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THE SALIVARY SECRETION AND CLEARANCE IN THE HORSE OF CHLORAL HYDRATE AND ITS METABOLITES

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Abstract—The salivary secretion of chloral hydrate and its metabolites in the horse have been studied. The concentration of chloral hydrate and trichlorethanol in saliva approximated to that in the plasma, but saliva levels of trichloracetic acid were much lower than plasma levels.

Chloral hydrate and trichlorethanol were quickly absorbed after oral administration. About 60 per cent of the dose of chloral hydrate was excreted in the urine as the glucuronide of trichlorethanol; traces of free trichlorethanol and trichloracetic acid were also detected. After chloral hydrate administration the main plasma constituents were trichlorethanol, trichloracetic acid, and chloral hydrate. Trichloracetic acid persisted longest in the plasma. Chloral hydrate could only be detected in the plasma for four or five hours after its administration whereas the other two metabolites persisted for much longer.

Methods for the determination of trichlorethanol and chloral hydrate by gas–liquid chromatography using electron capture have been described. Trichloracetic acid was measured using the Fujiwara reaction.[6]

THE SECRETION of drugs into the saliva of the horse has received little attention despite the widespread practice of collecting saliva for drug screening procedures. In this work ponies with parotid fistulae were used to investigate the salivary secretion of chloral hydrate and its metabolites. At the same time the excretion of these compounds in the urine was studied.

The fate of chloral hydrate in the dog has been investigated by Butler[1] and subsequently by Marshall and Owens[2] who extended their observations to man. The latter authors showed some interesting differences in the metabolism of chloral hydrate between man and dog, and it seemed probable therefore that some differences might exist in the way in which this drug was metabolized by the horse.

MATERIALS AND METHODS

Animal experiments

1. *Chloral hydrate.* Two ponies of 150 kg weight were each given 25 g of chloral hydrate by stomach tube. Samples of blood were taken from the jugular vein at fixed intervals into heparin and separated immediately by centrifugation. Samples of saliva and urine were taken as and when these fluids were passed. Parotid saliva only

flows during mastication.[3] The methods for the collection of urine and parotid saliva have been described.[3]

2. *Trichlorethanol.* 20 g of trichlorethanol was administered with water by drenching gun to a 220 kg gelding. Urine was collected in this experiment by the method of Clabby *et al.*[4]

Samples of body fluids from the chloral hydrate experiments were immediately deep-frozen and transported from Edinburgh to Newmarket in solid carbon dioxide, in specially made insulated containers.

Analysis

Drugs and metabolites were extracted from body fluids with ether and concentrations measured using gas–liquid chromatography with electron capture for trichlorethanol and chloral hydrate. For trichloracetic acid, the method of Friedman and Cooper,[5] based on the Fujiwara reaction[6] was employed. Urochloralic acid (trichlorethanol β-glucuronide) was hydrolysed enzymatically and the liberated trichlorethanol estimated.

Extraction

All samples except cells were made strongly acid with hydrochloric acid, saturated with ammonium sulphate, and extracted at least 3 times with half volumes of ether. The final extract was checked for completeness of extraction by gas–liquid chromatography. Plasma samples were usually diluted with an equal volume of water to keep them fluid. To avoid emulsions, solvent and body fluids were rotated in 15 ml stoppered tubes on a Matburn blood mixer, at an angle of about 8° to the horizontal for 15 min at 30 rpm.

Blood cells were extracted five times with half volumes of ether. No pH adjustment or ammonium sulphate saturation was used.

To separate trichloracetic acid from trichlorethanol and chloral hydrate, the ether extract was extracted with N/2 sodium hydroxide, and this exhaustively extracted with ether. The aqueous solution remaining was used for the determination of trichloracetic acid.

Urochloralic acid was hydrolysed (β-glucuronidase (Seravac) 2000 Fishman units/ml urine, pH 4·5, 18 hr at 45°) and the liberated trichlorethanol extracted and determined. The enzyme contained some sulphatase activity.

