surement was determined by making six replicate injections of a single standard preparation. The overall precision was evaluated by making one injection for each of six separate sample preparations and calculating the result by comparison with the six replicate single injections. Most of the error resulted from the response ratio measurement (Table I), which can be reduced by replicate injections.

Instrument setup and preparation of the standard required ~2 hr. Individual analyses, including calculations, can be carried out at a rate of more than one sample per hour.

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Nonvariance of LD_{50} Values of Drugs in Gravid and Nongravid Mice

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Abstract □ This study showed that the LD_{50} values for morphine sulfate, cobalt chloride, and phenytoin sodium did not vary significantly on Day 9 of gestation in CF-1 mice when compared to values of nongravid animals.

Keyphrases □ LD_{50} testing—gravid and nongravid mice, morphine sulfate, cobalt chloride, phenytoin sodium, teratological study □ Teratological study—gravid and nongravid mice, morphine sulfate, cobalt chloride, phenytoin sodium, LD_{50} testing □ Morphine sulfate—LD_{50} testing, gravid and nongravid mice □ Phenytoin sodium—LD_{50} testing, gravid and nongravid mice □ Cobalt chloride—LD_{50} testing, gravid and nongravid mice

The LD_{50} value is the dose of a drug that proves lethal to 50% of a population. Despite the frequent use of this parameter to determine dosage schedules in teratological studies, it is often utilized with reservation due to its variance in gravid mice.

Therefore, to determine the LD_{50} variance in gravid and nongravid mice, it was decided to utilize drugs from different classes (morphine sulfate, cobalt chloride, and phenytoin sodium) and to subject the mice to a typical LD_{50} screen. Gravid mice were challenged on Day 9 of gestation, which corresponds to the midpoint in mouse pregnancy and also with the appearance of overt birth defects (1).

EXPERIMENTAL

Male and female CF-1 mice[1], ≥25 g, were utilized. Males were caged individually, and females were maintained in groups of 10 in large aggregate cages until exposure to the males. All mice were allowed water and food[2] *ad libitum*.

For morphine sulfate[3] and cobalt chloride[4] testing, 40 nongravid females were assigned to one of five groups of eight using a table of random numbers. Fifty-five animals, 11 per group, were used for phenytoin sodium testing[5] in the same manner. Dosage levels then were assigned to the groups (Tables I–III). Each mouse was weighed to the closest 0.1 g, caged individually, and administered the respective drug subcutaneously on the medial side of the right inguinal region.

Morphine sulfate (2%) and cobalt chloride (1 or 2%) were prepared daily in double-distilled water; phenytoin sodium (1%) was prepared in 70% propylene glycol in 0.9% NaCl. If the injected volume exceeded 1 ml, it was divided into two equal portions and was injected in separate inguinal regions. After injection, each mouse was observed for 24 hr, at which time the fatalities were observed (Tables I–III).

Breeding was accomplished as follows. Two females were placed

Table I—Observed Deaths [a] with Morphine Sulfate after 24 hr

Mice	Dosage, mg/kg				
	250	400	450	500	750
Nongravid	0/8	2/8	2/8	5/8	8/8
Gravid	0/8	1/8	2/8	5/8	7/8

[a] Deaths per number of mice tested.

Table II—Observed Deaths [a] with Cobalt Chloride after 24 hr

Mice	Dosage, mg/kg				
	100	125	150	200	300
Nongravid	0/8	2/8	4/8	6/8	8/8
Gravid	0/8	1/8	3/8	5/8	8/8

[a] Deaths per number of mice tested.

Table III—Observed Deaths [a] with Phenytoin Sodium after 24 hr

Mice	Dosage, mg/kg				
	200	250	300	325	400
Nongravid	0/11	3/11	7/11	10/11	11/11
Gravid	0/11	6/11	9/11	9/11	11/11

[a] Deaths per number of mice tested.

[1] Charles River Breeding Laboratories, Wilmington, Mass.
[2] Purina Laboratory Chow.
[3] USP, Merck & Co., Rahway, N.J.
[4] Lot 713262, Fisher Scientific, Fair Lawn, N.J.
[5] Lot PE338, Parke-Davis Co., Detroit, Mich.

