Biochemical Pharmacology, 1966, Vol. 15, pp. 521–531. Pergamon Press Ltd., Printed in Great Britain.

THE METABOLISM OF ACETOPHENETIDIN AND N-ACETYL-*p*-AMINOPHENOL IN THE CAT*

R. M. WELCH, A. H. CONNEY and J. J. BURNS

The Wellcome Research Laboratories, Burroughs Wellcome & Co., (U.S.A.) Inc., Tuckahoe, N.Y., and Albert Einstein College of Medicine, New York, N.Y., U.S.A.

(*Received* 28 *September* 1965; *accepted* 18 *November* 1965)

Abstract—3-Methylcholanthrene (3-MC), given to cats 24 hr before administration of acetophenetidin, stimulated the metabolism of acetophenetidin *in vivo* to N-acetyl-*p*-aminophenol (APAP). However, 3-MC pretreatment did not stimulate the metabolism of APAP *in vivo* in this species. Studies in rats indicated that pretreatment with 3-MC stimulated the metabolism of acetophenetidin to APAP *in vivo* and *in vitro* by liver enzymes. When urine from APAP-treated cats was hydrolyzed with β-glucuronidase or sulfatase, it was found that less than 6 per cent of the APAP was excreted in urine as the glucuronide and less than 2 per cent as the sulfate. Administration of 3-MC to cats failed to increase the amount of APAP excreted in the urine as the glucuronide or sulfate. Man and dog, unlike the cat, excreted 50–60 per cent of administered APAP as the glucuronide. Treatment of urine from APAP-injected cats with an enzyme preparation (Glusulase), obtained from the intestinal juices of the snail *Helix pomatia*, resulted in the liberation of APAP. These results suggested that the cat forms an unknown conjugate that is cleaved by Glusulase treatment.

Cats given either APAP or acetophenetidin excreted primary aromatic amines in the urine, which amounted to 10 per cent of the dose when calculated as aniline. Further studies revealed that this material was not *p*-phenetidine or *p*-aminophenol. Dogs given APAP excreted about 3 per cent of the dose as aromatic amines, whereas less than 0·5 per cent of the dose was excreted as an aromatic amine in the urine of humans who ingested APAP. Acetophenetidin and APAP produced the same degree of methemoglobinemia in cats, and this effect was not altered by 3-MC treatment.

SEVERAL investigators have found the cat unusually sensitive to foreign compounds.[1] Although many studies have been carried out on the metabolic fate of foreign compounds in man and experimental animals, little is known about the metabolic fate of drugs in the cat. Studies of the metabolism of drugs in the cat may provide a possible explanation for the marked sensitivity of this species to drug administration. Indeed, reports have appeared, indicating that very little glucuronide formation occurs in the cat,[2–4] which can explain the marked toxicity of phenols in this animal. Because of these observations, it was of interest to determine whether other pathways of drug metabolism are also impaired in the cat.

Acetophenetidin is a drug metabolized mainly by O-dealkylation to N-acetyl-*p*-aminophenol[5] (APAP), which is then conjugated as the glucuronide in man[6] and in rabbit.[7] Since certain polycyclic hydrocarbons stimulate the dealkylation and glucuronidation of drugs in the rat,[8, 9] we have studied the effect of 3-methylcholanethrene

* A preliminary report of these studies was given at the Spring Meeting of the Society for Pharmacology and Experimental Therapeutics in April 1964 in Chicago, Ill.

(3-MC) on the ability of the cat to metabolize acetophenetidin to APAP as well as on its ability to form APAP glucuronide. The present study demonstrates that 3-MC markedly stimulates the conversion of acetophenetidin to APAP; however, negligible amounts of acetophenetidin or APAP were metabolized to APAP glucuronide in normal or 3-MC-treated cats. In addition, the data indicate the relative potency of acetophenetidin and APAP in producing methemoglobinemia in the cat before and after treatment with 3-MC.

MATERIALS AND METHODS

Animals and solutions. Male cats ranging in weight from 2·5 to 5·2 kg, male dogs weighing 8·5 to 10 kg, and male Sprague-Dawley rats weighing 50 to 60 g were used in this study. The cats were fed Puss'n Boots cat food (Quaker Oats Co., Chicago, Ill.) and milk; the dogs were fed Thrivo dog food (Thrivo Co., Philadelphia, Pa). and Gaines meal (General Foods Corp., White Plains, N.Y.); the rats were fed a synthetic diet as previously described.[10] Unless otherwise indicated, all drugs were administered intraperitoneally. A parenteral solution containing 2% APAP was prepared in a vehicle of 20% ethanol in saline. Cats received a 5-ml suspension of acetophenetidin in saline containing 10% Tween 80. 3-Methylcholanthrene was dissolved in 5 ml corn oil and injected into cats. Immature male rats (60 g) received 2 mg 3-MC dissolved in 0·5 ml corn oil. Urine from dogs and cats treated with acetophenetidin or APAP was collected for 24 hr in a vessel stored in an insulated bucket containing ice. The urine was either analyzed immediately or stored frozen for future use.