Gas chromatography

Instrument:	Pye Panchromatograph
Detector:	Electron Capture
Detector voltage:	15 V (giving full scale deflection with a signal of 3×10^{-10} A).
Detector temp:	180°
Column:	5′ 10% polyethylene glycol adipate on celite, 100/120 mesh.
Column temp:	175°
Carrier gas:	Nitrogen, dried with Linde molecular sieve (Type 5A).
Flow rate:	25·5 ml/min (column pressure 300 mm Hg).
Retention times:	Ether = 54 sec, trichloracetic acid = 67 sec, chloral

hydrate = 74 sec, trichlorethanol = 199 sec, hexachlorbutadiene = 132 sec.

Relative response for equi-molar quantities: Chloral hydrate = 1·0, trichlorethanol = 0·25, trichloracetic acid = 0·04, hexachlorbutadiene = 0·6.

Final ether extracts were diluted so that the compound being measured and the interal standard (hexachlorbutadiene) each gave about 50 per cent full-scale deflection when 10 μl. were injected using a Hamilton syringe. Mean ratios of peak heights were used for calculation, several aliquots being used for each measurement. Standard mixtures giving peak heights within ± 5 per cent of the solution for measurement were injected alternately between injections of extracts.

In some extracts, the concentration of compounds to be measured was too low to allow dilution with interal standard. In such cases, constant volumes were injected and calculations were based on absolute peak heights. A standard peak height curve was prepared at the same time, which was accurate to within ± 1 per cent.

Under these conditions, a single analysis took 4 min, except for the urine extracts, for which it was necessary to allow purging of normal constituents with retention times of 9 and 12 min respectively.

As an approximate check on the quantity of urochloralic acid, total urine glucuronic acid was measured by the method of Nir.[7]

Reagents

All reagents used were analytical grade with the exception of ether, which was Howard's Anaesthetic ether, and pyridine which was Hopkin and Williams' G.P.R. Chloral hydrate was B.P. grade; it was free from trichlorethanol, but contained 5·6 μg/gm trichloracetic acid. Trichlorethanol was free from chloral hydrate and trichloracetic acid.

RESULTS

The dose of chloral hydrate was sufficient to make the ponies inco-ordinate, and for $\frac{1}{2}$–1 hr after dosing they were unable to stand unsupported. The dose of trichlorethanol caused slight inco-ordination.

Table 1 shows the plasma concentrations of trichlorethanol and trichloracetic acid after the oral administration of trichlorethanol. A trace of chloral hydrate was detected in the plasma during the first hour after administration. Tables 2 and 3 show the results of urine analysis following a single dose of chloral hydrate and one of trichlorethanol respectively. However, in a preliminary experiment in which urine samples were not frozen in transit, 30 per cent of the trichlorethanol was found to be unconjugated. The presence of free trichlorethanol may thus be due to hydrolysis of the conjugate after excretion. No chloral hydrate was found in the urine at any time. The increase in the amount of glucuronic acid excreted was 10–20 per cent higher than could be accounted for by the amount of trichlorethanol conjugated.

Plasma and saliva concentrations of chloral hydrate, trichlorethanol and trichloracetic acid following chloral hydrate administration are shown in Fig. 1(a), (b) and (c).

The concentration of chloral hydrate and trichlorethanol in plasma and blood cells was approximately equal. Trichloracetic acid levels were not measured in cells, as no reliable method could be found.

TABLE 1. PLASMA CONCENTRATIONS OF TRICHLORETHANOL AND TRICHLORACETIC ACID AFTER ORAL ADMINISTRATION OF TRICHLORETHANOL

Time after administration of approximately 20 g trichlorethanol (hr)	Trichlorethanol (γ/ml plasma)	Trichloracetic acid (γ/ml plasma)
0	0	0
0·25	80·1	0·82
0·5	43·6	1·14
0·75	21·6	1·46
1·2	15·0	1·61
1·7	9·2	2·06
2·1	6·7	2·42
4·2	2·6	2·82
7·0	1·2	2·35
25·5	0	1·93
49·75	0	0·93