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Table IV—Statistical Comparison [a] of Drugs Tested in Gravid and Nongravid CF-1 Mice

Test Group	LD_{50}, mg/kg	Slope	Slope Ratio	Slope Ratio Function	Potency Ratio	Potency Ratio Function
Morphine sulfate			1.11	1.20	1.08	1.22
Nongravid mice	480 (428–538)	1.17				
Gravid mice	520 (488–603)	1.30				
Cobalt chloride			1.04	1.60	1.11	1.26
Nongravid mice	157 (138–179)	1.33				
Gravid mice	174 (144–210)	1.28				
Phenytoin sodium			1.09	1.33	1.11	1.19
Nongravid mice	273 (250–298)	1.16				
Gravid mice	246 (214–283)	1.26				

[a] Values calculated by the method of Litchfield and Wilcoxon (2).

overnight in cages containing one male. On the next morning (Day 0), all females were observed for the presence of coagulated intravaginal semen, which was the criterion used for determining pregnancy. Then all those deemed pregnant were weighed and placed in individual cages. All of the nonpregnant mice were returned to the aggregate cages for later use.

On Day 9, gravid mice were randomly assigned to one of the appropriate dosage groups, and the respective solutions were administered subcutaneously; the same number of animals were used as for the nongravid groups. After 24 hr, fatalities were recorded. All gravid mice then were cervically dislocated and laparotomized to confirm pregnancy by the presence of fetal swellings.

RESULTS AND DISCUSSION

The teratogenic effects of morphine sulfate, cobalt chloride, and phenytoin sodium were reported previously (1, 3, 4). The determination of dosage schedules in these studies was made under the assumption that the LD_{50} of each drug did not vary between gravid and nongravid mice.

The data in Tables I–III were subjected to probit analysis according to the method of Litchfield and Wilcoxon (2). Because the slope ratios were less than the slope ratio functions, it can be concluded, with 95% confidence, that the dose–response curves did not deviate significantly from parallelism (Table IV). Furthermore, because the potency ratios were calculated to be less than the potency ratio functions, it can be concluded, with 95% confidence, that the LD_{50} of the respective drugs did not change significantly on Day 9 of gestation in CF-1 mice.

The range of the conclusions in this study is limited by the strain of mouse and the peculiarities of the drugs. However, because the drugs from three different classes did not vary in gravid and nongravid mice with respect to their LD_{50} curves, these results possibly can be extrapolated to other drugs. Hence, one LD_{50} curve obtained from nongravid mice may serve as a valid reference to determine the dose of an agent to be tested for teratogenicity.

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Rapid Colorimetric Analysis of Chlorhexidine in Pharmaceutical Preparations

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Abstract □ A colorimetric determination of chlorhexidine is described. The method is based on the formation of a yellow complex between the drug and bromcresol green. The absorption peak of this complex, extracted by chloroform, is at 410 nm, and linear response is obtained from 2.5 to 30 μg of chlorhexidine/ml. The accuracy and reproducibility of this rapid method make it useful for chlorhexidine determination in the manufacturing control of pharmaceutical mixtures.

Keyphrases □ Chlorhexidine—analysis, colorimetric determination in commercial dosage forms □ Antimicrobial agents—chlorhexidine, colorimetric determination in commercial dosage forms □ Colorimetry—analysis of chlorhexidine in commercial dosage forms

Chlorhexidine (I), an antimicrobial agent, is a common ingredient in antiseptic preparations. The presence of chlorhexidine as a preservative in other pharmaceutical dosage forms (eye drops) and cosmetics necessitated a rapid, economical, and sensitive determination.

BACKGROUND

The primary method available previously was a colorimetric procedure (1), but several components in pharmaceutical formulations interfere in the determination. Another colorimetric assay using *N*-(1-naphthyl)-ethylenediamine was reported for the determination of chlorhexidine in biological fluids (2), but it is time consuming and has background interference. A direct spectrophotometric assay of chlorhexidine using bromthymol blue was reported recently (3). Chlorhexidine is estimated based on differences in the absorbance before and after complexation with the acid dye. These techniques yield rather large errors.

Polarographic estimation (4) and an automated liquid chromatographic determination (5) also were reported recently. Chlorhexidine also has been determined successfully by GLC after hydrolysis to *p*-chloroaniline and suitable derivatization to *p*-chloroiodobenzene (6, 7), but this technique is time consuming and unsuitable for multiple analyses.

This paper describes a rapid colorimetric method that is based on the solvent extraction of a dye complex and is suitable for chlorhexidine analysis in pharmaceutical mixtures and cosmetics.

EXPERIMENTAL

Instrumentation—A double-beam spectrophotometer[1] and a shaker[2]

[1] Perkin-Elmer model 554.
[2] Lab-Line.

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