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COMPARATIVE AND AGE-RELATED PHARMACOKINETICS FOR
SINGLE AND MULTIPLE DOSES OF o-PHENYLPHENOL

by

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B.A. Kansas State University
Manhattan, Kansas 1972

A MASTER'S THESIS

submitted in partial fulfillment of the
requirements for the degree

MASTER OF SCIENCE

Department of Anatomy and Physiology
College of Veterinary Medicine

KANSAS STATE UNIVERSITY
Manhattan, Kansas
1977

Approved by:

[Signature]
Major Professor
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Of course, to my parents and immediate family whose moral and financial support made my efforts possible, I owe incalculable debt.
COMPARATIVE AND AGE-RELATED PHARMACOKINETICS
FOR SINGLE AND MULTIPLE DOSES OF o-PHENYLPHENOL

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Short Title: SINGLE AND MULTIPLE o-PHENYLPHENOL DOSES

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THIS BOOK CONTAINS NUMEROUS PAGES WITH THE ORIGINAL PRINTING BEING SKEWED DIFFERENTLY FROM THE TOP OF THE PAGE TO THE BOTTOM.

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Randomly selected immature (6 weeks of age) and adult (greater than 6 months of age) dogs and cats received 3.7 mg UL-14C o-phenylphenol (OPP) singly or repeated every other day for 25 doses per os. Plasma and urine samples were collected periodically and measured for radioactivity; kinetic values for plasma disappearance were computed. Animals were observed for clinical signs and necropsied at the conclusion of the trial. Tissue residues were determined. Plasma half-life of OPP was longer in felines than in canines and was longer in young than older dogs. Urinary excretion was greater in both singly- and multiply-dosed canines. Adult animals excreted more OPP in urine and feces than did young. No effect on urinary excretion of OPP was noted in singly-dosed individuals due to age. Cats tended to accumulate higher residues than dogs, and immature dogs accumulated more chronically-administered OPP than mature dogs. No significant difference was observed between the ability of immature and adult cats to excrete or to accumulate OPP. No serious overt or post mortem toxicity was observed in any of the OPP-dosed groups of dogs and cats.
o-Phenylphenol (OPP) is a germicide commonly employed in household spray disinfectants (Deichmann and Keplinger, 1963). Because the use of spray disinfectants is so widespread in our aesthetically conscious society, human and animal exposure to the chemical occurs unavoidably. Such exposure may be either to single or repeated doses.

Early studies using rats showed that feeding the compound at levels of 2, 20, and 200 mg/kg per day neither impeded the growth of the rats nor caused any ill effects on health in general, hemoglobin, or white cell levels (MacIntosh, 1945). However, a later study revealed that rats could not tolerate 2% OPP in their diets for an extended period of time. Those on the 2% diet evidenced retarded growth, marked dilation of kidney tubules, and slight OPP kidney tissue residues. On the other hand, dogs fed OPP at 0.02, 0.2 and 0.5 g/kg per day for one year had no adverse effects as judged by body weights, gross appearance, hematologic values, urinary sugar and protein values, organ weights, and histopathological evaluation (Hodge et al., 1952).

Recent studies have underscored important species differences in response to OPP. Whereas dogs readily survived doses of 1 and 3 g OPP/kg, cats succumbed to the low dose in 15 hr and the higher dose within 6 hr. Further, dogs were more readily able to excrete the compound in urine. Urinary excretion was both more rapid and more complete in dogs. OPP tissue residues in dogs and cats were highest in lung, liver,
kidney, spleen, bile, and feces. Gross and microscopic pathology resulting from single large doses of OPP were similar in dog and cat; the pathology varied only in degree. Noted grossly was a hemorrhagic gastroenteritis. Microscopic lesions were found primarily in the kidneys with toxic tubular nephrosis evident. Clinical signs of acute OPP intoxication in dogs and cats have been reported as incoordination, mild muscular fasciculations, depression leading to an ever deepening coma with respiratory and circulatory depression. Three times more OPP was required to produce signs in canines than felines (Oehme, 1971).

Considerable controversy has arisen as to whether the potential biological effect of OPP on various species (including man) and varying ages receiving single or multiple exposures to OPP would be similar. There is little doubt that species differences in biological and metabolic response to phenolics exists (Miller, 1973; Oehme and Davis, 1970; Oehme, 1971).

Also well known is that multiple exposure may lead to increased body burdens (Greenblatt and Koch-Weser, 1975a, b). It is commonly agreed that the ability of an animal to biotransform and excrete foreign chemicals is largely dependent on enzyme systems. The efficacy of these enzyme systems varies with age (Short and Davis, 1970; Yaffe and Jachau, 1974). Quantitative information on the kinetics of OPP during single and multiple exposures of animal models of varying age and species is specifically lacking.

The present study investigates the biological response, pathologic changes, kinetics, and tissue residues resulting from single and repeated doses of OPP in immature and adult dogs and
Recently weaned, randomly selected mixed breed puppies and domestic short haired kittens of less than six weeks of age were chosen for the immature group. Adult beagle-type dogs (greater than six months old) and domestic short haired cats (greater than six months old) were randomly selected for the mature group. A total of 44 animals was divided into species-age groups as follows: 11 immature dogs, 11 adult dogs, 11 immature cats, and 11 adult cats. Of the 22 canines, 11 were male and 11 were female. Likewise, the 22 felines were equally divided as to sex. Within each species-age group of 11, 6 were selected randomly for chronic multiple-dose administration of OPP while 5 were held for single exposure kinetic studies (Fig. 1).

It was determined stoichiometrically that the actual amount of OPP in a 4-second burst of aerosol disinfectant was 3.7 mg. A single dose was designed to contain 3.7 mg chemically-pure OPP plus a tracer quantity of UL-\textsuperscript{14}C-OPP together in a gelatin capsule. Regardless of weight, animals on the chronic study received a single dose every other day for 25 doses. Plasma samples were collected from these chronically exposed animals immediately prior to and three hours after each dose. Because of the impracticality of an extremely large number of closely spaced venipunctures in very young kittens, the three-hour after-dosing sample was not attempted in the chronically dosed immature feline group. Subsequent to the 25th dose (final), plasma
samples were collected periodically for two weeks to determine kinetic parameters. Animals on the single dose regimen received only one administration. Plasma samples were obtained periodically following this single administration.

All animals were housed in metabolism cages, fed dry food once each morning, given water ad lib, and monitored continually for adverse effects. Urine samples were collected daily from the chronically dosed groups and every 12 hr from the single-dose kinetic groups. Urine samples were analyzed for OPP levels using standard liquid scintillation techniques. Degree of activity in urine was calculated, plotted, and statistically analyzed as percent of total dose administered. Plasma OPP levels reflecting body burden were also determined using liquid scintillation. Kinetic values for plasma disappearance in each age-species group were determined. The data from each species-age group was analyzed first with a monophasic plasma disappearance routine, then with a biphasic disappearance routine, and finally with a triphasic routine. The analysis that yielded the highest regression was considered to be most nearly representative. Values for the first plasma disappearance constant ($k_{d1}$) were compared statistically.

All animals on the multiple-dose regimen were sacrificed and examined post mortem two weeks after the 25th (final) dose. Using liquid scintillation procedures, tissue residues at necropsy were determined in kidney, liver, bile, lung, heart, spleen, brain, muscle (striped), fat, and feces. At necropsy each organ was weighed. A small random sample of each organ was weighed, digested in NCS solubilizer, and counted. Counts were
then extrapolated to total organ weight. Figures for the total weight of body muscle and fat were adapted from previous compendia (Altman and Dittmer, 1962). Species-age group tissue residue differences were analyzed statistically on both a gram of organ and total organ weight basis. Major organs were studied histologically. The five members of each of the four species-age groups of singly-dosed animals were sequentially killed and examined at post mortem 12, 24, 48, 72, and 120 hr following administration of OPP. Gross pathology and histopathology were noted.

A rough estimation of fecal excretion of the chronically administered compound was obtained by sampling, weighing, and counting the feces of the four chronically exposed species-age groups for one week during the middle of the experiment.

Student's t test was used to compare all urinary excretion data, tissue residues, and fecal excretion data (Snedecor and Cochran, 1967). Values for plasma disappearance constant (kd) were compared using the method of proportional parts (Hoel, 1971).

RESULTS

Single-Dose Kinetic Studies

Pathology and clinical signs. No animal exhibited malaise. Only one of the adult cats and one of the adult dogs had a mild gastroenteritis post mortem. All immature dogs exhibited a mild enteritis prominent in the distal small intestine. No immature cats evidenced post mortem pathology. All lesions were sub-
Plasma levels. Figs. 2-5 display plasma levels of OPP subsequent to administration of a single 3.7 mg dose of OPP. Both adult and immature canines achieved peak levels faster than their feline counterparts. All canine OPP levels peaked within two hr; immature and adult cats required 12 and 36 hr, respectively, to achieve peak plasma concentrations.

The steepness of the single dose lines is reflected in the plasma disappearance constant (kd₁) and plasma half-time (t-1/2) values presented in Table 1. Although kd₁ values between adult and weanling cats and between adult and weanling dogs were not significantly different at the 95% confidence level, interspecies kd₁ values are grossly different. Immature dog plasma levels dropped far faster after peak than did the corresponding immature cat levels (P < 0.01). Levels in mature dogs dropped faster after peak than in mature cats (P < 0.01). Half-time in adult cats was almost 80 times greater than in adult dogs, and t-1/2 in immature cats was 9 times longer than in immature dogs. Biphasic plasma disappearance was noted in the adult cat group. Both immature groups were monophasic. In the biphasic case kd₁ was greater in absolute value than kd₂. The kd₂ value for the adult cat group was insignificant (< 0.001) and yielded a t-1/2 of 17.290 hr.

Urinary excretion. Urinary excretion of single doses of OPP was not as severely affected by age as by species (Fig. 6). On the whole, adult and immature dogs excreted approximately twice as much of the original dose as their feline counterparts.
At the end of 120 hr, dogs excreted more than twice as much of the compound in urine as cats.

**Multiple-Dose Studies**

*Pathology and clinical signs.* No feline exhibited any clinical abnormality during the course of the 25-dose study. Neither gross nor microscopic pathology was detected at post mortem. All chronically-dosed adult dogs exhibited signs of mild gastroenteropathy. Sporadic loose stools and intermittent vomiting were observed. Spontaneous recovery always occurred, and no malaise, anorexia, dysphagia, or tenesmus was noted. Necropsy revealed a mild catarrhal enteritis that was most prominent in the proximal small bowel. However, clinical signs in the chronically-dosed immature dogs were rare and insignificant. Necropsy of the immature multiply-dosed dogs revealed no pathology.

*Plasma levels.* Plasma OPP accumulation over the 25-dose regimen varied between species and between age groups within species. Fig. 7 displays chronic plasma concentrations before each dose and 3 hr after each dose for immature dogs. Dosing every other day produced a gradual increase in plasma OPP level. Fig. 8 displays plasma levels in adult dogs. Pre-dose plasma levels do not show the gradual increase observed in the immature dogs. Levels three hr after dosing remain lower than in immature dogs.

Immature cats on the 25-dose regimen (Fig. 9) displayed quickly rising pre-dose plasma levels. On any given day, levels
were many times higher in immature cats than immature dogs. Adult cats (Fig. 10) had a pattern similar to immature cats. On any given day the pre-dose adult cat OPP plasma level was slightly higher, generally, than the corresponding pre-dose immature cat plasma level.

Table 2 summarizes the plasma kd and t-1/2 values derived from plasma samples taken periodically for 2 weeks following the final 25th dose. These values were reflected in the steepness of the plasma level lines following final dosing (Figs. 7-10). Plasma disappearance in adult dogs was not significantly more rapid than in immature dogs at the 95% confidence level. Plasma disappearance in adult dogs was significantly more rapid (P < 0.01) than in adult cats; likewise, plasma levels decreased more rapidly in immature dogs than immature cats (P < 0.01). Differences in kd between immature and adult cats were not significant at the 95% confidence level. The data of the species-age groups immature dogs and adult cats best fit the biphasic model. Disappearance from plasma of immature cats was monophasic. As was the case for the single-dose study, kd2 values were much smaller in absolute value than kd1; in fact, the kd2 value for adult cats was insignificant (< 0.001) and produced a theoretical t-1/2 of 22,170 days.

Urinary excretion. Urinary excretion of the chronically-dosed compound varied between species-age groups both in pattern of excretion and percent of total dose recovered.

Fig. 11 depicts the percent of total dose excreted by immature and adult dogs and cats. Immature dogs reached their
level of maximum excretion more rapidly than immature cats. On any given day the immature dogs voided approximately twice as much OPP in the urine as immature cats. Ultimately, the immature cats were able to excrete only 31% of the total administered dose of OPP in their urine. Excretion in immature dogs approached 47%. The difference in total urinary excretion between immature dogs and cats was significant at the 99% confidence level.

Prominent species differences were seen between adult dogs and cats. Maximum excretion occurred later in the feline. On any given day levels of dosed OPP in the urine of adult dogs was approximately 1.5 times greater than levels in adult cats. Total urinary excretion in the multiply-dosed adult dog approached 56%; excretion in the multiply-dosed cat approached 41%. The ability of the adult dog to void OPP in the urine was significantly greater ($P < 0.05$) than in the adult cat.

Statistical comparisons of age groups within species were not consistent. While chronically-dosed mature dogs excreted significantly more OPP in urine than immature ($P < 0.05$), adult cats on the chronic study were not able to excrete significantly more OPP in urine than immature cats ($P > 0.05$).

Table 3 tabulates the values for total urinary excretion of OPP in the various chronically-dosed species-age groups.

**Fecal excretion.** Table 4 represents the estimated percent of OPP excreted in the feces of the four species-age groups on the 25-dose study. Older dogs and cats excreted significantly more OPP in feces than their immature counterparts ($P < 0.05$). Species comparisons between adult cats and adult dogs, as well
as between immature cats and immature dogs, showed no significant differences at the 95% confidence levels.

**Tissue residues.** Table 5 presents the per gram of tissue residues determined at post mortem two weeks after the final 25th dose. Highest residues were generally found in lung, bile, heart, kidney, and liver.

Adult dogs on the chronic study had residues only in brain and muscle. Immature dogs displayed per gram residues in all tissues, with kidney, bile, liver, and lung highest. Differences between adult and immature dogs in per gram tissue residues were significant at 95% or greater confidence levels in all tissues except brain.

With the exception of muscle and fat, adult cats displayed higher per gram tissue residues than chronically-dosed immature cats. Both the adult and young felines evidenced OPP residues in all tissues. However, only the kidney OPP level was statistically different at the 95% confidence level with adult cats having higher per gram tissue residues in this organ.

Immature dogs and cats generally had similar per gram residues. However, per gram brain OPP levels were significantly greater in immature dogs ($P < 0.05$).

Since mature dogs displayed residues only in brain and muscle, adult dog tissue levels were generally significantly ($P < 0.01$) lower than adult cats on the chronic regimen. Exceptions to the statistically significant difference were spleen, brain, and muscle.

More revealing were residues on a total tissue weight
basis resulting from chronic OPP administration. Table 5 lists the total tissue residues. Percent of total dose recovered in all body tissues and percent of total dose recovered in muscle only are shown in Table 6. Residues found in muscle account for as much as 93% of total recovered OPP tissue activity (e.g. adult dogs).

Statistical evaluation of chronic total tissue residues generally showed more significant differences between the four species-age groups than did analysis on a per gram tissue basis. An exception is comparison of adult and immature dogs whole tissue residues which revealed significantly higher residues in all the tissues, other than brain and muscle, of the chronically-dosed immature dogs (P < 0.05). On per gram analysis only the brain level was not significantly different from that found in the other groups.

Statistically significant differences between immature and adult cat whole tissue residues occurred in kidney, liver, lung, and heart, with the adults having greater residues (P < 0.05).

