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A STUDY OF STRUCTURE–ACTIVITY RELATIONSHIPS IN REGARD TO SPECIES DIFFERENCE IN THE PHENYLBUTAZONE SERIES*

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Abstract—Physicochemical properties of phenylbutazone analogues were correlated with their physiological disposition, in particular with reference to species difference. While in man there exists a direct relationship between p*K*a and half-life, no such correlation was observed in dogs. Half-life in dog appears to depend on factors such as fat/buffer partition coefficient (K_p), plasma protein binding, tissue distribution and drug-metabolizing enzyme activity.

Rate of metabolism of analogues, based on plasma level decline, varied extensively in both species. In man it ranged from 1 to 72 hr; in the dog from 0·5 to 33 hr. There are some striking species differences for certain compounds. For instance, oxyphenbutazone has a half-life of 0·5 hr in the dog whereas in man it is 72 hr. On the other hand, the half-life of G-15140 (*p,p'*-dichloro analogue of phenylbutazone) is of the same order of magnitude for both species.

An anomaly to the p*K*a hypothesis, G-34208 (tertiary-butyl analogue of oxyphenbutazone, p*K*a 7·1), has been shown to be excreted as a glucuronide. This conversion provides a ready explanation for the short half-life of G-34208, since glucuronides have p*K*a values of about 3.

Another sterically hindered analogue, G-13838 (isopropyl analogue of phenylbutazone), was found to have a volume of distribution twice that of phenylbutazone both in man and dog.

The results are discussed in relation to the importance of these factors in the search for new drugs.

INVESTIGATIONS with human subjects have shown[1, 2] that the p*K*a of phenylbutazone analogues is a major factor, influencing their physiological disposition and uricosuric activity. While species difference in rate of metabolism had been demonstrated for phenylbutazone,[3] it was of interest to evaluate whether p*K*a could be correlated with physiological disposition in another species. Since renal clearance studies have been carried out with a number of phenylbutazone analogues both in man and dog,[2] the latter was the logical species to use. To extend the knowledge of structure-activity relationships in this series, half-life of certain analogues in man was also determined. In addition, other metabolic and physicochemical studies were undertaken with analogues selected because of their particular structural features.

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METHODS AND MATERIAL

The human subjects with whom half-life studies were carried out were ambulatory in-patients in a chronic disease hospital; they were without any overt liver or kidney disease. Human bile was collected from a subject with a cholecystostomy. The analogues (for structures see Table 1) were given to humans i.v., with the exception of G-35716 and G-28300 which were administered orally. In general, the drugs were given in a single 800-mg dose. In the case of G-13838, G-15140 and G-34208, the dose was 400 mg; for G-29665 it was 600 mg. Mongrel dogs used in this study (5–20 kg) were maintained on Kibbled dog food (Big Red Co.). They received single 50-mg i.v. doses of the analogues/kg except for G-15140 (25 mg/kg) and where otherwise stated. All metabolism studies in man were carried out in at least two different subjects. Similarly, at least two different dogs were used in each half-life study. Because of the possibility of induction of drug-metabolizing enzymes, each dog was studied only once. To collect dog bile, large dogs (20–37 kg) were anesthetized with sodium pentobarbital (30 mg/kg i.v.). A 4-in. subcostal incision was made on the right side, and the duodenum was exposed and then gently pulled up. The common bile duct was located and a small transverse incision made in its distal portion, approximately $\frac{1}{2}$ in. from the ampulla of Vater. A polyethylene catheter (ext. dia. 0·062 in.) was inserted to the junction of the cystic and common duct, and a double ligature was made. After squeezing the gall bladder, 'blank' bile was collected for 20–60 min, and the drug was then injected i.v. into the saphenous vein as a single dose.

