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RESEARCH ARTICLES

Elimination of 4-*n*-Butoxyphenylacethydroxamic Acid (Bufexamac) in Man

D. R. BOREHAM, A. J. CUMMINGS▲, D. DELL, and B. K. MARTIN

Abstract □ A GLC determination showed that about 80% of a dose of bufexamac (125–500 mg.) can be recovered from urine after acid hydrolysis as 4-*n*-butoxyphenylacetic acid. Excretion is apparently complete within 24 hr. Not more than 1% of the dose is excreted as free bufexamac or 4-butoxyphenylacetic acid. Enzymic hydrolysis indicated that about 75% of the dose is excreted with the hydroxamic function intact and that the elimination of bufexamac is mainly by conjugation, probably with glucuronic acid. About 6% of the dose was recovered from urine as 4-butoxyphenylacetic acid glucuronide. Bufexamac is fairly rapidly absorbed and eliminated, the peak rate of excretion of total 4-butoxyphenylacetic acid occurring 3–6 hr. after dosage. 4-Butoxyphenylacetic acid glucuronide is less rapidly excreted, and the ratio of bufexamac conjugate to 4-butoxyphenylacetic acid glucuronide decreases steadily with time after dosage. The rate of excretion of total 4-butoxyphenylacetic acid could not be interpreted as log-linear during the period (16 hr.) of the kinetic studies.

Keyphrases □ 4-*n*-Butoxyphenylacethydroxamic acid (bufexamac) —absorption, metabolism, urinary excretion rates, man □ Bufexamac—absorption, metabolism, urinary excretion rates, man □ Excretion rates, urinary—4-*n*-butoxyphenylacethydroxamic acid (bufexamac), man

4-*n*-Butoxyphenylacethydroxamic acid (bufexamac)[1] is an anti-inflammatory drug which has been used clinically in doses up to 2.0 g. daily (1, 2). The metabolism of the ^{14}C-labeled compound has been studied in both animals and man (3, 4). In man, about 80% of the dose was recovered in the urine as radioactive material within 72 hr. From results obtained using ion-exchange chromatography, Roncucci *et al.* (5) concluded that the hydroxamic function was largely degraded *in vivo*, leading possibly to the formation of the corresponding amide (4-butoxyphenylacetamide) or carboxylic acid (4-butoxyphenylacetic acid).

The present report describes studies undertaken to gain further information on the absorption and metabolism of bufexamac in man.

[1] Supplied by Continental Pharma S.A., Belgium.

Table I—Percentage of Dose Recovered from Urine as Total 4-Butoxyphenylacetic Acid after the Administration of Graded Doses of Bufexamac to Nine Men[a]

Dose, mg.	Dose Recovered, %			
125	—	72	86	85
250	85	78	89	88
500	—	64	94	80

[a] Urine was collected for 48 hr. after dosage.

EXPERIMENTAL

Drug Administration—Male volunteers received a single dose of 500, 250, or 125 mg. of bufexamac in hard gelatin capsules without added excipient. The dose was administered either at 9:30 a.m. or 11:00 p.m.

One subject received, on separate occasions, doses of 53 and 50 mg. of 4-*n*-butoxyphenylacetic acid in hard gelatin capsules.

Urine Collection—A complete collection of urine was made at timed intervals for up to 72 hr. after dosage. Urine was stored at 0–4° until assayed.

Stability of Bufexamac in Solution—Solutions of bufexamac (0.225 m*M*) were prepared in: (*a*) 0.01 *N* hydrochloric acid, pH 2.0; (*b*) 0.1 *M* phosphate, pH 7.2; and (*c*) 0.01 *N* sodium hydroxide, pH 11.8. The solutions were maintained at 37° for 4 days, and samples were withdrawn at intervals and assayed for bufexamac by the method described here.

Determination of Free Bufexamac and Free 4-Butoxyphenylacetic Acid in Urine—Urine (10 ml.) was acidified with *N* hydrochloric acid (1 ml.) and immediately extracted with chloroform (25 ml.). An aliquot of the chloroform extract (20 ml.) was evaporated to dryness under reduced pressure and assayed simultaneously for bufexamac and 4-butoxyphenylacetic acid by the GC procedure of Dell *et al.* (6). Standard solutions of bufexamac and 4-butoxyphenylacetic acid in urine were treated by the same procedure to provide the appropriate calibration plots.