Interspecies differences occurred in the total tissue weight basis comparisons. The immature, chronically-dosed dogs evidenced greater tissue residues in brain, heart, kidney, spleen, fat, and liver than their feline counterparts (P < 0.05).

Total tissue weight comparisons between adult chronically-dosed dogs and their feline equivalent mimicked the per gram results. That is, the tissue levels in adult cats were significantly greater (P < 0.05) in all tissues except spleen, brain, and muscle than the levels in adult dogs.
DISCUSSION

The present investigation confirmed previous work which demonstrated a lack of serious clinical signs attributable to chronic low doses of OPP (Macintosh, 1945; Hodge et al., 1952). The mild signs of gastroenteropathy noted in the present study might have been due to the inherent ability of phenolics to irritate the gastrointestinal tract (Patty, 1963). The fact that no feline in the current study displayed any clinical sign or post mortem lesion detracts from the theory of direct irritation.

The presence of mild gastroenteric lesions in the adult dogs of the current chronic study and the absence of such lesions in felines could be explained by incrimination of the enterohepatic circulation. The ability of the dog and the inability of the cat to metabolize and excrete phenolics has been well documented (Miller, 1973; Oehme and Davis, 1970; Oehme, 1971; Oehme and Smith, 1972). Also well documented has been the ease with which the canine forms glucuronic acid conjugates of phenol and OPP (Oehme, 1971; Oehme and Smith, 1972; Williams, 1971). The bile provides an avenue of excretion for liver-made glucuronide conjugates (Smith, 1971; Smith, 1973). Once the conjugated compound in bile is in the intestine, the phenolic conjugate will be either excreted in feces or split by β-glucuronidase, thereby releasing the free phenolic. The free form may damage the intestinal mucosa by direct contact. It may also be reabsorbed via the portal system to the liver to begin the process of conjugation anew. Since cats cannot efficiently conjugate phenolics
to glucuronic acid, and since other cat conjugation routes are limited (Mandel, 1971), body burdens rise. Liver excretion into bile and, hence, intestinal irritation are minimal in the feline, as observed in our study.

Further support for incrimination of the enterohepatic circulation as the mechanism responsible for the sub-clinical canine lesions is the fact that the chronically-dosed adult dogs had gastrointestinal post mortem lesions, whereas chronically-dosed immature dogs did not. Since enzyme systems develop with age (Short and Davis, 1970; Yaffe and Jackau, 1974), glucuronide conjugating enzymes might not have matured in time to promote significant enterohepatic circulation in the immature dogs.

The fulminating gastrointestinal and nervous signs of high dose OPP administration previously reported (Oehme, 1971) were not evident in the current study although post mortem lesions exhibited by dogs in the present study did approximate a mild form of the fulminating lesions. Previously reported toxic changes in kidney tubules of chronically-dosed rats (Hodge et al., 1952) did not occur in any of the presently studied animals.

The present single dose kinetic study confirmed previous work which indicated that OPP given to dogs reached peak plasma levels faster and was excreted more rapidly in urine than OPP given to cats (Oehme, 1971). Present chronic multiple-dose studies showed highest plasma burdens in adult cats, followed in decreasing order by immature cats and immature dogs. Adult dogs had little tendency to accumulate OPP.

The inability of cats to remove OPP from plasma during the studies may be a reflection of saturation kinetics (Bates and
Gibaldi, 1970; Cohn, 1971; Harper, 1973; Notari, 1971). This implies that limited biotransforming enzyme activity relative to substrate may exist. Support for the existence of a saturated system may further be inferred from the fact that chronically-dosed immature dogs excreted OPP less readily than single-dosed immature dogs. Enzyme systems responsible for the creation of water-soluble metabolites may be deficient in the immature dog as compared to the adult dog. That the ability of felines to remove OPP from plasma was not altered by multiple exposure reflects that all cats—adult or immature—are grossly deficient in their ability to metabolize phenolics (Oehme, 1971). It might then be inferred that some of the factors responsible for plasma disappearance and excretion of OPP have not completely developed in either cats or young dogs.

Since biotransformation systems for phenolics favor the formation of water-soluble metabolites, the species best able to form these metabolites will excrete OPP in urine with greatest efficiency. Urinary excretion data gathered currently confirmed that both chronically and single-dosed dogs with their relatively efficient metabolic machinery for excreting phenolics showed higher urinary levels of OPP than their feline counterparts.

Factors other than biotransformation affect the distribution of foreign chemicals (Butler, 1971; Doley and Davies, 1975; Jenkins, 1971). Prominent among these many factors are protein binding and tissue sequestration. That sequestration of OPP occurred was reflected in the consistently high muscle residue levels noted in all chronically-dosed species-age groups. The fact that many species-age groups generated biphasic plasma
disappearance data with small absolute \( kd_2 \) as compared to \( kd_1 \) values might well be due to binding and sequestration (Davison, 1971). As half-times increase, and biotransformation and excretion decrease, tissue residues will increase.

The present studies indicate that cats are generally less able than dogs to remove OPP from plasma and to excrete OPP in urine. Accordingly, cats tended to accumulate generally higher tissue residues than dogs. Immature dogs were less able to remove chronically-administered OPP than adult dogs and showed generally higher residues. No general significant difference existed between the ability of immature and adult cats to excrete or accumulate OPP.

Animals exposed to single or multiple low doses of OPP were not significantly affected clinically. Normal household or environmental use of the compound should have no serious or debilitating effect on either immature or adult dogs or cats.
ACKNOWLEDGEMENTS

The assistance of Drs. L. Penumarthy and T. Gopal and Mrs. A. Donahy and Mr. S. Galitzer is gratefully acknowledged. This study was supported by a grant from Lehn and Fink Products Co., a Division of Sterling Drug, Inc.
REFERENCES


1 A preliminary report of this paper was presented at the 15th Annual Meeting of the Society of Toxicology, March 14-18, 1976, in Atlanta, GA.

2 Carl Metzler NONLIN Computer program for parameter estimation, The Upjohn Company.

3 Amersham/Searle, Des Plaines, IL.
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<th>Rodenticide</th>
<th>Time (hr)</th>
<th>Regression</th>
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</thead>
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<tr>
<td>Immature dogs</td>
<td>$k_d_1 = -0.072 (\pm 0.002)$</td>
<td>9.6</td>
<td>0.993</td>
</tr>
<tr>
<td>Adult dogs</td>
<td>$k_d_1 = -0.243 (\pm 1.110)^a$, $k_d_2 = -0.086 (\pm 0.022)$</td>
<td>2.8</td>
<td>0.925</td>
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<td>Immature cats</td>
<td>$k_d_1 = 0.008 (&lt; 0.001)$</td>
<td>84.8</td>
<td>0.994</td>
</tr>
<tr>
<td>Adult cats</td>
<td>$k_d_1 = -0.003 (\pm 0.048)$, $k_d_2 = \text{insignificant}$</td>
<td>226.2</td>
<td>0.905</td>
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* $^a$ ± 1 standard deviation.
### TABLE 2.

Mean Kinetic Constants ($k_{d1}$, $k_{d2}$) and Half-times ($t_{1/2}$) for o-Phenylphenol (OPP) Disappearance from Plasma in Various Age Groups Previously Exposed to 25 Doses of 3.7 mg OPP

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<thead>
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<th>$k_d$ (day$^{-1}$)</th>
<th>$t_{1/2}$ (days)</th>
<th>Regression</th>
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<tbody>
<tr>
<td>Immature dogs$^b$</td>
<td>$k_{d1} = -0.989$ ($\pm$ 0.087)$^a$</td>
<td>0.7</td>
<td>0.995</td>
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<tr>
<td></td>
<td>$k_{d2} = -0.015$ ($\pm$ 0.004)</td>
<td>46.4</td>
<td></td>
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<tr>
<td>Adult dogs$^c$</td>
<td>$k_{d1} = -3.263$ ($\pm$ 0.258)</td>
<td>0.2</td>
<td>0.987</td>
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<tr>
<td>Immature cats$^c$</td>
<td>$k_{d1} = -0.075$ ($\pm$ 0.001)</td>
<td>9.3</td>
<td>0.993</td>
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<tr>
<td>Adult cats$^c$</td>
<td>$k_{d1} = -0.148$ ($\pm$ 0.354)</td>
<td>3.6</td>
<td>0.939</td>
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<tr>
<td></td>
<td>$k_{d2} = $ insignificant</td>
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$^a$ ± 1 standard deviation.

$^b$ $N = 5$.

$^c$ $N = 6$. 
<table>
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<tr>
<th>Species</th>
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</thead>
<tbody>
<tr>
<td>Immature dogs</td>
<td>44.8 ± 5.0</td>
</tr>
<tr>
<td>Adult dogs</td>
<td>53.7 ± 5.8</td>
</tr>
<tr>
<td>Immature cats</td>
<td>31.3 ± 10.6</td>
</tr>
<tr>
<td>Adult cats</td>
<td>41.7 ± 7.0</td>
</tr>
</tbody>
</table>

\( ^a \pm 1 \) standard deviation.

\( ^b \) N = 5.

\( ^c \) N = 6.
### TABLE 4

Mean Estimated Percent of Total o-Phenylphenol (OPP) Dose Excreted in Feces of Various Species and Age Groups Receiving 25 Doses of 3.7 mg OPP

<table>
<thead>
<tr>
<th>Category</th>
<th>Percent  ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immature dogs</td>
<td>14.8 ± 3.4</td>
</tr>
<tr>
<td>Adult dogs</td>
<td>23.3 ± 9.1</td>
</tr>
<tr>
<td>Immature cats</td>
<td>16.0 ± 1.1</td>
</tr>
<tr>
<td>Adult cats</td>
<td>21.4 ± 2.6</td>
</tr>
</tbody>
</table>

- **a** ± 1 standard deviation.
- **b** $N = 4$.
- **c** $N = 3$. 
THIS BOOK CONTAINS NUMEROUS PAGES WITH DIAGRAMS THAT ARE CROOKED COMPARED TO THE REST OF THE INFORMATION ON THE PAGE.

THIS IS AS RECEIVED FROM CUSTOMER.
<table>
<thead>
<tr>
<th>Tissue</th>
<th>Immature Dogs&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Adult Dogs&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Immature Cats&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Adult Cats&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X 10&lt;sup&gt;3&lt;/sup&gt; dpm/g</td>
<td>X 10&lt;sup&gt;6&lt;/sup&gt; dpm/</td>
<td>X 10&lt;sup&gt;3&lt;/sup&gt; dpm/g</td>
<td>X 10&lt;sup&gt;6&lt;/sup&gt; dpm/</td>
</tr>
<tr>
<td></td>
<td>tissue</td>
<td>total tissue</td>
<td>tissue</td>
<td>total tissue</td>
</tr>
<tr>
<td>Kidney</td>
<td>16.4 ± 8.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.4 ± 0.1</td>
<td>ND&lt;sup&gt;d&lt;/sup&gt;</td>
<td>ND</td>
</tr>
<tr>
<td>Liver</td>
<td>20.5 ± 15.1</td>
<td>3.0 ± 2.0</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Bile</td>
<td>18.3 ± 11.9</td>
<td>---</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Lung</td>
<td>16.9 ± 14.3</td>
<td>0.6 ± 0.5</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Heart</td>
<td>17.2 ± 7.8</td>
<td>0.5 ± 0.2</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Spleen</td>
<td>18.4 ± 15.2</td>
<td>0.3 ± 0.2</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Brain</td>
<td>12.2 ± 5.7</td>
<td>0.8 ± 0.3</td>
<td>10.4 ± 20.8</td>
<td>0.8 ± 1.7</td>
</tr>
<tr>
<td>Muscle</td>
<td>11.4 ± 2.8</td>
<td>19.7 ± 5.0</td>
<td>1.9 ± 3.8</td>
<td>15.9 ± 31.8</td>
</tr>
<tr>
<td>Fat</td>
<td>11.8 ± 5.2</td>
<td>5.0 ± 2.0</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

<sup>a</sup> ± 1 standard deviation.
<sup>b</sup> N = 5.
<sup>c</sup> N = 6.
<sup>d</sup> No residue detected.
TABLE 6

Mean Percent of Total o-Phenylphenol (OPP) Dose Recovered in all Tissues and in Muscle of Various Species and Age Groups Receiving 25 Doses of 3.7 mg OPP

<table>
<thead>
<tr>
<th></th>
<th>Total dose recovered in tissue (%)</th>
<th>Total dose recovered in muscle (%)</th>
<th>Fraction of total recovery found in muscle a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immature dogs b</td>
<td>5.2</td>
<td>3.4</td>
<td>0.65</td>
</tr>
<tr>
<td>Adult dogs c</td>
<td>2.9</td>
<td>2.7</td>
<td>0.93</td>
</tr>
<tr>
<td>Immature cats c</td>
<td>3.8</td>
<td>3.1</td>
<td>0.82</td>
</tr>
<tr>
<td>Adult cats c</td>
<td>4.6</td>
<td>3.2</td>
<td>0.70</td>
</tr>
</tbody>
</table>

a % of total dose recovered in muscle/% of total dose recovered in all tissues.

b N = 5.
c N = 6.
Fig. 1. Experimental design for the study of o-phenylphenol in dogs and cats.
Fig. 2. Mean plasma disappearance of radioactive o-phenylphenol (OPP) in five immature dogs following the single oral administration of 3.7 mg OPP to each animal.
ADULT DOGS

Fig. 3. Mean plasma disappearance of radioactive o-phenylphenol (OPP) in five adult dogs following the single oral administration of 3.7 mg OPP to each animal.
Fig. 4. Mean plasma disappearance of radioactive o-phenylphenol (OPP) in five immature cats following the single oral administration of 3.7 mg OPP to each animal.
Fig. 5. Mean plasma disappearance of radioactive o-phenylphenol (OPP) in five adult cats following the single oral administration of 3.7 mg OPP to each animal.
Fig. 6. Excretion in the urine of radioactive o-phenylphenol (OPP) in adult dogs, immature dogs, immature cats, and adult cats after the oral administration of 3.7 mg OPP to 5 animals in each group.
Fig. 7. Mean plasma radioactive o-phenylenediamine (OPP) levels in 6 immature dogs during and after the oral administration of 3.7 mg OPP per animal every other day for 25 doses. Plasma disappearance lines for $k_1$ and $k_2$ are illustrated for the time period following the last dose (day 48).
**ADULT DOGS**

- Immediately prior to dosing
- 3 hr after dosing or after final dose

---

**Fig. 8.** Mean plasma radioactive o-phenylphenol (OPP) levels in 6 adult dogs during and after the oral administration of 3.7 mg OPP per animal every other day for 25 doses. The plasma disappearance line is illustrated for the time period following the last dose (day 48).
Fig. 9. Mean plasma radioactive o-phenylphenol (OPP) levels in 6 immature cats during and after the oral administration of 3.7 mg OPP per animal every other day for 25 doses. The plasma disappearance line is illustrated for the time period following the last dose (day 48).
ADULT CATS
- Immediately prior to dosing
- 3 hr after dosing or after final dose

Fig. 10. Mean plasma radioactive o-phenylphenol (OPP) levels in 6 adult cats during and after the oral administration of 3.7 mg OPP per animal every other day for 25 doses. Plasma disappearance lines for $k_1$ and $k_2$ are illustrated for the time period following the last dose (day 48).
Fig. 11. Excretion in the urine of radioactive o-phenylphenol (OPP) in adult dogs, immature dogs, adult cats and immature cats during and after the oral administration of 3.7 mg OPP every other day for 25 doses. Six animals comprised each group. The values depicted are the total percents of the cumulative dose administered that were excreted by each time frame.
Index Terms: o-Phenylphenol, Dogs, Cats, Immature, Adult, Single dose, Multiple dose, Clinical effects, Necropsy lesions, Plasma, Urine, Kinetics, Excretion, Tissue residues
CLINICAL CONSIDERATIONS FOR SMALL ANIMAL EXPOSURE
TO POTENTIALLY TOXIC PHENOLIC DISINFECTANTS

Marc A. Rachofsky, DVM, MS
and
Frederick W. Oehme, DVM, PhD

SUMMARY

Phenol and its derivatives have long found use as disinfectants. o-Phenylphenol (OPP) is commonly employed in household and spray disinfectants. Humans and pets may be exposed to single or multiple doses in routine use; hence, information on the toxicity and kinetics of biotransformation of the compound in various species of different ages is important. Randomly selected immature (less than 6 weeks of age) and adult (greater than 6 weeks of age) dogs and cats were dosed per os in a single or multiple dose regimen. Doses of 3.7 mg (the amount of OPP in a 4-second burst of spray disinfectant) and $[\text{UL-}^{14}\text{C}]-\text{OPP}$ were either given singly or repeated every other day for 25 doses. Plasma and urine samples were collected periodically and measured for radioactivity. Kinetic values for plasma disappearance were computed. Urine excretion was monitored. Animals were observed

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for clinical signs during the trial and necropsied at the conclusion of the trial. In general, plasma half-life of OPP was greater in felines than in canines and was greater in young than older dogs. Urinary excretion was greater in both singly and multiply dosed canines; adults excreted more OPP than young. No effect on urinary excretion was attributable to age. No generally significant difference was observed between the ability of immature and adult cats to excrete OPP in urine. No serious overt or post-mortem toxicity was observed in any of the OPP-dosed groups of dogs or cats.