Drug analysis. Acetophenetidin, *p*-phenetidine, N-acetyl-*p*-aminophenol, and *p*-aminophenol were measured as described by Brodie and Axelrod.[5, 11] Methemoglobin was determined by the method of Evelyn and Malloy.[12]

Aromatic amines present in the urine were determined by a modification of the Bratton and Marshall techniques[13] as follows. A 0·1-ml aliquot of urine was added to 2 ml of 0·1 N HCl, followed by 0·5 ml of 0·2% sodium nitrite. After standing in ice for 10 min, 0·5 ml of 1·0% ammonium sulfamate was added and allowed to stand at room temperature for 3 min. Then 1 ml of 50% sodium acetate was added, followed by 0·5 ml of a 0·2% N (1-naphthyl)ethylenediamine dihydrochloride solution. After 20 min, 0·5 ml of concentrated HCl was added and the optical density of the resulting dye measured in a Beckman DU spectrophotometer at 540 mμ.

O-Dealkylation of acetophenetidin in vitro. Livers from four rats were pooled and a 33% homogenate made in 1·15% KCl. The homogenate was centrifuged at 9000 *g* for 15 min and the supernatant fluid used as a source of enzyme. The total reaction mixture (5·5 ml) was incubated for 30 min at 37° in 50-ml Erlenmeyer flasks. The incubation mixture contained liver enzyme (1 ml), acetophenetidin (1 ml, 2 μmoles), glucose-6-phosphate (0·4 ml, 3·4 mg), NADP (0·3 ml, 600 μg), ATP (0·2 ml, 1 mg), 0·6 M niacinamide solution (0·2 ml), 2 M KCl solution (0·1 ml), 0·1 M $MgCl_2$ solution (0·1 ml), and 0·1 M KH_2PO_4–K_2HPO_4 buffer (2·2 ml, pH 7·4). After the incubation period or at zero time, a 5-ml aliquot was analyzed for APAP according to the method indicated above. Two micromoles of acetophenetidin per incubation was selected as the substrate concentration, since at this concentration the rate of APAP formation was maximal.

Determination of urinary conjugates of N-acetyl-p-aminophenol. The APAP glucuronide present in urine was determined after incubating 1 ml urine with 1 ml (5000

Fishman units) beef liver β-glucuronidase (Ketodase, Warner Chilcott, Morris Plains, N.J.) and 3 ml 0·2 M sodium acetate buffer (pH 5·0) for 24 hr at 37°. Different concentrations of APAP in urine were found to be stable for at least 24 hr when incubated under these conditions. When 0·5 ml of a 0·01 M phenolphthalein glucuronide solution was added to 1 ml urine and incubated in the same way, complete hydrolysis of the glucuronide was obtained in 5 hr. The APAP glucuronide present in urine was also determined chemically by the carbazole method for uronic acid.[14]

The APAP conjugated as sulfate was determined after incubating 20 units of sulfatase (Limpets type III, Sigma Chemical Co., St. Louis, Mo.) with 0·1 ml urine in 10·0 ml 0·2 M sodium acetate buffer (pH 5·0) for 24 hr at 37°. When 20 units of sulfatase was incubated with 800 μg of *p*-nitrocatechol sulfate in the presence of 0·1 ml of cat urine as described above, complete hydrolysis was obtained in 24 hr. It was observed that the presence of 0·05, 0·1, and 0·5 ml of cat urine in the incubation medium produced 45%, 62%, and 80% inhibition respectively of *p*-nitrocatechol sulfate hydrolysis after a 4-hr incubation. Therefore, a 24-hr incubation period was used, and the concentration of cat urine in the incubation mixture did not exceed 1% of the incubation mixture.

In some studies, the APAP conjugates present in human, dog, and cat urine were hydrolyzed by the intestinal juice of the snail *Helix pomatia* (Glusulase,† Endo Labs., Garden City, N.Y.) by adding 0·1 ml Glusulase to 4 ml 0·2 M sodium acetate buffer (pH 5·0) and 1 ml urine. The mixture was incubated for 4 hr at 37°, and the APAP liberated from the conjugate was then assayed by the method for APAP indicated above.

