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Studies of the Interaction of Phenylbutazone, Oxyphenbutazone and Methandrostenolone in Man* (33338)

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It was previously reported (1) that methandrostenolone caused an increase of plasma levels of oxyphenbutazone in human subjects. To extend these investigations, similar studies were carried out with phenylbutazone, of which oxyphenbutazone is a major metabolite in man (2). In the present work an interaction was observed among phenylbutazone, oxyphenbutazone, and methandrostenolone.

Materials and Methods. Eight ambulatory subjects (2 with osteoarthritis and 6 with rheumatoid arthritis) were selected from a population treated at an arthritis clinic. All medication was stopped at least 1 week prior to the study. Two types of studies were undertaken. In one (half-life study) a single oral dose of oxyphenbutazone (Tandearil) or phenylbutazone (Butazolidin) was administered. The dose of the former was 400 mg, the latter 400–600 mg. Plasma concentrations were determined at suitable intervals up to 1 week after dose. In the other type of study, oxyphenbutazone or phenylbutazone were given continuously over a period of several days; the daily oral dose of oxyphenbutazone was 300 mg; that of phenylbutazone, 400 mg, except for subject 3 who received 300 mg. The doses were divided as follows: 100 mg at 8 a.m., 1 p.m. and 7 p.m.; or 100 mg 8 a.m., 1 p.m., and 200 mg at 7 p.m. Blood was drawn at 11 a.m. Methandrostenolone (Dianabol) was given in oral doses of 2.5 mg each at 8 a.m. and 7 p.m.

In the absence of phenylbutazone, oxyphenbutazone concentrations in plasma, albumin, or buffer solutions were determined by a previously published procedure (2). Phenylbutazone was measured either by a published procedure (3) or by a modification which involved, after the initial extraction from acid, two washes of the organic phase with 15 ml of 0.15 *N* HCl. When oxyphenbutazone was determined in the same sample used for the phenylbutazone assay, after the heptane extractions (3), 2 ml of the 3.5 ml aqueous acidic phase was extracted (2) and correction was made for the loss (24% of oxyphenbutazone) by the heptane extractions. Equilibrium dialysis experiments were performed at 37° as described (4). In brief, 5 ml of plasma or 5% (w/v) albumin solution was added to each bag and 1 ml of buffer, pH 7.4 was added inside; 17 ml of buffer were the outside phase. Human plasma (albumin 4.5 g/100 ml, globulin 2.6 g/100 ml) was diluted in the same manner. Methandrostenolone (23 μg) was added to the dialysis bag in 0.15 ml of ethanol. The same amount

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TABLE I. Lack of Effect of Methandrostenolone on Plasma Levels.

Subject	Age[b]	Days of steroid therapy	Drug	Phenylbutazone and oxyphenbutazone (mg/liter)[a]		
				Before steroid	During steroid	After steroid
3	50	4	P	158 (157–159)	150 (142–157)	147 (142–152)
			O	17 (17– 18)	16 (15– 18)	19 (18– 20)
4	40	7	P	113 (102–126)	104 (102–106)	109 (101–123)
			O	26 (22– 29)	25 (24– 25)	23 (22– 24)
5	52	6	P	62 (61– 62)	59 (56– 63)	44 (41– 47)
			O	14 (12– 16)	14 (10– 18)	11 (10– 11)
6	42	7	P	82 (73– 92)	79 (73– 85)	75 (66– 81)
			O	15 (13– 17)	11 (9– 13)	10 (9– 12)
7	58	8	P	103 (96–114)	109 (107–111)	85 (73– 93)
			O	17 (16– 19)	16 (15– 17)	18 (15– 22)
8	66	7	P	129 (124–138)	137 (130–138)	143 (139–149)
			O	25 (22– 28)	31 (30– 33)	34 (33– 34)

[a] Figures represent average (range in parentheses) levels during period indicated (minimum of 3 values/period determined every 1–3 days). P = levels of phenylbutazone; O = oxyphenbutazone. Phenylbutazone given weeks prior to steroid and 1 week after.

[b] Subjects 3, 4, and 8 were females: 3, 5, 6, and 7 were white Caucasians and 4 and 8 were Negro. All had a diagnosis of rheumatoid arthritis except 8, who had osteoarthritis.

of ethanol was also added to control bags. Crystalline human albumin low in lipids (5) was obtained from Pentex, Inc., Kankakee, Illinois. Serum of all subjects was subjected to electrophoresis before and after each change of drug administration.