Phenol and its derivatives have been used widely in the production of explosives, fertilizers, coke, illuminating gases, lampblack, paints, paint removers, and wood preservatives. Phenol is also found in petroleum, leather, soap, dye, and agricultural products. After Lister's first use of phenol in 1867 as a disinfectant, medicinal use of the compound has expanded to antipruritics, spray disinfectants, cauterizing agents, and insecticides. Toxic effects of phenol in animals vary with amount and type of exposure. Skin exposure may result in eczema, inflammation, and skin necrosis with sloughing and gangrene. Oral exposure may yield corrosion and necrosis of mucosae, lung hyperemia and bronchopneumonia. Prolonged exposure by any route may cause parenchymatous nephritis or nephrosis. High acute doses of phenol act primarily on the spinal cord and cause both reflex twitching and clonic convulsions. High doses further act on both the heart to cause myocardial depression and on the
peripheral vasculature to cause blood pressure increase. This increase in blood pressure is usually followed by a drastic decrease due to failure of the vasomotor centers.\(^4,\text{13}\)

Species, age, and regimen of exposure affect the ability of mammals to cope with phenols. Excretion studies of phenol in rats and rabbits showed that phenol is excreted primarily through conjugation with glucuronic and sulfuric acids. Little is excreted as free phenol in these species. The absolute manner in which the phenol is handled, however, is dose dependent. Small doses in rabbits are wholly excreted within twenty-four hours and 80% is present as conjugate. Following administration of lethal doses, phenol is present chiefly as free compound. Therefore, the amount of conjugate product increases with time.\(^3,\text{16,17,22,24}\)

Cats have been reported to be especially sensitive to phenolic compounds.\(^1,\text{6,13,14,23}\) Recent metabolic studies have indicated that cats have a reduced ability to form glucuronide and glycine conjugates of phenol.\(^2,\text{11,13}\) Previous work has shown glucuronyl transferase lacking in the cat liver.\(^5\)

It may be rationalized that since the cat lacks the primary enzyme necessary to excrete phenol, the feline may be exceptionally susceptible to its effects.

That age affects the ability of an animal to biotransform chemicals is well documented.\(^1\text{8,25}\) Studies in perinatal pigs have shown that the ability to perform oxidation increases rapidly from birth to week 4. Between weeks 4 and 10, however, the increase in oxidating ability is far less dramatic. Reductive enzyme capability increases markedly the first 6 weeks of life, after which plateau occurs. Microsomal capability increases
thirty times the first three weeks before it plateaus.\textsuperscript{18}

Multiple dosing of chemicals increases the body burden so long as the rate of excretion does not exceed the rate of accumulation. When accumulated drug concentration overpowers enzyme systems, the half-time of the drug in the body increases.\textsuperscript{10,12}

\textit{o}-Phenylphenol (OPP) is the active moiety in many household spray disinfectants. Little previous work on either the potential toxicity or the kinetics of this compound has been carried out in domestic animals. One study demonstrated that rats could not tolerate more than 2\% OPP in their diets for an extended period of time without showing growth retardation and kidney lesions.\textsuperscript{8} The same study found that dogs tolerated dietary OPP at a dose of 0.02, 0.2, and 0.5 g/kg/day for one year without ill effects. More recent studies indicated that dogs readily survive oral doses of 1 and 3 g/kg of chemically pure OPP dissolved in olive oil. Cats, on the other hand, succumbed to the low dose in 15 hours and to the high dose within 6 hours. In monitoring plasma levels in dogs and cats, the investigator found that levels in dogs receiving 3 g/kg peaked in 1.5 hours, whereas levels in cats rose until death. Dogs excreted most of the compound in their urine within 12 hours; cats excreted little before their death.\textsuperscript{13,15} When given OPP-containing disinfectant at dosages of 3 and 9 ml concentrated disinfectant/kg bodyweight (3.75 X 10^{-3} and 1.125 X 10^{-2} g OPP/kg), toxicity was similar to giving pure OPP. While all low dosage dogs survived, the average cat succumbed in 57 hours. Average dogs given the higher dose level died within 50 hours while average high dose cats died within 6 hours. Plasma concentration rose swiftly in cats and
slowly in dogs. Urinary excretion was rapid in dogs and slow in cats with dogs excreting two-thirds in one day. In cats less than one-third of the initial dose was removed in three days.\textsuperscript{13} The pathology attributed to single doses of OPP has been described as typically phenolic—hemorrhagic gastroenteritis and toxic tubular nephrosis. Clinical signs of OPP intoxication have been described as those of incoordination, muscle fasciculations, and respiratory and cardiac depression.\textsuperscript{13}

The present work studies the potential toxicity and kinetics of OPP in both young (immature) and adult dogs and cats as it is used commonly around the house.

**MATERIALS AND METHODS**

Randomly selected mixed-breed puppies and domestic short hair kittens of less than 6 weeks of age were used for the immature groups. The adult groups consisted of mixed-breed Beagle dogs and domestic short hair cats greater than 6 months old. A total of 22 dogs and 22 cats were divided into species-age groups of 11 immature dogs, 11 adult dogs, 11 immature cats, and 11 adult cats. Within each species age group, six were randomly selected for chronic OPP administration while five were selected for single exposure studies (Fig 1).

The amount of OPP in a four-second burst of commercial spray disinfectant was determined stoichiometrically to be 3.7 mg. Tracer quantities of $[\text{UL}-^{14}\text{C}]$-OPP were added to chemically pure OPP in gelatin capsules containing the 3.7 mg dose. Animals on the single dose kinetic study received a single
3.7 mg OPP plus tracer \([\text{UL}^{-14}\text{C}]\)-OPP administration. Plasma samples on kinetic trials were collected every fifteen minutes for two hours following administration and then periodically. Animals on the chronic accumulation study received a standard dose of 3.7 mg OPP and a tracer quantity of \([\text{UL}^{-14}\text{C}]\)-OPP orally via gelatin capsule regardless of body weight every other day for 25 doses. Plasma samples were collected immediately prior to and three hours after chronic dosing. Following the 25th (final) dose on chronic trials, plasma samples were collected every 15 minutes for two hours and then periodically until sacrifice.

All animals were housed in metabolism cages, fed dry food once each morning, given water ad lib., and observed closely for adverse effects. Urine output was collected daily and together with plasma samples was assayed for OPP levels by standard liquid scintillation techniques. Plasma OPP levels reflecting biological accumulation of plasma disappearance following administration of the last chronic or single kinetic dose were determined. Urinary OPP concentration and degree of urinary excretion as percent of total OPP dose were calculated. Half-time values for plasma disappearance of OPP were computed.

All animals receiving multiple doses were killed and examined post mortem 2 weeks after administration of the last dose. Representative animals from groups receiving single doses of OPP were individually sacrificed at 12, 24, 48, 72, and 120 hours following their single OPP administration. All tissues were studied for both gross and microscopic pathology.

Toxicologic, kinetic, and pathologic results were analyzed
statistically and significant data was plotted and interpreted. Students' t test was used to analyze data on urinary excretion. The method of proportional parts was used to compare rates of removal from plasma.

RESULTS

Single Dose Studies

Clinical Effects and Pathology. Overt signs of toxicity were not observed in any of the immature or adult dogs or cats receiving single 3.7 mg doses of OPP.

All immature dogs showed subclinical enteritis post mortem. Twenty percent of the adult dogs and cats had similar lesions. No immature cats evidenced any lesion post mortem.

Plasma Levels Following Single Doses. Fig 2 displays plasma disappearance following a single dose of OPP in the four species-age groups. Table 1 displays the time to peak level and the half-times of the four species-age groups. Both adult and immature canines achieved peak plasma levels faster than their feline counterparts. The relatively short half-times of OPP in dogs is reflected in the steepness of their curves in Fig 2.

Statistically, immature dogs' plasma levels dropped faster after peak than did corresponding immature cat levels ($P < 0.01$). Too, levels in mature dogs dropped faster than mature cats ($P < 0.01$). Differences in disappearance rates after peak between adult and immature feline, as well as between adult and immature canine, were not significant at the 95% confidence level.
Urinary Excretion of Single Doses. Urinary excretion of OPP was not as severely affected by age as by species (Fig 3). Little difference between immature and adult dogs, as well as between immature and adult cats, in rapidity of excretion and total percentage of activity ultimately recovered in urine was observed. Major differences, however, were noted when dogs and cats were compared. Immature dogs excreted the administered OPP more rapidly and completely than did immature cats. While adult canines and felines had similar excretory patterns, the adult dogs again excreted OPP more rapidly than did adult cats.

Multiple Dose Studies

Clinical Effects and Pathology. No chronically dosed feline or immature canine exhibited any clinical, post mortem, or histopathology. However, all adult dogs exhibited signs of mild gastroenteropathy. Sporadic loose stools and intermittent vomiting were observed. No malaise, anorexia, dysphagia, or tenesmus accompanied the signs; recovery was always spontaneous. Necropsy revealed a mild catarrhal enteritis most prominent in the proximal small bowel.

Plasma Levels During Multiple Dose Regimen. Fig 4 illustrates the levels of OPP radioactivity during and following the administration of the 25 every-other-day doses in the immature and adult dogs and cats. Levels depicted on the left-hand portion of the illustration are those detected during the course of the 25 doses. Plasma disappearance lines for each group following administration of the last dose are depicted on the right. Table 2 displays the half-time for elimination
of the 25th (final) dose in the four species-age groups.

A small but gradual increase in plasma level occurred with the repeated OPP administration to immature dogs. Plasma levels during chronic administration to adult dogs remained insignificant. Following administration of the final dose, a steep decline in the disappearance curve portends the short half-life presented for adult dogs in Table 2. Plasma values for immature chronically dosed cats rose acutely and remained high throughout the course of OPP administration. Too, a much less rapid decline following the final dose portends a long half-time of disappearance. Adult cats also maintained continually high levels of OPP during multiple dosing and, like immature cats, only gradually relieved themselves of OPP following final administration.

Plasma disappearance rate in adult dogs was significantly more rapid than in adult cats ($P < 0.01$). Although differences in plasma disappearance between immature dogs and immature cats were significant ($P < 0.01$), differences in disappearance rates between immature and adult cats, as well as between immature and adult dogs, were not significant at the 95% confidence level.

**Urinary Excretion.** Table 3 tabulates the percent of total radioactivity recovered in the urine of the four species-age groups following chronic administration. Urinary excretion of OPP varied between species and age groups both in pattern of excretion and percent recovered. Fig 5 displays the urinary excretion patterns of the four species and age groups during chronic administration. Immature felines have a gradual development of OPP excretion while immature canines reach maximum excre-
tion levels promptly. Both immature dogs and cats eventually reach excretion plateaus with the kittens excreting approximately 25% of the total administered dose in the urine while the dogs were able to excrete 41%. The difference between adult dogs and cats is more prominent. Adult cats plateau more gradually and excrete approximately 31% of the total dose in urine. Adult dog excretion approaches 56%.

Total urinary excretion of OPP by adult dogs was significantly greater than adult cats (P < 0.01); mature dogs excreted more OPP in urine than did immature (P < 0.01). Differences in total urinary excretion of OPP by immature and adult felines was not significant at the 95% confidence level.

CONCLUSION AND DISCUSSION

The present study complements previous work which attributed little clinical malaise to chronic low doses of OPP. The mild signs of gastroenteropathy noted in the present study may be due to either the inherent ability of phenolics to irritate mucosae or to incrimination of the enterohepatic circulation. The bile provides an avenue of escape for liver-made glucuronide conjugates. Once in the intestine, the conjugates will be split by β-glucuronidase thereby releasing active free drug form. The fact that no cat in the present chronic or acute studies exhibited any clinical signs could well be due to the cat’s inability to form glucuronide conjugates and to excrete them in bile.

Fulminating gastrointestinal and nervous signs following
high level dosing of OPP previously reported\textsuperscript{13} were not evident in the current study although post mortem lesions exhibited by dogs in the current chronic study did approximate a very mild form of the high dose lesions. Previously reported toxic changes in the tubules of rats subjected to chronic low doses of OPP\textsuperscript{8} did not occur in any of the presently studied dogs or cats.

The present single-dose kinetic study confirms previous work which indicated that oral OPP given to dogs reached peak plasma levels faster, disappeared more rapidly from plasma, and was excreted more rapidly in urine than OPP given to cats \textit{per os}.\textsuperscript{13} Present multiple dose studies showed highest plasma burdens in adult cats, followed in order by immature cats and immature dogs. Adult dogs had little tendency to accumulate OPP. The fact that cats removed OPP from plasma less rapidly than their canine counterparts in both of the current studies, and the fact that immature dogs on the chronic study removed OPP from plasma less rapidly than immature dogs on the single-dose study, points to the presence of a saturated system \textsuperscript{7,10,12} since biotransforming enzyme levels differ between species.\textsuperscript{2,5,11,13}

That biotransformation systems generally favor the formation of water soluble metabolites is commonly known. The present study confirms previous work\textsuperscript{13,15} which found that canines, with their ability to form water soluble glucuronide and other conjugates more readily than felines, excrete OPP in urine with greater facility than felines.