Chromatography of N-acetyl-p-aminophenol. N-Acetyl-*p*-aminophenol liberated by treatment of cat urine with Glusulase was extracted into organic solvents and identified as APAP by comparing its mobility with authentic APAP on thin-layer chromatography plates (adsorbent ALOGF, Brinkman Instruments, Inc., Westbury, N.Y.); the upper phases were used from mixtures of benzene:toluene:H_2O:acetic acid (2:2:1:2), benzene:ethanol:H_2O:acetic acid (20:20:20:1), and the lower phases from mixtures of chloroform:methanol:H_2O:acetic acid (20:10:20:1). These systems were deve[oped by Dr. Albert Klutch in our laboratory.

RESULTS

Effect of 3-methylcholanthrene on the metabolism of acetophenetidin in the cat

The administration of 200 mg acetophenetidin per kg to cats resulted in high plasma levels of acetophenetidin and its O-dealkylated product, N-acetyl-*p*-aminophenol. Two weeks later, the same cats were treated with 50 mg 3-MC/kg 24 hr before another dose of acetophenetidin, and the plasma levels of APAP and acetophenetidin were again determined. The results of this study, in which each cat served as its own control, are indicated in Fig. 1. Treatment of cats with 3-MC enhanced the disappearance of acetophenetidin from the plasma and increased the plasma level of its major metabolite, APAP, indicating that the cat metabolizes acetophenetidin to APAP and that pretreatment with 3-MC stimulates this biotransformation. The enhanced metabolism of acetophenetidin in cats pretreated with 3-MC is further indicated in Fig. 2 where the acetophenetidin data from Fig. 1 are presented in a form suitable for the estimation

† Glusulase contains 100,000 (Fishman) units/ml β-glucuronidase and 50,000 units/ml sulfatase (*p*-nitrophenyl sulfate) as well as unknown hydrolytic enzymes.

of the plasma half-life. As indicated in Fig. 2, pretreatment of cats with 3-MC shortened the half-life of acetophenetidin from 80 to about 30 min.

In addition to stimulating the metabolism of acetophenetidin in the cat, 3-MC also enhanced the metabolism of acetophenetidin in the rat. Rats were treated with 40 mg 3-MC/kg 24 hr before an oral dose of 200 mg acetophenetidin/kg. One hour

