The Oral Toxicity of Clindamycin in Laboratory Animals

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The Oral Toxicity of Clindamycin in Laboratory Animals. Gray, J. E., Weaver, R. N., Bollert, J. A., and Feenstra, E. S. (1972). Toxicol. Appl. Pharmacol. 21, 516-531. The potoxicities of clindamycin hydrochloride and clindamycin palmitate have been extensively studied in mice, rats and dogs. For the hydrochloride, the ip mouse and po rat LD50 values were 361 and 2618 mg/kg, respectively. The values for the palmitate ester were >2500 and >5000 mg/kg; in a formulated syrup the po LD50 was reduced to 1950 mg/kg.

The maximum daily tolerated dose of the hydrochloride in the dog and rat for as long as 1 yr was greater than 300 mg/kg but less than 600 mg/kg. Significant changes in bioclinical data in dogs treated with 300 mg/kg were confined to sporadic elevations in serum transaminase levels. At 600 mg/kg, in short-term studies, the effects of focal irritation were recognized in the mucosa of the stomach and gallbladder of the dog. No teratogenic effect or developmental retardation in the offspring of mouse or rat dams, or effect on the breeding performance of male and female rats was observed.

In a comparable series of studies with the palmitate ester, essentially no effects due to the antibiotic were detected. A daily dose of 600 mg/kg was well tolerated in the dog and rat for 6 mo.

Clindamycin hydrochloride, a new semisynthetic antibiotic, is produced by chlorination of lincomycin (Birkenmeyer and Kagan, 1970). Sun (1970) has shown that clindamycin administered po at 100 mg/kg is rapidly and quantitatively absorbed in the Sprague-Dawley rat and in the beagle. The antibiotic and its metabolites are rapidly excreted in urine (roughly 1/3) and in bile (roughly 2/3). In the rat, 53% of the urinary radioactivity was intact clindamycin, 31% was the sulfoxide and 15% was N-demethyl clindamycin. In the dog, 36% of the urinary radioactivity was unchanged clindamycin, 28% was the sulfoxide, 28% was the glucuronide conjugate and 9% was N-demethyl clindamycin. The major difference between the 2 species was the presence of the glucuronide conjugate in the urine of the dog.

Toxicologic data in animals has been obtained through studies of conventional design. Major emphasis has been placed on the use of near maximum tolerated doses in both short- and long-term treatments. Ultrastructural effects in the hepatocyte were monitored in the rat and dog (for as long as a year during treatment in the dog) (Gray et al., 1971). In the teratologic studies the mouse was substituted for the rabbit, which is peculiarly intolerant to any antibiotic which alters the intestinal flora as a result of activity against gram-positive organisms (Gray and Lewis, 1966).

The results of a similar series of studies which have been conducted on the po pediatric

1 Cleocin® is the registered trademark of the Upjohn Company for clindamycin.

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formulation of the 2-palmitate ester of clindamycin hydrochloride are also presented. A major consideration in accomplishing these studies was the tolerated volume of the formulated syrup vehicle rather than the antibiotic itself.

**METHODS**

**Clindamycin Hydrochloride**

LD$_{50}$ determinations (ip, iv in mice; po in rats; sc in newborn and adult rats). For all LD$_{50}$ determinations, 7-chloro-7-deoxylincomycin hydrochloride (88.6% base equivalence) was dissolved in sterile water. White Swiss mice (20 g) of the ICR line and Upjohn Sprague-Dawley (TUC/SD) rats (175 g) were fasted overnight prior to weighing and dosing, after which they were observed for 7 days. The sc route of administration was used to establish the comparative toxicity in newborn (6 g) and adult (400 g) rats. The Spearman-Karber method (Finney, 1952) was employed to determine the LD$_{50}$.

Five-day tolerance studies in the rat and dog. Single aqueous doses of 500 mg/kg were given by gastric intubation of 5 TUC/SD male rats (average 100 g) for 5 days. During the test period, body weights, food consumption and hematologic values of these rats and those of a comparable group of control rats were obtained. Necropsy was performed on the day following the last treatment.

Similarly, 3 female beagles were treated po with the antibiotic in capsules. Serum samples were obtained for assay of clindamycin bioactivity following administration of 113 mg/kg (100 mg/kg base equivalence) on the first day. On the following 4 days, a single dose of 500 mg/kg was given to each dog. The day after the last dose, blood and urine samples were obtained for biochemical determinations as in the subacute and chronic studies.