Because no debilitating overt or post mortem toxicity was observed in any of the OPP-dosed groups of dogs or cats in the
present study, OPP should be considered relatively safe for routine household or office use around dogs and cats.
REFERENCES


TABLE 1--Time of Peak Plasma Level and Half Times for Disappearance from Plasma Following Single Administration of 3.7 mg o-Phenylphenol

<table>
<thead>
<tr>
<th></th>
<th>Time to peak (nearest hr)</th>
<th>Half-time (hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immature dogs*</td>
<td>3</td>
<td>9.6</td>
</tr>
<tr>
<td>Adult dogs</td>
<td>2</td>
<td>2.8</td>
</tr>
<tr>
<td>Immature cats</td>
<td>16</td>
<td>84.8</td>
</tr>
<tr>
<td>Adult cats</td>
<td>36</td>
<td>226.2</td>
</tr>
</tbody>
</table>

* N = 5 in all groups.
TABLE 2--Half-time of Disappearance from Plasma for the 25th (Final) Dose of 3.7 mg o-Phenylphenol (OPP) in Various Species-Age Groups

<table>
<thead>
<tr>
<th></th>
<th>Half-time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immature dogs*</td>
<td>0.7</td>
</tr>
<tr>
<td>Adult dogs**</td>
<td>0.2</td>
</tr>
<tr>
<td>Immature cats**</td>
<td>9.3</td>
</tr>
<tr>
<td>Adult cats**</td>
<td>3.6</td>
</tr>
</tbody>
</table>

* N = 5.  ** N = 6.
TABLE 3—Mean Percent (± 1 Standard Deviation) of Total Radioactivity from $^{14}C$-o-Phenylphenol Doses Excreted in Urine of Various Species-Age Groups Receiving 25 Doses of 3.7 mg Each

<table>
<thead>
<tr>
<th></th>
<th>Urinary excretion (% of dose)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immature dogs*</td>
<td>44.8 ± 5.0</td>
</tr>
<tr>
<td>Adult dogs**</td>
<td>53.7 ± 5.8</td>
</tr>
<tr>
<td>Immature cats*</td>
<td>31.3 ± 10.6</td>
</tr>
<tr>
<td>Adult cats**</td>
<td>41.7 ± 7.0</td>
</tr>
</tbody>
</table>

* N = 5.  ** N = 6.
Fig 1—Experimental design for the study of o-phenylphenol in dogs and cats.
Fig 2 -- Plasma disappearance of radioactive o-phenylenediamine (OPP) in immature cats, adult cats, immature dogs, and adult dogs following oral administration of 3.7 mg OPP to 5 animals in each group.
Fig 3 -- Excretion in the urine of radioactive o-phenylphenol (OPP) in adult dogs, immature dogs, immature cats, and adult cats after the oral administration of 3.7 mg OPP to 5 animals in each group.
Fig 4 -- Plasma radioactive o-phenylphenol (OPP) levels in immature cats, adult cats, immature dogs and adult dogs during and after oral administration of 3.7 mg OPP every other day for 25 doses. Plasma disappearance lines for each of the groups (n = 6/group) are illustrated for the time period after the last dose (day 48).
Fig 5 -- Excretion in the urine of radioactive o-phenylphenol (OPP) in adult dogs, immature dogs, adult cats and immature cats during and after the oral administration of 3.7 mg OPP every other day for 25 doses. Six animals comprised each group. The values depicted are the total percents of the cumulative dose administered that were excreted by each time frame.
AN OVERVIEW OF DRUG RECEPTOR INTERACTION, SAFETY MARGINS, PHARMACODYNAMICS, AND DOSAGE REGIMEN

by

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Comparative Toxicology Laboratory
Kansas State University
Manhattan, Kansas 66506

Confronting the practitioner daily are problems of drug dosage regimens, tissue residues, and drug toxicities. He seeks to maximize both safety and efficacy. In evaluating drugs, toxicologists and pharmacologists report their data in terms of actions at receptor sites, safety margins, biological half-life, and volumes of distribution. This review is provided as an aid to the practitioner so that he might better find his way to safe and effective drug use.

DRUG-RECEPTOR INTERACTION

It is apothegmatic that intraspecies' response to a drug varies. There are several reasons for this biological difference. Within each animal there is a population of receptors sensitive to a given drug. These receptors may exist in one organ or may be located at more than one site. The occupancy theory of receptor activation states that no matter where located, each receptor within a given population of receptors has its own threshold drug concentration at which activation occurs. Drug effect is proportional to the fraction of receptors occupied by a drug, and the response elicited is a physiological function of the site where the receptors are located. The whole population of receptors in a tissue
displays a normal range of sensitivities. That is, a small percentage of receptors will be extremely sensitive and, hence, have very low threshold concentrations; too, a small percentage will be refractory and have high threshold concentrations. However, most receptors will be of average sensitivity. At any given moment the concentration of a drug at its action site is a function of the whole organism's ability to absorb, distribute, and eliminate the drug (2,4,9).

Add now the hypothetical complication that effective drug action requires 60% receptor activation and that toxic effects occur at or above 80% activation. To provide efficacy without toxicity, therefore, a therapeutic dosage regimen should achieve activation of greater than 60% but fewer than 80% of receptors. The greater the difference between effective dose and adverse effect, the greater the margin for clinical error in dosing an individual. A drug which activates fewer than 60% of the receptor will elicit too little effect to be efficacious.

The concept of varying a dose to activate a greater or lesser number of receptors, and thereby achieve a greater or lesser response, is known as dose-response. The dose-response relationship is depicted on a hypothetical dose-response curve (Fig. 1). Doses must be greater than A but less than B to be both safe and efficacious. Increasing the dose between A and B produces greater and greater response.

The dose-response curves for a given drug are not identical in every individual of a given species. Some animals are either hyporeactive or hyperreactive to a therapeutic drug dose. Fig. 2 depicts the distribution of quantitatively identical responses
of individual animals. Point A (curve 1) represents the median response. Half the individuals will be more sensitive and half less sensitive to a given drug dose. Points B and C represent the hyper- and hyporeactive responses, respectively. A relatively small number of individuals fit into these categories. Curve 2 depicts the cumulative frequency response for the population and provides a measure of variation in threshold dose needed to produce a stated response. Any dose along the curve gives the percent of animals responding to that and all lower doses.

Another way of looking at the concept of hyper- and hyporeactivity is depicted by the three dose-response curves in Fig. 3. The hyperreactive Dog 1 may have a whole population of receptors which are inherently very sensitive. Further, Dog 1 may require engagement of a lower fraction of receptors to induce effect. Doses A and D are the effective and toxic doses respectively for Dog 1. Dog 2 represents a median dog; dose B would be sufficient to achieve desired response in Dog 2, while dose E would be toxic. Hyporeactive Dog 3 may have either very resistant receptors or may require activation of an inordinately large percentage of receptors to induce effect. Doses C and F are the effective and toxic doses, respectively, for Dog 3. Administration of dose A to Dog 3 would have little effect on its innately refractory receptor. Conversely, administration of dose C to Dog 1 or 2 would produce an adverse effect.

There is yet another set of circumstances whereby an individual's receptor distribution may make it exceptionally susceptible to drug toxicity. If the dose-response curve of Fig. 1 were so steep vertically that it became impossible to
titrate the dose, it would be difficult to administer the effective dose A without the great risk of approaching the toxic level B. Unfortunately, it is not possible to predict this receptor distribution in an animal (4,9).

EVALUATION OF DRUG SAFETY

The standard safety margin (SSM) and therapeutic index (TI) are parameters which measure and compare safety relative to efficacy. The SSM has dimensions of percent and compares the dose of a drug lethal (LD) to the most hyperreactive animal to the dose effective (ED) for the most hyporeactive. Mathematically,

$$SSM = \frac{LD_1 - ED_{99}}{ED_{99}} \times 100$$

To look at standard safety margin another way, it is the percentage by which the dose effective for 99% of the population (ED_{99}) has to be increased before the dose kills 1% (LD_1). The TI compares the dose toxic or lethal to 50% of all animals of a species (LD_{50}) to the dose effective in 50% of all animals of a species (ED_{50}). Mathematically, TI = LD_{50}/ED_{50}.

The greater the SSM or TI, the less likely that a drug will produce adverse effects in slight overdose. For example, pyrilamine, an antihistaminic drug, has a very high TI and is relatively safe. 6-Mercaptopurine, an anti-cancer drug, has a low TI (9). Greater care must be taken when administering a drug with a low SSM or TI. Proper administration of all drugs is to effect.
ABSORPTION AND MEMBRANE PERMEABILITY

Mathematically, the pKa of a drug is the negative log of its acid dissociation constant. Knowledge of a compound's pKa, together with knowledge of the pH of biological fluids in which the drug is dissolved, will allow prediction of how well an organic chemical will cross biological membranes or be absorbed from a depot. For absorption or membrane diffusion to take place, a drug must be in an undissociated, fat soluble, non-ionic form. In general, acid environments favor the absorption of weak acids; alkaline compartments favor the absorption of weak bases. The higher the pKa of a weak acid, the better its absorption in more acid media; the lower the pKa of a weak base, the better its absorption in more alkaline media (Figs. 4 and 5) (3,13,15-17).

It can readily be appreciated that weakly basic drugs, especially those with a high pKa, are poorly absorbed from the acid stomach. One value of parenteral administration is that it bypasses the low pH of the stomach. Once a compound has entered the blood, it is distributed according to its pKa and the blood pH. For example, during acidosis the unionized form of a weakly acid drug will increase in plasma at the expense of the ionized form. Since biological membranes are permeable to the unionized form of a drug, there will be a fall of the plasma concentration of the drug consequent to the drug's shift from extracellular to intracellular fluid. More drug can penetrate extravascularly and react with tissue receptors.

It is possible to treat barbituate toxicity by establishing
a pH gradient between plasma and cerebrospinal fluid. If the plasma is made more alkaline by sodium bicarbonate administration, the fraction of ionized barbituate in plasma increases at the expense of the unionized membrane permeable form. The ionized form is then "trapped" in the plasma and is excreted by the kidneys (9).

Consider the oral administration of a weakly acid sodium salt of a phenolic compound. The sodium is absorbed and finds its way into the body's sodium pool. Within the acid stomach the phenolic moiety is primarily in the undissociated form and rapidly penetrates mucosae and capillary endothelia. Once in the more alkaline blood, the ionized form of the drug will predominate and be trapped in the blood compartment until such time as it either becomes undissociated or is excreted. The undissociated fraction is free to penetrate extravascularly and to react with tissue receptors. As dissemination of the undissociated fraction takes place, a series of dynamic equilibria are established and a steady biological state is theoretically possible.

Any ion or molecule smaller than urea has the potential to permeate membranes along concentration gradients without regard to pH. Ions are filtered through membranes on the basis of their hydrated size. Although the sodium ion is smaller than the potassium ion, the charge density of the sodium ion is greater and therefore attracts more water. The attracted water increases the hydrated size of the sodium ion and decreases the ability of the sodium ion to penetrate membranes. Also important is that multivalent ions are generally of larger hydrated size than
monovalent and therefore penetrate cell membranes with greater difficulty than monovalent ions (5).

**VOLUME OF DISTRIBUTION**

Apparent volume of distribution is a mathematical constant which provides an estimate of the extent to which a drug is distributed through the body's fluid compartments and of the drug's uptake by tissues (10). It equates concentration of a drug in plasma with the amount of drug in the body. Despite its units of liter/kg, the apparent volume of distribution does not represent a real body space. On the one hand it may be smaller than the plasma volume; or it may be as large as several hundred liters depending on how much drug is either bound to albumin in plasma or sequestered in body tissues. Accordingly, a large apparent volume of distribution implies wide distribution, extensive tissue uptake, or both. Drugs with low volumes of distribution would likely be limited in dissemination. Those drugs with volumes of distribution many times larger than the actual volume of the body must have tissue concentrations far higher than plasma concentrations (10). A drug which exists primarily in the charged state at plasma pH will have low volume of distribution. This is due first to the fact that the drug will be largely unable to pass membranes and distribute into fluid compartments other than plasma and, second, the drug will be rapidly excreted by the kidneys. Chemicals, such as DDT, which are exceptionally fat soluble and uncharged at plasma pH readily permeate membranes and sequester in fat.
BIOLOGICAL HALF-LIFE

The biological half-life of a drug is the time the body requires to deplete itself of one-half of the quantity of the drug. In most cases the half-life of a drug is independent of the dose, concentration, or route of administration (6). Compounds which have large volumes of distribution and exist in the body fluids largely in undissociated, fat soluble forms tend to have long biological half-lives. The body rapidly eliminates compounds with low volumes of distribution and highly ionic character; these rapidly excreted compounds have short biological half-lives.

Biological half-life is dependent on the many physical and chemical reactions that constitute metabolism, distribution and excretion. Many drugs, especially fat soluble ones, are highly protein bound in plasma (18). Plasma proteins, in essence, act as a sponge binding drug in the vascular compartment. This confining of drug in the vascular space can affect kinetic parameters. For instance, it can lead to falsely low volumes of distribution. This is because volume of distribution is calculated not on the basis of free drug in plasma, but rather on total concentration. Only the free drug can penetrate extravascularly (1,10).

In vivo metabolism of compounds generally follows first or zero order kinetics. In first order the rate of biotransformation is proportional to the concentration of foreign chemical present. When the concentration is high, the rate of elimination (amount of drug per unit time) is rapid. When the blood concentration is low, the rate of elimination is
slow (8,13,14).

A possible explanation for the order phenomenon lies within metabolizing enzyme systems (1). Rate of enzyme-catalyzed reactions can increase as long as an abundance of enzyme relative to substrate exists; first order implies such an enzyme excess. At drug concentrations so high that the enzyme is saturated, the amount of drug biotransformed per unit time is constant and maximum. Since rate is no longer dependent on the concentration of reactant drug, the rate is termed a zero-order process (Fig. 6) (12). Increasing the concentration of a drug in the body will not change the drug's half-time until the point of enzyme saturation is achieved. Any renal or pre-renal urinary pathology will affect clearance, biological half-life, and volume of distribution. Drug toxicities are not uncommon in animals with kidney failure.

**DOSAGE REGIMEN**

The biological half-life, the dosage interval, and the quantity of a drug dose must all be considered when developing a dosage regimen. Repeated dosing of drug results in accumulation unless the interval between dosing greatly exceeds the biological half-life for elimination (11). Fig. 7 displays the relative plasma concentration resulting from three hypothetical drugs with varying half-lives when each is given at the same dose every 4 hours. The drugs with the longer half-lives display greater tendency to accumulate. For example, at time = 4 hours, the drug with the half-life of 2 hours shows a concentration only one-half that of the drug with a
half-life of 4 hours.

In general, steady-state plasma concentrations are high when doses are large, the interval between doses is small, when drug elimination is slow, or when volume of distribution is low (8,11). As an example, Fig. 8 displays a hypothetical result of varying dose size. The higher the dose, the greater the accumulation. The Dose = 20 curve rapidly accumulates. Note that the concentration at any given time for the Dose = 20 curve is exactly 4 times that of Dose = 5 curve and twice that of the Dose = 10 curve. Increasing dose can increase steady-state concentration.

At some time after each dose the serum concentration reaches a maximum, point C (Fig. 9). At this point the rate at which the drug enters the sampled compartment is equal to the rate at which the drug is being removed. Past C, the rate of removal exceeds absorption and serum concentration falls toward a minimum (D) reached just after the next dose. At this minimum, the rate of drug absorption again equals the rate of removal. From D to C, rate of entry exceeds rate of elimination (11,13).

The time taken to reach a steady-state concentration depends almost entirely on the biological half-life. Clinically, it requires 5 half-lives to reach 95% steady-state concentration. Since this delay may be unacceptable, a loading dose may be given. As a general rule, if a given efficacious dosing interval is equal to the half-life for elimination, the appropriate loading dose is twice the maintenance dose. This loading dose will bring plasma concentration to eventual steady-state level (11).
SUMMARY

Drug response in an individual is proportional to the number of receptors engaged. Higher doses yield greater responses. However, there may be significant differences in response from individual to individual. Biologic membranes are permeable to fat-soluble, non-charged forms of drugs. A drug's state of dissociation is determined by both its pKa and by the pH of the media into which the drug is placed. Volume of distribution, half-time, and renal clearance measure dissemination and removal of drugs from the body. Large volumes of distribution, long half-times, and low renal clearance favor accumulation. Proper dosage regimen is a function of half-life of the drug, dosage interval, and quantity of dose. In general, plasma steady-state concentrations are high when doses are large, when the interval between doses is small, when drug elimination is slow, or when volume of distribution is low. Time to steady-state concentration depends almost entirely on half-life. It takes 5 half-lives to reach 95% steady-state concentration.
REFERENCES


Receptors Activated

(Toxic Level)

(Minimum Efficacy Level)

80%

60%

Log Dose

A

B

Fig. 1 Dose-Response curve
Curve 1
Number of Individuals manifesting quantitatively identical responses

Curve 2
Cumulative % of population responding

Log Dose

Fig. 2

Dose response curves for hyper- and hyporeactive individuals
Fig. 3

Responses observed in 3 dogs of differing sensitivity to a drug
Effect of pH on the absorption of weak acids and weak bases
Enzymatic rates of reaction with increasing substrate concentration
Fig. 7 Relative plasma concentrations resulting from three drugs with varying half-lives when each is given at the same dose every 4 hours.
Fig. 8 Relative plasma concentrations resulting from the same drug ($t_{1/2} = 4$ hours) given every 4 hours at varying dosages.
Fig. 9

Biologic concentrations of a drug observed in an ideal dosage regimen
Complex physical, chemical, and biological factors determine the action of foreign chemicals on receptor sites. These factors include route of administration, efficiency or absorption, distribution of the chemical throughout the body, protein binding, sequestration in tissue, biotransformation, rate and efficiency of biotransformation and excretion. Other factors, such as age, sex, and species variation may, in turn, alter biological effect of drugs or foreign chemicals.