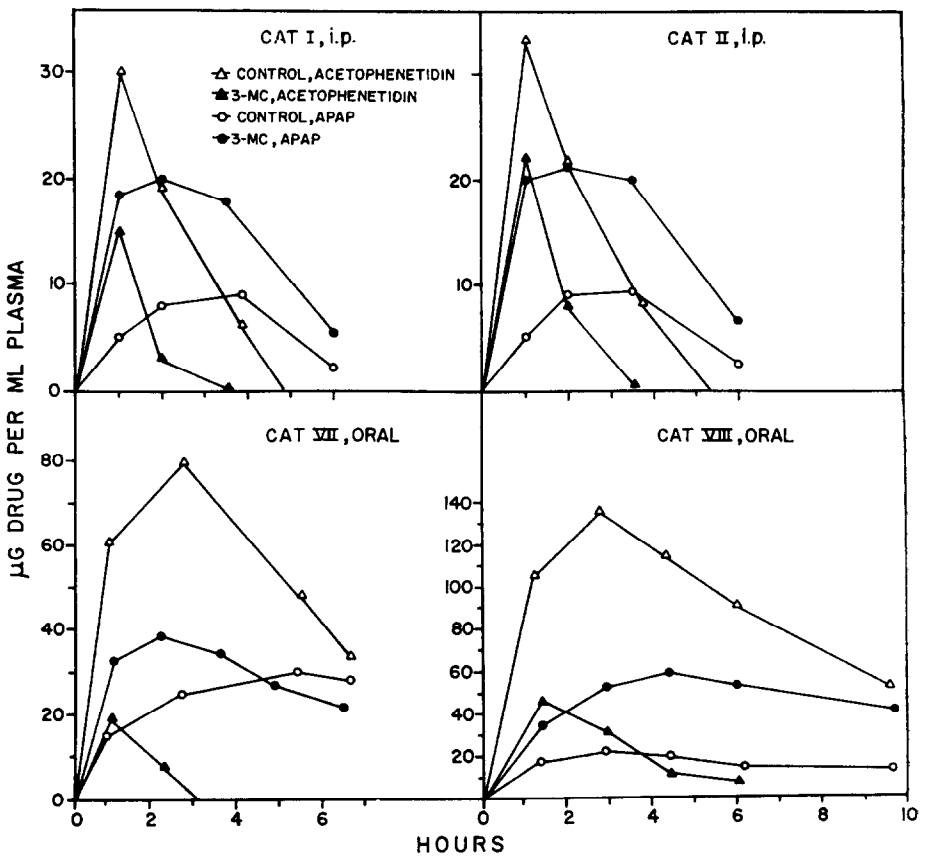

FIG. 1. Plasma levels of acetophenetidin and N-acetyl-*p*-aminophenol (APAP) after the administration of acetophenetidin to cats before and after treatment with 3-MC. Cats I and II were given 50 mg acetophenetidin/kg i.p. Cats VII and VIII were given 200 mg/kg orally. Two weeks later the cats were treated with 50 mg 3-MC/kg i.p. 24 hr before a second dose of acetophenetidin.

after the acetophenetidin, when peak plasma levels of drug were attained, the rats were sacrificed and the plasma levels of acetophenetidin and APAP determined. Pretreatment of rats with 3-MC markedly lowered the acetophenetidin plasma level while slightly elevating the APAP level (Table 1). Additional evidence that the low plasma levels of acetophenetidin present in plasma of 3-MC-treated rats are due to enhanced acetophenetidin metabolism was obtained from studies with liver homogenates *in vitro*. Rats were treated with 3-MC (40 mg/kg i.p.) 24 hr before sacrifice and the liver tested for ability to metabolize acetophenetidin to N-acetyl-*p*-amino-

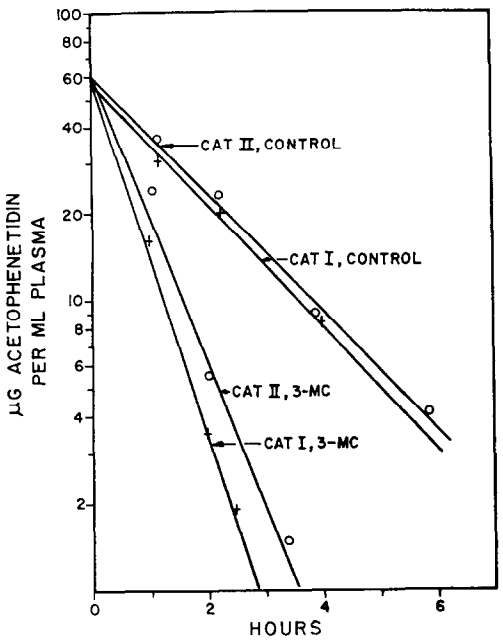

FIG. 2. Plasma half-life of acetophenetidin in cats before and after treatment with 3-MC. Cats were given 50 mg acetophenetidin/kg i.p. Two weeks later the cats were treated with 50 mg 3-MC/kg i.p. 24 hr before a second dose of acetophenetidin.

phenol. As indicated in Fig. 3, liver from rats treated with 3-MC metabolized acetophenetidin to APAP three times more rapidly than did liver from control rats.

Because APAP is the major metabolite of acetophenetidin, it was of interest to determine whether 3-MC could stimulate the metabolism of APAP in the cat. Cats were given 50 mg APAP/kg i.p. and the plasma levels determined. Two weeks later

TABLE 1. PLASMA LEVELS OF ACETOPHENETIDIN AND N-ACETYL-*p*-AMINOPHENOL IN RATS TREATED WITH 3-METHYLCHOLANTHRENE

Treatment	No. of rats	Acetophenetidin (μg/ml ± S.D.)	APAP (μg/ml ± S.D.)
Control	7	37·1 ± 7·1	15·7 ± 3·3
3-MC	7	6·3 ± 2·4	23·7 ± 2·7

Acetophenetidin (200 mg/kg) was given orally 24 hr after the the i.p. injection of corn oil or 40 mg 3-MC/kg in corn oil. Plasma was taken at 1 hr after the acetophenetidin and analyzed for acetophenetidin or N-acetyl-*p*-aminophenol (APAP).

the same cats were pretreated with 3-MC 24 hr before another dose of APAP, and the APAP plasma levels were determined again. The results shown in Fig. 4 indicate that 3-MC treatment does not stimulate the metabolism of APAP in the cat. These results are markedly different from those observed for acetophenetidin metabolism in

3-MC-treated cats and suggest that APAP conjugation, a major route of APAP detoxication, was not increased by 3-MC. It should also be noted in Fig. 4 that, unlike acetophenetidin, APAP disappears from cat plasma according to zero-order kinetics. The reason for this unusual type of decline in APAP plasma levels is unknown.