Methods of analysis

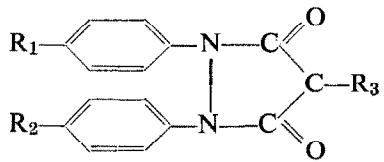
Previously described methods of analysis of plasma concentrations of the drugs were used: phenylbutazone,[3] oxyphenbutazone and G-28231,[4] sulfinpyrazone,[5] *p*-hydroxy sulfinpyrazone (G-32642) and G-33378,[6] G-28234,[7] G-25671,[8] G-29701,[9] and G-15140, G-13838, G-32567, G-32170.[2] G-25592 and G-28300 were analyzed by the same method used for phenylbutazone.[3] G-29665 was analyzed by the method employed for oxyphenbutazone except that 1·5% isoamyl alcohol was added to ethylene dichloride. G-34208 and G-35716 were determined in plasma by the same method used for oxyphenbutazone.[4] For analysis of G-34208 in urine after the extraction of the compound with ethylene dichloride from acidified urine, the solvent was shaken with an equal volume of Clark and Lubs borate buffer, pH 8·0.[10] This removes urine 'blank' without significant loss of drug.*

For the determination of G-34208 β-glucuronide, the following procedure was used: 0·5 ml 3 N HCl was added to 3 ml urine; unchanged G-34208 was removed by extracting with 20 ml ethylene dichloride. The organic phase was either discarded or analyzed for G-34208. The acidic aqueous phase was adjusted to pH 5·0 with solid sodium acetate, and aliquots were incubated with β-glucuronidase (Ketodase, Warner-Chilcott, 1 ml). For control purposes, instead of glucuronidase, 1 ml 0·2 M acetate buffer, pH 5·0, was added to another aliquot. After a 2-hr incubation at 37°, the resulting solutions were analyzed in the same manner as that used for unchanged drug. Aliquots of 24-hr urines collected prior to drug administration were analyzed by the identical procedure because the β-glucuronidase increases 'blank'. The method

* The u.v. absorption peaks used in the analytical procedures are as follows: G-29665, 255 mμ; G-28300, 265 mμ; G-34208, 255 mμ; G-25592, 263 mμ; and G-35716, 254 mμ. The limit of detectability was found to be 1 μg/ml.

TABLE 1. COMPARISON OF PHYSICOCHEMICAL AND PHYSIOLOGICAL PROPERTIES

(Compounds listed in descending order of p*Ka*)



Identification	Compound Structure*			Physicochemical Properties							$T_{1/2}$	
							Binding to plasma					
							Man		Dog		Man†	Dog†
	R_1	R_2	R_3	p*Ka* I*	Kp	Solubility in pH 7·4 buffer (mg/ml)†	Equilibrium concentration inside bag (mg/l)	Bound (%)	Equilibrium concentration inside bag (mg/l)	Bound (%)	(hr)	
G-34208	OH	H	$C(CH_3)_3$	7·1	8·0	0·02	81	99	54	87	1	1
G-35716	OH	H	$CH(CH_3)_2$	5·8	0·4	1·5	220	96	47	79	12	1
G-13838	H	H	$CH(CH_3)_2$	5·5	3·4	0·16	95	98	93	96	72 (2)	5
Oxyphenbutazone	OH	H	$(CH_2)_3CH_3$	4·7	0·6	10 (14)	98	99	77	89	72 (4)	0·5
Phenylbutazone	H	H	$(CH_2)_3CH_3$	4·5	2·2	2·2 (14)	100	99	80	92	72 (3)	7 (3)
G-32170	F	F	$(CH_2)_3CH_3$	4·5	1·0	9·2	96	99	102	91	40 (2)	47
G-29665	OH	H	$(CH_2)_2CHOH\ CH_3$	4·3	0·01	>30	87	83	46	63	4	2
G-25592	H	H	$(CH_2)_2OC_6H_5$	4·2	2·4	0·03	86	99	130	98		7
G-33378	OH	H	$(CH_2)_2SC_6H_5$	4·1	0·4	5·8	195	99	165	90	17 (6)	3
G-28231	H	H	$(CH_2)_2CHOH\ CH_3$	4·0	0·6	>30	67	94	60	85	12 (4)	2
G-15140	Cl	Cl	$(CH_2)_3CH_3$	4·0	83	0·09 (14)	161	99	80	99	20 (2)	33
G-25671	H	H	$(CH_2)_2SC_6H_5$	3·9	1·6	1·6 (14)	120	99	90	99	3 (8)	7
G-28234	NO_2	H	$(CH_2)_3CH_3$	3·2	1·0	13·5	100	98	74	98	20 (7)	12
G-32642	OH	H	$(CH_2)_2SO\ C_6H_5$	3·1	0·01	>30	92	98	70	93	1 (6)	0·5
Sulfinpyrazone	H	H	$(CH_2)_2SO\ C_6H_5$	2·8	0·5	24	100	99	46	90	3 (5)	2
G-32567	CH_3SO_2	CH_3SO_2	$(CH_2)_3CH_3$	2·6	0·6	>30	93	98	197	87	1 (2)	0·5
G-29701	OH	H	$CO(CH_2)_2CH_3$	2·3	0·4	>30	42	99	121	91	8 (9)	3