The purpose of the first part of this review (I) is to provide an overview of drug disposition, biotransformation, and excretion as determined by the many factors mentioned above. The second part of the review (II) examines o-phenylphenol, its history, toxicity, and biotransformation.
I. THE ABSORPTION, BIOTRANSFORMATION, AND EXCRETION OF FOREIGN CHEMICALS

MEMBRANE STRUCTURE AND TRANSPORT

No matter the route of administration of a foreign chemical, a drug must pass one or more membrane barriers to reach its site of action.18,26 Surface epithelia, capillary endothelia, plasma membranes of parenchymal cells, and intracellular membranes of organelles all offer impedance to foreign chemicals.23 Further, chemical distribution, biotransformation, and excretion all involve membrane passage.15 Since chemical concentration at receptor sites modifies response intensity, and since chemicals must cross membranes to approximate receptors, it follows that membrane permeability is a determinant in chemical response.

Classical observations were made by Overton in the Nineteenth Century. He found that esters, aldehydes and ketones penetrated cell membranes very rapidly; more polar urea, glycerol, and amino acids penetrated more slowly. Years later (1933) Collander and Barlund correlated easy membrane passage with a high oil/water partition coefficient. They postulated the presence of a "lipoid-sieve" in the plasma membrane to explain the rapid penetration of small charged molecules with low oil/water partition coefficients.11,26 The "lipoid-sieve" was most permeable to compounds of low molecular volume. It is now known that the "lipoid-sieve" (now called a "pore") is merely a continuous aqueous phase permeating the plasmalemma. With minor exceptions, water soluble solutes with molecular weight
greater than 100 are excluded. Measurements of the dimensions of pore radii vary from $4^{\circ}$ to $7.4^{\circ}$ in intestinal mucosal cells to $7.4^{\circ}$ in the canine red cell.

Chemically, cell membranes are lipoid with associated protein. Cellular and subcellular membranes contain large amounts of phospholipids, cholesterol, and neutral lipids associated with protein. For example, rat liver plasma membrane contains approximately 40% lipids and 60% proteins. Other plasma and organelle membranes have their own characteristic proportion of lipid and protein.

As yet, experts have not been able to agree on the precise structural arrangement of membranes. The classical Davidson and Danielli model proposes that the membrane is composed of a lipid bilayer; each of the two lipid layers is arranged perpendicular to the cell surface. The polar ends of the lipid molecules are directed outwards, and the lipid core is covered on each side with an ionicly bound layer of protein. Since different membranes do differ in chemical composition, their structures may be different. Recent models place emphasis on the protein or lipoprotein portions of the membranes. Fluid mosaics have been proposed as well. In any event, it now appears certain that protein does extend all the way through the plasma membrane; thereby, a means of penetration is provided for charged substances.

Mucosae, epithelia, and capillary endothelia are merely aggregates of individual cells. Like an individual cell's plasma membrane, the multicellular barriers are relatively permeable to lipid soluble solutes and hydrophilic solutes whose molecular weights are less than 100. However, the barrier qualities of
the multicellular structures must take into account openings between cells. Large aqueous pores between cells in the epidermis and rumen epithelium may allow solute to bypass cell membranes. Typical capillary bed endothelium, such as that of striped muscle, contains pores so large that passage of drugs up to the size of plasma proteins may occur. Therefore, drugs should and do exchange readily between capillary plasma and interstitial fluid unless the drug is protein bound in plasma or interstitial fluid. Hepatic sinusoids, comparatively, have large pores; plasma protein-bound drugs can reach and interact with hepatic parenchymal cells. In a like manner, the glomerular pores of the kidney are relatively large allowing all molecules smaller than albumin to pass provided the molecules are not plasma protein bound. The CNS vessels, on the other hand, contain virtually no endothelial pores. Thus, only lipid soluble molecules may pass.

**Processes By Which Chemicals Cross Membrane Barriers**

There are two basic groups of processes by which chemicals cross membrane barriers. In passive processes, the first major group, substances pass through lipoid membranes down concentration gradients; the cell membrane is inert. Special transport systems comprise the second major group; here the membrane actually participates in moving solute molecules.

**Passive Processes.** There are two passive processes—simple diffusion and filtration. In simple diffusion solute transfer is directly proportional to and is determined by concentration gradients. However, there is disagreement on the mathematical
model which describes simple passive diffusion. Some favor Fick's equation which states that the diffusion rate of a compound across a membrane is directly proportional to the concentration gradient \((C_1 - C_2)\), the surface area available for transfer \((A)\), and a diffusion constant \(k(d)\). In the Fick equation, rate of transfer is inversely proportional to the thickness of the membrane \((T)\). Mathematically,

\[
\text{Rate of Diffusion} = \frac{k(d) A (C_1 - C_2)}{T}. \tag{44}
\]

At least one author eschews Fick's equation in favor of Ussing's flux equation:

\[
\frac{M_{12}}{M_{21}} = \frac{A_1}{A_2} \exp \left(\frac{ZF}{RT}\right) \text{PD} \tag{23}
\]

Here \(M_{12}\) and \(M_{21}\) are undirectional fluxes from solution 1 to 2 and 2 to 1, respectively; \(A_1\) and \(A_2\) represent the thermodynamic activity of the solute particles; \(Z\) is valence, \(F\) is the Faraday constant; \(R\) is the gas constant; \(T\) the absolute temperature; and PD the potential difference. \(^2\)

Both lipid soluble compounds and small polar compounds can move by passive diffusion. The higher the lipid/water partition coefficient of a chemical, the greater the dissolution of drug in membrane and the more rapid the diffusion. Yet, once a steady state is achieved, the concentration of chemical on both sides of the membrane will be theoretically identical. \(^15\) However, most agents of toxicological interest are weak electrolytes. At a given physiological pH drugs will exist in both undissociated and
dissociated forms. The extent of dissociation is a function of the chemical nature of the compound. Compounds which are undissociated penetrate membranes more rapidly than those that are dissociated. This lack of ionized form penetration is due to the fact that the hydrated ions are usually too large to traverse pores.\textsuperscript{8,23,27} An ion of a particular drug may distribute itself, then, unequally on two sides of a membrane. Because of pH differences across and Donnan equilibrium phenomena, ion distribution across membranes may be yet more unequal.\textsuperscript{27}

Filtration involves the bulk flow of water through pores; hydrostatic or osmotic gradients provide the force for the bulk flow. Molecular size permitting, any molecule in solution can pass pores. Obviously, large molecules pass with more difficulty than small. In general, molecules smaller than albumin pass multacellular membrane pores easily; molecules smaller than urea pass unicellular pores easily.\textsuperscript{32}

At least one investigator has determined that ions pass membrane pores on the basis of their hydrated size. The larger the hydrated ion, the less facile the filtration.\textsuperscript{8} Other investigators indicate that diffusion is charge rather than size dependent.\textsuperscript{7} In actuality, however, the theories are similar. For example, monovalent ions have smaller hydrated size than divalent, and, therefore monovalent pass pores more rapidly.

**Special Transport Systems.** There are at least five distinct special transport types: active transport, facilitated diffusion, counter-transport or exchange diffusion, augmented diffusion, and pinocytosis.\textsuperscript{32} Traditionally, an active transport system is defined as one in which a chemical is moved against a concentra-
tion or electrical gradient. A component of the cell membrane (a carrier) binds the solute molecule on one side of the membrane and transports the solute molecule actively to the other side of the cell membrane. At this point the carrier releases the solute and returns, its duty completed, to its original location to begin the process anew. Active transport requires energy, is selective, has a transport maximum, can be saturated, and can be completely inhibited. Active transport systems function in both absorption and excretion processes in the body.

Like active transport, facilitated diffusion uses a carrier system and evidences saturation kinetics. Unlike active transport, the system operates in the direction of the concentration gradient and achieves the same concentration on either side of the membrane as simple diffusion. Further, facilitated diffusion requires no energy and is not inhibited by metabolic poisons. Facilitated diffusion does not have as great a role in drug transport as active transport. However, facilitated diffusion is vital to amino acid, glucose, and nucleoside transport in various cells and tissues. For example, glucose absorption into the blood from the g.i. tract, into red cells from the blood, and into the CNS from the blood occurs via facilitated diffusion.

The kinetic model of active transport and facilitated diffusion is not difficult. The model in effect varies with conditions. Theoretically, only a limited number of carrier molecules exist. At increasing solute concentrations, more and more of the carrier molecules will be actively engaged in transport. The rate of transport will increase until all carrier molecules are
active. After this point of saturation, further increase in solute concentration will not increase rate of transfer. As long as the system remains saturated, the transport rate will be constant. Zero order kinetics will prevail. However, when carrier molecules far outnumber solute molecules, the rate of transfer will be far less than maximum. Increasing the solute concentration from here will yield increasing rate of transfer. When the transfer rate is proportional to the concentration of transferable drug, the system is displaying first-order kinetics. This system is analogous to traditional Michaelis-Menten treatment of enzyme kinetics. In Michaelis-Menten, the zero-order transport maximum is referred to as \( V_m \)--the maximum velocity of an enzyme catalyzed reaction. The Michaelis-Menten \( K_m \) is the substrate concentration at which the reaction velocity is one-half maximum. At substrate concentrations far below \( K_m \), rate is determined primarily by substrate concentration. This is analogous to first-order transport kinetics.\(^2,11,21,40\)

Counter transport or exchange diffusion may occur where facilitated diffusion occurs, for the system is bi-directional. In this system a high concentration of substance A on side one of a membrane drives the flow of substance B from low to high concentration (side one to side two).\(^23,27\)

Augmented diffusion is an anomalous phenomenon peculiar to the gut epithelium. It has long been established that compounds traverse the gut passively in relation to their chemical properties and in relation to the pH of their environment. In the case of certain compounds or combinations of compounds, however, measured plasma levels exceed that predicted by pH partition
hypothesis. One investigator postulates the existence of a microenvironment at the surface of the gut epithelium where the pH is maintained different from that of the gut.

Pinocytosis and phagocytosis are processes in which the cell membrane actually flows around particles of solvent or solute, respectively. These processes are vital to cell nutrition and removal of particulate matter from lungs.

Summary of Membrane Structure and Transport

In the case of cell membranes, lipid structures traverse passively more readily than hydrophilic because the membrane itself can dissolve lipids. Present within membranes are pores. Pores vary in size according to cell type and multicellular membrane type. Through these pores hydrophilic substances pass according to their charge and molecular weight. There are several special transport systems including active transport, facilitated diffusion, counter diffusion, augmented diffusion, and pinocytes (phagocytosis). Active transport and phagocytosis are the most important in toxicology.

THE ABSORPTION OF DRUGS AND FOREIGN CHEMICALS

Physical Factors Affecting Absorption

Since absorption of a chemical can usually occur only when the chemical is in solution, orally administered solids must first dissolve before they are available for absorption. Rate of dissolution is an important determinant in bioavailability and is influenced, in part, by dosage form, particle size, diffusion from
dosage form, and hydration. Drugs given in solid form as capsules or tablets disaggregate as follows:

Drug in Capsule → Dispersion → Solid Drug Particle → Dissolution

Drug in Tablet → Disintegration → Available for Absorption

Drug in Solution → Available for Absorption

The greater the surface area of the drug or chemical (the smaller the particle size) the faster that dissolution takes place. Soluble drug forms generally diffuse rapidly, and anhydrous forms tend to be more soluble. In general aqueous solutions and syrups have the fastest release rate. Suspensions, powders, capsules, tablets, and coated tablets release at progressively slower rates.

Chemical Factors Affecting Absorption

As previously stated, unionized chemicals pass membranes more readily than do ionized chemicals. The proportion of a weak acid or base in ionized form depends on the compound's pKa and the pH of the environment in which the drug is dissolved. The Henderson-Hasselbalch equation describes this relationship.

For a weak acid,
\[ \text{pH} = pK_a + \log \frac{[\text{ionized acid}]}{[\text{unionized acid}]} \]

For a weak base,
\[ \text{pH} = pK_a + \log \frac{[\text{ionized base}]}{[\text{unionized base}]} \]
The pKa in both equations is the negative log of the acid dissociation constant.\textsuperscript{51,52}

Chemicals that penetrate biological membranes by simple diffusion do so according to their degrees of ionization, the charged state of their ionized form, and the extent to which they are macro-molecule bound in bulk solution. A non-protein bound unionized drug will have the same equilibrium concentration on both sides of a membrane. In contrast, partially ionized, unbound chemicals may distribute unevenly according to Donnan effect or pH difference on the two sides of the membrane. The Donnan effect maintains charge balance and is largely a result of non-diffusible ions, such as proteins.\textsuperscript{51}

pH differences on two sides of a membrane alter equilibrium distribution of drugs because pH has a great effect on the charged state of weak acids and bases (discussed above under the Henderson-Hasselbalch equation). A weakly acid compound in low pH medium will exist primarily in undissociated, lipid soluble, membrane permeable form. The identical chemical in alkaline medium will exist in dissociated, water soluble, impermeable form. Since membranes are permeable to the unionized form of bulky chemical molecules, the steady state concentration of unionized, unbound chemical will be the same on both sides of a membrane regardless of pH. However, the concentrations of the ionized form will vary according to pH.\textsuperscript{51} The ratio of total chemical on either side of a membrane is given by the equation:

$$\text{Ratio} = \frac{1 + \text{Antilog} \ (\text{pKa}-\text{pH}_1)}{1 + \text{Antilog} \ (\text{pKa}-\text{pH}_2)}$$
Since most biological membranes are impermeable to proteins, the binding of solute molecules to proteins has profound effect on drug distribution. For example, plasma proteins bind many drugs tightly and prevent their diffusion.\textsuperscript{51, 66}

**Buccal.** The mucous membranes of the buccal cavity behave as a typical lipid barrier. Chemicals penetrate by simple diffusion. Only limited roles are attributed to active transport, pinocytosis, and filtration through pores. Here, too, lipid soluble unionized forms pass most readily. Previous discussions regarding pH partitioning apply. The pH of saliva is around 6 in man.\textsuperscript{4}

Chemicals which are acid labile or rapidly metabolized by liver are good candidates for buccal administration, for the buccal route bypasses both the acid stomach and the portal circulation.