Subacute and chronic studies in the rat. Aqueous solutions of 500 mg/kg of clindamycin hydrochloride were given to Spartan Sprague-Dawley rats daily for 1 yr. At the onset, 10 male and 10 female rats were assigned to each of 4 groups, 0, 30, 100 and 300 mg/kg, respectively. A fifth group of 20 rats was treated with 600 mg/kg/day for 6 mo. The beginning body weight averages were 100–105 g. Each rat was caged individually and given free access to water and BA dry mash diet (Evans and Gray, 1959). Clinical examinations were made daily; body weight and food consumption computations were done each week. Hematologic data (hemoglobin, hematocrit, total and differential white count) were obtained after 1 mo, periodically at 5 intervals and terminally at 12 mo. Gross and microscopic examinations were made on 2 males and 2 females of each of the 4 groups after 1 mo of treatment. Examinations were completed on most of the rats which died during the study and on the survivors, which were killed by electrocution at the termination of the study. The liver, kidneys, adrenals, heart and testes were weighed and examined. Paraffin sections of these and other major organs were stained with hematoxylin-eosin; selected tissues were stained with Oil Red 0. To detect any phototoxic property, 4 rats from each group were exposed to direct sunlight during the 8th mo (June) for 2½ hr. A positive control group was pretreated po with 50 mg/kg of 8-methoxypsoralen.

Subacute and chronic studies in the dog. Initially, clindamycin was given po in capsule to 3 groups of 6 beagles each at 30, 100 and 300 mg/kg daily, and 5 beagles served as controls. After 1 mo, 2 dogs from each treatment group and 2 of the control dogs were
sacrificed for interim pathologic examination. Two dogs in the 300 mg/kg group were sacrificed after 3.5 mo. Four other dogs were treated with 600 mg/kg/day for 2 wk; 1 was sacrificed at that time, 1 was treated for 3 more wk before sacrifice, and 2 were removed from treatment and allowed to recover for 2 wk before they were killed. During the study each of the 28 dogs was caged individually and fed Purina Lab Chow daily.

Clinical examinations were made daily and each dog was weighed each week. Twice prior to treatment, at monthly intervals and terminally, the following clinical laboratory data were obtained from each dog: hemogram, blood glucose, urea, creatinine, total protein, prothrombin time and urinalyses (which included concentration test, specific gravity, pH, albumin, glucose, bile and microscopic examination). Hepatic function tests, which included bromosulfalein retention (BSP), serum transaminases (SGOT, SGPT) and alkaline phosphatase (K-A units) were done from 19 to 24 times on the same days liver biopsies were taken for electron microscopy. SGPT determinations were done by a modified Reitman-Frankel procedure with Enzatrol\(^2\) as a control sample.

At the termination of the study after 1 yr, the remaining 10 treated and 3 control dogs were sacrificed by electrocution, and complete necropsies were performed. The weights of the spleen, adrenals, kidneys, liver, ovaries, testes, prostate, thyroid, heart, brain and pituitary were recorded. Paraffin sections stained with hematoxylin-eosin that were examined microscopically included the brain, eye, tonsil, salivary glands, esophagus, stomach, pancreas, duodenum, ileum, lymph nodes (mandibular, bronchial, mesenteric), liver, gallbladder, heart, aorta, lung, adrenals, pituitary, spleen, thyroids, kidneys, urinary bladder, prostate, testes, epididymis, ovary, uterus, skeletal muscle and sternal bone marrow. Formalin-fixed sections of the heart, liver, kidney and adrenal were stained with Oil Red O.

Terminally, serum and bile samples were assayed for microbiologic activity using the clindamycin standard in pooled commercial dog serum. Urine samples were diluted 1:3 and 1:10, and bile 1:100, with 0.1 M, pH 7.0, potassium phosphate buffer, and assayed against the standard in buffer. A disk agar-diffusion method was employed utilizing *Sarcina lutea* UC-130 in BBL seed agar.

**Teratogenic and reproduction studies.** In the first study, clindamycin was given po at 100 mg/kg to 14 pregnant TUC/SD rat dams between days 6 and 16 of gestation. Also, 13 pregnant rats dosed with the aqueous vehicle during the same period served as controls. The 20-day fetuses from 7 of the treated dams and 6 of the control dams were examined grossly, and transverse slices of fixed fetuses and alizarin red-stained fetuses from the same litter were studied under the stereoscopic microscope for soft tissue and bone abnormalities. The litters of the remaining dams (7 treated, 7 controls) were observed for livability, sex distribution and postnatal development.

In the second study, clindamycin was administered po in the food at 30 mg and 60 mg/kg to determine the effect upon reproductive performance of male and female rats for 1 generation. The 5 groups (2 dose levels with treated-untreated and reciprocal matings; 1 control group) each consisted of 10 males and 20 females of the Upjohn Sprague-Dawley line. Treatment was started in the males at 40 days of age and in the proven breeder females 14 days before breeding. In both sexes treatment was terminated

\(^2\) Dade Reagents, Inc. Miami, Florida 33152.
at the weaning of the F1 generation. Livability, sex ratios, body weights and clinical condition of the offspring were determined at weekly intervals from birth to weaning.