* Compounds where R_1 is OH have p*Ka* II of 10·0 ± 0·2.
† Parentheses indicate references to previously published data.

was shown to be at least 80 % specific by the procedure of partition coefficients.[11] In the case of analogues having measurable p*K*a I and p*K*a II, the specificity was further established by the determination of the 'apparent' dissociation constants of the corresponding urine extracts of subjects and dogs having received the drugs. Analysis for β-glucuronide of oxyphenbutazone was made in the same manner as for G-34208 glucuronide, but the buffer wash consisted of citrate (0·1 M)–disodium phosphate (0·2 M) buffer, pH 4·8 (McIlvaine), and specificity was also established by the technique of partition coefficients. The 'glucuronide of G-35716' was analyzed in the same manner as G-34208 glucuronide except that a McIlvaine buffer, pH 6·0, was employed. In order to determine specificity, aliquots of urines of human subjects given G-35716 were extracted to remove unchanged drug, and the remaining aqueous phase was treated with β-glucuronidase. It was found that the product of hydrolysis consisted not only of G-35716 but also of one or more metabolites, which was demonstrated by the technique of partition coefficients. Assumed that the metabolites have molecular extinction coefficients of the same order of magnitude as the drug, the amount of drug and metabolites in the form of glucuronides can be estimated.

Phenylbutazone, oxyphenbutazone, and sulfinpyrazone concentrations in bile were analyzed by the same methods as those used for plasma except that, just as in the analysis of urines, the organic phase was washed with an equal volume of McIlvaine buffer, pH 4·8. The pentobarbital used for anesthesia did not interfere with the analytical methods. Specificity of the phenylbutazone method for bile was shown by isolation, mixed melting-point determination, partition coefficient, and u.v. and i.r. analysis. Specificity of the analysis for sulfinpyrazone in bile was demonstrated by countercurrent distribution carried out in the same manner as described for the urine method.[2] Based upon the concentration in the three middle tubes and application of the principles described by Williamson and Craig,[12] the method was found to be at least 95 % specific. Further proof was obtained by the isolation of the drug from bile and determination of the mixed melting point and u.v. and i.r. absorption spectra. The specificity of the method for oxyphenbutazone and its glucuronide in bile was demonstrated by the partition coefficient technique.[11]

Determination of physicochemical properties of the analogues

The measurement of p*K*a was carried out at room temperature in dilute buffer solutions by a spectrophotometric procedure based on the differences in u.v. absorption of the ionized and non-ionized forms.[13] Solubility was measured as follows: 100–600 mg of the compound was dissolved in a minimum of 1 N NaOH (4–6 ml), and then 6 ml of Sörenson buffer, pH 7·00 (0·066 M Na_2HPO_4, KH_2PO_4) was added. The acidity was brought to pH 7·00 ± 0·02 with buffer constituents, and the final volume was adjusted to 20 ml by adding the buffer. After standing overnight at room temperature, the pH was checked and, if necessary, readjusted with the constituents of the buffer. After centrifugation (200 *g*) for 10 min, the supernatant was filtered and analyzed. The solubility of some of the analogues as determined by this method had been previously communicated by Brodie and Hogben.[14]

Partition coefficients for the system—peanut oil and Sörenson buffer, pH 7·4—were measured by the method used by Mark *et al.*,[15] with the following modifications: when the partition coefficient was below 0·4, 10 ml oil and 5 ml buffer were used; when the coefficient was above 10, 1 ml oil and 20 ml buffer were used. In each case,

4 mg drug was distributed; the buffer phase was analyzed by the double extraction procedures indicated above.

