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PHARMACOLOGIC IMPLICATIONS OF ALTERATIONS IN THE METABOLISM OF CHLORAMPHENICOL*

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Abstract—In the present study, it was shown that in rats the glucuronide conjugation of chloramphenicol was reduced by 18- to 24-hr fasting and by pretreatment with *o*- and *p*-toluic acids. This was reflected in the increased availability of chloramphenicol to produce liver microsomal enzyme inhibition. Eighteen- to 24-hr fasting and administration of *o*- and *p*-toluic acids delayed the disappearance of chloramphenicol from the plasma and from the liver and reduced the levels of chloramphenicol glucuronide in the plasma and in the liver tissue. These changes in the inactivation profile were accompanied by potentiation of chloramphenicol-induced prolongation of hexobarbital sleeping time. In fasted rats, glucose administration antagonized the changes observed in chloramphenicol metabolism and its effect on hexobarbital sleeping time. Pretreatment with *o*-toluamide, which is not conjugated with glucuronic acid, did not have a significant effect on glucuronide conjugation of chloramphenicol. It was also shown that thiamphenicol-induced inhibition of liver microsomal enzymes *in vivo*, measured in terms of its effect on the prolongation of hexobarbital sleeping time, was not affected either by fasting or by administration of *o*- and *p*-toluic acids. Thiamphenicol, an analog of chloramphenicol, is not metabolized by glucuronide conjugation, but is excreted unchanged. These observations indicate that the potentiation of chloramphenicol-induced prolongation of hexobarbital sleeping time in fasted and in *o*- or *p*-toluic acid-treated rats was due to the availability of higher amounts of chloramphenicol producing a greater degree of microsomal inhibition.

CHLORAMPHENICOL is a broad spectrum antibiotic that is inactivated predominantly by conjugation to the glucuronide.[1] It is also a potent inhibitor of liver microsomal drug-metabolizing enzymes.[2] In rats and mice, chloramphenicol has been shown to prolong oxybarbiturate anesthesia[3] and in man to prolong the biologic half-life of diphenylhydantoin, tolbutamide and dicoumarol.[4] A case of chloramphenicol-induced hypoglycemic collapse in a tolbutamide-treated patient has also been reported.[4] It has been shown that the chloramphenicol-induced microsomal enzyme inhibition is dose related and appears to decrease in intensity with a time course similar to that of unchanged chloramphenicol from liver tissue.[2,3] In light of these findings, the present study was undertaken to determine how alterations in the glucuronide conjugation of chloramphenicol would affect the intensity of chloramphenicol-induced prolongation of hexobarbital anesthesia. Pretreatments, such as

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fasting and administration of alternate substrates, which are commonly encountered in laboratory situations were investigated.

EXPERIMENTAL

All studies were performed on 200–220 g male Wistar or Sprague–Dawley rats maintained for at least 1 week at (23·5 ± 1°) under artificial illumination, with a "light–dark" cycle of 12 hr. Unless otherwise indicated, animals were fed and watered *ad lib* until the time of the experiment.

Chloramphenicol and thiamphenicol (D-*threo*-analogs) were dissolved in 5% ethanol in water. Chloramphenicol succinate (Chloromycetin) dissolved in glass distilled water was used in some experiments.

Statistical significance of difference between two means was evaluated by the Student's *t*-test. Analysis of variance for the 2 × 2 factorial design was done on an Olivetti–Programma 101 desk computer.

Sleeping times. These studies were designed to evaluate how certain experimental conditions would alter the intensity of chloramphenicol-mediated prolongation of hexobarbital sleeping time. Hexobarbital sodium (90 mg/kg) was administered intraperitoneally and sleeping time was measured as the interval between the time the animal loses and regains its righting reflex. Chloramphenicol or thiamphenicol was administered 45 min prior to hexobarbital. This pretreatment interval was found to produce maximal prolongation. The doses of chloramphenicol chosen were on the linear portion of the relationship between chloramphenicol dose and chloramphenicol-mediated prolongation of hexobarbital sleeping time.

Collection of plasma and tissue samples. Rats were sacrificed, at various intervals after chloramphenicol, by decapitation and exsanguinated. Blood was collected in heparinized beakers and centrifuged at 1600 *g* for 20 min. Plasma samples were stored at −20° until assayed.

After laparotomy, a liver lobe was excised, blotted free of blood, placed in an aluminium foil envelope and frozen rapidly in dry ice–ethanol mixture. The samples were stored at −20° until assayed.

Measurement of glucuronide conjugation of o- *and* p-*toluic* acids *in the rat.* Two groups of rats ($N = 3$) were given 5·0 ml of water by gastric intubation followed by either *o*- or *p*-toluic acid, 350 mg/kg, i.p. Animals were kept in metabolic cages for collection of urine. At the end of a 12-hr period they were sacrificed by crevical dislocation, and their bladders were tapped and washed for residual urine with a syringe. Urine and bladder washings were combined and stored frozen until analyzed for free and conjugated *o*- and *p*-toluic acids, as described below.