Phenobarbital alone was measured according to a published procedure (6) but in presence of its metabolite by another method (7). The *p*-hydroxyphenobarbital was assayed in samples containing phenobarbital by removing the phenobarbital with benzene; subsequently the remaining aqueous phase was analyzed by a published procedure (8). The metabolite, *p*-hydroxyphenobarbital was measured and synthesized as described (8). Its purity was established by various criteria including thin-layer chromatography (9) and NMR spectra.

Results. In order to test whether in our patient population methandrostenolone would increase the plasma levels of oxyphenbutazone, two subjects (1 and 2) were given oxyphenbutazone daily throughout the study. This resulted in a plateau of plasma levels, as was previously described for phenylbutazone and oxyphenbutazone (1, 3). Subsequently methandrostenolone was given for 10 days, while continuing oxyphenbutazone. The plasma levels of oxyphenbutazone increased from an average of 80 mg/liter to an average 140 mg/liter in one case and from 110 to 140 in the other. After methandrostenolone was discontinued, oxyphenbutazone levels declined, which confirmed previous observations (1). The half-life of oxyphenbutazone was determined prior to this study in subject 1 after a 400-mg dose, and found to be 57 hr; the interpolated intercept was 30 mg/liter. Two months after the initial half-life study and 1 month after the continuous therapy study ended, the subject was pretreated for 5 days with methandrostenolone. At that time no oxyphenbutazone could be detected. Then she was given a single oral dose of 400 mg of oxyphenbutazone, and serum determinations made over a period of 3 days while continuing methandrostenolone therapy. The half-life was 150 hr and the interpolated intercept 55 mg/liter. These results are in contrast to those obtained previously (1) where no change in half-life was observed, but only an increase in the volume of distribution.

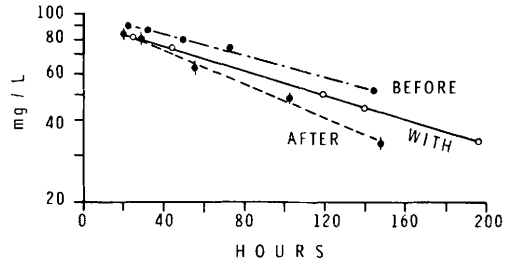

FIG. 1. Subject 3 was given a single oral 400-mg dose of phenylbutazone (before); after 3 days of pretreatment with methandrostenolone, 400 mg of phenylbutazone was given while methandrostenolone was continued for 6 days (with); 6 days after last dose, 400 mg of phenylbutazone was given (after).

Six subjects were given phenylbutazone and a plateau of plasma concentrations was established (Table I). Then methandrostenolone was administered without interrupting phenylbutazone administration. No significant change in phenylbutazone or oxyphenbutazone concentrations due to methandrostenolone was observed (Table I). To further elaborate this point, the possible effect of the steroid on the half-life of phenylbutazone was studied in two subjects. There is no major difference in half-life (Figs. 1, 2); this was expected from the continuous dose studies.

During the entire study, serum total protein and albumin remained virtually unchanged and were within normal limits. Except for Subject 1, there was no change in liver function tests. In all subjects no change in hematocrit, WBC, or differential count was observed.

The results of dialysis experiments are given in Table II. It was found for dog plasma,

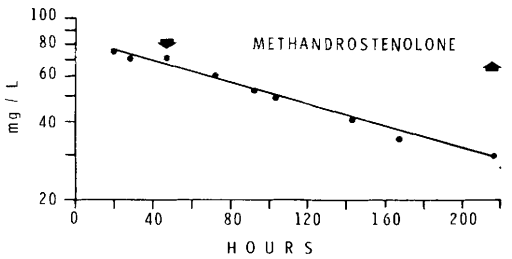

FIG. 2. Subject 4 was given 600 mg of phenylbutazone and 48 hr later (arrow) methandrostenolone was given for 1 week. End of steroid dose is indicated by an arrow.

human albumin, and plasma that oxyphenbutazone and phenylbutazone compete with each other for binding. The latter is more tightly bound and at equal concentrations displaced oxyphenbutazone. However, when the concentration of oxyphenbutazone is much higher than that of phenylbutazone, the latter was displaced. Methandrostenolone had no effect on the binding of oxyphenbutazone or phenylbutazone.

TABLE II. Competition for Binding.