The effect of clindamycin in pregnancy was also studied in the rat (CD/spf) and mouse (CD-1/spf) at Huntingdon Research Center, Huntingdon, England (Palmer and Searles, unpublished data). In the rat study, daily doses of 50 and 200 mg/kg (22 dams/group) were administered po from day 6 to day 15 of gestation. The incidence of major malformations, minor anomalies and distribution of skeletal variants as well as the effect on litter size, fetal loss, and litter and mean pup weight were determined. Mouse dams (24/group) received po daily dosages of 20, 50 and 200 mg/kg from day 6 through 15 of gestation. Similarly, standard teratologic methods and reproduction parameters were utilized. Untreated groups of pregnant dams were included in both studies.

**Clindamycin Palmitate**

*LD50* determinations (ip in mice; ip and po in rats; sc in newborn and adult rats). The methods employed for these studies were the same as those for the hydrochloride. The po LD50 value was also determined for clindamycin 2-palmitate (as the HCl salt, 60% base equivalence) in formulated syrup (30.5 g sucrose and 6.0 g lactose/100 ml); the concentration of the antibiotic was 25 mg/ml.

*Seven day tolerance studies in the rat and dog.* Flavored granules were administered po as a syrup at 1000 mg/kg to 10 Upjohn Sprague-Dawley rats (5 males, 5 females) for 7 days. Clinical, hematologic and histologic examinations were made on these rats as well as those of a similar untreated group.

The same formulation was given po by gastric intubation to 3 beagles at 600 mg/kg for 7 days; the daily treatment was divided into 3 doses. Two pretest and terminal hemograms, blood chemistry determinations and urinalyses were obtained on each dog. The dogs were necropsied, and organ weights were obtained. Histologic observations were made on major organs.

*Chronic study in the rat.* The flavored granule formulation was given to 4 groups (1 control, 3 treated) of 20 rats each (10 males, 10 females) for 6 mo. The daily doses were 100, 300 and 600 mg/kg. At the end of 2 mo, 6 rats (3 males, 3 females) of each group were killed for interim pathologic examinations. Hemograms were obtained at 6 wk, 2 and 4 mo, and terminally. Other aspects of the study were similar to those of the chronic study of the hydrochloride in the rat.

*Chronic study in the dog.* The flavored granule formulation was given to 3 groups of 6 beagles each for 6 mo. The daily doses were 30, 100 and 300 mg/kg. Six beagles were used as controls; each received a volume of the sugary vehicle comparable to that given the 300 mg/kg group. After 2 mo of treatment, 2 dogs from each group were killed for interim pathologic examinations. Hemograms, blood chemistry determinations, liver function studies and urinalyses were obtained twice prior to treatment and at monthly intervals during treatment and terminally. Gross and microscopic observations were made and organ weights recorded on each dog utilized in the chronic study of the hydrochloride.

*Teratogenic and reproduction studies.* Pregnant rats, 2 groups of 10 each—TUC/SD (pf)—were treated from day 6 through 15 of gestation. The daily doses of the flavored granule formulation were 300 and 600 mg/kg; a third group received no treatment.
Clindamycin palmitate was also administered by gastric intubation to pregnant mice, TUC/ICR (spf), at doses of 150, 300 and 600 mg/kg. In these groups there were 20, 20 and 40 mice, respectively. A control group of 40 pregnant mice was included in the study. The teratogenic potential of the clindamycin palmitate and the reproductive performance of the dams of both species were evaluated by procedures described for similar studies of the hydrochloride.

Regimens of 100 and 300 mg/kg of the same formulation were used to evaluate the effect on reproductive performance during 1 generation of breeding rats, TUC/SD (spf). The conditions of the study and the duration of treatment of both sexes were essentially the same as those described for clindamycin hydrochloride.

RESULTS

Clindamycin Hydrochloride

LD50 determinations. The LD50 values obtained by various routes of administration are presented in Table 1. Mice injected parenterally became depressed following the administration of doses near or above the LD50 values. Death was always preceded by convulsions. Deaths occurred within 1 to 2 min after iv administration and from 15 min to 4 days, depending largely on the dose, after ip administration. Rats treated by the po or sc route usually died 1–2 days after a lethal dose was given.