Binding to plasma and protein fractions was determined as previously described.[3] Five ml plasma or a 5·0% solution of protein in Sörenson buffer, pH 7·4 were placed in a dialysis bag (Visking Co. cellophane tubing, size 23/32) and 1 ml of buffer containing the drug was added.* The bag was then placed in a tube containing 17 ml buffer and covered with Parafilm 'M' (American Can Co.) and incubated for 36–48 hr at 37°. In some experiments the previously determined equilibrium concentrations were placed inside and outside the dialysis bag; in these studies only 24-hr incubation was used. At the end of the experiments, an aliquot of the outside phase was tested for leakage with 10% trichloroacetic acid.

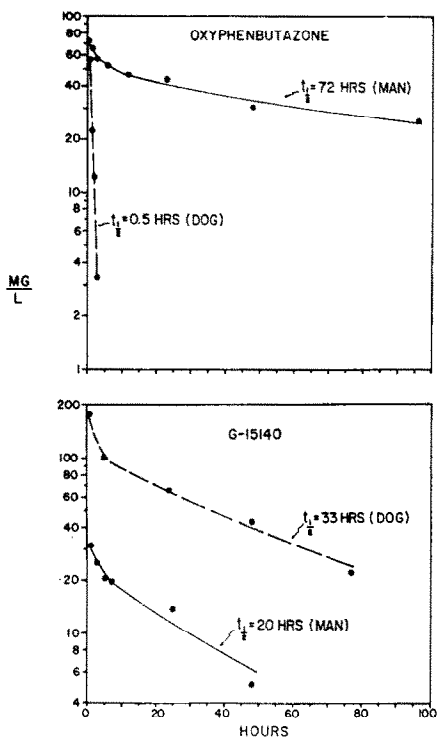


FIG. 1. Plasma levels in a typical man and a typical dog after the intravenous administration of the following drugs: oxyphenbutazone: man, 600 mg; dog, 50 mg/kg. G-15140: man, 400 mg; dog, 25 mg/kg.

RESULTS

Half-life studies in dogs

Half-lives were estimated on the basis of plasma level decay rate. In the case of the phenylbutazone series, sex differences did not affect rate of metabolism. The half-life values of analogues in dogs were found to vary extensively; they ranged from 0·5 hr for oxyphenbutazone to 47 hr for the *p,p'*-difluoro analogue (Table 1). The pattern was

* In order to determine the binding of poorly soluble analogues (Table 1), the desired concentrations in the inner phase were achieved by the initial addition of a dilute solution of the appropriate compound in pH 7·4 buffer to the outside phase.

different from the half-lives found in man.* For instance, oxyphenbutazone was slowly metabolized in man (Fig. 1) and rapidly metabolized in the dog; the reverse was true for the difluoro analogue.

Studies with G-13838 (isopropyl analogue of phenylbutazone) and its p-hydroxy analogue, G-35716

G-13838 has a volume of distribution about double that of phenylbutazone, both in man and dog. This was shown as follows: two human subjects were first given an i.v. dose of 400 mg phenylbutazone. A month later the same subjects received a 400-mg dose of G-13838 i.v., plasma levels of the analogue were about half those found with phenylbutazone, although the half-life was the same. A typical experiment is shown in Fig. 2. Similar results were observed in dogs.

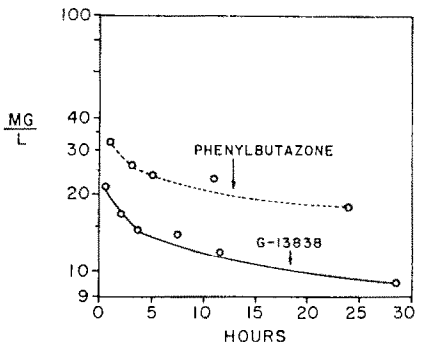


FIG. 2. Plasma levels of phenylbutazone and G-13838 in the same human subject after single intravenous doses. A 400-mg dose of G-13838 was followed four weeks later by a 400-mg dose of phenylbutazone.

The *p*-hydroxy analogue of G-13838, G-35716, is extensively converted to glucuronides in man. Two subjects given G-35716 excreted about 60% of the administered drug as glucuronides in 24-hr urine. Similarly, dogs given G-35716 excreted 7–8% of unchanged drug and 40–50% as glucuronides.