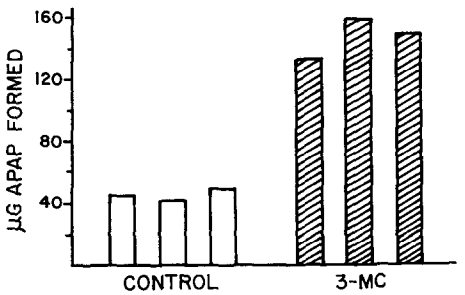

FIG. 3. Effect of 3-MC on the metabolism of acetophenetidin to N-acetyl-*p*-aminophenol (APAP) by rat liver. Liver enzyme was incubated with acetophenetidin for 30 min in the presence of a NADPH-generating system as described in Methods. Each bar represents the activity of pooled liver from three rats.

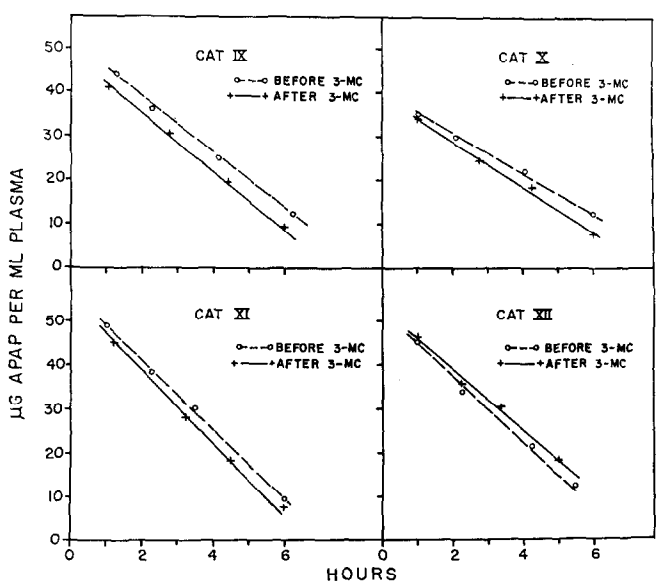

FIG. 4. Plasma levels of N-acetyl-*p*-aminophenol (APAP) in cats before and after treatment with 3-MC. Cats were injected i.p. with 50 mg APAP/kg. Fourteen days later the cats were treated with 50 mg 3-MC/kg i.p. 24 hr before a second dose of APAP.

*Effect of 3-methylcholanthrene on the methemoglobinemia caused by acetophenetidin and N-acetyl-*p*-aminophenol*

It has been shown[15] that acetophenetidin is more potent in causing methemoglobinemia in the cat than in man, dog, monkey, rabbit, and rat. In addition, it has been reported[16] that acetophenetidin and APAP are equally potent in causing methemoglobinemia in the cat. Since 3-MC increases the metabolism of acetophenetidin but

not APAP in the cat it was of interest to compare the relative potency of these two drugs in producing methemoglobinemia in the cat before and after 3-MC pretreatment. Acetophenetidin or APAP (50 mg/kg, i.p.) was given to cats and the methemoglobin determined over a 6-hr period. Two weeks later, 50 mg 3-MC/kg was administered i.p., and 24 hr later the study with acetophenetidin and APAP was

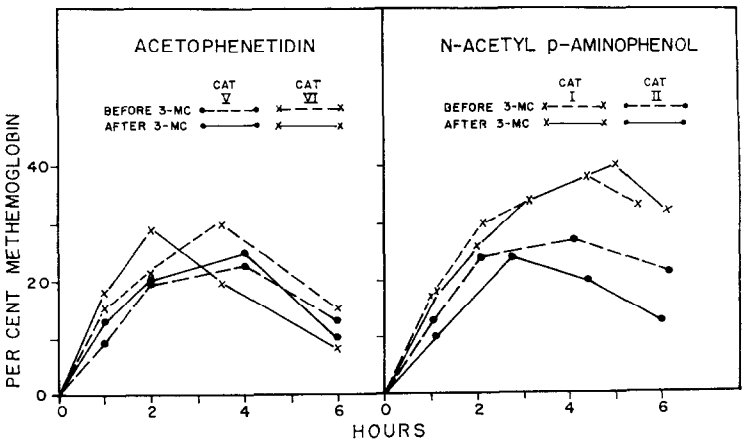

FIG. 5. Methemoglobin levels in cats after the i.p. administration of 50 mg acetophenetidin or N-acetyl-*p*-aminophenol (APAP) per kg before and after 3-MC treatment. Cats were given 50 mg acetophenetidin or APAP per kg i.p. Two weeks later the cats were treated with 50 mg 3-MC/kg i.p. 24 hr before another dose of acetophenetidin or APAP.