Determination of Total 4-Butoxyphenylacetic Acid in Urine—"Total 4-butoxyphenylacetic acid" is defined as the amount of bufexamac and its metabolites in urine which are hydrolyzed to 4-butoxyphenylacetic acid by the procedure described. Urine (10 ml.) and 9 *N* sulfuric acid (5 ml.) in a sealed glass ampul were heated in an autoclave at 107° for 1 hr. The hydrolysate (10 ml.) was extracted with chloroform (25 ml.). An aliquot (20 ml.) of the chloroform extract was assayed for 4-butoxyphenylacetic acid using the GC procedure.

Treatment of Urine with β-Glucuronidase—Urine (50 ml.) was adjusted to pH 5 with acetic acid. Then 0.2 *M* acetate, pH 5.0 (10 ml.), was added, followed by β-glucuronidase solution[2] which also contained sulfatase activity. The solution was maintained at 37°, and aliquots were withdrawn at intervals and assayed for bufexamac and 4-butoxyphenylacetic acid. Control studies were conducted with urine containing no added β-glucuronidase.

Determination of Conjugated 4-Butoxyphenylacetic Acid in Urine—Urine (7 ml.) was adjusted to pH 12 with 4 *N* sodium hydroxide and maintained at 37° for 1 hr. This solution was acidified to pH 2, adjusted to 10 ml. with water, and extracted with chloroform (25 ml.). An aliquot (20 ml.) of the chloroform extract was assayed for 4-butoxyphenylacetic acid.

Treatment of Urine with Hydroxylamine—Urine (10 ml.) was added to 5 *M* hydroxylamine, pH 7 (2.5 ml.), and the solution was adjusted to pH 7 and maintained at 37° for 24 hr. The solution was then acidified to pH 2–3 by dropwise addition of concentrated hydrochloric acid, diluted to 15 ml. with water, and extracted with chloroform (50 ml.). An aliquot (40 ml.) of the chloroform extract was assayed for bufexamac and 4-butoxyphenylacetic acid.

RESULTS

The urinary recovery of total 4-butoxyphenylacetic acid from subjects who received 125, 250, or 500 mg. of bufexamac corresponded to 64–94% of the dose (Table I). This result indicated that bufexamac is well absorbed from this dosage form and that an average of about 80% of the dose is excreted in the urine in one or more chemical forms which can be completely hydrolyzed to 4-butoxyphenylacetic acid by the method used.

[2] Sigma, Type H-2.

Table II—Amount of Bufexamac and 4-Butoxyphenylacetic Acid (BPA) Determined in Urine after β-Glucuronidase Hydrolysis (2700 units/ml.) for 14 Days at 37°[a]

Subject	β-Glucuronidase Hydrolysis: Bufexamac mmoles	Bufexamac Percent of Dose	BPA mmoles	BPA Percent of Dose	Acid Hydrolysis, Total BPA mmoles	Total BPA Percent of Dose
A	1.14	51.0	0.36	16.0	1.43	64.0
B	1.75	78.0	0.34	15.2	2.11	94.0
C	1.52	68.0	0.31	13.8	1.78	79.5

[a] The results relate to the urine collected for 48 hr. after dosage with 500 mg. (2.24 mmoles) bufexamac.

Free bufexamac and free 4-butoxyphenylacetic acid were each present in urine in amounts corresponding to less than 1% of the dose. The analytical procedure used is also capable of detecting traces of 4-butoxyphenylacetamide (6), but none was found. GC examination of extracts of urine and of hydrolyzed urine did not reveal the presence of other metabolites.

β-Glucuronidase Hydrolysis—Initial experiments were made with urine collected in the 0–7.5-hr. period after the administration of a 500-mg. dose of bufexamac. Treatment of this urine with β-glucuronidase (250–1000 units/ml.) produced a small amount of 4-butoxyphenylacetic acid and a much larger amount of bufexamac (Fig. 1). This finding suggested the presence of glucuronides of both 4-butoxyphenylacetic acid and bufexamac. The amount of 4-butoxyphenylacetic acid approached a maximum value within 3 days and increased only slightly beyond this time.