Five day tolerance studies in the rat and dog. Body weights and food consumption values of rats treated with 500 mg/kg daily and those of a comparable group of control rats are shown in Table 2. The major finding in the treated group was diarrhea; by the fifth day all 5 rats were affected. Hematologic values obtained on the first day after completion of the treatment schedule revealed no drug-related changes. No significant naturally occurring or drug related lesions were observed at necropsy.

The dogs vomited rather consistently 1–2 hr after a daily dose of 500 mg/kg, but not after a dose of 113 mg/kg on the first day. Otherwise the dogs remained in good clinical health during the test. No serious effects were produced, but this preliminary evidence suggested that the upper limit of tolerance in the dog was approached. Serum levels of clindamycin obtained during the first day of treatment (Table 3) indicated rapid absorption; peak levels were attained within 1 hr.

Subacute and chronic studies in the rat. Fecal pellets of test rats were slightly less well formed than those of the controls during the first mo of treatment. Thereafter they were indistinguishable except in the group treated with 600 mg/kg daily. No diarrhea was observed. Also, during the 1st mo only, inflammation of the lacrimal and salivary glands occurred in 29 of the 80 rats. Rales, weight loss and mortality associated with murine pneumonia occurred with increasing frequencies on an individual basis after the 6th mo.

Phototoxic effects, such as erythema and edema of the ears and dorsum of the feet, were observed in control rats treated with the psoralen compound and exposed to sunlight. Such effects did not occur in rats treated with clindamycin. Exposure was terminated when bulging and congestion of the eyeballs occurred in rats of all groups, including the controls. It was determined that severe periorbital inflammation had occurred as a result of excessive exposure to sunlight. Corneal opacity occurred in many of the exposed rats with occasional hypopyon and rupture.
### TABLE 1

**LD50 DETERMINATIONS** of Clindamycin Hydrochloride and Clindamycin 2-Palmitate

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mouse</th>
<th>Rat</th>
<th>Newborn: adult toxicity ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ip</td>
<td>Iv</td>
<td>Po</td>
</tr>
<tr>
<td>Clindamycin hydrochloride</td>
<td>361 (325–401)</td>
<td>245 (225–267)</td>
<td>2618 (2273–3014)</td>
</tr>
<tr>
<td>Clindamycin palmitate</td>
<td>&gt;2500 (1753–2182)</td>
<td>—</td>
<td>&gt;5000 (1956&lt;sup&gt;a&lt;/sup&gt;)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Spearman Karber method of calculating the LD50 with 95% confidence limits (Finney, 1952).

<sup>b</sup> Formulated in syrup.
TABLE 2

WEIGHT GAIN AND FOOD CONSUMPTION DATA OF SPRAGUE-DAWLEY RATS DURING TOLERANCE STUDIES OF CLINDAMYCIN HYDROCHLORIDE AND THE 2-PALMITATE ESTER

<table>
<thead>
<tr>
<th>Statistic</th>
<th>Clindamycin hydrochloride</th>
<th>Clindamycin palmitate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>500 mg/kg daily Control</td>
<td>1000 mg/kg daily Control</td>
</tr>
<tr>
<td>Starting weight (av g)</td>
<td>117.5</td>
<td>93.4</td>
</tr>
<tr>
<td>Terminal weight (av g)</td>
<td>136.3</td>
<td>148.6</td>
</tr>
<tr>
<td>Weight gain (av %)</td>
<td>+16.0</td>
<td>+59.1</td>
</tr>
<tr>
<td>Food consumption (av g)</td>
<td>62.0</td>
<td>96.8</td>
</tr>
<tr>
<td>Food conversion ratio</td>
<td>3.29</td>
<td>1.75</td>
</tr>
</tbody>
</table>

*a* Male rats were treated for 5 days with clindamycin hydrochloride, and male and female rats were treated for 7 days with the 2-palmitate ester.

*b* Grams food per gram weight gain.

TABLE 3

SERUM CLINDAMYCIN BIOACTIVITY* IN DOGS

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Sample time, Hr</th>
<th>Ratio* area under curve</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>4D-18</td>
<td>0</td>
<td>24.9</td>
</tr>
<tr>
<td>3M-22</td>
<td>0</td>
<td>12.9</td>
</tr>
<tr>
<td>4B-36</td>
<td>0</td>
<td>44.6</td>
</tr>
</tbody>
</table>

*a* As µg chlorolincomycin free base per ml.

*b* For area computation purposes, the 8-hr serum level was the last significant point, and all areas are only calculated for the first 8 hr.

The body weight gains of the treated rats were somewhat greater (3.6–14.4% in the 3 treated groups) than that of controls during the 1st mo. There was a tendency for treated rats to eat more and to gain slightly more than the controls. However, the respective food conversion ratios were regarded as comparable. During the latter half of the study, the presence of 1 or more rats with murine pneumonia in a group frequently skewed the average body weight and food consumption values temporarily for that group. The terminal values were somewhat variable, but the deviations from the control values were regarded as neither dose nor treatment related.