repeated. The results shown in Fig. 5 indicate that acetophenetidin and APAP are equipotent in producing methemoglobinemia in cats and that, as expected, stimulation of acetophenetidin metabolism to APAP with 3-MC did not alter the methemoglobin formation caused by a dose of acetophenetidin. In addition, 3-MC pretreatment, which does not stimulate APAP metabolism in the cat, does not influence the methemoglobin formation caused by APAP.

*Excretion of N-acetyl-*p*-aminophenol conjugates in the urine of various animal species*

The major route of APAP detoxication in man and rabbit is conjugation with glucuronic acid.[6, 7] It has been reported[3, 4] that the cat has an impaired ability to form glucuronides, and this defect has been attributed to the lack of the glucuronyl transferase enzyme in the liver.[17] This prompted us to compare how much of an administered dose of APAP was excreted in the urine as the glucuronide in man, dog, and cat and to determine whether 3-MC could stimulate APAP glucuronide formation in the cat. Humans were given 1·3 g APAP orally, and cats and dogs received 50 mg APAP/kg i.p. Urine was collected for 48 hr and an aliquot incubated for 16 hr with β-glucuronidase or sulfatase to determine the amount of APAP glucuronide and sulfate present. Table 2 indicates that when APAP is administered to man, dog, and cat, about 80 per cent of the total dose is excreted in a conjugated form which, upon acid hydrolysis, gives rise to *p*-aminophenol. In man and dog, approximately 50 per cent of the total dose administered was excreted as the glucuronide, with about 4 per cent as the sulfate. In contrast to these results, the cats excreted less than 6 per cent of the dose as the glucuronide (β-glucuronidase assay) and only trace amounts as the

sulfate conjugate (Table 2). Furthermore, the administration of 3-MC to cats 24 hr before APAP treatment failed to stimulate the conjugation of APAP with glucuronic acid or sulfate. The inability of the cat to conjugate APAP as the glucuronide was confirmed by analyzing the urine for glucuronic acid with the carbazole method for uronic acid. By this method it was found that, whereas the dog and man excreted 50–60 per cent of the dose as glucuronide, cats excreted less than 3 per cent as the glucuronide (Table 2).

TABLE 2. URINARY EXCRETION OF CONJUGATES OF N-ACETYL-*p*-AMINOPHENOL IN VARIOUS ANIMAL SPECIES TREATED WITH N-ACETYL-*p*-AMINOPHENOL

		Per cent of APAP dose in urine				
					APAP glucuronide	
Species	3-MC Treatment	Free APAP	Total conjugates* by acid hydrolysis	APAP sulfate	Enzyme assay†	Carbazole assay‡
Man I	–	1·8	91	4·6	63	66
Man II	–	1·4	88	4·2	52	48
Dog I	–	1·2	76	4·0	52	61
Dog II	–	1·0	79	4·2	55	58
Cat I	–	2·8	76	<2	<6	<3
Cat I	+	3·8	73	<2	<6	<3
Cat II	–	2·4	81	<2	<6	<3
Cat II	+	1·9	87	<2	<6	<3
Cat III	–	2·6	79	<2	<6	<3
Cat III	+	1·1	70	<2	<6	<3

* *p*-Aminophenol was determined after hydrolysis in 4 N HCl as described in Methods.
† N-Acetyl-*p*-aminophenol (APAP) was determined as described in Methods, after treatment of the urine with β-glucuridase or sulfatase.
‡ Uronic acid was measured by the carbazole method described in Methods.

TABLE 3. HYDROLYSIS OF UNKNOWN CONJUGATE OF APAP IN URINE OF CATS BY THE DIGESTIVE JUICE OF THE SNAIL *HELIX POMATIA* (GLUSULASE)

Cat no.	Per cent of dose recovered	
	Total conjugates by acid hydrolysis*	APAP liberated by glusulase treatment†
1	81	77
2	75	69
3	76	73

* *p*-Aminophenol was determined after hydrolysis in 4 N HCl as described in Methods.
† N-Acetyl-*p*-aminophenol (APAP) was determined as described in Methods, after treatment of the urine with Glusulase.