Biological material	No. of bags	Concentration inside bag (mg/liter)[a]		Bound (%)[a]	
		P	O	P	O
Human albumin	4	90	—	99	—
	3	—	105	—	98
	2	85	170	98	98
	3	127	180	99	94
	2	117	150	98	95
	2	117	105	98	94
	2	90	570	95	92
	2[b]	90	—	99	—
	2[b]	—	85	—	97
Human plasma	1	—	105	—	98
	2	108	32	98	97
	2	130	60	97	96
	2	82	550	94	88
	—	50–150	—	98[c]	—
	—	—	95	—	99[d]
Dog plasma	—	80	—	92[e]	—
	—	—	77	—	89[e]
	4	75	—	94	—
	4	58	2300	80	—

[a] Average; variation less than 1%. P = phenylbutazone; O = oxyphenbutazone.
[b] With methandrostenolone.
[c] From Ref. (3).
[d] From Refs. (2) and (4).
[e] From Ref. (4).

Another drug and metabolite pair, phenobarbital and parahydroxy phenobarbital was found not to appreciably compete for plasma protein binding sites. Phenobarbital binding to human albumin was found to be about the same as reported for human plasma (7). The binding of *p*-hydroxyphenobarbital was somewhat higher than for phenobarbital (Table III). The partition coefficient of *p*-hy-

TABLE III. Lack of Appreciable Competition of Binding for Albumin between Phenobarbital and Its Parahydroxy Metabolite.

Biological material	No. of bags	Concentration inside bag (mg/liter)[a]		Bound (%)[a]	
		PH	M	PH	M
Human albumin	2	70	—	48	—
	2	48	—	49	—
	2	—	6	—	65
	3	—	32	—	67
	4	—	84	—	67
	2	53	88	50	67
	2	60	150	53	58
Human plasma[b]	3	60	160	52	51
	—	83	—	46	—
Bovine albumin[c]	—	232	—	50	—

[a] Average, variation less than 1%. PH = phenobarbital; M = metabolite.

[b] From Ref. (7) at pH 7.6; these authors report similar values were obtained with human plasma.

[c] From Ref. (16).

droxyphenobarbital between benzene and 0.5 *N* HCl was less than 0.004, which allows analysis of the metabolite in presence of phenobarbital.

A simple, useful modification of the published method (3) for phenylbutazone was found; by applying two washes, less than 0.2% of oxyphenbutazone is co-extracted with phenylbutazone. This modification does not affect "blank" or recovery, and is necessary when significant concentrations of metabolite are present. The alcohol metabolite (2) of phenylbutazone is not as effectively removed (3.5% is co-extracted into heptane after washes), however, this metabolite does not accumulate since its half-life is comparatively short—12 hr in man (2).

Discussion. Oxyphenbutazone, which is a metabolite of phenylbutazone (2) is unusual in that its average half-life in man is fairly long (72 hr) and that this property is the same as for its parent drug, phenylbutazone (3). Both drugs are extensively bound to plasma albumin (4) and their urinary and biliary excretion is negligible (2–4, 10, 11). Thus, the half-life of these drugs in plasma is presumably proportional to the rate of metabolism. On continuous therapy constant plateau levels are established which are characteristic of a particular subject (1, 3, 12). That the half-life in an individual is a pharmacogenetic parameter has been recently found for phenylbutazone (13).

In a previous study (1) methandrostenolone increased oxyphenbutazone plasma levels of several subjects while on a continuous therapy. In two other subjects an increase in volume of distribution was demonstrated by means of single dose experiments, thus allowing the conclusion that the change of plateau level was not generally due to a metabolic effect.

In the present study, two subjects given continuous oxyphenbutazone therapy also displayed a marked increase of serum levels of oxyphenbutazone when the anabolic steroid was added to the therapy. In contrast, in one subject there was a marked increase not only in volume of distribution but also a decrease in the rate of metabolism. The results with this subject could be attributed to her alcoholism. Previously it had been reported that in cirrhotics the metabolism of phenylbutazone is not different from a normal group (14, 15).

The combination of methandrostenolone and phenylbutazone did not show the same pattern of plasma levels as the steroid plus oxyphenbutazone. Methandrostenolone did not cause an increase of levels during continuous phenylbutazone therapy, nor the prolongation of half-life in single dose experiments. Most striking was the finding that during continuous therapy with phenylbutazone, there was no increase in levels of the metabolite oxyphenbutazone, when the steroid was superimposed. The steroid dose used was one which previously was shown to have a definite effect on oxyphenbutazone; in fact the lower the level of oxyphenbutazone the more pronounced was the effect of the steroid (1). However, in this case a high plasma concentration of phenylbutazone exists in the presence of relatively lower concentrations of the metabolite.