Hematologic data obtained periodically remained in the normal range of values. From the first to the terminal set of data the packed cell volume decreased gradually about 5% for both sexes of all groups. The neutrophil:lymphocyte ratio of all groups also changed from approximately 1:6 to 1:4. These changes were believed to be related to the maturation of the rats during the 1 yr study.

At termination, the average weight of the liver and the kidneys of the treated groups, on a % body weight basis, was slightly higher than that of the control group.

Mortality data and the incidence of spontaneous lesions are presented in Table 4.
TABLE 4
MORTALITY DATA AND SPONTANEOUS LESIONS OF GROUPS OF SPRAGUE-DAWLEY RATS* TREATED FOR 1 YEAR WITH CLINDAMYCIN HYDROCHLORIDE

<table>
<thead>
<tr>
<th>Mortality data</th>
<th>0 mg/kg</th>
<th>30 mg/kg</th>
<th>100 mg/kg</th>
<th>300 mg/kg</th>
<th>600 mg/kgb</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>F</td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>F</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>---------</td>
<td>----------</td>
<td>-----------</td>
<td>-----------</td>
<td>------------</td>
</tr>
<tr>
<td>Mortality datac</td>
<td>0</td>
<td>2d</td>
<td>2</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Spontaneous lesions</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(1) Kidneys</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Progressive degeneration of older rats</td>
<td>6/8</td>
<td>0/6</td>
<td>3/6</td>
<td>3/6</td>
<td>3/5</td>
</tr>
<tr>
<td>(2) Respiratory System</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic murine pneumonia</td>
<td></td>
<td></td>
<td>3/6(1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic tracheitis</td>
<td></td>
<td></td>
<td></td>
<td>1/8</td>
<td></td>
</tr>
<tr>
<td>(3) Pancreas (acinar)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Degenerative change and/or inflammation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mild and focal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(4) Prostate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Suppuration</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(5) Mammary glands</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibroadenoma</td>
<td>(1)</td>
<td>(1)</td>
<td>1/8</td>
<td></td>
<td>1/5</td>
</tr>
<tr>
<td>(6) Pituitary gland</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chromophobe adenoma</td>
<td>1/6</td>
<td>1/6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(7) Periarteritis, nodular</td>
<td>1/6</td>
<td>1/6</td>
<td>1/5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
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</tr>
</tbody>
</table>
* Each group consisted of 10 males and 10 females. After 1 mo, 2 males and 2 females from each group (except 600 mg/kg group) were killed.
+ This group was treated with 600 mg/kg for 6 mo.
' During the year, 13/50 of each sex died. Diagnoses made on rats which died are indicated parenthetically. The other values represent the spontaneous findings which were obtained at the termination of the study. For dead rats unaccounted for under spontaneous lesions, either aspirated drug was detected in the lungs or no diagnosis could be made. In a few instances, more than 1 diagnosis was made on an individual rat.
* One female with "ocular sunburn," which developed in individuals selected for photosensitization study, was killed for pathologic examination.
Aspirated drug in the lungs or intubation accidents were considered to be the cause of death in 9 of the 26 rats which died prior to termination of the study; these losses occurred chiefly in the 300 and 600 mg/kg group. The majority of deaths in the 600 mg/kg group (10) occurred after 4–5 mo of treatment. Although no morphologic diagnosis could be made for 7 of these rats, a relationship to the high treatment regimen seemed probable.

No evidence was obtained from this rat study of a specific morphologic effect due to treatment with clindamycin hydrochloride. Progressive degeneration of the kidneys was more evident in the terminal study than chronic changes in the respiratory tract. Cast formations and atypical hyperplasia of renal epithelium appeared in some cases to be more marked than thickening of basement membranes. Focal and variable changes observed in the pancreas sections of 4 rats were regarded as being too small to affect the overall function of the acinar tissue.

Subacute and chronic studies in the dog. Dogs given 30 and 100 mg/kg daily remained clinically healthy throughout the study. No evidence of salivation, emesis, diarrhea or lassitude was obtained. The body temperatures remained normal. Appetites were regarded as good. After 3.5 mo, 2 dogs of the 300 mg/kg group were sacrificed. One of these dogs had high serum transaminase values (SGPT, 700; SGOT, 300), and was somewhat dehydrated but not clinically sick. A second dog of this group became abruptly sick and died with intussusception and peritonitis as a result of a punctured bile duct following liver biopsy.