After thawing, urine was fractionated[5] as shown in Figs. 1 and 2. The amount of toluic acid present in phase (III) and phase (IV) gave the fraction of the total dose excreted during the first 12 hr; the amount present in phase (IV) was the glucuronide conjugate. The amount found in phase (V) was that liberated by one 18-hr incubation with β-glucuronidase.

The *o*- or *p*-toluic acid was estimated by u.v. differential absorption spectrophotometry of the dissociated (pH 6) and the undissociated (pH 2) species. The p*K*a of *o*- and *p*-toluic acid is approximately 4·0. Two equal aliquots of the alkaline phase were titrated to pH 6 and to pH 2 with HCl (12 N) with a micropipette. Ultraviolet

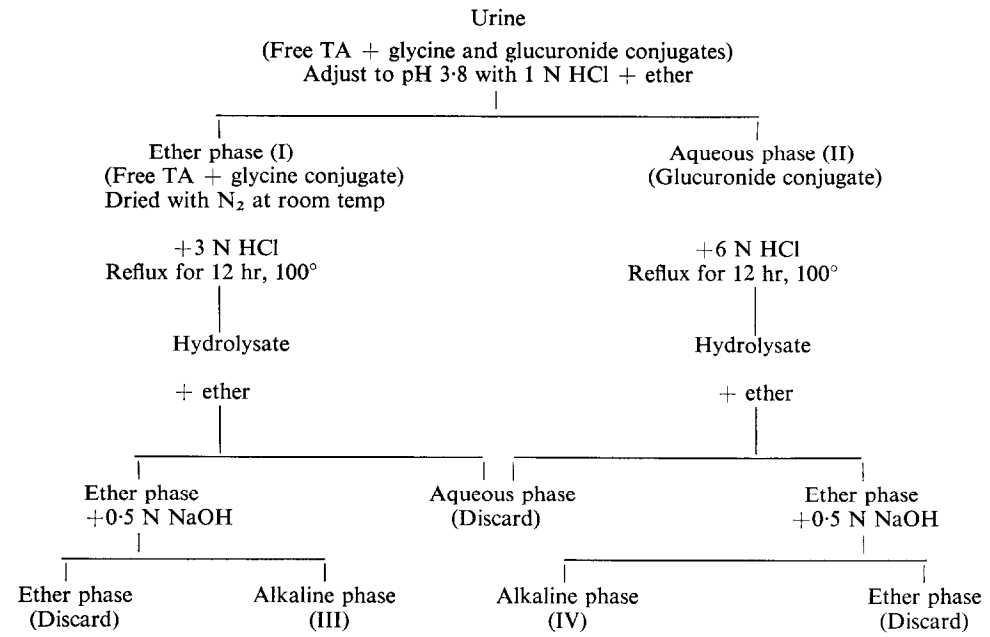

FIG. 1. Schematic representation of the fractionation procedure used to isolate glucuronide conjugate of *o*- and *p*-toluic acid (TA). TA in the alkaline phase (III) is equivalent to the amount excreted as free plus glycine conjugate, while TA present in the alkaline phase (IV) is the fraction that is excreted as the glucuronide conjugate during the 12-hr period.

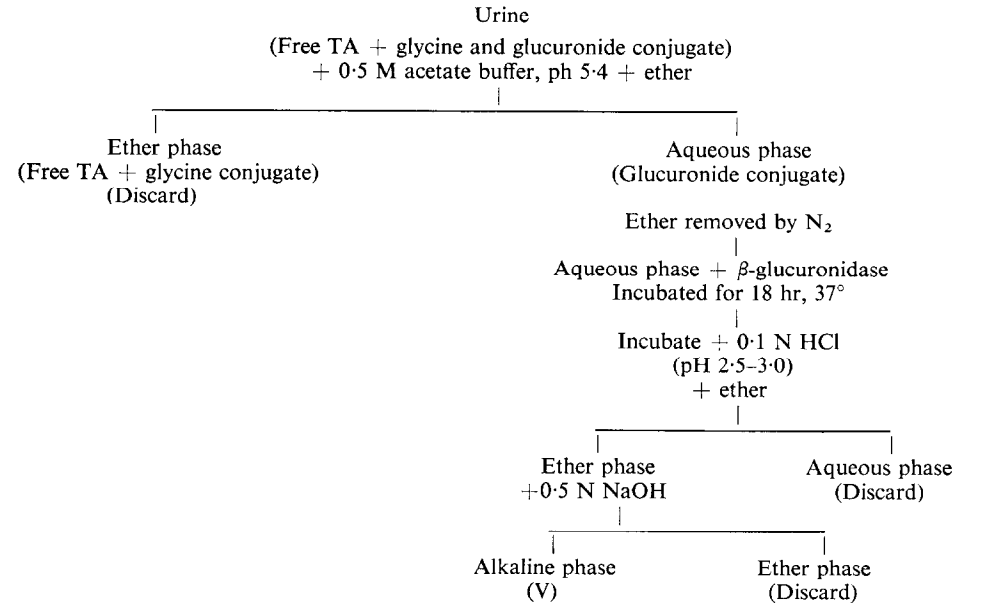

FIG. 2. Schematic representation of the procedure used to isolate and identify the glucuronide conjugate of *o*- and *p*-toluic acid (TA). TA in the alkaline phase (V) is the fraction that was released as free TA after one 18-hr incubation with β-glucuronidase.