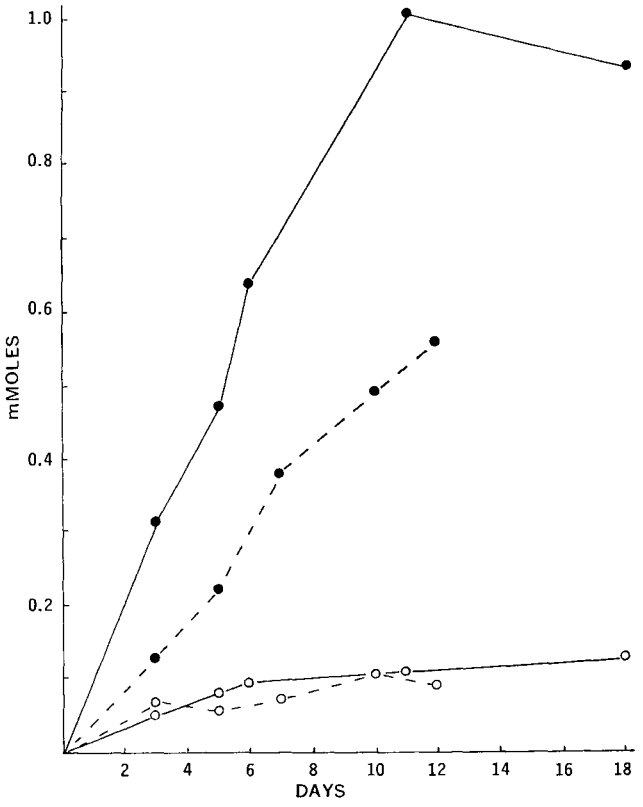

Figure 1—*Amount of bufexamac (●) and 4-butoxyphenylacetic acid (○) liberated by β-glucuronidase hydrolysis at 37° from urine collected (0–7.5 hr.) after dosage with 500 mg. bufexamac. Total 4-butoxyphenylacetic acid determined after acid hydrolysis = 1.13 mmoles. β-Glucuronidase concentration was: 1000 units/ml., - - -; and 2700 units/ml., ——.*

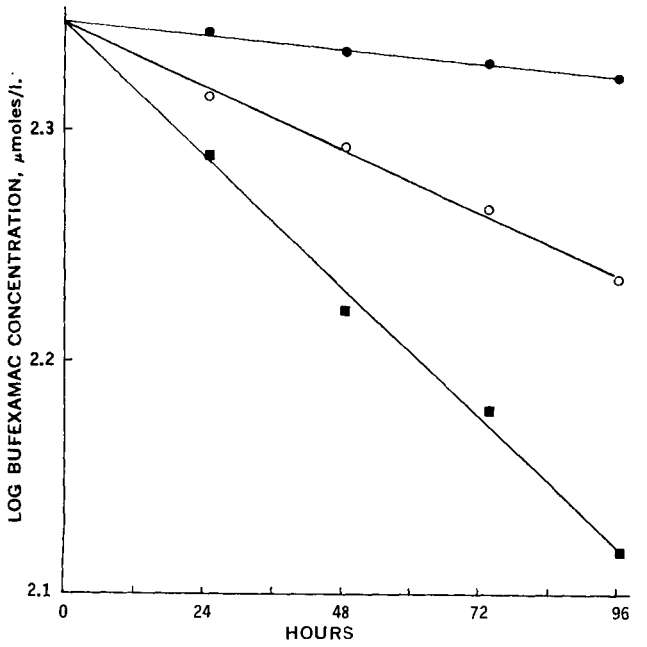

Figure 2—*Effect of pH on the rate of hydrolysis of bufexamac in aqueous solution at 37°. Key: pH 2* ($t_{0.5}$ = *11 days*), ○; *pH 7.2* ($t_{0.5}$ = *58 days*), ●; *and pH 11.8* ($t_{0.5}$ = *5 days*), ■.

