individually for 5 min. in an Eberbach homogenizer with a minimum quantity of distilled water (approximately 3 ml.). The homogenates were placed in Nalgene tubes and 0.5 ml. of a 70 mg. % phenol red solution was added to each tube. The tubes were then agitated slowly for 1 hr. at 37° in a gyrotory water bath shaker.

Gastric Pouch and Intestinal Sacs—Each segment was ligated at both ends and 0.5 ml. of a 70 mg. % phenol red solution was injected in the pouch or sac through the ligated end by means of a 1-ml. tuberculin syringe and blunt needle. No loss of phenol red was observed through the ligatures.

Each segment was then placed in 20 ml. of Ringer's solution in a culture tube. The tubes were agitated for 1 hr. at 37°. The sac or pouch was then removed and homogenized as described above. The serosal fluid was also retained for assay.

Assay—The assay procedure was essentially that of Reynell and Spray (2). Each tissue segment (or homogenate thereof) was homogenized for 5 min. with 1 ml. of 1 *N* sodium hydroxide and about 5 ml. of distilled water. The resulting homogenate was brought to 30 ml. volume with distilled water, centrifuged, and filtered through a Büchner funnel. A 10-ml. aliquot was taken and 1 ml. of a 30% w/v trichloroacetic acid solution was added to the aliquot to precipitate proteins. After centrifugation 5 ml. of the supernatant was removed and 1 ml. of a 1 *N* sodium hydroxide solution was added to develop the color to maximum intensity. The solution was further diluted, passed through a Millipore filter (0.45 μ pore size), and assayed with a Beckman DB-G spectrophotometer at 560 mμ.

RESULTS

Table I shows the recovery of phenol red after addition to homogenates of various gastrointestinal segments. Recovery ranged from 93% to 103% with little difference between homogenates of different segments. The average recovery of phenol

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TABLE I—RECOVERY OF PHENOL RED ADDED TO TISSUE HOMOGENATES

Gastrointestinal Segment	Rat No. 1	Rat No. 2
	%	
Stomach	93	93
Intestine		
Proximal	100	96
Middle	95	103
Distal	100	96

red from all segments of both rats was 97%. These findings are in agreement with those of Reynell and Spray (2) who observed quantitative recovery of phenol red from gastrointestinal tissue homogenates.

The recovery of phenol red after incubation in gastric pouches or intestinal sacs was in marked contrast to the recovery from homogenates. The data in Fig. 1, which represents *total recovery* (*i.e.*, from sac or pouch, its content, and serosal fluid) show a significant *apparent* loss of phenol red from the intestinal segments. The recovery of phenol red from the proximal intestinal segments was significantly different from the recovery from each of the other segments at the 99% confidence level. A progressive increase in recovery is observed from the first to the third intestinal segment. The average recovery of phenol red from all segments was 70%. In each case, 5–10% of the recovered phenol red represents material which permeated the sac to the serosal fluid.

A further experiment was devised to determine whether incomplete mixing of the phenol red with the tissue homogenate was responsible for the excellent recovery from these preparations compared to the recovery from the sacs. In two studies phenol red was homogenized with the intestinal segments for 5 min. to ensure intimate mixing. The homogenates were then incubated as described above for 1 hr. and assayed for phenol red. The results are shown in Table II. An average of 92% phenol red was recovered from the homogenates indicating that mixing does not account for the observed differences.

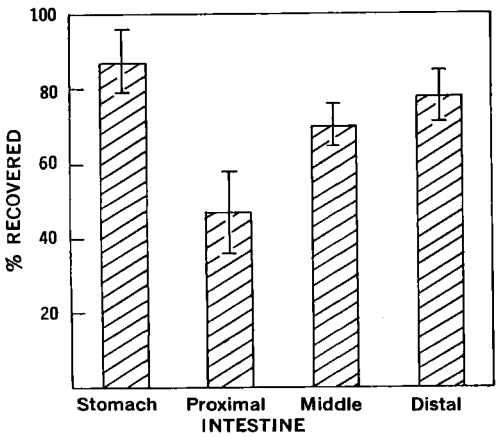

Fig. 1—Phenol red recovery from gastric pouch and intestinal sacs 1 hr. after intubation. Key: bar, average of 5 segments; vertical lines, standard deviations.

TABLE II—RECOVERY OF PHENOL RED HOMOGENIZED WITH TISSUE

Intestinal Segment	Rat No. 1	Rat No. 2
	%	
Proximal	86	81
Middle	94	94
Distal	98	100

DISCUSSION

The reason for the poor recovery of phenol red from intact sacs appears to be related to mucosal or tissue binding, since the possibility of mucosal metabolism has been ruled out by the work of Levine (3). Moreover, the degree of recovery of phenol red from the sacs was site dependent with increasing recovery in the distal direction. This finding is consistent with the amount of mucosal surface available for binding in various portions of the small intestine. If similar binding sites exist throughout the small intestine, then under the conditions of this experiment the greatest amount of phenol red would be bound to the segment having the largest mucosal surface area, *viz.* the proximal portion of the small intestine.