absorption of undissociated species was measured on a Beckman DK-1A recording spectrophotometer. Dissociated species were used as the reference. Under these conditions *p*-toluic acid exhibited a positive absorption peak at 247 nm and *o*-toluic acid at 232 nm.

Estimation of unchanged chloramphenicol and its metabolite. Chloramphenicol and its aryl nitro metabolites were determined essentially by the method of Bessman and Stevens.[6] The *p*-nitro group of chloramphenicol was reduced to p-NH_2 by boiling in an acidic solution of stannous chloride, then diazotized, and coupled with *N*-(1-naphthyl) ethylene diamine dihydrochloride by the method of Bratton and Marshall.[7] Absorbance was measured on a B & L Spectronic-20 photoelectric colorieter at 555 nm.

Plasma samples were deproteinized with 5% zinc sulfate [$ZnSO_4$-7 H_2O] and 4·5% barium hydroxide [Ba $(OH)_2$-8 H_2O]. Differential separation of unchanged chloramphenicol from its metabolites was accomplished by the procedure of Glazko *et al.*[8] as follows. Aliquots of filtrates after deproteinization were partitioned between 0·3 M sodium phosphate buffer (pH 6·0) and ethyl acetate (EA). The buffer phase containing metabolites was discarded. The EA layer was washed twice with buffer saturated with EA and was evaporated to dryness by forced air at room temperature. The residue was dissolved in glass distilled water for analysis. The procedure extracts 80–82 per cent of unchanged chloramphenicol present in the samples. All values for unchanged chloramphenicol have been corrected for this recovery. With each set of unknowns, a set of standards were run simultaneously through the entire procedure.

The total concentration of unchanged chloramphenicol plus its metabolites was determined by assaying the filtrate without extraction with EA. The concentration of chloramphenicol metabolites was calculated as the difference between the values obtained for the total chloramphenicol and for the unchanged chloramphenicol, and is expressed as chloramphenicol equivalents. The concentration of endogenously formed aryl amine metabolites appearing in the filtrate was determined by running an "aryl amine blank" in which reduction with stannous chloride was omitted. It was found that the level of endogenous aryl amine was always well under 5 per cent of the total aryl nitro compounds in plasma and, therefore, was not considered separately.

Frozen liver tissue samples were thawed at 4°. A 1 : 2 homogenate was made in glass distilled water, strained through gauze and centrifuged at 1600 *g* for 20 min. The supernatant was deproteinized by the barium hydroxide–zinc sulfate method and the filtrate was assayed for unchanged chloramphenicol and its metabolites as described for plasma. Like plasma, the concentration of the endogenous aryl amine metabolite in the liver tissue was found to be negligible.

Identification of chloramphenicol metabolite. To determine whether the material estimated as chloramphenicol metabolite by difference (CAP-M) was predominantly the glucuronide conjugate, the following experiments were conducted.

Plasma samples from control rats were divided into three aliquots. Unconjugated and total chloramphenicol were determined in the deproteinized filtrates of two aliquots as described earlier. The third aliquot was buffered with an equal volume of 0·5 M acetate buffer, pH 5·4, and was incubated with β-glucuronidase at 37° for 18 hr. Chloramphenicol was determined in the EA extract of the deproteinized filtrate of the incubate.

A 1 : 2 homogenate of liver, made in 0·5 M acetate buffer, pH 5·4, was shaken with a

small amount of ether to terminate any inherent enzyme activity and ether was removed by passing nitrogen. Two aliquots from each sample were deproteinized and the filtrate was assayed for unconjugated and total chloramphenicol as described earlier. A third aliquot was incubated with β-glucuronidase at 37° for 18 hr, deproteinized and chloramphenicol was determined in the EA extract of the filtrate.