The possibility of competition of both drugs for protein binding sites was consid-

ered. It is known that barbiturates such as thiopental and pentobarbital can compete for albumin *in vitro* (16) and that even structurally unrelated drugs can compete for albumin (17). Competiton for albumin by drugs has been demonstrated *in vivo* in animals (17) and man (18, 19). It is of interest that compounds in the phenylbutazone series compete with steroids (20) and that polymers can displace oxyphenbutazone from albumin (21).

The phenomenon of competition between drug and metabolite for plasma protein binding may be fairly ubiquitous, but in most instances it is not important *in vivo*, because the metabolite is usually more rapidly eliminated from the body than the parent drug, resulting in lower concentrations of the metabolite compared to the parent drug. In fact, competitive binding may contribute to this situation. Furthermore, the metabolite is generally more polar than the parent drug, and may have a different pK_a, which may result in slower diffusion through lipid membranes (as in passive tubular reabsorption) and finally the affinity constant for albumin is generally lower. A combination of factors results in a significant interaction between phenylbutazone and oxyphenbutazone. First, the drug and metabolite have about the same half-life, resulting in significant plasma concentrations of the latter. Secondly, the relative affinities for albumin are not markedly different. Thirdly, there is only one major and one very minor binding site on albumin (N. P. Saltzman and B. B. Brodie, personal communication). Fourthly, the binding by globulins is generally of a lower order (4). It is of interest, however, that it has been shown that a drug can influence the metabolism of its metabolite (22), and vice versa (23).

While methandrostenolone presumably tends to cause an increase of oxyphenbutazone levels in subjects receiving phenylbutazone, the effect is opposed by the presence of phenylbutazone, which acts in the opposite direction, by competing for albumin binding sites. Because of a lack of an effect of steroid on oxyphenbutazone levels, in face of an effect shown *in vitro*, the data suggests that there is an interaction between all three drugs at the plasma level. Thus, the effect of one interaction appears obliterated by another.

For purposes of comparison, the possible competition in another drug–metabolite pair (phenobarbital vs *p*-hydroxyphenobarbital) was studied. It was chosen because the pK_a of drug and metabolite were within 0.2 pH units as in the previous case (4, 6-8). However, no appreciable competition was observed even with relatively higher and unphysiological concentrations of metabolite. With physiological concentrations, the binding of oxyphenbutazone changed from 98 to 97%. This represents a 50% increase of the free form, which is the determinant of distribution. Phenobarbital and its metabolite are less extensively bound than phenylbutazone and there are probably many binding sites; thus a change in binding is more difficult to detect. Since *p*-hydroxyphenobarbital has a very short half-life, compared to the parent drug phenobarbital (8), plasma levels of the metabolite after phenobarbital administration are likely to be low. Therefore, the displacement observed with high concentrations of *p*-hydroxyphenobarbital are not significant *in vivo*. For these reasons, there is no likelihood of an effect of methandrostenolone on the fate of *p*-hydroxyphenobarbital.

Summary. The action of methandrostenolone on phenylbutazone and its major metabolite, oxyphenbutazone, has been studied in man by determining the half-life and plateau plasma levels of the antirheumatic drugs. The anabolic steroid caused an increase in the plasma level of oxyphenbutazone, confirming previous studies. This effect was previously attributed to a change in volume of distribution. In the present study, in addition to the effect on volume of distribution, a prolongation of half-life of oxyphenbutazone was observed in one subject. In contrast, in six patients receiving continuous therapy with phenylbutazone, plasma levels were unchanged when methandrostenolone was superimposed. The plasma levels of oxyphenbutazone, determined in the same samples, were unaffected. The half-life of phenylbutazone in two patients was not changed upon the

administration of methandrostenolone, showing that no alteration had occurred in the metabolism of phenylbutazone. Equilibrium dialysis experiments indicated competition between phenylbutazone and oxyphenbutazone, and that the former has a relatively greater affinity for albumin. These findings suggest that phenylbutazone masks the influence of methadrostenolone on plasma levels of oxyphenbutazone, presumably by displacing oxyphenbutazone from plasma protein binding sites. Comparable *in vitro* studies with phenobarbital and *p*-hydroxyphenobarbital indicate the absence of significant competition for albumin binding sites.

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Effect of Pralidoxime on Electrical Activity of the Cat Brain* (33339)

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Pralidoxime (pyridine-2-aldoxime methiodide, 2-PAM) is an ionic compound of a type that is not expected to penetrate the blood-brain barrier easily. However, the evidence on this point is conflicting (1). Rutland (2) found that 2-PAM reactivated blood cholinesterase but not brain cholines-

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