Interestingly, treatment of cat urine with Glusulase, an enzyme mixture previously believed to hydrolyze primarily glucuronide and sulfate conjugates, rapidly split the unknown conjugate of APAP to yield free N-acetyl-*p*-aminophenol (Table 3). The Glusulase was found to be as effective as acid hydrolysis in liberating the unknown conjugate. After treatment of the cat urine with Glusulase, the urine was extracted

with a mixture of 1·5 per cent isoamyl alcohol in ethyl ether, and an aliquot of the organic solvent was chromatographed in the four thin-layer chromatography systems described in Methods. Another aliquot was analyzed for APAP as described under Methods. The chromatographic results indicated the presence of APAP in the amounts indicated by the chemical method.

Presence of primary aromatic amine in urine of various species treated with N-acetyl-p-aminophenol

After the intraperitoneal administration of 50 mg acetophenetidin/kg to cats, urine was collected for 24 hr and analyzed for various metabolites. Although less than 0·1 per cent of the dose was present in cat urine as acetophenetidin or *p*-phenetidine, a considerable amount of a primary aromatic amine which coupled with N-naphthylethylenediamine was found in urine. This metabolite, when coupled with N-naphthylethylenediamine, had an absorption maximum at 540 mμ. When aniline or *p*-phenetidine was added to urine and coupled to N-naphthylethylenediamine, the optical density maxima were at 540 mμ and 565 mμ respectively. From 8–10 per cent of the acetophenetidin dose was excreted in cat urine as primary aromatic amine when calculated as aniline (Table 4). After the administration of APAP or acetophenetidin

TABLE 4. PRIMARY AROMATIC AMINE PRESENT IN URINE OF VARIOUS ANIMAL SPECIES GIVEN N-ACETYL-*p*-AMINOPHENOL OR ACETOPHENETIDIN

Species	Drug administered		Per cent of dose recovered		
	Acetophenetidin (mg/kg)	APAP (mg/kg)	Free APAP	Conjugated APAP*	Aromatic amine†
Man		19	1·8	91	0·13
Man		16	1·4	88	0·32
Dog		50	1·2	76	3·8
Dog		50	1·0	79	3·5
Cat		50	1·9	74	12·7
Cat		50	2·3	72	10·2
Cat		50	2·8	73	12·4
Cat	50		1·5	68	8·1
Cat	50		2·1	67	10·3
Cat	50		2·4	71	9·7

* Measured as *p*-aminophenol after acid hydrolysis.

† An aliquot of urine was analyzed directly (without organic solvent extraction) for free amine as described in Methods. The results are calculated on the basis of an aniline standard.

to cats, about 70 per cent of the total dose was excreted as conjugated APAP, 2 per cent as free APAP, and 11 per cent as primary aromatic amine (Table 4). Two weeks later, the same cats were treated with 50 mg 3-MC/kg, followed in 24 hr by another 50 mg/kg dose of either acetophenetidin or APAP. Urine was collected for 24 hr and the analysis of urinary metabolites repeated. Treatment of cats with 3-MC before a dose of acetophenetidin or APAP did not change the amounts of acetophenetidin, conjugated APAP, free APAP, *p*-phenetidine, or aromatic amine excreted in the urine. In the human, only trace amounts of aromatic amine were detected in urine, whereas in the dog 3–4 per cent of a dose of APAP was excreted as aromatic amine

(Table 4). The aromatic amine present in dog and cat urine after a dose of APAP could not be extracted from unhydrolyzed urine into a mixture of 1·5 per cent isoamyl alcohol in ethyl ether, which suggests that the amine is very polar. The amine in cat urine was unstable, since it was destroyed by heating the urine in 6 N HCl at 100° for 30 min, or by treating the urine with Glusulase at 37° for 2 hr.