Experimental design. These experiments were designed to determine the pharmacologic implications of alterations in glucuronide conjugation of chloramphenicol *in vivo*. Changes in chloramphenicol glucuronidation were monitored by measuring: (i) the decline of unchanged chloramphenicol from the plasma; (ii) the concentration of unchanged chloramphenicol and its metabolites in the plasma; and (iii) the concentration of unchanged chloramphenicol and its metabolites in the liver tissue. Changes in pharmacologic effect were evaluated by measuring the potentiation of chloramphenicol-induced prolongation of hexobarbital sleeping time under various experimental conditions. A 2 × 2 factorial design was used so that the effect of two variables could be evaluated in a single experiment, and it was also possible to evaluate the interaction between the two variables. The following experimental conditions were used to alter the glucuronide conjugation of chloramphenicol: (i) rats were fasted for 18–24 hr; (ii) fasted rats were given glucose to counteract the effects of fasting; and (iii) *o*- or *p*-toluic acid was given to non-fasted rats. These two compounds undergo extensive glucuronidation in rats, rabbits and dogs.[9,10] In certain experiments, *o*-toluamide was also used. *o*-Toluamide is not conjugated with glucuronic acid since the site of glucuronidation is blocked by an amide group. The toluic acids or *o*-toluamide were given intraperitoneally 45 min prior to chloramphenicol. Due to some central nervous system depressant activity, the effect of *o*-toluamide on chloramphenicol-induced prolongation of hexobarbital sleeping time could not be evaluated. After chloramphenicol administration, rats were either (i) sacrificed for collection of plasma or liver tissue samples for chemical analysis, or (ii) given hexobarbital sodium and sleeping times determined. The effect of fasting and pretreatment with *o*- and *p*-toluic acid on thiamphenicol-induced prolongation of hexobarbital sleeping time was also evaluated.

RESULTS

Hexobarbital sleeping time. Fasting produced 120 per cent prolongation of hexobarbital sleeping time (Fig. 3) in chloramphenicol-treated rats, but in untreated animals the prolongation seen was only 30 per cent. Analysis of variance for the 2 × 2 factorial design showed that fasting significantly potentiated the chloramphenicol-induced prolongation of hexobarbital sleeping time. Glucose administration, to a great extent, counteracted the effect of fasting on chloramphenicol-induced prolongation of hexobarbital sleeping time and brought it within the range of the non-fasted control group (Fig. 4). Both toluic acid isomers enhanced chloramphenicol-mediated prolongation of hexobarbital sleeping time (Figs. 5 and 6). Administration of toluic acids alone did show some effect on hexobarbital sleeping times in animals not given chloramphenicol. *o*-Toluic acid by itself reduced sleeping time by 17 per cent, while *p*-toluic acid potentiated it by 14 per cent. However, analysis of variance for the 2 × 2 factorial design indicated that the effect of the toluic acids on chloramphenicol-prolonged sleeping time was of a much greater magnitude than their effect on the sleeping time of control animals.

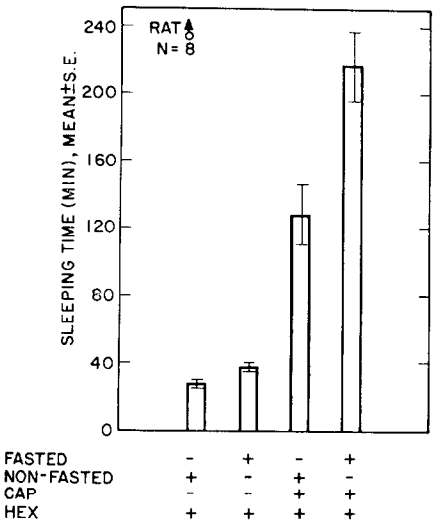

FIG. 3. Effect of 18-hr fasting on the prolongation of hexobarbital (HEX) sleeping time by chloramphenicol (CAP). Chloramphenicol succinate, 80 mg/kg, i.p., 45 min prior to hexobarbital sodium, 90 mg/kg, i.p.; 2 × 2 analysis of variance: fasted vs non-fasted, $P < 0.01$; control vs CAP, $P < 0.01$; interaction, $P < 0.01$.

Fasting produced approximately a 40 per cent potentiation of hexobarbital sleeping time of both the thiamphenicol-treated and control animals. *o*-Toluic acid by itself reduced sleeping time by 20 per cent, while *p*-toluic acid enhanced it by 16 per cent. Analysis of variance confirmed that fasting and administration of either *o*- or *p*-toluic acid had no significant effect on thiamphenicol-prolonged sleeping time (Table 1).

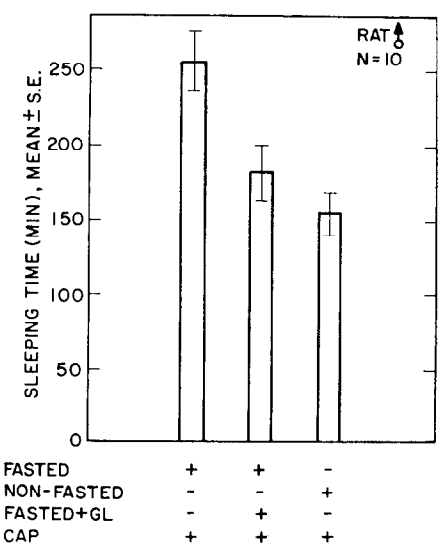

FIG. 4. Effect of glucose administration on the prolongation of hexobarbital (HEX) sleeping time by chloramphenicol (CAP). Glucose (GL), 2·5 ml of 50 per cent solution/rat, p.o., 30 min prior to chloramphenicol succinate, 80 mg/kg, i.p. Fasted vs non-fasted, $P < 0.01$; non-fasted vs fasted + glucose, NS.