Under these conditions, the amount of bufexamac did not approach an asymptotic value within the 12 days of the experiment and it was necessary to increase the β-glucuronidase concentration to 2700 units/ml. to achieve this value. Samples of complete urine collections (0–48 hr.) from three subjects were, therefore, treated with β-glucuronidase (2700 units/ml.) for 14 days. This procedure yielded bufexamac equivalent, on the average, to 66% of the dose, which together with the 4-butoxyphenylacetic acid closely corresponded to the total 4-butoxyphenylacetic acid. These results are shown in Table II.

It was concluded that the major route of elimination of bufexamac involves the formation and urinary excretion of a bufexamac conjugate, the present evidence suggesting a glucuronide. Data pertaining to the stability of bufexamac (Fig. 2) indicate that during the 14-day period of incubation, some of the bufexamac liberated will be hydrolyzed to 4-butoxyphenylacetic acid. This probably accounts for the observation that the amount of 4-butoxyphenylacetic acid continued to increase (Fig. 1) after hydrolysis of its conjugate was complete (Table III). The resulting amount of bufexamac, therefore, provides only a minimum estimate of the amount of conjugated bufexamac excreted, while the value for 4-butoxyphenylacetic acid overestimates the amount of conjugated 4-butoxyphenylacetic acid present.

Elimination of 4-Butoxyphenylacetic Acid—The demonstration that 4-butoxyphenylacetic acid is a metabolite of bufexamac suggested a study of the urinary excretion of metabolites after the direct administration of this drug. After a small dose (50 mg.) of 4-butoxyphenylacetic acid, 32% of the dose was present in the urine as total 4-butoxyphenylacetic acid within 24 hr. In a second study, 49% of the dose was recovered after 33 hr., at which time excretion was proceeding at a very low level.

Table III—Hydrolysis of 4-Butoxyphenylacetic Acid (BPA) Conjugate in Urine by β-Glucuronidase (2000 units/ml.) and by Alkali (pH 12)[a]

Time of Incubation at 37°, hr.	BPA in Urine, μmoles	
	β-Glucuronidase	pH 12
0	5	5
1	—	81
2	—	80
3	—	82
60	83	—

[a] Urine was collected for 8 hr. after dosage with 50 mg. (254 μmoles). Total 4-butoxyphenylacetic acid determined after acid hydrolysis = 82 μmoles.

Table IV—Amount of Total 4-Butoxyphenylacetic Acid (BPA) in the Urine at Timed Intervals after the Administration of a Single Dose of Bufexamac to Eight Men

Interval of Urine Collection, hr.	Dose: 2240 μmoles (500 mg.)				Dose: 1120 μmoles (250 mg.)			
	Total BPA in Urine, μmoles							
0–1.5	27	24	13	197	—	—	—	49
1.5–3.0	215	385	150	266	—	—	—	51
3.0–4.5	529	473	218	377	—	—	—	170
4.5–6.0	366	347	295	472	—	—	—	64
6.0–7.5	125	155	244	106	—	—	—	29
7.5–9.0	48	86	136	57	—	—	—	—
9.0–10.5	30	42	—	30	27	19	67	—
10.5–12.0	24	28	—	—	19	11	19	—
12.0–13.5	14	—	—	—	12	8.4	14	—
13.5–15.0	—	—	—	—	6.3	5.2	6.0	—
15.0–16.5	—	—	—	—	4.0	0	5.5	—
16.5–18.0	—	—	—	—	0	0	6.3	—

After dosage with 50 mg. 4-butoxyphenylacetic acid, the urine (0–8-hr. collection) contained a small amount (1 mg.) of free drug; but after treatment with β-glucuronidase, the amount increased to a value equal to the total 4-butoxyphenylacetic acid content (Table III). This finding indicated the presence of a glucuronide conjugate. When treated with alkali (pH 12) at 37° for 1 hr., the urine gave rise to an amount of 4-butoxyphenylacetic acid equal to the total 4-butoxyphenylacetic acid content (Table III). Hydrolysis of the conjugate is, therefore, complete in 1 hr.