Studies with G-34208 (tertiary-butyl analogue of oxyphenbutazone)

Dogs were given G-34208 i.v., and 24-hr urines were collected. The excretion of unchanged drug ranged from 0·2 to 4·3%, whereas 22–29% of the dose was excreted as G-34208 β-glucuronide. Subsequently, three human subjects were given the drug i.v. As in the case of the dog, very little unchanged drug was excreted in urine (less than 2% in 24 hr), whereas 9·3–46% was excreted as a glucuronide, most of which was excreted in 6 hr. Upon oral administration of 800 mg of G-34208 to two subjects, practically no absorption could be observed; this was determined by the absence of significant blood levels (concentrations below 1 mg/l) and finding less than 1·5% of the dose as a glucuronide in 24-hr urines. The low solubility in pH 7·4 buffer of the drug (Table 1) may in part be responsible for its poor absorption. Similarly, when dogs were given G-34208 in 100 mg oral doses/kg, no appreciable plasma levels (less than

* While for any particular analogue the range of half-life in different human subjects varied slightly, in dogs moderate randomness was observed. For example, for oxyphenbutazone, the half life ranged from 0·25–0·75 hr, for phenylbutazone, 3·5–12 hr, and for G-32170, 40–53 hr.

2 mg/l) could be detected. The tertiary-butyl analogue of phenylbutazone (G-28300; p*K*a 6·8) was given orally both to dogs (100 mg/kg) and to human subjects (800 mg), and no measurable plasma levels were found. Due to the fact that G-28300 has an even lower solubility in water than G-34208, no i.v. studies were attempted. The p*K*a of G-34208 is the highest observed in our series (Table 1). Laubach and Bloom[16] have recently suggested, based upon studies with molecular models, that this high p*K*a is primarily due to crowding of the oxygen substituents of the pyrazolidinedione ring.

Studies with oxyphenbutazone

Two dogs were given 100 mg of oxyphenbutazone i.v./kg, and no ill effects were observed. They excreted 10·5 and 15% of the unchanged drug and 18 and 28% respectively as the glucuronide in 24 hr. The presence of glucuronide was further confirmed by chemical treatment of the dog urine, according to a modification of the procedure of Bray *et al.*[17] without actual isolation, and subsequent incubation with glucuronidase at 37° at pH 5·0 (Dayton and Perel, unpublished observations). In contrast, when 50 mg phenylbutazone/kg was given orally to two other dogs, less than 2% of the dose was found in 24-hr urine as unchanged drug; 19·8 and 28% were found as mixtures of metabolite I (oxyphenbutazone) and metabolite II (G-28231). When human subjects were given 800 mg orally of oxyphenbutazone, they excreted 1–5% of the dose as a glucuronide in 24 hr. Since the rate of metabolism of the drug is slow, about 20%/day, the small amount of glucuronide excreted by man becomes significant. A conversion of 5%/day to the conjugate suggests that as much as 25% of oxyphenbutazone could be transformed to a glucuronide. Previously, no glucuronide could be detected in urines of subjects receiving phenylbutazone.[3]

The sulfate of oxyphenbutazone is probably not a major metabolite. It hydrolyzes in water at room temperature (personal communication, Dr. F. Häfliger) and since less than 2% of oxyphenbutazone is excreted in 24 hr,[2] this would represent the maximal conversion of oxyphenbutazone to the sulfate.

A dihydroxy analogue, G-29665, was also studied in man and dog. This compound, by virtue of the position of the hydroxyl groups, is directly related to both known metabolites of phenylbutazone.[4] G-29665 has a much shorter half-life in man than either of the two phenylbutazone metabolites. The short half-life is due mainly to its extensive urinary excretion—more than 50% of the drug is excreted in 24 hr. The excretion may be correlated with lower binding and the low lipid solubility of the analogue, the latter factor reducing the possibility of non-ionic back-diffusion in the kidney in contrast to phenylbutazone and oxyphenbutazone.[2]

Bile excretion studies

Two experiments were carried out in dogs with each of three drugs—phenylbutazone, sulfinpyrazone, and oxyphenbutazone. In the case of phenylbutazone and sulfinpyrazone, considerable amounts of the drug were excreted in bile, as much as 20% of the dose in 6 hr for sulfinpyrazone. However, only 1 to 2% oxyphenbutazone, was excreted, which is attributed to the shorter half-life of this analogue in the dog. For all three compounds, concentrations in bile were 10 to 15 times higher than simultaneous plasma concentrations. In contrast, practically no drug or known metabolites could be detected in human bile after an 800-mg dose i.v. of phenylbutazone in 24 hr. Thus, biliary excretion is not an important factor in the fate of phenylbutazone in man.