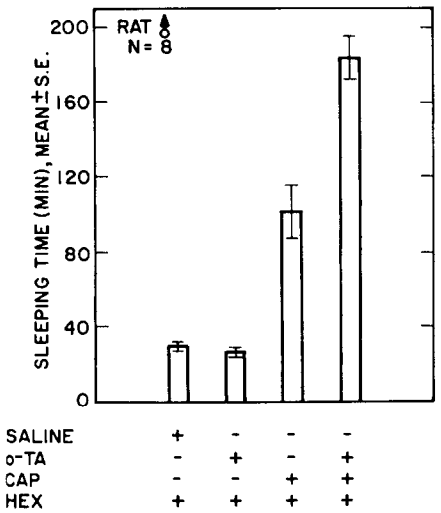

FIG. 5. Effect of pretreatment with *o*-toluic acid (*o*-TA) on the prolongation of hexobarbital (HEX) sleeping time by chloramphenicol (CAP). *o*-TA, 350 mg/kg, i.p., 45 min prior to CAP. Chloramphenicol, 72 mg/kg, i.p., 45 min prior to hexobarbital sodium, 90 mg/kg, i.p.; 2 × 2 analysis of variance: control vs *o*-TA, $P < 0{\cdot}01$; control vs CAP, $P < 0{\cdot}01$; interaction, $P < 0{\cdot}01$.

Glucuronide conjugation of toluic acids. During the first 12-hr period, 44 per cent of the dose of *p*-toluic acid and 41 per cent of the dose of *o*-toluic acid were excreted in the urine. Results obtained with acid hydrolysis (Fig. 1) showed that in the case of *p*-toluic acid 53 per cent of this fraction was in the form of glucuronide while with *o*-toluic acid it was 69 per cent. Seventy-one per cent (p-TA) and 74 per cent (o-TA) of this acid hydrolyzable fraction was liberated as free toluic acid after one 18-hr

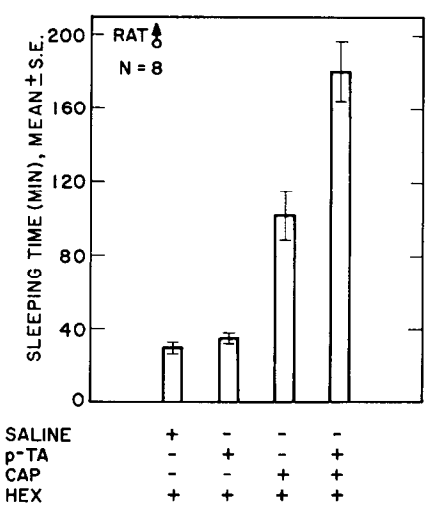

FIG. 6. Effect of pretreatment with *p*-toluic acid (*p*-TA) on the prolongation of hexobarbital (HEX) sleeping time by chloramphenicol (CAP). *p*-TA, 350 mg/kg, i.p., 45 min prior to CAP. Choramphenicol, 72 mg/kg, i.p., 45 min prior to hexobarbital sodium, 90 mg/kg, i.p.; 2 × 2 analysis of variance: control vs *p*-TA, $P < 0{\cdot}01$; control vs CAP, $P < 0{\cdot}01$; interaction, $P < 0{\cdot}01$.

TABLE 1. EFFECT OF 18-HR FASTING OR PRETREATMENT WITH TOLUIC ACIDS ON THE PROLONGATION OF HEXOBARBITAL SLEEPING TIME BY THIAMPHENICOL IN THE MALE RAT*

	Experimental condition	Sleeping time (min), mean ± S.E.
(A)	Non-fasted	32·7 ± 3·1 (10)†
	Fasted	44·0 ± 4·7 (10)
	Non-fasted + thiamphenicol	64·7 ± 4·2 (10)
	Fasted + thiamphenicol	89·7 ± 4·1 (10)
(B)	Saline	28·2 ± 1·0 (6)
	o-Toluic acid	22·6 ± 1·0 (6)
	Thiamphenicol	84·0 ± 8·0 (6)
	o-Toluic acid + thiamphenicol	74·5 ± 4·9 (6)
(C)	Saline	28·3 ± 3·1 (6)
	p-Toluic acid	32·6 ± 2·8 (6)
	Thiamphenicol	64·6 ± 4·2 (6)
	p-Toluic acid + thiamphenicol	70·0 ± 4·4 (6)

† Number in parentheses indicated the number of animals in each group.
* *o*-Toluic acid and *p*-toluic acid, 350 mg/kg, i.p., 45 min prior to thiamphenicol. Thiamphenicol, 72 mg/kg, i.p., 45 min prior to hexobarbital Na, 90 mg/kg, i.p. Analysis of variance: (A) Fasted vs non-fasted, $P < 0.01$; control vs thiamphenicol, $P < 0.01$; interaction, NS. (B) Control vs *o*-toluic acid, NS; control vs thiamphenicol, $P < 0.01$; interaction, NS. (C) Control vs *p*-toluic acid, NS; control vs thiamphenicol, $P < 0.01$; interaction, NS.

incubation with β-glucuronidase (Fig. 2). These results indicate that in the rat *o*- and *p*-toluic acids are conjugated with glucuronic acid to a considerable extent. These acids, therefore, can function as "alternate substrates" for the glucuronide conjugation mechanism.