Elimination of Bufexamac—The information gained from studies involving the administration of 4-butoxyphenylacetic acid was then applied to elucidate further the metabolism of bufexamac. When urine collected after dosage with bufexamac was treated with alkali, a rapid increase in the amount of 4-butoxyphenylacetic acid was observed, attaining a constant value in 1 hr. (Table III); no increase in the amount of free bufexamac was observed. Under these

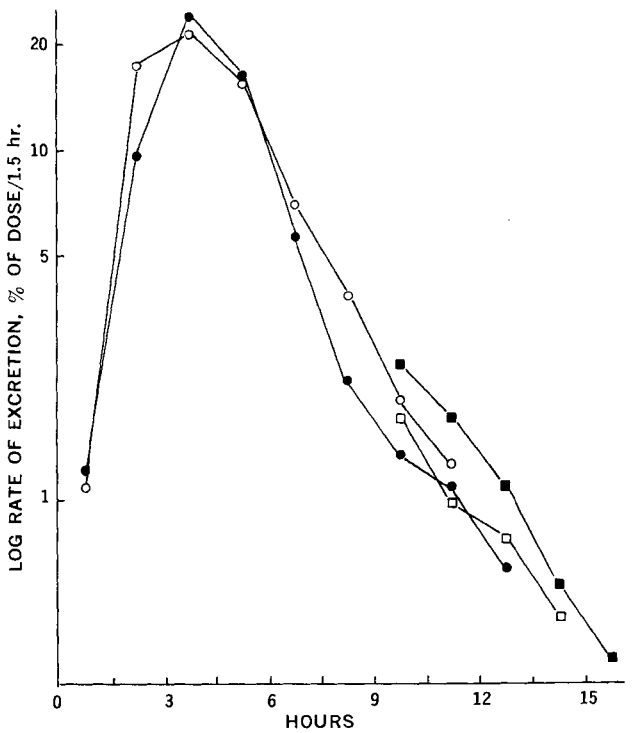

Figure 3—*Plots of log rate of excretion of total 4-butoxyphenylacetic acid from results in Table I. Key: Subject A,* ●; *Subject B,* ○; *Subject E,* ■; *and Subject F,* □. *Results are expressed as percentage of dose excreted per 1.5 hr.*

Table V—Amount of Total 4-Butoxyphenylacetic Acid (BPA), Conjugated 4-Butoxyphenylacetic Acid, and Conjugated Bufexamac Excreted in the Urine at Timed Intervals after Dosage with 250 mg. Bufexamac[a]

Time of Urine Collection, hr. after Dosage	Total BPA	Conjugated BPA	Conjugated Bufexamac (by Difference)	Ratio Conjugated Bufexamac to Conjugated BPA
1.5	7.7	1.2	6.5	5.4
3	78.2	1.7	76.5	43.8
4.5	169.1	4.2	164.9	39.2
6	213.7	5.9	207.8	35.2
7.5	185.4	7.8	177.6	22.7
9	146.6	8.0	138.6	17.2
10.5	83.4	8.3	75.1	9.1
12	23.7	6.0	17.7	3.0
13.5	8.8	3.8	5.0	1.4
15	6.5	2.3	4.2	1.8
24	25.4	19.6	5.8	0.3
Total μmoles	948.0	69.0	880.0	
Percent of dose	84.6	6.1	78.6	

[a] The difference between the total 4-butoxyphenylacetic acid and conjugated 4-butoxyphenylacetic acid is interpreted as conjugated bufexamac.

conditions, bufexamac is relatively stable, the rate of hydrolysis being less than 1%/hr. (Fig. 2). It was, therefore, concluded that the conjugated bufexamac in urine is stable under the conditions employed and that the increase in 4-butoxyphenylacetic acid is a measure of the amount of conjugated 4-butoxyphenylacetic acid present.

The rate of elimination of bufexamac in man was assessed from urinary excretion data (Table IV); some results are expressed graphically in Fig. 3. The maximum rate of excretion of total 4-butoxyphenylacetic acid occurred between 3 and 6 hr. after dosage. The decline in the rate of excretion of total 4-butoxyphenylacetic acid did not, however, exhibit a simple log-linear pattern. The rate of excretion at first declines rapidly, but the decline is slower in the terminal period. The total 4-butoxyphenylacetic acid is derived from two metabolites, and the rate at which they are excreted is governed by their respective rate constants. The rates of excretion of the two metabolites could then decline at different rates, and this would explain the complex pattern of excretion of total 4-butoxyphenylacetic acid. Therefore, it is not possible to assign a value to any particular rate constant from these data, since at no time during the period of these studies could a plot of log rate of excretion against time be interpreted as linear. However, at the time when excretion was proceeding near its maximum rate, 50% of the total 4-butoxyphenylacetic acid ultimately recovered was excreted within 3 hr. This was observed in six instances and implies that bufexamac has a half-life of less than 3 hr.