Binding studies

The results of binding studies with human and dog plasma are shown in Table 1. In man most of the analogues[2] are highly bound (about 98%). The generally higher binding of phenylbutazone or its analogues to human plasma compared to dog plasma should be a significant factor in accounting for some of the species differences.

Results of studies with plasma fractions are shown in Table 2. It was found that albumin has a greater affinity for phenylbutazone than has γ-globulin.

TABLE 2. BINDING STUDIES BY EQUILIBRIUM DIALYSIS

Drug	Protein solution	Equilibrium concentration inside bag (mg/l)	Bound (%)
Phenylbutazone	5% Human albumin	80	99
	5% Human γ-globulin	59	< 5
	5% Dog albumin	69	97
Oxyphenbutazone	5% Human albumin	85	99
	5% Human γ-globulin	29	<25
Sulfinpyrazone	5% Human albumin	79	99
	5% Human γ-globulin	28	<25

DISCUSSION

The importance of species difference with regard to variation in drug metabolism has recently been reviewed.[18] Some of the data included in the present study have been cited in the aforementioned article.

A considerable spread in rate of plasma decline among different phenylbutazone analogues in man has been observed; the half-life range is from 1 to 72 hr. Similarly in the dog, the range of half-life is from 0·5 hr to 47 hr.

In previous studies in man, p*K*a was correlated to half-life, urinary excretion, and uricosuric activity.[1, 2] In man, compounds with p*K*a of 4·5 to 5·5 were found to be excreted only to a small extent (less than 2% of the administered dose in 24 hr.) These compounds had long half-lives, slight uricosuric effect, and generally had potent antirheumatic activity. Analogues with p*K*a of 2·3–3·1 were rapidly excreted in urine (more than 35% in 24 hr) and had short half-lives; however, they had potent uricosuric activity. The differences in the behavior of the analogues, in particular concerning their renal handling, was attributed to differences in tubular secretion and non-ionic back-diffusion. Partition coefficient, determined between HCl and a mixture of ethylene dichloride and normal heptane, could not be correlated with the fate of the analogues.[2] However, the present evidence indicates that partition coefficient between peanut oil and buffer of pH 7·4 (K_p), correlates to a certain extent with their physiological disposition. For instance, of the compounds having a K_p less than 0·6, five have a half-life of 12 hr or less and only one, G-33378, has a longer half-life (17 hr), whereas when K_p is above 0·6, with few exceptions the half-life is longer.

In view of the present results, the structure-activity generalizations for man can now be extended. The tertiary-butyl analogue of oxyphenbutazone (G-34208, p*K*a 7·1) has been found to have a very short half-life, about 1 hr, and is excreted as a glucuronide. This seeming anomaly can be explained within the framework of the p*K*a hypothesis. Glucuronides have p*K*a's of the same order of magnitude as free

glucuronic acid (p*K*a 3·2[19]) but frequently are even more acidic.[20] Furthermore, G-34208 has a high K_p. Thus, according to the concepts of Quick,[21] Brodie and co-workers,[14, 22] and Williams,[20] this analogue should readily enter liver cells and be conjugated to a glucuronide and then be rapidly excreted. Another compound, G-35716, has been shown to be extensively converted to glucuronides. This conversion, however, takes place more slowly, which may be due to the relatively lower K_p and p*K*a. Oxyphenbutazone in relation to G-34208, with an even lower p*K*a, is also conjugated, but at a still slower rate.