The nature of the binding requires further investigation. However, it would appear to require the mucosa and the phenol red to be in intimate contact. Dilution with fluids or tissue mass, as in the homogenate studies, greatly minimizes the degree of binding. Although the mucosal-bound phenol red is resistant to aqueous extraction, it is unlikely that the binding is covalent since Levine has effected quantitative extraction with ethanolic solutions (3).

The difference in recovery from sac homogenates and intact sacs indicates clearly the possible error involved in assuming the recovery of drug added to homogenates reflects the ultimate efficiency of the tissue assay procedure. This is further emphasized by considering the recovery of phenol red after administration to intact rats.

The authors have recently reported an average recovery of 80% of a dose of phenol red from the gastrointestinal tract, 0.5 hr. after gastric intubation in rats (4). Levy and Jusko (5) report an average recovery of 81% after oral administration of phenol red to rats. In view of the recovery of phenol red from tissue homogenates, one might assume that 15–20% of the dose of phenol red is absorbed from the tract. This value, however, is a significant overestimate. Levine (3) has found that less than 7% of a phenol red dose is absorbed from the gastrointestinal tract. Schanker (6) and Tidball (7) have observed still poorer absorption in the range of 1–2%. On the basis of these reports one must conclude that about 15% of the dose of phenol red is bound to mucosa or tissue *in vivo* and not recovered by the aqueous extraction procedure. The results of these incubation studies with gastric pouches and intestinal sacs are qualitatively consistent with the *in vivo* binding of phenol red.

No previous reports known to the authors have considered the possibility of nonequivalent extraction efficiency after incubation of drug with homogenates or intact tissue. Hence, the extent to which this problem does exist is now known. Nevertheless,

the possibilities and ramifications are manifest. The approach of incubating the drug with intact tissue before homogenization and assay may be a useful one and should be considered even when quantitative extraction from a homogenate is obtained.

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Keyphrases

Drug estimation—biologic tissue
Intestinal homogenates—phenol red recovery
Intestinal sacs, intact—phenol red recovery
Colorimetric analysis—spectrophotometry

Method for Testing the Efficacy of Topical Sunscreen Preparations

By W. M. KOOYERS

A method has been developed for testing the efficacy of topical sunscreen preparations using photosensitive albino rats. The method involves pretreatment of one hind paw with a sunscreen preparation, followed by an oral dose of a photosensitizing agent and exposure to direct sunlight. The delayed reaction and resulting difference between the treated and untreated hind paw weight is an objective index of the protection afforded by the preparation tested.

INTEREST IN topical sunscreen preparations was stimulated by the successful treatment of light-sensitive patients at the University of Minnesota Hospital. Fusaro and Runge (1–3) reported clinical data supporting the hypothesis that topical treatment of the stratum corneum can reduce or minimize the harmful effect of ultraviolet radiation.

The researcher's never-ending quest for new or better means to treat human diseases has led to the development of biological systems which simulate these diseases. As the diabetic rat is used as a screen for antidiabetic drugs (4), and the adjuvant arthritic rat for testing anti-inflammatory drugs (5), this method uses a photosensitive rat to test the efficacy of topical sunscreen preparations. A sunscreen preparation (SSP) is defined as a formulation which, when applied topically, protects the treated area from sunburn.

Albino rats are not hyperphotosensitive; however, rats treated orally with 25 mg./Kg. of 8-methoxypsoralen (8-MOP) become very photosensitive (6–9). Subsequent exposure of 3–7 hr. of direct sunlight will initiate a delayed reaction that progressively worsens. The resulting erythema, edema, and blindness will incapacitate these rats after 5–7 days. Rats photosensitized with 8-MOP, which have only part of their extremities exposed to direct sunlight, will show evidence of photosensitivity on the exposed areas only (9).

This photosensitive reaction is evident 2 or 3 days after exposure and can be visually evaluated by scoring the affected extremities from 0 to 4 depending upon the severity of the erythema and edema. A more objective index is the comparison of hind paw weights of photosensitive rats and normal rats. This is a measurement of the edema caused by the reaction. This objective index is used in this method.

METHOD

Five male albino rats[1] per group, weighing 180–200 Gm., were restrained in stocks while each left hind paw was dipped into the SSP. The restraint was maintained until the treated area was completely dry. This minimized the possibility of systemic effect that could be caused by ingestion if the rats were allowed to clean the treated area.

After application of the SSP, the sunscreen groups and one control group were dosed orally with the photosensitizing agent, 8-MOP[2] at 25 mg./Kg., and were then placed in direct sunlight for 5 hr. Each group was housed in a cage designed to allow maximum exposure to the sunlight. Normal controls were exposed simultaneously.

Three to seven days after exposure, the rats were sacrificed, body weights recorded, and both hind paws were uniformly severed (10) using a suitable apparatus.[3] The weight of each hind paw was recorded. The weight difference between the untreated right hind paw and the treated left hind

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[1] Spartan Research, Sprague-Dawley strain.
[2] Five mg./ml. of 8-MOP as a suspension containing per ml.: 5 mg. sodium carboxymethylcellulose; 4 mg. polysorbate 80; 9 mg. sodium chloride; and 9 mg. benzyl alcohol NF.
[3] Harvard Apparatus Company's decapitator.