In an attempt to resolve the complex pattern of excretion of total 4-butoxyphenylacetic acid, urine collected over intervals of 1.5 hr. was assayed for total and conjugated 4-butoxyphenylacetic acid, the difference between the two being interpreted as conjugated bufexamac. The results (Table V) show that, with the exception of the first interval, the ratio of rate of excretion of conjugated bufexamac to that of conjugated 4-butoxyphenylacetic acid progressively decreased from 39 to 0.3; about 6% of the dose of bufexamac was eliminated in the urine as conjugated 4-butoxyphenylacetic acid.

Table VI—Amount of Bufexamac and 4-Butoxyphenylacetic Acid (BPA) Formed when Urine Collected after Dosage with 50 mg. 4-Butoxyphenylacetic Acid Was Incubated with 1 *M* Hydroxylamine (pH 7) at 37°[a]

Time of Incubation, hr.	Bufexamac, μmoles	BPA, μmoles
0	0	4
2	54	—
4	55	—
6	59	—
22	58	10

[a] Total 4-butoxyphenylacetic acid determined after acid hydrolysis = 73 μmoles.

The rate of excretion of bufexamac glucuronide declined rapidly and in a log-linear manner, corresponding to a half-life of less than 1 hr. This probably reflects the half-life of bufexamac. Whereas nearly all of the bufexamac conjugate was excreted within 12 hr., only a little more than half of the conjugated 4-butoxyphenylacetic acid was excreted within that time. Urinary excretion beyond this time consisted almost entirely of conjugated 4-butoxyphenylacetic acid; this finding may be related either to a slower elimination of this acid or to the slower elimination of its conjugate.

Studies with Hydroxylamine—The authors have studied the reaction of certain acyl glucuronides with hydroxylamine to yield hydroxamic acids[3]. In the present study, urine collected after dosage with 4-butoxyphenylacetic acid, when treated with hydroxylamine, gave rise to a considerable amount of bufexamac and a small increase in free drug, the sum of the two then being equivalent to the total 4-butoxyphenylacetic acid content of the urine (Table VI). Under the conditions selected, about 90% of the conjugated 4-butoxyphenylacetic acid in urine reacted with hydroxylamine to form bufexamac, while about 10% was hydrolyzed to the parent acid (Table VI). Therefore, the reaction does not lead exclusively to the formation of hydroxamic acid.

When urine from subjects dosed with bufexamac (250 mg.) was treated with hydroxylamine, a considerable amount of bufexamac and some 4-butoxyphenylacetic acid were formed. The sum of the amounts of these two substances present closely corresponded to the total 4-butoxyphenylacetic acid content of the urine (Table VII). With respect to bufexamac glucuronide, there are, therefore, two reactions involved: the hydroxylamine not only converts conjugated 4-butoxyphenylacetic acid to bufexamac but also effectively hydrolyzes the conjugated bufexamac to the free drug. The hydrolysis of bufexamac glucuronide by hydroxylamine is particularly notable.

DISCUSSION

The results show that bufexamac is relatively rapidly absorbed and that absorption of the dose approaches completion. The evidence for these findings is based on the recovery of total 4-butoxyphenylacetic acid from the urine corresponding to 64–94% of the dose. The percentage recovered was independent of dose within the range of 125–500 mg. (Table I), whereas a reduction might be expected with an increase of dose if absorption were slow or incomplete. The rapid absorption of bufexamac is also indicated by the occurrence of peak rates of excretion between 3 and 6 hr. after dosage (Table IV).

About 75% of the dose is excreted in the urine as a bufexamac conjugate; therefore, the hydroxamic acid function in the bufexamac molecule is not extensively degraded. This finding is directly at variance with the conclusion of Roncucci *et al.* (5) that the hydroxamate function is largely degraded in man.