An analogue of oxyphenbutazone, G-29665, having a hydroxy group in the gamma position, is of particular interest. Although this analogue has a p*K*a of 4·3, its half-life is 4 hr and it is extensively excreted in urine. Thus this compound is an exception to the p*K*a relationship, which can be rationalised in terms of its extremely low K_p and lesser binding; the former would not permit facile non-ionic back-diffusion in the kidney. Both p*K*a and K_p thus appear to be needed for a good correlation with the concentration of the diffusible form of the drug, if it is assumed that passive non-ionic transport is of major consequence. The passive diffusion of phenylbutazone analogues probably occurs at most cell boundaries, including the microsomal boundaries. The passive reabsorption of these drugs in the distal portion of the renal tubule and the importance of reabsorption with regard to their physiological fate has been pointed out.[2] Assuming that, in this series, renal mechanisms in the dog are similar to man, active transport occurs in the human proximal tubule and appears to be p*K*a-dependent.[2] This is in agreement with the general concepts expressed by Fisher *et al.*,[23] who found a relation between p*K*a and fate of sulfonamides. In the phenylbutazone series, p*K*a, K_p, and other factors such as binding and enzyme activity have contrived to make possible a good correlation between p*K*a and parameters such as half-life, urinary excretion, and pharmacologic activities in man.[2] Based upon the concepts suggested by Albert,[24] it would be attractive to postulate that phenylbutazone analogues enter cells in the non-ionized form but that they act intracellularly in the ionized form.

Although the more acidic phenylbutazone analogues are secreted to a greater extent by the renal tubules of the dog,[2] there is no obvious relationship between p*K*a and rate of plasma decline. One possible reason for the poor correlation between half-life in the dog and p*K*a is that in some instances more drug is in the free form, as compared to man, and thus is made more available to the drug-metabolizing enzymes. However, there is some correlation between half-life and plasma binding in the dog; the more highly bound analogues generally have longer half-lives. The finding of a long half-life in the dog for the highly fat-soluble analogue G-15140 supports this correlation; this analogue is more highly bound to dog plasma than is phenylbutazone, and one possible route of metabolism, hydroxylation in the para-position, has been blocked. In some instances, hydroxy groups reduce the ability of the compound to bind to plasma proteins. One of the factors which seems to contribute to the lesser binding of the hydroxy compounds is their lower lipid solubility. A precedent has been observed in the barbiturate series, which are also enolic in character—the poorly bound barbiturates have lower lipid solubility.[15, 25] Two hydroxy compounds, G-28231 and G-29665, are also less bound to human plasma. Their lower degree of binding could be attributed not only to their poor lipid solubility but also to their existence in two molecular forms (Fig. 3).[26] However, since substitutions with other polar

groups in the gamma position of the side chain of phenylbutazone analogues do not affect binding, it would seem that formation of the lactone reduces drug–protein interactions. Interestingly enough, it has been reported that phenylbutazone analogues having hydroxy groups cause lesser inhibition of *p*-amino hippuric acid uptake by kidney slices, as compared to the parent compounds.[27]

The binding of phenylbutazone analogues to plasma is generally higher in man than in dogs. Actually most of the plasma binding appears to take place with albumin[28]

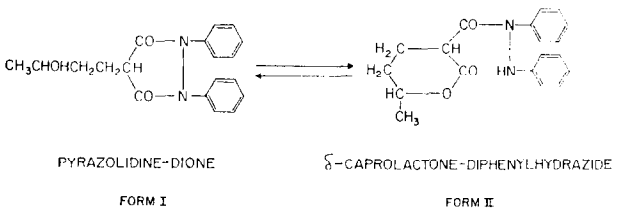


FIG. 3. Molecular rearrangement of G-28231 in aqueous solution.[26]

(Table 2). Large variations in binding to albumin have been reported for the same drug with different species.[29] Owing to the generally high binding found for the analogues in the phenylbutazone series, a considerable fraction of the drug is bound to albumin.*

Two other related factors have to be considered to account for the variation in rate of metabolism in diverse species: the possibility of different schemes of metabolism and the concentration and types of drug-metabolizing enzymes in each species. Differences in metabolism may be marked[20, 24] or rather subtle. Posner *et al.*[31] and Parke[32] have shown that the ratio of positional isomers formed in various species is not constant. For instance, one species hydroxylates aniline primarily in the para-position, whereas another species introduces the hydroxy group mainly in the ortho-position. Even when rate and pathways of metabolism are known for a compound, an analogue may behave in an unexpected manner. Striking differences in half-life have been observed in the phenylbutazone series for species other than dog[3, 33–35] (see Table 3). For comparison three other compounds are listed for which half-life was determined in man and in several experimental animals.[36, 37] Of special interest is biscoumacetate, which is rapidly metabolized by man and slowly by the dog.