From the start, dogs given 600 mg/kg vomited and did not eat readily; supplemental meat and vitamins helped their condition only temporarily. After 2 wk they were dehydrated, and 3 of the 4 were clinically sick, having black, tarry feces. One of the sick dogs was sacrificed and necropsied, 2 others were taken off treatment and allowed to recover; treatment was continued in the fourth dog for another 3 wk, despite increasing intolerance.

No significant loss of body weight, either transient or continuing, occurred in any of the dogs treated for 1 yr. One dog of the 300 mg/kg group had a moderate weight loss (16.5%) during the 1st mo, after which time the dog was necropsied.

Hematologic data from the treated dogs remained essentially normal throughout the study. At the 4th mo, mild transient elevations (2/10 values) of the total leukocyte count were recorded in dogs of the 100 and 300 mg/kg groups. Hemoconcentration was found in certain dogs which became dehydrated (600 mg/kg group; 1 of 300 mg/kg group). Higher than normal prothrombin time values in some dogs without respect to level of treatment were regarded as not drug related.

Significant changes in the blood chemistry data throughout the year of treatment were confined largely to sporadic elevations of the SGPT values. The SGOT values were consistently lower than the corresponding SGPT value (Fig. 1). Elevations of SGPT values were first observed in dogs of the 300 mg/kg group about 2 wk after treatment started. The values obtained from some dogs, particularly those of the high group, fluctuated considerably during the year. From month 7 to 9, transient elevations occurred in dogs of all 3 treated groups. Elevations above 50 R-F units were also recorded in the control dogs during the same period, though to a lesser degree. Other tests (BSP, alkaline phosphatase) gave no indication of altered hepatic function.

In the group started on 600 mg/kg, there were similar elevations in SGPT values after
1–2 wk of treatment in 3 of the 4 dogs. Mild increase (2.2–3.2%) in BSP dye retention was noted in 2 of these dogs. Qualitative determinations of monthly urine samples from the treated dogs were similar to those of the controls. No drug related effects were detected in organ weights of dogs sacrificed after 1 mo and terminally.

Lesions related to the po administration of clindamycin hydrochloride in the dog were confined principally to the mucosa of the stomach and the gallbladder. These findings were fully assessable only in dogs which were treated with 600 mg/kg daily.

With the exception of changes in 1 dog of the 300 mg/kg group sacrificed after 3.5 mo, dogs given 30, 100 and 300 mg/kg remained free of pathologic changes regarded as drug-related. Bile from the gallbladder after 12 mo treatment revealed variable but generally high concentrations of clindamycin. The average concentration was about 275 µg of base/ml, and the highest value was 620 µg/ml. The concentration in the bile was 20–50 times that in the serum on the last day. The gallbladder of these dogs, however, appeared normal at necropsy.

In dogs treated with 600 mg/kg and necropsied at the termination of 2–5 wk of treatment, the lining of the gallbladder was marked by 1 to several bile-stained ulcers.
These varied from punctate (1 mm) to irregular coalesced lesions several mm across. Microscopic sections revealed foci of coagulative necrosis which penetrated into the muscular layers (Fig. 2). The inflammatory cell response about the base of the ulcer was mild and mixed. Mucosal folds adjacent to the necrotic areas appeared hyperplastic and became occluded so that microcysts, filled with mucuslike content, were often formed. This type of lesion was not found in dogs which had been allowed to recover for 2 wk.

Minute focal erosions of the gastric mucosa were more easily evaluated upon microscopic examination than by gross inspection. The eroded areas varied in size and depth; in some foci, gland cells and lamina propria as well as surface epithelium were necrotic (Fig. 3). Congestion and small hemorrhages were usually present. Inflammatory cells were generally scarce.

No distinctive light microscopic changes were observed in paraffin sections of the liver.

Teratogenic and reproduction studies. In observations of 116 rat fetuses for soft tissue and 51 for bone abnormalities, only a few minor deviations from normal morphology were noted. The litters of both treated and control dams were large (average 13.1 and 12.8, respectively), and the fetuses were uniform. Resorption sites in the dams of both groups were few (3.75 and 7.1% of implanted ova) and well within the usually observed incidence in the Sprague-Dawley rat. The sex distributions of the fetuses of both groups were regarded as similar. No evidence of teratogenic effect of clindamycin
was found; neither did treatment affect the fecundity of the dams or the development of the offspring.

The reproductive performance of male and female rats during 1 generation was not significantly affected. Treated females conceived at only a slightly lower rate, their young were slightly smaller in size at birth and were slightly smaller at weaning than the untreated controls.