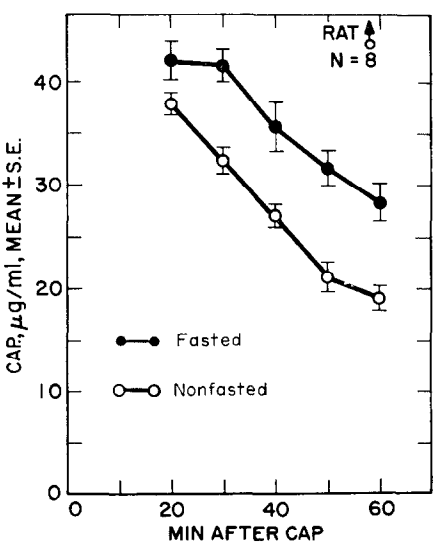

FIG. 7. Effect of 18-hr fasting on the decline of plasma concentration of unchanged chloramphenicol (CAP). Choramphenicol, 72 mg/kg, i.p.

Decline of plasma chloramphenicol. The concentration of unchanged chloramphenicol in the plasma was measured at 20, 30, 40, 50 and 60 min after its administration (Fig. 7). Fasted animals showed higher concentrations of unchanged chloramphenicol in the plasma than those seen in non-fasted controls at all but the 20-min time interval. Fasted animals receiving glucose showed significantly lower levels of unchanged chloramphenicol in the plasma than the fasted group receiving saline. When non-fasted animals were treated in a similar manner, no difference in the levels of unchanged chloramphenicol in plasma was seen between those receiving saline and those receiving glucose. Animals pretreated with *o*- and *p*-toluic acid showed significantly higher plasma levels of unchanged chloramphenicol than saline controls at all but the 20-min time interval (Fig. 8).

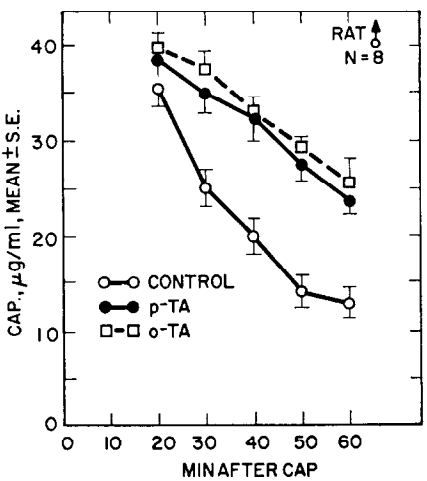

FIG. 8. Effect of *p*-toluic acid (p-TA) and *o*-toluic acid (o-TA) on the decline of plasma level of unchanged chloramphenicol (CAP). Chloramphenicol, 72 mg/kg, i.p., 45 min after p-TA or o-TA, 350 mg/kg, i.p.

Concentration of unchanged chloramphenicol and its metabolites in the plasma and liver. The concentration of unchanged chloramphenicol and its metabolites in the plasma and in the liver was measured 40 min after its administration (Table 2). Fasted animals showed significantly lower metabolite concentrations in the plasma and in the liver than those found in non-fasted controls. The levels of unchanged chloramphenicol in the plasma and in the liver were significantly higher in fasted animals when compared with those found in non-fasted animals. In fasted animals, glucose significantly reduced the levels of unchanged chloramphenicol and significantly increased the levels of chloramphenicol metabolites in the plasma and in the liver tissue in comparison with respective controls. Pretreatment with toluic acid isomers significantly lowered the concentration of chloramphenicol metabolites in the plasma and in the liver tissue in comparison with saline controls, while the concentration of unchanged chloramphenicol in the plasma and in the liver tissue was significantly higher in toluic acid-treated groups. Animals receiving *o*-toluamide produced no significant effect on the level of unchanged chloramphenicol or its metabolites in the plasma.