**Subcutaneous and Intramuscular Injection.** By definition, subcutaneous and intramuscular administration of chemicals penetrate epithelial barriers. In both subcutaneous and intramuscular injections, the injected solution becomes disseminated solely in the connective tissue ground substance. An increased blood flow to the area results from an aseptic inflammation caused by needle and fluid pressure.\textsuperscript{55}

The addition of hyaluronidase to injected solutions increases rate of absorption because hyaluronidase enhances chemical diffusion in the extravascular hyaluronic acid gel. The absorptive area is, thus, increased. Absorption of vehicle and chemical are two independent events occurring simultaneously. In general, the absorption rate of low molecular weight compounds
is more rapid than high.\textsuperscript{55}

Dissolved chemicals are free to diffuse through the tissues and capillary walls; water solubility favors diffusion and uptake is both molecular weight and blood flow dependent. Lipid solubility favors endothelial passage.\textsuperscript{55}

Percutaneous. The skin is not highly permeable to foreign chemicals because of its many barrier layers. To be absorbed through skin, a compound must pass either through epidermal or appendage cells. Since epidermal cells comprise the majority of surface area, passage through them is primary. All materials move across the stratum corneum by simple diffusion. Like absorption from subcutaneous depots, the rate of passive diffusion of non-polar drugs and toxicants is related directly to lipid solubility and indirectly to molecular weight. The dermis is less dense than epidermis and passage into systemic circulation from the dermis is dependent on effective blood flow, interstitial fluid movement, and lymphatics. It is possible to enhance skin absorption by incorporating chemical molecules into dimethyl sulfoxide (DMSO).\textsuperscript{32}

Absorption Through the Lungs. Chemicals absorbed across lung mucosae are usually gases, vapors of volatilizable liquids, and aerosols. The size of the active particle in aerosols is extremely important. Particles greater than 10\textmu m impact with the pharynx and fail to reach alveoli. Particles between 1 and 5\textmu m sediment within bronchioles and fail to reach lung parenchyma; particles less than 1\textmu m, however, are free to diffuse within alveoli. Until a particle reaches the alveoli it can be removed by mucus and reverse ciliary action. Soluble compounds are best
removed by translocation from alveoli to blood or removal via the
bronchi to the g.i. tract. The lymphatic route of macrophage-
mediated removal is best for particulate matter.\textsuperscript{32}

The absorption of liquid toxicants in aerosol suspension
occurs by passive diffusion; therefore, lipid soluble compounds
are most easily absorbed. Gases diffuse across lung membranes
according to their partial pressure.\textsuperscript{32}

\textbf{Corneal Absorption.} Not surprisingly, chemicals traverse
the cornea in proportion to their lipid solubility. Highly polar
drugs, such as antibiotics, cross poorly; organic bases, such as
tropine, cross readily.\textsuperscript{27}

\textbf{Gastrointestinal Exposure.} The gastrointestinal tract is
commonly exposed to toxicants. The absorption of chemicals may
take place along the whole length of the g.i. tract. Two mem-
brane barriers exist which separate the g.i. tract from the blood
stream: the mucosal epithelium and the capillary endothelium.
Again, lipoid substances are the most readily permeable.\textsuperscript{27,50}

Weakly basic strychnine does not exert its toxic effect so
long as it is kept in the milieu of the highly acid stomach.
However, when the stomach is made alkaline, the toxicity of
strychnine evidences itself. Weakly basic electrolytes, then,
are best absorbed in the less acid portions of the g.i. tract.
On the other hand, weakly acid compounds are well absorbed in
the most acid portion of the gut.\textsuperscript{49,52} Because of marked pH
differences between gastric juice and plasma, an uneven distribu-
tion of chemical across the mucosal-endothelial membrane can
occur. Weakly basic compounds become trapped in the acid milieu
of the gut because they are largely ionized at gut pH. On the
other hand, weakly acid compounds are undissociated at gut acid pH; hence, they cross readily into the plasma where they, in turn, are trapped because of relatively high plasma pH. That is, at the high plasma pH, weakly acid compounds exist mainly in ionized form and cannot escape the endothelial barrier.\textsuperscript{49}

Absorption from the small intestine is not really different from absorption from the stomach. Acids with a pKa greater than three and bases with a pKa of less than seven or eight are best absorbed. It has been suggested that the measured pH (6.6) of the human small intestine does not accurately reflect and explain true intestinal passage of drugs. Rather, investigators postulate that the true effective pH of the gut at the absorptive surface is 5.3. In comparison to small intestine, absorption from colon is less rapid. Otherwise colonic absorption mimics that of small intestine. It is certainly worth noting that changes in the colonic pH affect permeation of chemicals.\textsuperscript{27}

Both augmented diffusion and active transport occur in the gut epithelium. Tetracycline is complexed in the gut epithelium in its charged form and its absorption is augmented.\textsuperscript{27} Compounds whose structures resemble naturally occurring substrates sufficiently can be actively transported across intestinal epithelium. For example, 5-florouracil is transported by the uracil mechanism.\textsuperscript{54} The kinetics of this process are those of active transport.

\textbf{Physiological, Pharmacological, and Pathological Factors Which Affect the Absorption of Chemicals}

\textit{Oral Exposure}. Orally administered compounds are subject
to a variety of physiological conditions which may affect their absorption. Most obvious is that the morphology of the g.i. tract varies markedly from mouth to anus. Fick's law of diffusion takes surface area into account; therefore, it is only logical that the small intestine with its folds of Kerckring, villi, and microvilli has the greatest capacity to allow passive diffusion. Yet, the stomach is an effective surface for compounds whose absorption is favored by low pH. Although the large intestine may absorb compounds, an oral dose that passes through both stomach and small intestine will, in general, be poorly absorbed in the colon. It is certainly worth noting that some compounds are so unstable at the low pH of the stomach or, to a lesser extent, the small intestine, that they are destroyed before absorption can occur.

The pH change from mouth to anus is great. The mouth saliva has pH 6; the stomach pH 1-3, the duodenum pH 5-6, the colon pH 8. Stomach pH is influenced by feeding: the pH of the fasted stomach is in the range of 1.2-1.8; the pH of the non-fasted increases to 3 or above. In a like manner the pH of the intestines is affected by food. The acidity of the duodenum is increased by the presence of acid stomach chyme. So, there are three explanations of the fact that some chemicals are absorbed better from small intestine than from stomach: the compound in pKa favors the less acid media; a specific augmented transfer system exists; a large surface area is required.

A decrease in the rate at which compounds leave the stomach may have a pronounced effect on duodenal absorption.
With small liquid meals there is an initial phase of slow gastric emptying followed by rapid emptying. If a large liquid meal is introduced, a rapid initial rate of removal is followed by a first-order volume dependent phase. Warm and viscous foods empty slowly, as well. Any factor which increases the acid content of the small intestine will reflexly cause the pylorus to contract and thereby delay emptying.\textsuperscript{2} In general, the presence of bulk food in the stomach delays emptying.

Other dietary factors delay emptying. Fasting for a period of twenty hours decreases blood flow to the intestine. Food present in the g.i. tract may decrease the amount of biological fluid, thereby decrease the rate of chemical dissolution. It is likewise conceivable that viscosity could become so great that diffusion to mucosal surfaces could be delayed. Not unlikely, too, is that components of the diet may physically or chemically adsorb or interact with a chemical. Overall ingestion of a meal increases the blood flow through splanchnic vessels by 30\%.\textsuperscript{2}

Physiological peristaltic movement provides for intimate contact between chemical and intestinal mucosae. Mobility also enhances the fluid movement in the gut and increases dissolution. Passage time through the intestine varies because bulk enhances peristalsis.

The entire tract also contains enzymes, mucin, and bile salts. Enzymes could conceivably either activate or inactivate drugs. Metabolizing enzymes occur in the gut epithelium as well as in the lumen.\textsuperscript{45} Mucin normally interferes little with drug absorption.\textsuperscript{48} Bile salts, on the other hand, may enhance the
absorption of poorly soluble compounds by nature of their surface-
active effect.³ Age also plays a role in absorption. The period
immediately after birth is marked by high gut permeability. Too,
β glucuronidase activity is very high neonatally while glucuronyl
transferase activity is lacking. Therefore, the breakdown and
reabsorption of glucuronide conjugates is favored.⁶⁵

Any agent which alters the pH of the g.i. tract will alter
absorption. Acetylsalicylic acid, acetaminophen, fats, fatty
acids, anti-cholinergics, and antacids are only a few examples.
Further, many of these same drugs affect emptying and motility.

Many poorly absorbed compounds have been shown to complex
with gastric mucin or intestinal bile salts. Others complex
with concomitant food or milk.² On the other hand, some chemi-
cals, such as EDTA which are themselves complexing agents,
actually can enhance the intestinal absorption of normally
poorly absorbed lipid insoluble substances.⁴⁹

Any process which causes a breakdown in the structure of
cell membranes, such as inflammation, inhibition of normal
metabolism, and ulcer formation will lead to the loss of
intact absorption barriers.²⁷ Ulcer formation favors decreased
pH.² Hypermotility and hypomotility of the g.i. tract decrease
and enhance absorption, respectively.

Parenteral Exposure. Absorption through skin may be
enhanced by the addition of highly permeable solvents, such as
DMSO. Inunction and iontophoresis also may enhance percutaneous
absorption. Hyaluronidase can increase the diffusion of i.m.
or s.c. administered compounds.²⁷ In general, increasing or
decreasing the blood supply to an absorptive surface will,
respectively, enhance or retard absorption. Too, any desquamation or destruction of absorptive surface will enhance absorption.

Absorption Kinetics

At least one author describes a method for determining the $k$ absorption of an orally administered chemical. Begin by plotting plasma concentration vs. time on semi-log scale. Extrapolate the terminal linear portion of the resulting graph back to time zero ($t_0$). Determine the difference between measured experimental plasma concentrations and the extrapolated line at several points. A semi-log plot of these residual differences ("residuals") vs. time has a slope of $-k$ absorption/2.303.35 The time ($t_p$) required for maximum absorption to take place is described by the equation:

$$t_p = \frac{2.303}{ka-k} \log \frac{ka}{k}$$

where $ka$ is the absorption constant and $k$ the constant of elimination from a one compartment system.16

Summary of the Absorption of Drugs and Foreign Chemicals

Some membranes are most permeable to unionized, lipid soluble substances; chemicals which exist primarily in lipid soluble form are rapidly absorbed. Whether a weakly acid or weakly basic drug exists in unionized form is a function of the compounds's pKa and pH of the milieu into which it is placed. Chemicals may become ion-trapped.

In general, a large area of contact with absorptive surfaces enhances absorption as does high blood flow to the surface.
Many pharmacological, physiological, and pathological conditions exert effect and interreact to cause altered drug absorption patterns.

THE DISTRIBUTION OF DRUGS AND FOREIGN CHEMICALS

It is empirical that a chemical must reach its site of action for receptor activation to occur. Enroute, it must overcome pH gradients, plasma protein and cell binding, and tissue sequestration.

Blood is the most convenient tissue for sampling. Because of the obvious fact that blood circulates throughout the entire body, some approximation of equilibrium can be expected between plasma and tissue. It is assumed, sometimes fallaciously, that chemical concentrations to this plasma is proportional to its concentration at tissue receptor sites. Most chemicals, however, do not distribute equally throughout body fluids. Compounds that penetrate freely through cell membranes become disseminated throughout body water while those that can traverse endothelium but not parenchymal cell membranes become distributed through extracellular fluid only. Some chemicals cannot even escape the vascular system. The apparent volume of distribution (Vd) of a compound is defined as the volume in which the total amount of the compound would be distributed uniformly in order to evidence an observed plasma concentration.

Mathematically,

\[ V_d = \frac{\text{Dose}}{\text{Concentration}} \]
With dose measured in mg/kg, and with concentration measured in mg/ml, Vd assumes the units ml/kg which can be considered a fraction of body weight. Implied in the above equation is that Vd does not change over wide ranges of dose and concentration. It is most desirable to measure volume of distribution under conditions of drug equilibrium between tissue and plasma. When a drug is administered, some time elapses before equilibrium is achieved; however, metabolism of the drug begins immediately upon absorption. Because metabolism favors drug alteration or loss, the drug loss to metabolism must be determined so that the loss might be subtracted from the original dose to arrive at the total amount of drug that is present in the body at the time when Vd is calculated.

\[ \frac{dc}{dt} = -kC; \]
\[ C = C_0e^{-kt}; \]
\[ \ln C = \ln C_0 - kt \]

C is concentration after measured time (t); Co is concentration at to. A plot of ln C vs. time evidences a linear relationship after absorption is complete. The slope of the line is \(-k\). The relationship of k to half-time—the time required for the measurable concentration of the drug in the body to decrease by one-half—is defined by \( t \frac{1}{2} = \ln 2 / k \).

A practical method of estimating the amount of chemical actually in the body is to extrapolate the linear segment of the disappearance curve back to \( t_0 \). This process implies that the loss of chemical before equilibrium follows the same decay
as that before equilibrium. The formula becomes:

\[ V_d = \frac{\text{Dose administered}}{\text{Concentration at } t_0} \]

A more referred method of calculating has been described. For a chemical that disappears from the body at a rate proportional to its plasma concentration, the rate loss at any time is \( kVdC \) where \( k \) is the disappearance constant, \( Vd \) the volume of distribution and \( C \) the concentration of a drug at a given time. The amount lost between times \( t_1 \) and \( t_2 \) is \( kVd \int_{t_1}^{t_2} Cdt \). If one considers all times from \( t_0 \) to \( t_\infty \) and plots concentration vs. time, then the area under the generated curve will equal the entire amount of absorbed chemical. That is:

\[ kVd \int_{t_0}^{t_\infty} Cdt = \text{Absorbed drug}, \text{ or} \]

\[ \text{Absorbed drug} = kVd \text{ Area}, \text{ or} \]

\[ V_d = \frac{\text{Dose}}{k \cdot \text{Area}} \]

Because cell membranes provide a barrier, chemicals will distribute themselves throughout the fluid pools in accordance with their abilities to traverse the barriers. Substances which are highly bound to plasma albumin, which have very high molecular weight, or which are excreted very rapidly seldom escape plasma water. A substance which penetrates endothelial cells but not parenchymal cell membranes will distribute throughout 20% of the body weight. A compound which penetrates all membranes may distribute to 60% and more of body weight.9
General Factors Affecting Chemical Distribution

Recently a phenomenon called "first pass" has been invoked to explain the varying degrees of efficacy attained by the same chemical administered by different routes. Oral drugs are the most susceptible to "first pass". Chemicals administered orally are absorbed into the mesenteric veins; from here they pass to the liver via the portal veins. Therefore, a chemical actually encounters the major organ of metabolism before arriving at the systemic circulation. In the liver the chemical may be bound, activated, or inactivated. Other routes may show "first pass" effects to varying degree. A chemical extensively bound to protein will distribute little after intramuscular injection. Still other compounds bind heavily to plasma proteins on intravenous administration. It is important to note that "first pass" does not alter the elimination of a compound once the compound is distributed through the systemic circulation; therefore, a chemical may still have a long half-time if its volume of distribution is great.13

Chemical molecules may interact with fat. The molecules distribute into depot fat on the basis of their oil-water partition coefficients. If this coefficient is high, the percent of chemical localized or sequestered in fat may be high for two reasons: high fat solubility and low blood perfusion of fat. It is the low blood perfusion that delays any establishment of equilibrium between blood and fat. The volume of distribution of highly fat soluble compounds may well exceed one.

Many compounds have the capacity to bind to plasma albumin. If the extent of binding is great (>90%), the majority of body
burden will be in plasma. Although volume of distribution is affected only slightly by albumin binding, half-time may increase markedly with increased binding. This increase is due to the fact that glomerular filtration cannot occur.\textsuperscript{9} It is logical that as concentration of albumin decreases, binding decreases. As chemical concentration increases, the bound fraction decreases. In general, lipid soluble compounds are most bound. Protein binding varies between species.\textsuperscript{66}

There are cogent toxicological implications in protein binding. Drugs and endogenous compounds compete for binding sites. An example occurs in kernicterus where sulfonamides displace bilirubin from binding sites. If two exogenous chemicals compete for binding sites, the one more tightly bound will displace the other yielding potentially dangerous concentrations of the displaced compound. For example, many acidic drugs will displace warfarin from plasma proteins. Free fatty acids, too, compete for binding sites, and conditions, such as pregnancy or starvation decrease binding of chemicals.\textsuperscript{66}

Not uncommon is the binding of chemicals to blood cell membranes and to hemoglobin within red cells. Sulfaguanidine is an example. Phagocytes have the ability to distribute chemicals in oil emulsion. Some chemicals such as highly basic substances, may bind nucleoproteins or other basophilic cell components. For example, chlorotetracycline becomes localized in liver and kidney mitochondria. Other drugs of ionic nature may bind mucopolysaccharides of connective tissue. In a like manner bone may bind heavy metals, and sympathetic neurons may sequester biogenic amines.\textsuperscript{28} Since only free chemical can equilibrate
across membranes, distribution is influenced greatly by tissue sequestration.