Quinn *et al.*[38] have correlated half-life of certain drugs with the activities of drug-metabolizing enzymes in liver microsomes. After accounting for differences of sensitivity of the organism to the drug, they were also able to correlate duration of action with enzyme activity. Interestingly, of all the species tested (man, dog, rat, rabbit and mouse) man and dog had the longest half-life. On the basis of the work of Quinn *et al.* and the present study one can infer that in general the drug-metabolizing activity in man is less than that of the dog. Direct information may come from studies in progress with human liver microsomes.[39] Exact comparisons at this stage are difficult because certain drugs exhibit variations in rate of drug metabolism, dependent on the dose.[36]

* For example, when a human subject was given 600 mg oxyphenbutazone (Fig. 1), a plasma level of 70 mg/l was observed initially. By assuming a 3-liter plasma volume it can be estimated that about one third of the drug is in the plasma, of which 99% is bound to albumin. This estimate represents only a minimal value for the amount of bound drug, since the major fraction but not all of albumin is intravascular.[30]

TABLE 3. METABOLISM OF DRUGS IN DIFFERENT SPECIES

Drug	Species										
	Rat			Rabbit			Dog			Man	
	$T_{1/2}$ (hr)	Dose (mg/kg)	S.R.*	$T_{1/2}$ (hr)	Dose (mg/kg)	S.R.	$T_{1/2}$ (hr)	Dose (mg/kg)	S.R.	$T_{1/2}$ (hr)	Dose (mg/kg)
Phenylbutazone	6	100	12	3	100	24	7	50	10	72	15
Oxyphenbutazone	6	50	9	4	50	18	0·5	50	144	72	15
Sulfinpyrazone	3	50	1				3	50	1	3	15
Phenobarbital	11·5		3·7	17		2·5	55		0·8	42	
Barbital	<24	150	< 3	17		3·8	21		3	64	
Biscoumacetate				3	50	0·6	33	75	0·06	2	20

* S.R. (species ratio) $= \frac{\text{mean } T_{1/2} \text{ (man)}}{\text{mean } T_{1/2} \text{ (experimental animal)}}$.

In this series acute toxicity is presumably related to half-life. Domenjoz[34] found that the i.v. LD_{50} in rats for the following drugs is: sulfinpyrazone, 154 mg/kg; phenylbutazone, 150 mg/kg; oxyphenbutazone, 68 mg/kg. In the same species sulfinpyrazone was found to be more potent than oxyphenbutazone in inhibiting formalin edema.[34] Thus the therapeutic index would favor sulfinpyrazone. However, while sulfinpyrazone has potent uricosuric activity in man, it has only weak antirheumatic activity.[5] Although oxyphenbutazone is more toxic in rats than is phenylbutazone, it has potent antirheumatic activity in man and fewer undesirable side effects.[40] This anomalous finding may be explained by the fact that in rats the half-life of oxyphenbutazone is longer than that of phenylbutazone, whereas in man the half-lives are about the same (Table 3). For ease of comparison of half-life differences among various species, the ratio of half-life in man to half-life in an experimental animal (species ratio) has also been included in Table 3.

The magnitude and variation of half-lives found in the present investigation show that the projection of toxicity and pharmacologic activity from experimental animals to man may be even more difficult than in the comparisons made by Domenjoz, unless information about physiological disposition is also made available. Further evidence for this difficulty is provided by the present observation that the half-life of oxyphenbutazone in man is about 144 times longer than in the dog* and that, in contrast to the rat, essentially no toxicity in the dog was observed at higher than LD_{50} doses for the rat. Evaluation of chronic or subchronic toxicity is even more complex because of the possibility of induction of drug-metabolizing enzymes.[43-45]

The present study supports the growing belief that in the development of new drugs the methods of classical pharmacology and biochemical pharmacology must complement each other. Conceivably, one should study fewer analogues of a 'lead compound' in animals but should investigate these in depth. Also, it may be erroneous to reject a series of drugs based on a clinical trial with only one analogue, unless adequate information of the fate of the drug in man is available.[46]

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* These variations of species difference approach in magnitude those observed in studies of the susceptibility to cardiac glycosides.[41, 42]

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