In the Huntingdon studies, embryonic and fetal development in the rat were unaffected by treatment. At 200 mg/kg the clinical appearance of the dams became slightly inferior to that of the control dams or of the dams given 50 mg/kg. Similarly, no teratogenic effect or developmental retardation was observed in the offspring of mouse dams treated with either 20 or 50 mg/kg. Inasmuch as effects upon litter parameters were recorded only at the dose toxic to the dam (200 mg/kg), there was no evidence of a selective embryopathy in the mouse.

**Clindamycin Palmitate**

*LD50 determinations*. Values within practical limits of dosing are shown in Table 1. In contrast to the po LD50 of >5000 mg/kg for the ester alone, the LD50 for the ester formulated in syrup was reduced to 1950 mg/kg. At necropsy, the stomach of a rat treated with a single formulated dose of 2000 mg/kg or higher showed congestion of the mucosa but was not hemorrhagic; the intestinal tract, including the cecum, was distended with fluid. Histologically, remarkable findings were hydropic degeneration of mainly parietal cells in the gastric mucosa, and low grade necrobiosis of Peyer patches and the lamina propria of the intestinal villi. Both hepatic and proximal renal...
tubule cells were vacuolated. These changes were readily reproduced by giving 12 ml of the sugar vehicle alone to a 125-g rat.

Seven day tolerance studies in the rat and dog. The flavored granule formulation was well tolerated at 1000 mg/kg in the rat. The fecal pellets of the treated rats remained essentially unchanged from those of the control rats. The increase in body weights of both sexes during the 7-day study, as well as the food consumption ratios, were regarded as being similar in both the treated and control groups (Table 2). Terminal hematogetic values, and gross and histologic examinations gave no indications of any drug-induced effect.

The treatment regimen of 600 mg/kg for 7 days was well tolerated by 3 beagles. A marginal increase in alkaline phosphatase values in 2 of the dogs could not be readily correlated with the steady levels of serum transaminases and the lack of morphologic evidence of drug effect in the liver of these dogs. No unusual deviations were found in the urinalyses and organ weight data.

Chronic study in the rat. All 3 treatment levels were well tolerated for 6 mo. Terminally, the body weight gains of females of the 300 and 600 mg/kg groups were superior (13.4 and 22.3%, respectively) to those of the control females. Gains of the 4 male groups were similar. The food conversion ratios of the control groups and the 600 mg/kg groups were better than those of the 2 lower treatment groups. In this respect, it is probably significant that the control groups received a daily volume of the syrup vehicle equivalent to that of the high dose groups; the lower 2 treatment groups received proportionally less of the syrup vehicle.

Hemograms gave no indication of a drug effect. Weights of liver, kidneys, adrenals, gonads (testes) and heart were similar to that of the respective controls. No drug related findings were observed upon necropsy of all 80 rats. In the 32 rats examined microscopically (8/level), the mucus-secreting cells of the mixed portion of the submaxillary gland revealed a somewhat distorted nuclear pattern. Nuclei were observed commonly to be hyperchromatic, enlarged, malpositioned and fused. The atypical appearance was considered to be a sequel to the widely spread sialodacryoadenitis in all 4 groups during the first mo of the study.

Chronic study in the dog. All dogs were healthy throughout the study. Body weights were relatively uniform with the exception of 2 dogs in the control and 300 mg/kg groups, which gained 22.2 and 26.7%, respectively, during the 6-mo period. No gastrointestinal disturbance (emesis, diarrhea) was encountered in spite of the high volume (120-140 ml) of the sugary vehicle which was administered daily to the control and high dose groups. The large volume of the vehicle was regarded as a limiting factor in extending the treatment levels upward.

No significant deviations related to the administration of the drug were found in the hematology, blood chemistry and urinalysis data. Alkaline phosphatase values were consistently higher in 3 dogs (2 controls; 1 300 mg/kg) than those usually obtained; the pretest values in these dogs also ranged higher than usual. No relationships to treatment were recognized in the organ weight data. Microscopically, a variety of low grade inflammatory and degenerative lesions were observed. Since the incidence of these findings was similar for the various groups, no morphologic response to the treatment regimens was apparent.

Teratogenic and reproduction studies. Clindamycin palmitate was not teratogenic
when given to pregnant rat and mouse dams. By the visceral and skeletal methods of examination, the fetuses of treated dams were found to be comparable to those of the controls. The reproductive performance of the treated dams was also comparable to that of the controls. No drug-related effect was apparent in treated males.