DISCUSSION

The results presented here indicate that treatment of rats or cats with 3-MC stimulates the metabolism *in vivo* of acetophenetidin to APAP. These results are in accord with the observation that liver preparations from 3MC-treated rats were more active than control liver in metabolizing acetophenetidin to APAP. Although 3-MC treatment stimulated the metabolism *in vivo* of acetophenetidin in the cat, it has no effect on the metabolism of APAP, suggesting that the conjugation mechanism for APAP detoxication in the cat is not enhanced by 3-MC. The analysis of urine from APAP-treated cats, employing either the enzyme β-glucuronidase or the chemical carbazole method for uronic acid, indicated that the cat excreted little or no APAP glucuronide and that this pathway was not stimulated by 3-MC. In contrast to the cat, excretion of APAP glucuronide by man and dog ranged between 50–60 per cent of the APAP dose. Negligible amounts of the total APAP conjugate in man, dog, and cat were excreted as the sulfate conjugate in urine. Of special significance was the observation that the digestive juice of the snail *H. pomatia* (Glusulase) was capable of hydrolyzing the unknown APAP conjugates in cat urine. This finding indicates that cats conjugate APAP with substances other than glucuronic acid or sulfate and that Glusulase contains enzymes other than β-glucuronidase and sulfatase. It is of interest that the cat can form small amounts of glucuronide conjugates of N-2-fluorenylacetamide and testosterone metabolites.[18, 19] A recent report[20] has shown that after the administration of acetophenetidin to humans, 2 per cent of the dose was excreted in the urine as a cysteine conjugate of APAP. It is possible that in the cat, a large portion of the APAP conjugate present in the urine is conjugated with cysteine.

The data presented here, indicating that APAP and acetophenetidin are equally potent in causing methemoglobin formation in the cat, agree with the results of Doll and Hackenthal,[16] and are supported further by the finding that 3-MC stimulates the metabolism of acetophenetidin to APAP without influencing the methemoglobinemia caused by acetophenetidin.

An earlier study[5] has indicated that after the administration of acetophenetidin to man, no free aromatic amines were observed in urine other than *p*-phenetidine, which did not exceed 0·12 per cent of the dose. Our studies with cats indicate that a considerable quantity of diazotizable material (about 10 per cent of the dose) which couples with N-naphthylethylenediamine is present in cat urine after the administration of acetophenetidin or APAP. In contrast to the cat, much less of this material appears in dog urine, and only trace amounts appear in human urine. Interestingly, the excretion of diazotizable amine in the urine of these three species parallels the ability of acetophenetidin to cause methemoglobinemia in these animal species.[15] The results suggest that further work be carried out to identify and determine the pharmacological and toxicological properties of the primary aromatic amines present in the urine of animals treated with acetophenetidin and APAP.

REFERENCES

1. W. S. SPECTOR, *Handbook of Toxicology*, Vol. 1. Saunders, Philadelphia (1956).
2. K. J. W. HARTIALA, *Ann. Med. exp. Fenn.* **33**, 239 (1955).
3. S. BORRELL, *Biochem. J.* **70**, 727 (1958).
4. D. ROBINSON and R. T. WILLIAMS, *Biochem. J.* **68**, 23P (1958).
5. B. B. BRODIE and J. AXELROD, *J. Pharmac. exp. Ther.* **97**, 58 (1949).
6. R. SCHMID and L. HAMMAKER, *New Eng. J. Med.* **260**, 1310 (1959).
7. J. N. SMITH and R. T. WILLIAMS, *Biochem. J.* **44**, 239 (1949).
8. J. K. INSCOE and J. AXELROD, *J. Pharmac. exp. Ther.* **129**, 128 (1960).
9. A. H. CONNEY and J. J. BURNS, *Advanc. Pharmac.* **1**, 31 (1962).
10. R. R. BROWN, J. A. MILLER and E. C. MILLER, *J. biol. Chem.* **209**, 211 (1954).
11. B. B. BRODIE and J. AXELROD, *J. Pharmac. exp. Ther.* **94**, 22 (1948).
12. K. A. EVELYN and H. T. MALLOY, *J. biol. Chem.* **126**, 655 (1938).
13. A. C. BRATTON and E. K. MARSHALL, *J. biol. Chem.* **128**, 537 (1939).
14. G. ASHWELL, in *Methods in Enzymology*, (Eds. S. P. COLOWICK and N. O. KAPLAN), Vol. 1, p. 92. Academic Press, New York (1957).
15. D. LESTER, *J. Pharmac. exp. Ther.* **77**, 154 (1943).
16. G. DOLL and E. HACKENTHAL, *Arzneimittel-Forsch* **13**, 68 (1963).
17. G. J. DUTTON and C. G. GREIG, *Biochem. J.* **66**, 52P (1957).
18. J. H. WEISBURGER, P. H. GRANTHAM and E. K. WEISBURGER, *Biochem. Pharmac.* **13**, 469 (1964).
19. S. E. H. ARCHER, T. SCRATCHERD and W. TAYLOR, *Biochem. J.* **94**, 778 (1965).
20. O. R. JAGENBURG and K. TOCAKO, *Biochem. J.* **92**, 639 (1964).