The conjugated bufexamac is tentatively regarded as consisting predominantly of a glucuronide[4]. The β-glucuronidase preparation used to effect the hydrolysis also contained sulfatase, and the presence of a sulfate conjugate of bufexamac cannot be excluded. Roncucci *et al.* (5), using the ^{14}C-labeled drug, were able to separate a fraction from urine which was radioactive and contained sulfate; however, this fraction corresponded to only 5–10% of the dose. It is noteworthy that the conjugated bufexamac in urine is only very slowly hydrolyzed by β-glucuronidase. In this study, it was essential to establish that the amount of bufexamac formed by the enzymic hydrolysis had approached an asymptotic value and that failure to adopt this procedure could have lead to gross underestimation of the amount of this conjugate.

Certain hydroxamic acids are eliminated in the rabbit as glucuronides, including *N*-acetyl-*N*-phenylhydroxamic acid (7) and *N*-acetyl-*N*-2-fluorenylhydroxamic acid (8). These differ from bufexamac by having two substituent groups on the nitrogen atom;

[3] Unpublished work of A. J. Cummings, D. Dell, and B. K. Martin.
[4] Work subsequently to be reported supports this view.

Table VII—Amount of Bufexamac and 4-Butoxyphenylacetic Acid (BPA) Formed when Urine Collected after Dosage with Bufexamac (250 mg.) Was Incubated at 37° with 1 *M* Hydroxylamine (pH 7) for 24 hr.

Period of Urine Collection, hr. after Dosage	Bufexamac, μmoles	BPA, μmoles	Total BPA (Acid Hydrolysis), μmoles
0–1.5	39	4	49
1.5–3.0	481	18	511
3.0–4.5	155	15	170
4.5–6.0	56	13	64
6.0–7.5	26	3	29
Total (7.5 hr.)	757	53	823

moreover, their glucuronides are readily hydrolyzed by alkali. The bufexamac glucuronide is relatively resistant to alkali and, in this respect, more closely resembles the ether-type glucuronides.

The conjugate of 4-butoxyphenylacetic acid excreted in the urine is readily hydrolyzed by β-glucuronidase. Other evidence suggesting that the conjugate is a typical ester-type glucuronide is afforded by its very rapid hydrolysis by alkali at 37° and by its facile reaction with hydroxylamine to form the corresponding hydroxamic acid, that is, bufexamac. This interpretation conforms with the general pattern, because while phenylacetic acid is eliminated in man as a glutamine conjugate, other types of conjugation predominate with certain substituted phenylacetic acids (9, 10).

The direct administration of a small dose of 4-butoxyphenylacetic acid gave rise to the urinary recovery of less than 50% of the dose. This finding suggests that whereas about 6% of a dose of bufexamac is excreted in the urine as 4-butoxyphenylacetic acid metabolites, a larger amount of 4-butoxyphenylacetic acid may in fact be formed in the body, with some being eliminated by a different route (*e.g.*, in the bile) or excreted in the urine in a form that is not assayed. This may account for the failure to obtain a complete recovery of a dose of bufexamac and also for the variation in the proportion of the dose recovered from the urine (Table I).

The rate of conversion of bufexamac to 4-butoxyphenylacetic acid *in vivo* appears to exceed the observed rate of hydrolysis *in vitro* at pH 7.4; therefore, metabolic processes are most probably involved. Two alternative routes may be considered. One route, which has been investigated in respect to certain hydroxamic acids (11), consists of the reduction of the hydroxamic acid to the amide and its subsequent conversion to the acid; this route does not involve the liberation of hydroxylamine. Fishbein and Streeter (12) reported on the metabolism of aliphatic hydroxamic acids to the corresponding amide in the mouse and expressed the view that this is the general pathway in mammalian species. McIsaac and Williams (13) showed that both salicylhydroxamic acid and 5-bromosalicylhydroxamic acid are extensively degraded in man to the corresponding amides as the major metabolites.

The present studies employed a sensitive and specific method for the detection of 4-butoxyphenylacetamide in urine, but none was found. The alternative route involves direct hydrolysis of the hydroxamic acid and the consequent liberation of an equivalent amount of hydroxylamine; Bernheim (14) demonstrated the presence of enzymes in animal liver preparations able to effect this hydrolysis *in vitro*.

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