TABLE 2. EFFECT OF 18-HR FASTING OR PRETREATMENT WITH TOLUIC ACIDS AND *o*-TOLUAMIDE ON THE LEVELS OF UNCHANGED CHLORAMPHENICOL (CAP) AND CHLORAMPHENICOL METABOLITES (CAP-M), IN PLASMA AND LIVER TISSUE OF THE MALE RAT, MEASURED 40 min AFTER CHLORAMPHENICOL (72 mg/kg, i.p.)*

Experimental condition	*N*	Plasma (μg/ml, mean ± S. E.)		Liver (μg/g, mean ± S. E.)	
		CAP	CAP-M	CAP	CAP-M
Fasted	12	39·1 ± 1·1	9·0 ± 1·4	88·4 ± 4·2	21·4 ± 3·3
Non-fasted	12	29·6 ± 1·2†	16·0 ± 1·3†	51·0 ± 3·9†	38·0 ± 2·5†
Fasted	12	34·6 ± 2·2	7·7 ± 1·1	90·0 ± 3·1	18·0 ± 2·0
Fasted + glucose	12	25·2 ± 0·9†	15·1 ± 1·3†	60·4 ± 4·1†	28·0 ± 2·6†
Non-fasted	12	28·5 ± 1·4	17·7 ± 1·1	53·2 ± 4·4	30·7 ± 4·3
Non-fasted + *o*-toluic acid	12	43·0 ± 1·1†	10·9 ± 0·5†	105·0 ± 5·1†	12·6 ± 3·7†
Non-fasted + *p*-toluic acid	10	40·0 ± 1·2†	12·6 ± 1·2†	100·0 ± 4·3†	13·9 ± 3·2†
Non-fasted	8	23·8 ± 1·7	15·7 ± 2·0		
Non-fasted + *o*-toluamide	8	24·0 ± 2·7	13·9 ± 2·4		

* *o*-Toluic acid, *p*-toluic acid or *o*-toluamide, 350 mg/kg, i.p., 45 min prior to chloramphenicol. Glucose, 1·5 ml of 50 per cent solution/rat, i.p., 30 min prior to chloramphenicol.
† $P < 0·01$ when compared with respective controls.

TABLE 3. LEVELS OF UNCHANGED CHLORAMPHENICOL (CAP), CHLORAMPHENICOL METABOLITE (CAP-M), TOTAL CHLORAMPHENICOL (CAP-T) AND CHLORAMPHENICOL GLUCURONIDE (CAP-G) IN PLASMA AND LIVER TISSUE OF UNFASTED CONTROL RATS, MEASURED 40 min AFTER CHLORAMPHENICOL (72 mg/kg, i.p.)

Sample	Chloramphenicol equivalents (μg/ml or μg/g, mean ± S. E.) (*N* = 3)				
	CAP-T_1*	CAP	CAP-M	CAP-T_2†	CAP-G‡
Plasma	46·3 ± 2·6	27·0 ± 2·1	18·3 ± 0·9	42·3 ± 2·3	15·3 ± 0·3
Liver	91·7 ± 5·5	59·0 ± 3·2	32·7 ± 2·7	87·0 ± 5·7	28·0 ± 3·0

* CAP-T_1 = total chloramphenicol determined without ethyl acetate extraction.
† CAP-T_2 = total chloramphenicol determined after one 18-hr incubation with β-glucuronidase.
‡ CAP-G = chloramphenicol glucuronide calculated as the difference between CAP-T_2 and CAP.

Nature of chloramphenicol metabolite. The results obtained after incubation of control plasma and liver homogenate with β-glucuronidase are shown in Table 3. In the case of plasma, after one incubation with β-glucuronidase, 82 per cent of the metabolite fraction was liberated as chloramphenicol, while in the case of liver it was 86 per cent. Second incubation with the enzyme liberated additional free chloramphenicol (up to 10 per cent), but was not added to the levels reported because of the large variation in the values obtained. Based on this information, it was concluded that the metabolite measured by difference was predominantly chloramphenicol glucuronide.

DISCUSSION

Chloramphenicol-mediated inhibition of microsomal enzymes has previously been shown to be dose related and, therefore, may be further enhanced by increasing the

effective concentration of chloramphenicol at the site of its action. This implies that the microsomal inhibition due to chloramphenicol would change in intensity with changes in the rate of chloramphenicol decline from its site of action. A rapid rate of chloramphenicol metabolism would then be reflected in a reduction and a decreased rate in a potentiation of the prolongation of hexobarbital sleeping time. The present investigation was designed to determine whether the degree of chloramphenicol-mediated prolongation of hexobarbital sleeping time is influenced by factors that affect the plasma and the liver concentration of the antibiotic through alterations of its metabolic inactivation.

Several indices of chloramphenicol metabolism were used to obtain an estimate of the effective concentration of chloramphenicol available for microsomal enzyme inhibition. These indices were: (i) the decline of unchanged chloramphenicol from the plasma; (ii) the concentration of unchanged chloramphenicol and its metabolites in the plasma; and (iii) the concentration of unchanged chloramphenicol and its metabolites in the liver. The changes in these indices are used to explain the effect of various experimental conditions designed to alter glucuronide conjugation on the chloramphenicol-induced prolongation of hexobarbital sleeping time.