Worth at least casual note is that body water in the young represents a relatively greater percentage of body weight. Therefore, the relative amount of protein binding in the young may be low. 16

Certainly the most important factor in chemical distribution is pH. Since most chemicals of biological interest are weak electrolytes, they penetrate membranes according to Fick's law in proportion to their lipid solubility. As previously discussed, the lipid solubility of a weak electrolyte is determined by the pH of the chemical's environment, the pKa of the chemical, and the chemical's weakly acidic or basic properties. The pH gradient from extracellular fluid to intracellular fluid is only 0.4 (7.4-7.0) that from plasma to CSF is only .1 (7.4-7.3). Nevertheless, these gradients do have profound influence on chemical disposition.

Repeated dosing of a chemical results in accumulation unless the compound is biotransformed or sequestered before a subsequent dosing occurs. After several dosings at regular intervals, a steady state will be reached. When doses are large and closely spaced, steady state concentrations are high. When doses are low or infrequent, steady state concentrations are small. There is a point after each dose where serum concentration reaches a maximum. At this point rate of entry into the sampled plasma compartment is the same as rate of removal from the plasma. During the rest of the dosage interval, concentration falls until just after the next dose when chemical absorp-
tion rate of the new dose equals chemical removal rate of the old dose(s). Rate of accumulation, in general, is dependent primarily on rate of post-distribution removal.\textsuperscript{19,20} It is empirically obvious that accumulation means high blood and tissue concentration and high concentration of chemical at receptor sites.

Specific Barriers

The Blood-Brain Barrier. The blood-brain barrier is a result of the fact that capillary endothelial cells are joined together by tight intercellular junctions. The junction is so tight that any penetration of drug from blood to brain must be through the capillary endothelial cells themselves; no endothelial membrane pores exist as they do in glomerulus or striped muscle. Too, the cells of the choroid plexus are joined together with continuous tight junctions. The only exceptions to CNS lack of endothelial pores occurs in specific locations, such as the area postrema, where no tight junctions exist. Likewise, the barrier is not complete in the neonate; chemicals, therefore, may enter and effect brain receptors in larger than adult quantities.\textsuperscript{47} Highly ionized or plasma protein-bound substances will not penetrate the barrier well and will not usually reach effective concentrations in the CNS. Pathological conditions, such as meningitis, uremias, severe intoxications, and neoplastic processes may increase the permeability of the barrier.

Placental Transfer. Factors that affect placental transfer and fetal disposition are identical to those previously discussed. These factors include blood flow, protein binding of the chemical,
lipid solubility of chemical at various physiological pH, molecular weight of the chemical, and the surface area of the membranes.¹

Summary of the Distribution of Drugs and Foreign Chemicals

Chemicals distribute themselves throughout the body in proportion to their ability to penetrate membranes. Thus ability to penetrate membranes is dependent on the lipid solubility of the drug. However, plasma protein binding, red cell binding, tissue sequestration, and special membrane barriers may prevent a compound from distributing completely. Too, distribution may be enhanced or depressed by competition for albumin binding sites. Several mathematical methods have been devised for determining the extent to which chemicals distribute themselves throughout body fluids.

BIOTRANSFORMATION

A primary purpose of chemical detoxication and biotransformation mechanisms is the formation of water-soluble derivatives with low pharmacological activity. In general, water-soluble moieties are easily excreted by kidney and liver. Metabolites are water-soluble because they either contain more hydrophilic function groups than their parent or are conjugated with hydrophilic moieties. Generally true, too, is that metabolites tend to be more ionized at physiological pH than parent compounds. Chemical metabolism does not necessarily imply detoxication. In fact, some compounds become more toxic or active. Examples include the "pro-drugs" α-methyldopa and parathion.³⁷
Drug detoxication occurs in two phases. Phase I results in oxidation, reduction, or hydrolysis; these processes can either inactivate a chemical, convert an active compound to a different active chemical, or convert a chemical to an active metabolite. Phase II reactions are synthetic; usually they convert active compounds to inactive excretory products.

\[
\text{Chemical} \xrightarrow{\text{PHASE I ACTIVATION}} \xrightarrow{\text{and/or HYDROLYSIS}} \xrightarrow{\text{PHASE II INACTIVATION}} \text{synthetic or conjugation product}
\]

Phase I reactions often result in the addition of functional groups, such as OH, COOH, or NH₂. These groups having been added, phase II reactions may then occur. Typical phase I microsomal oxidations include: the oxidation of aromatic rings, the oxidation of alkyl chains, oxidative dealkylation, N-oxidation, sulfoxidation, replacement of S by O, and epoxidation. Phase I microsomal reductions include: the reduction of nitro compounds to amines, the reduction of azo compounds to amines, and the reduction of ketones to secondary alcohols. Typical hydrolyses are described by the hydrolysis of esters and amides.\(^{39,44,63}\)

There are several different phase II conjugations. Since glucose is readily available, glucuronide conjugation is very common. What actually happens in glucuronide conjugation is a multi-step reaction.
Glucose-1-Phosphate + UTP pyro-phosphotase \[ \rightarrow \]
\[
\text{UDP glucose + pyrophosphate}
\]

\[
\text{UDP glucose + NAD}^{+} + \text{H}_2\text{O} \xrightarrow{\text{UDPGL}} \text{UDP glucuronic acid + 2NADH + 2H}^{+} \quad (\text{UDPGL})
\]

\[
\text{UDP glucuronic acid + RZH} \xrightarrow{\text{Glucuronyl transferase}} \text{RZ glucuronic acid + UDD}
\]

where Z is O, C- O\(^{-}\), NH, or S and R is an organic radical.\(^{37}\)

Interestingly, the enzyme mediating UDPGA formation is not microsomal. Glucuronyl transferase is, however, microsomal; this transferase is found in liver, kidney, GI tract, and skin. Implied is that both microsomal and mitochondrial oxidative processes need to be functioning. Alcohols and phenols commonly form glucuronides. Endogenous substrates such as steroids, bilirubin, and thyroxin also form glucuronides. Glucuronides are usually stronger acids than their parent compound; they are highly ionized at physiological pH and are excreted in urine and bile. It is interesting to note that at least one gut enzyme, β-glucuronidase, exists which can break a glucuronide conjugate to its agluconic form.\(^{37}\) Glucuronide formation takes place in most species; however, the cat has limited glucuronide forming capability because of only limited transferase activity.\(^{14}\)
Like glucuronide conjugation, sulfate conjugation is multi-step.

\[ \text{SO}_4^{2-} + \text{ATP} \xrightarrow{\text{ATP sulfurylase}} \text{Adenosine-5' phosphosulfate (APS)} \]

\[ \text{APS} + \text{ATP} \xrightarrow{\text{APS phosphokinase}} \text{3'-phosphoadenosine 5'-phosphosulfate (PAPS) + ADP} \]

\[ \text{PAPS} + \text{RZH} \xrightarrow{\text{sulfokinase}} \text{R-Z-SO}_3^2 + 3\text{-phosphoadenosine-5'-phosphate (PAP)} \]

where Z is O or NH.

The enzyme which transfers sulfate to phenol is found only in the soluble fraction of liver, kidney and intestines.\(^\text{37}\)

Several metabolic routes favor the interaction of acid and amine to form amide. In all cases the acid moiety must be converted to an active form using acetyl Co-A.

\[ \text{RCOOH} + \text{ATP} \xrightarrow{\text{Acylsynthetase or thiokinase}} \text{RCO} - \text{AMP} + \text{pyrophosphate} \]

\[ \text{RCO-AMP} + \text{CoASH} \xrightarrow{\text{Acylthio-kinase}} \text{RCO-S-CoA} + \text{AMP} \]

\[ \text{RCO-S-CoA} + \text{R'NH}_2 \xrightarrow{\text{transacylase}} \text{RCONHR'} + \text{CoASH} \]

This is a normal mitochondrial reaction of liver and kidney. The most common example is the condensation of aromatic and heterocyclic carboxylic and drugs with glycine. The glycine pool, however, is limited. Zero-order kinetics may prevail in this reaction. Another common example occurs when exogenous amide compounds, such as sulfonamides, acetylate. Neither
phenols nor alcohols form acetyl derivatives.\textsuperscript{37}

Chemicals which form mercapturates usually contain a nitro group or active halogen.

\[
RX + \text{glutathione} \xrightarrow{\text{GS-S-ARYLTRANSferase}} CO-NH-CH_2-COOH
\]

\[
R-S-CH_2-C-H \xrightarrow{\text{Glutathionase}} R-S-CH_2-CH
\]

\[
\xrightarrow{\text{peptidase}} CO-NH-CH_2-COOH
\]

\[
\xrightarrow{\text{acetylase}} R-S-CH_2-COCH_3 \xrightarrow{\text{acetylase}} COOH
\]

where RX is an aromatic ring, a halide, or a nitro compound.

The transferase enzymes exist primarily in the soluble fraction. Benzene metabolism closely parallels this route.\textsuperscript{37}
Methylation is a minor metabolic pathway which, unlike other pathways, favors the formation of active products.

\[ \text{ATP} + \text{methionine} \xrightarrow{\text{transferase}} \text{S-adenosylmethionine} + \text{pyrophosphate} + \text{phosphate} \]

\[ \text{S-adenosylmethionine} + \text{RZH} \xrightarrow{\text{methyl transferase}} \text{RZCH}_3 + \text{S-adenosyl homo-cysteine} \]

where \( Z \) is 0, NH, or S. Although unequal, methylation of phenols does occur. More usual is the methylation of endogenous compounds, such as epinephrine and histamine. 32

**Microsomal Enzyme Systems**

Many of the metabolizing enzymes are located in the microsomal function of liver cells. The microsomal fraction contains the endoplasmic reticulum (ER). The rough ER is concerned with protein synthesis; the smooth contains metabolizing enzymes. Both NADPH and \( O_2 \) are required for the activity of most oxidative and a few reductive microsomal processes. Electron transport is involved and requires carriers. NADPH is oxidized to NADP+ by NADPH cytochrome \( C \) reductase; this carrier is then reoxidized by cytochrome P-450 reductase. Immediately prior to this step, the compound to be metabolized binds firmly to oxidized cytochrome P-450. The chemical-oxidized cytochrome P-450 complex is reduced by interaction with cytochrome P-450 reductase; the reduced complex binds one molecule of oxygen. One atom of oxygen goes to oxidize drug and one serves to form water. Oxygen re-
leased, the cytochrome P-450 returns to its natural oxidized state.\textsuperscript{31}

Lipid solubility is the single most important factor in determining whether a chemical will undergo microsomal oxidation. Lipid soluble compounds penetrate microsomal membranes with little difficulty. Reactions catalyzed by the microsomal mixed function oxidase system includes N and O dealkylation, aromatic ring and side chain hydroxylation, sulfoxide formation, N-oxidation, N-hydrogenation, deamination of primary and secondary amines, desulfuration, and glucuronide formation.\textsuperscript{18,44}

The cytochrome P-450 system is highly inducible. It can be stimulated by a myriad of exogenous chemicals, endogenous steroids, and drugs. Some substances, on the other hand, inhibit the enzyme system. Lead is an example, as is SKF 525A.\textsuperscript{29}

Further, there is much competition for biotransformation among chemicals at this site. The use of either an enzyme inducer or inhibitor can have profound effect on drug action; for example, the chronic administration of phenobarbital as an inducer will result in enhanced metabolism and excretion of other microsomally metabolized drugs.

**Enzyme Development**

Studies of non-microsomal liver enzyme activity in perinatal pigs have shown that the ability to perform oxidations increases rapidly from birth to week four. The increase is linear. Between the fourth and the tenth week, however, there is a slow, steady increase in oxidating ability. Prenatal levels of oxidating enzymes are negligible. Reductive and sulfating,
enzymes, however, are present in significant quantities at birth. Reductive enzymes increase by three times within the first 6 weeks of life at which point they plateau. Whereas non-microsomal enzymes increase to approximately 3-4 times birth quantity the first several weeks of life, microsomal glucuronyl transferase increase approaches thirty times birth quantity the first 3 weeks. Soon after this precipitous increase, plateau occurs. Too, transferase levels are negligible in the fetus.\textsuperscript{56} Interestingly, pretreatment of pregnant females with phenobarbital has the ability to increase glucuronide conjugation in the neonate. Oxidative and reductive microsomal pathways can be induced, as well. Induction in the newborn may last 3-4 weeks. In the adult the induction may last less than one week.\textsuperscript{56,65}

**Species Difference in Biotransformation**

Great differences exist in the ability of the various species to biotransform specific drugs. For example, dogs are unable to acetylate aromatic amines; cats form glucuronides with great difficulty. Different species may be forced to use alternate metabolic pathways. For example, the sulfate pool is usually quite limited; therefore glucuronide conjugation is preferred in species with glucuronide forming capability.\textsuperscript{29,64}

**Other Factors Influencing Biotransformation**

High levels of female pregnancy hormones may compete for drug metabolism sites and delay biotransformation. In addition, some chemicals selectively affect one sex more than the other. Nutritional states must be adequate for maximum enzyme activity to take place. Further, liver pathology will reduce biotrans-
formation, as well as other major organ dysfunction. 29

Summary of Biotransformation

Chemical detoxication mechanisms generally favor the production of water soluble, biologically inactive molecules. Detoxification often involves both phase I and phase II reactions. Phase I reactions include oxidation, reduction, and hydrolysis. Phase II reactions are conjugations. Deformation of conjugates is usually an energy requiring process. Many detoxication steps occur in the microsomes. There they are mediated by inducible and inhibitable enzyme systems. Species, age, and sex all may influence biotransformation pathways.

EXCRETION OF DRUGS AND FOREIGN CHEMICALS

The excretion of a foreign compound is the cumulative definition of all the many processes that occur from time of exposure to time of depletion. Consider the path of a typical drug administered orally. If a weak electrolyte is lipid soluble at intestinal or stomach pH, and if the drug is not bound to food particles or intestinal protein, and if the drug has sufficient contact with the intestinal epithelium, and if the drug is not acid labile or destroyed by intestinal flora, then the drug may be absorbed. Absorption then takes place according to Pock's Law. Metabolism in the intestinal cells may or may not occur, and absorption into the portal system takes place. "First pass" effects may be felt.

Once in the systemic circulation, chemicals are distributed according to qualities, such as pKa, extent of protein binding,
fat solubility, etc. Chemicals with very high volumes of distribution are widely disseminated not only in body lipid pools, but they also may be sequestered in tissue.

Liver and kidney metabolism of the chemical begins right away. If the compound is water soluble in the blood, it may be directly excreted by the kidneys. If the chemical is not water soluble, it will probably be metabolized to more soluble form by either mitochondrial or microsomal biotransformations to water soluble form.

Pharmacokineticists have developed at least two major models which define the absorption, distribution, and excretion of a chemical. The purpose of each of the models is to define $k$ values for the absorption, tissue distribution, and excretion phases of the journey of chemical through the body. To determine the half-times, then, is the ultimate goal of the kinetic models. The half-time is, in effect, a summary of the many physiological processes which go to make up its numerical value.