DISCUSSION

Some aspects of the interpretation of the serum transaminase values in the 1 yr po study of clindamycin hydrochloride in the dog deserve further consideration. SGPT or alanine transaminase has been shown to be an enzyme of high specificity. In the liver, which is its principal source, the enzyme is almost wholly confined to the cell sap or soluble fraction (Wilkinson, 1970). Leakage from the liver into the blood, it is believed, may occur with only very slight damage or effect on the cytoplasmic membrane of the hepatic cell. The transaminase value is generally not regarded as a measure of liver function but relates more, perhaps, to the membrane efficiency of the hepatic cell with respect to its external environment. Elevated transaminase values, at least the commonly encountered lower levels of increase, are not generally regarded as indicative of irreversible damage or necrosis of the liver cell. The average pretreatment or control value in the beagle in our laboratory has been 22 ± 5.8 R-F units/ml of serum, yet empirically a value of 50 units has acquired greater acceptance with respect to the onset of clinical significance of hepatic injury. Even so, sporadic values of 50 and higher are recorded among values from control dogs.

There has been rather widespread utilization in animal toxicity studies of the SGPT value as the most sensitive expression of initial intolerance of the liver to a drug. The value of this sensitivity may be offset, particularly in the case of slight or marginal increases, by aberrant values. From Fig. 1, in which all SGPT values from the control group are plotted, there is an unexplained tendency for the values to average in a higher, yet "normal" range (35-50 R-F units) and to vary more toward the end of the study. A second type of aberrant response is readily apparent in the scatter diagrams of the treated groups as well as the control group from months 7-9 of the study. The upward swing of values in all groups appeared at least partially to be unrelated to treatment. An intercurrent factor, perhaps viral infection, was considered as a possible explanation; however, no clinical signs supporting this were observed.

The SGPT patterns of elevations, even for dogs on the same regimen, defy sharp definition. Because of the wide variations, the month-to-month values from the same dog could not be shown readily on the scatter diagrams of the treated groups. Only for the 2 surviving dogs of the 300 mg/kg group can the disparity in fluctuations be visualized. In 3 of the 4 dogs of the 600 mg/kg group, the SGPT value rose rather rapidly and was accompanied by clinical signs of intolerance. In contrast, the elevations in the fourth dog of this group and in some dogs of the 300 mg/kg group remained fairly low, and no signs of physical discomfort of these dogs from the drug were detected. In other dogs a significant or an abrupt increase was delayed or was not apparent until after several months of treatment. A further characteristic of the SGPT values during the year of study was the marked fluctuation from a near normal to a high value which later receded in succeeding intervals of measurement. The latter type of fluctuation was particularly conspicuous in dogs of the 300 mg/kg group, and also in the single
dog of the 50 mg/kg group which developed a high, unexplainable level of SGPT during the 7th mo. It should also be noted that the terminal values in dogs of all treated groups were among the lowest observed during the year.

Perhaps the most definitive conclusion obtained from the SGPT measurements in this study was the recognition that significantly elevated values were characteristic of the group given 300 mg/kg. Inasmuch as hepatic ultrastructural changes were found in only 1 dog of this group, and these changes were shown to be mild and transient (Gray et al., 1971), the SGPT value might perhaps be reckoned as a more sensitive indicator of drug-related effect in the liver than morphologic change. Unfortunately the elevated transaminase value and ultrastructural change were not necessarily found to be predictive of each other. No unmistakable morphologic correlate for an elevated SGPT value could be shown. It may be that the ultrastructural whorled figures produced in the liver by a high level of clindamycin hydrochloride (600 mg/kg), and the elevated SGPT value obtained from the same dog, are independent phenomena which may or may not occur simultaneously.

The possibility that a substance which appeared in the bile might accumulate in the gallbladder in concentrations not attained in the bile was demonstrated with methylene blue by Halpert and Hanke (1929). The evidence that roughly \( \frac{2}{3} \) of the dose of clindamycin is excreted in the bile as clindamycin and its metabolites, and that the terminal assays of bile from dogs treated with 30, 100 and 300 mg/kg were high, would strongly implicate clindamycin as the primary irritant in the ulcers of the gallbladder observed in dogs treated with 600 mg/kg. It should be emphasized that these unusual lesions were produced only by exceeding the maximum daily tolerated dose, which is somewhat above 300 mg/kg. Dogs given 600 mg/kg ate very poorly during the 10-day to 2-wk period of treatment, presumably because of drug related gastric irritation. The secondary relationship of depressed function of the gallbladder to low food intake, coupled with the high accumulation of clindamycin, would seem pertinent to the pathogenesis of ulcers in the mucosa of the gallbladder of these dogs. The drug concentration necessary to produce an ulcer would most likely exceed 620 \( \mu \text{g base/ml} \), the high terminal value from a dog without detectable change in the mucosa. Bile salts and cholesterol are considered to be potent irritants and may have been contributing factors.

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