As has been shown by earlier investigators,[1,11] the results obtained in this study demonstrated that, in the rat, chloramphenicol is mainly metabolized by glucuronide conjugation. The present studies also showed that fasting significantly increased the plasma level of unchanged chloramphenicol and this difference was maintained as long as 60 min after chloramphenicol administration. If this higher level of chloramphenicol was due to reduction in glucuronide conjugation of chloramphenicol, it should reflect in reduction in the plasma level of the chloramphenicol metabolites, almost all of which is chloramphenicol glucuronide. Actually, this was found to be true. Further, in the liver tissue, the site of microsomal enzyme inhibition by chloramphenicol, the concentration of unchanged chloramphenicol was significantly higher and that of chloramphenicol metabolites significantly lower in fasted animals when compared to non-fasted controls. The significant potentiation of chloramphenicol-mediated prolongation of hexobarbital sleeping time observed in fasted rats is most probably due to this increased availability of unchanged chloramphenicol for a longer period of time, producing prolonged inhibition of microsomal enzymes. When chloramphenicol was replaced by thiamphenicol, an analog of chloramphenicol which is not conjugated with glucuronic acid to any significant extent,[11,12] fasting did not modify the prolongation of hexobarbital sleeping time beyond that produced in controls. These results with thiamphenicol further strengthen the conclusion that fasting produces its effect on chloramphenicol by altering glucuronide conjugation of chloramphenicol.

Short-term fasting has previously been shown to reduce glucuronide conjugation. One or more of the following factors may be responsible for this effect: (i) a decrease in UDP-glucose dehydrogenase activity;[13] (ii) inhibition by glucose 1-phosphate of glucose 1-phosphate pyrophosphorylase;[14] (iii) reduction in the glucuronyl transferase activity associated with glycogen depletion; and (iv) a change in the $NAD^+/NADH$ ratio in the direction of reduction so as to reduce the amount of UDP-glucuronic acid (UDPGA) made available for conjugation.[15]

Glucose administration to fasted rats restored the profile of plasma concentration–time curve back to the one seen in non-fasted animals. The plasma and liver concen-

trations of unchanged chloramphenicol in these animals were significantly lower than those seen in fasted rats at corresponding intervals after chloramphenicol administration. At the same time, metabolite concentrations in the plasma and in the liver were higher in rats receiving glucose. This decrease in the availability of unchanged chloramphenicol for the microsomal enzyme inhibition is reflected in the hexobarbital sleeping time. Chloramphenicol-induced prolongation of hexobarbital sleeping time in fasted rats given glucose is not significantly different from that observed in non-fasted animals. The exact mechanism by which glucose increases glucuronidation in fasted animals is not known, but it may be due to an increased availability of glucose for the synthesis of UDP-glucose. When glucose was administered to non-fasted rats, it had no significant effect on the decline of chloramphenicol level from plasma. So also, glucose administration to *o*- and *p*-toluic acid-pretreated animals did not restore the profile of the plasma concentration–time curve back to the one seen in control animals.

Glucuronide conjugation has been shown to be saturable or "capacity limited".[16,17] This means that administration of an "alternate substrate" for the glucuronyl transferase might result in decreased conjugation of the primary substrate. This might be due to a competition for UDPGA or for the active sites of the transferase. Both *o*- and *p*-toluic acids undergo glucuronidation to a significant extent in the rabbit, the dog, and as shown in the present study, also in the rat. Administration of the toluic acid isomers resulted in significantly higher plasma concentrations of unchanged chloramphenicol at several time intervals after its administration. It also produced significantly lower plasma levels of the chloramphenicol metabolites, almost all of which is glucuronide. Higher levels of unchanged chloramphenicol and lower levels of metabolites were also found in the liver tissue. This increased availability of free chloramphenicol for the inhibition of microsomal enzymes in animals treated with either of the toluic acid isomers was reflected in the potentiation of chloramphenicol-mediated prolongation of hexobarbital sleeping time. When chloramphenicol was replaced by thiamphenicol, pretreatment with *o*- or *p*-toluic acid did not modify the prolongation response. Further, when the toluic acid isomers were replaced by the amide derivative that does not have the carboxyl moiety available for glucuronide conjugation, levels of unchanged chloramphenicol and its metabolites in the plasma were not significantly different from control levels. Results obtained with thiamphenicol and with *o*-toluamide strengthen the conclusion that the toluic acids produce their effect on chloramphenicol metabolism and on chloramphenicol-induced prolongation of hexobarbital sleeping time by reducing chloramphenicol glucuronide conjugation.

The implication of the studies presented here is that, at a given dose, the intensity of chloramphenicol-mediated prolongation of hexobarbital sleeping time is dependent, at least in part, on the glucuronide conjugation of chloramphenicol. Glucuronide conjugation can be reduced by conditions of short-term fasting or by administration of substances which act as alternate substrates for glucuronyl transferase, and this indirectly can potentiate the microsomal enzyme inhibition well beyond that produced by chloramphenicol alone. These findings suggest that the intensity of inhibition of liver microsomal enzymes due to chloramphenicol may be difficult to assess in uncontrolled situations.

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