**The Two-Compartment Model**

In the two compartment model, chemical contained in the body behaves as though it were in two kinetically distinguishable compartments. Compartment one is the vascular tree, and compartment two represents the many other body tissues. Body depletion comes from the central vascular or peripheral tissue compartments. Any chemical placed directly into the blood (IV) undergoes both elimination and distribution simultaneously. The distribution and diffusion into tissue is reversible. If dog blood concentration is plotted against time at many times, a
biphasic curve results. The several k values definable include:

\[ k_1 \] - rate of chemical absorption

\[ k_{12} \] - rate of distribution from the central blood compartment to extracellular tissue

\[ k_{21} \] - rate of distribution from tissue back to the central blood compartment

\[ k_{10} \] - rate of elimination from the body via the peripheral compartment

\[ k_{20} \] - rate of elimination from the central vascular compartment

When chemicals are administered IV, the concentration in the plasma \( (P) \) at any time \( T \) is:

\[ P = Ae^{-at}Be^{-bt} \]

Where \( A \) and \( B \) are the two intercepts on the concentration axis of the biphasic semi-log plot, and \( a \) and \( b \) are the slopes of the two disappearance phases, the second half of the equation represents the straight portion of the semi-log disappearance curve. The first half of the equation represents the tissue distribution portion of the curve. The distribution portion of the curve is determined by a method of residuals called "feathering."\(^{35,40}\)

There are three basic types of bicompartent systems. The first has body elimination only from the tissue compartment; the second has elimination from both tissue and blood compartments; the third has elimination only from blood compartment. The usual assumption is that the third bicompartent model holds
true. The $k$ values can be determined as follows:

$$k_{21} = \frac{Ab + Ba}{A+B}$$

$$k_{10} = \frac{ab}{k_{21}}$$

$$k_{12} = a + b - k_{21} - k_{10}$$

Further, the biological half-life is:

$$t_{1/2} = \frac{0.693}{b}.35,40$$

The One-Compartment Model

If the distribution of chemical throughout the body is extremely rapid in comparison to the rate of elimination from the central compartment, then $a >> b$ and $P$ approaches $Be^{-bt}$. Plotting this graph as concentration vs. time yields an apparent monophasic disappearance. Since absorption terminates long before disappearance, the monophasic assumption is valid for much of the time the chemical is in the body. Here $b$ approaches $k_{20}$.40

It is worth mentioning that three-compartment mathematics have been described. In this model there exists both a central, deep, and shallow compartments.16

Multiple Doses

Multiple dosing of chemicals increases the body burden so long as the rate of excretion does not exceed the rate of accumulation. As compounds accumulate the probability that they will meet with receptors increases. When accumulated drug concentra-
tion overwhelms and saturates enzyme system, zero-order kinetics comes into effect. Half-time and volume of distribution increase accordingly.

Pathways of Chemical Elimination

The normally functioning kidney provides a ready route of escape for non-protein bound, polar compounds. The rate of kidney excretion is determined by glomerular filtration, passive tubular transfer, and active secretion. In glomerular transfer, any molecule smaller than albumin will be filtered provided that it is not protein bound. Like all membranes, the renal epithelium acts as a lipid soluble barrier; therefore, ion water-soluble compounds can be reabsorbed after passing through the glomerulus. In a like manner, chemicals may traverse this tubular epithelial barrier from the opposite direction. That is, chemicals may follow a concentration gradient into the lumen of the tubule from the peritubular capillaries. Active tubular secretion involves the active transport of organic anions and cations from blood to tubule lumen. Other paths of elimination include the lungs which provide a gas and lipid soluble barrier across which compounds may diffuse passively, and salivary excretion which is exceptionally important in ruminant animals for two reasons. First, large quantities of saliva are produced, and second, the pH of saliva is one unit higher in ruminants than in other species. Again, transfer depends on pKa, lipid solubility, concentration gradient and molecular size. Sweat is similar in excretory properties to saliva except that sweat is a very minor route.
The 6.7 pH of milk favors ion trapping of many organic bases. Passive diffusion occur into and out of milk on the basis of lipid solubility of large molecules. Small molecules pass through pores and equilibrate between milk and plasma.30

The bile provides an avenue of excretion for compounds of high molecular weight. Excretion of compounds in the bile with molecular weight less than 300 is uniformly low. Further, for extensive biliary excretion to occur, a compound should have at least one polar or potentially ionizable group. Anionic polar groups must generally have a pKa of less than 5. Glucuronide and sulfate conjugation increase both the molecular weight and polarity of compounds. Therefore, these conjugation favor biliary removal. The liver bile and the kidney urine complement but cannot replace each other's excretory functions. Blocking urinary excretion of low molecular weight compounds does not increase their biliary excretion. As might be expected, species which lack the ability to conjugate glucuronides have only limited biliary excretion of compounds biotransformed by glucuronide formation. Too, the threshold molecular weight for biliary excretion varies between species.57 It goes without saying that lipid soluble chemicals find their way most easily into liver parenchymal cells. Once made polar by the cells, active transport causes the moiety into bile.58 Enterohepatic circulation may also occur; that's a substance that may be re-absorbed into portal circulation after biliary excretion into the intestine. The presence of a gallbladder favors enterohepatic circulation because the gallbladder itself favors intermittent release of large quantities of bile. Once in the
intestine, the compound in bile may be either excreted in feces or recirculated. Since many compounds are eliminated as glucuronides, sulfates, and glycine conjugates, their enterohepatic circulation is dependent on splitting enzymes, such as β-glucuronidase. These splitting enzymes are especially high in the neonate and are produced by gut flora in all species.\textsuperscript{65} Reabsorption into the portal circulation may be either uneventful or, as some suggest, a complex with glucuronic acid may form in the gut wall.\textsuperscript{46} Little of this formed glucuronide escapes into the systemic circulation. The free chemical, having been absorbed passes either to liver or to kidney for excretion or to tissue for sequestration. Retention of compounds in the enterohepatic circulation favors increased half-time and low renal clearance.\textsuperscript{58}

**Pathological Favors Influencing Excretion**

Any condition which reduces glomerular filtration, such as glomerulonephritis, shock, or congenital renal disease will decrease renal excretion. Too, many forms of liver damage, such as ascarid migration or hepatitis will decrease metabolism and biliary excretion. Intestinal hyper- or hypomotility can speed or delay passage so that enterohepatic circulation may be decreased or increased accordingly.

**Summary of Excretion**

Excretion in the summary of the many body events which influence a compound before excretion occurs. Likewise, the parameter half-time is a summary of excretory processes. Two kinetic models— one-compartment and two-compartment—are generally
used to describe distribution and excretion of chemicals. Routes of excretion include kidney, bile, saliva, sweat, air (lungs), feces, and milk. The enterohepatic circulation may become involved with compounds excreted in bile. Any kidney, liver, or gut pathology may affect excretion. A delay in excretion causes increased half-lives and volumes of distribution.
II. O-PHENYLPHENOL

CHEMISTRY

o-Phenylphenol has the chemical formula C₁₂H₁₀O. The C content is 84.68%; the H content is 5.92%; the O content is 9.40%. Its molecular weight is 172.20. It is a white, flaky crystal with a mild odor. It melts between 55.5° and 57.5° C and boils between 280° and 284°. OPP is practically insoluble in water but it is soluble in most organic solvents. It was originally marketed as Dowicide I.⁵⁹

A sodium tetrahydrate salt has been synthesized. It, too, has a white flake form. In contrast to the non-hydrated form, the tetrahydrate is soluble in water.⁵⁹

USES OF THE COMPOUND

o-Phenylphenol has been used as a fungicide, germicide, and household disinfectant; it has served as an intermediate for dyes and as a preservative in oil-water emulsion. The water soluble salt is used for protecting water extendable points against decomposition, as well as for preserving decomposable adhesives.⁴⁵

Recently, OPP has been used in the treatment of citrus fruit to prevent mold, in a fungistic wax for coating vegetables, and for dipping paper and similar containers employed for storage of foods.⁴⁵
TOXICITY OF ANHYDROUS FORM

Little previous work on OPP toxicity has been carried out. A 1945 study reported a mean lethal dose of OPP dissolved in nut oil to be 0.5 g/Kg for cats and 3 g/Kg for rats. The same study indicated that OPP fed to rats at 2, 20, 200 mg/Kg per day neither impeded growth nor caused any ill effects on health in general, hemoglobin, or white cell level. Another 1945 study reported that there would be 20-30 mg of OPP dissolved in a typically disinfected orange peel.

A 1955 study showed that while the cutaneous administration of 5% OPP solution in sesame oil caused no primary initiation on skin, the sodium tetrahydrate salt was irritating in concentration of less than 1%. Acute toxicity data agreed with those gathered in 1945; chronic studies, however, yielded interesting results. Rats could not tolerate more than 2% OPP in their diets for an extended period of time, though rats given less than 2% tolerated the diet for a period of two years with no ill effects. Rats on a 2% diet showed slight growth retardation, tubular dilation of kidney tubules and small amounts of OPP kidney tissue. In the same study, dogs were fed 0.02, 0.2, and 0.5 gm/Kg OPP per day for a period of one year. No adverse influence was attributed to the compound.

No further work on the toxicity of the compound occurred until 1971. This investigator found that dogs readily survived oral doses of 1 and 3 g/Kg of chemically pure OPP dissolved in olive oil. Cats, on the other hand, succumbed to the low dosage in about 15 hours and to the high dosage within 6 hours. In
monitoring plasma levels in dogs and cats, the investigator found that levels in dogs receiving 3 g/Kg peaked in 1.5 hours. Levels in cats rose continually until death. Urine monitoring revealed that dogs excreted most of the compound in 12 hours; cats excreted little before death. \(^{41,43}\)

When given OPP containing disinfectant orally at dosages of 3 and 9 ml concentrated disinfectant/Kg body weight (3.75 \(\times\) 10\(^{-3}\) and 1.125 \(\times\) 10\(^{-2}\) g/Kg), toxicity was similar to giving pure OPP. All low dosage dogs survived; despite low concentration, the average cat died within 57 hours. Average dogs given the higher dose died within 50 hours; high dosed cats died within 6 hours. Peak low dose plasma levels occurred at 2 hours in the dog and 10 hours in the cat. Concentrations reduced swiftly in dogs and slowly in cats. Urinary excretion in dogs was rapid with 2/3 excreted within a day; however, in cats less than 30\% was recovered in three days. \(^{41}\)

The pathology caused by OPP is typically phenolic--hemorrhagic gastroenteritis with pinpoint ulcers down the tract, toxic tubular nephrosis with glomerular protein leakage. Clinical signs of OPP intoxication include incoordination, mild muscular fasciculations, depression leading to an ever deepening coma with respiratory and cardiac depression. Three times as much OPP was required to produce signs in dogs than in cats. Highest tissue residues were found in lung, liver, kidney, and spleen. In addition to urine, bile and feces contained prominent residues. \(^{41}\)
METABOLISM AND KINETICS OF O-PHENYLPHENOL

Urinary metabolites of OPP were shown to be unchanged OPP, OPP-glucuronide and -sulfate conjugates, and phenol from both OPP rings in the dog. A study which administered pure phenol IV to dogs showed free phenol, phenylglucuronide, and phenylsulfate as urinary metabolites. With increasing dose, increasing amounts of injected phenol were excreted within 24 hours. Phenylglucuronide excretion increased with dose; phenylsulfate excretion increased but not in proportion to dose. One feline study showed saturation kinetics. Phenylsulfates and phenylglucuronide were the only metabolites of phenol identified in the cat after doses of 10, 30, and 50 mg/Kg. The proportion of phenylsulfate decreased with increasing dose while phenylglucuronide formation increased with increasing dose. In another study cats were given phenol IV at a 20 mg/Kg dose. Phenolic metabolites in this study included only phenylsulfate and quinol sulfate. When 40 mg/Kg were administered, quinol and pure phenol were found in urine.

Plasma disappearance of pure phenol required far longer in the cat than in the dog. At high doses dogs showed great ability to increase glucuronide synthesis. At low dosages, cats showed great ability to form sulfate.

One investigator reported results of phenol metabolism in the rabbit. The metabolic results resemble the cat with sulfate conjugation decreasing with increasing dose due to the limited availability of sulfate.
Several authors report similar schemes for phenol metabolism.

![Chemical Diagram]

Only one investigator indicated that liver and kidney are not the principal sites of phenol metabolism. He stated that free phenol was not transported from the gut lumen to the liver. Rather he felt that the conjugated form only was absorbed. He did admit, however, that liver conjugation did occur if oral routes were bypassed.46

SUMMARY

o-phenylphenol is a white flaky crystal which is soluble in many organic solvents. It has been used as a germicide and preservative. Concentrations of less than 2% of diet were shown to be non-toxic to rats. Studies on dogs showed them to be more resistant to the toxic effects of OPP than cats. Although no metabolic studies have been carried out with OPP, pure low doses of phenol are excreted primarily as glucuronides in dogs and sulfate conjugates in cats. With high doses, sulfate conjuga-
tion in proportion to glucuronide conjugation increases slowly in the dog and decreases in the cat. Cat maintain non-lethal blood levels of both phenol and OPP longer than dogs and, further, cats excrete the metabolites of the compounds more slowly in urine.
III. BIBLIOGRAPHY


36. Macintosh, F. C.: The Toxicity of diphenyl and o-phenyl-

chemical Conjugations. In: Fundamentals of Drug
Metabolism and Drug Disposition. Edited by B. N. LaDu,
H. G. Mandel, and E. L. Way. The Williams and Wilkins

38. Miller, J. J., G. M. Powell, A. H. Olavesen, and C. G.
Curtis: The Metabolism and Toxicity of Phenol in Cats. 
Biochemical Society of London Transactions, 1 (1973):
1163-1165.

cology: The Science of Poisons, Edited by L. J.

40. Notari, R. E.: Biopharmaceutics and Pharmacokinetics. An
Introduction. Marcel-Dekker, Inc., New York, N.Y.,
1971.

41. Oehme, F. W.: New Information on the Toxicity of Phenolic
Compounds in Small Animals. Paper presented at the
Twenty-first Gaines Veterinary Symposium. Gaines Dog
Research Center, White Plains, N.Y., (October 20, 1971):
8-15.

42. Oehme, F. W. and L. E. Davis: The Comparative Toxicity
Biotransformation of Phenol. Toxicol-Appl. Pharmacol.,

43. Oehme, F. W. and T. Smith: The Metabolism and Urinary Ex-
cretion of o-phenylphenol in Dogs and Cats. Toxicol-

44. Parke, D. V.: The Biochemistry of Foreign Compounds.

45. Patty, F. A., ed. Industrial Hygiene and Toxicology, Vol.
II. Edited by D. W. Fassett and D. D. Irish. John

46. Powell, G. M., J. J. Miller, A. H. Olanssen, and C. G.
Curtis: Liver as a Major Organ of Phenol Detoxication? 

47. Rall, D. P.: Drug Entry into Brain and Cerebrospinal Fluid. 
In: Fundamentals of Drug Metabolism and Drug Disposi-
tion. Edited by B. N. LaDu, H. G. Mandel, and E. L.
Way. The Williams and Wilkins Company, Baltimore, Md.,


COMPARATIVE AND AGE-RELATED PHARMACOKINETICS FOR SINGLE AND MULTIPLE DOSES OF o-PHENYLPHENOL

by

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Randomly selected immature (6 weeks of age) and adult (greater than 6 months of age) dogs and cats received 3.7 mg UL-14C OPP singly or repeated every other day for 25 doses per os. Plasma and urine samples were collected periodically and measured for radioactivity; kinetic values for plasma disappearance were computed. Animals were observed for clinical signs and necropsied at the conclusion of the trial. Tissue residues were determined. Plasma half-life of OPP was longer in felines than in canines and was longer in young than older dogs. Urinary excretion was greater in both singly and multiply dosed canines. Adult animals excreted more OPP in urine and feces than did young. No effect on urinary excretion of OPP was noted in singly-dosed individuals due to age. Cats tended to accumulate higher residues than dogs, and immature dogs accumulated more chronically administered OPP than mature dogs. No significant difference was observed between the ability of immature and adult cats to excrete or to accumulate OPP. No serious overt or post mortem toxicity was observed in any of the OPP-dosed groups of dogs and cats.