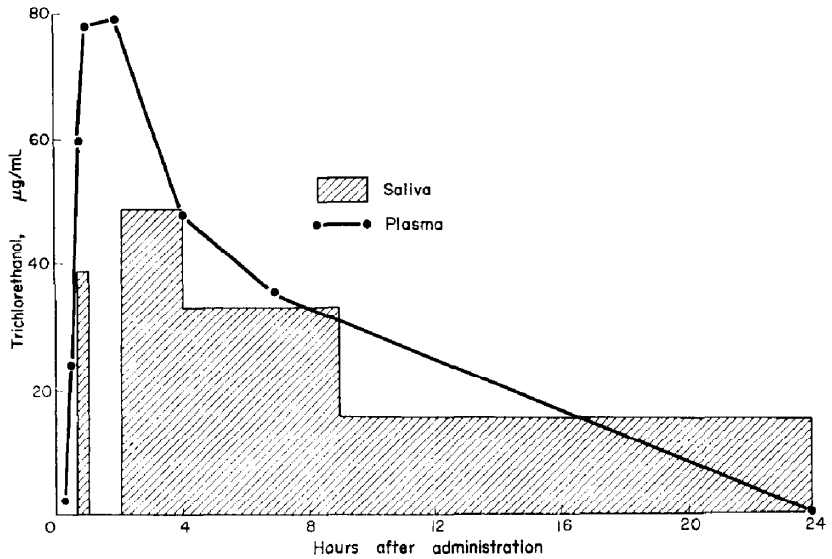
TABLE 2. URINARY EXCRETION OF TRICHLORETHANOL AND TRICHLORACETIC ACID AFTER ORAL ADMINISTRATION OF CHLORAL HYDRATE

Time after administration of 25 g chloral hydrate (hr)	Volume of urine produced (L)	Amounts excreted (mg)				Urochloralic as % of total trichlorethanol excreted
		Free in urine		Total (after β-glucuronidase hydrolysis)		
		Trichlorethanol	Trichloracetic acid	Trichlorethanol	Trichloracetic acid	
28·5–29·5	0·820	20·4	3·4	10100	3·4	99·8
32–48	2·120	44·8	2·9	4280	2·2	99·0
Total 0–48	2·940	65·2	6·3	14380	5·6	

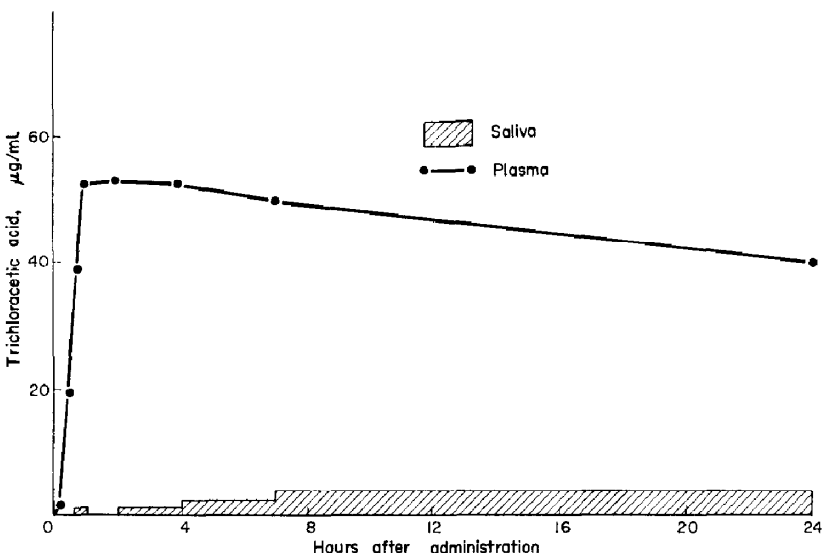
Recovery of all three metabolites in the urine was equivalent to 63·7 per cent of the administered chloral hydrate.

TABLE 3. URINARY EXCRETION OF TRICHLORETHANOL AFTER ORAL ADMINISTRATION OF TRICHLORETHANOL

Time after administration of approximately 20 g trichlorethanol (hr)	Volume of urine produced (L)	Amounts of trichlorethanol excreted (mg)		Urochloralic acid as % of total trichlorethanol excreted
		Free in urine	Total (after β-glucuronidase hydrolysis)	
minus 1·5	2·175	0	0	—
3·5–4	1·076	7·7	4900	99·8
7·5–22·5	2·320	7·4	3280	99·8
34	0·820	2·1	143	98·5
34–46·5	0·758	1·2	52	97·7
55·5	0·820	3·2	25	87·2
55·5–70·5	1·317	5·1	22	76·8
Total 0–70·5	9·286	26·7	8422	



1 (a)



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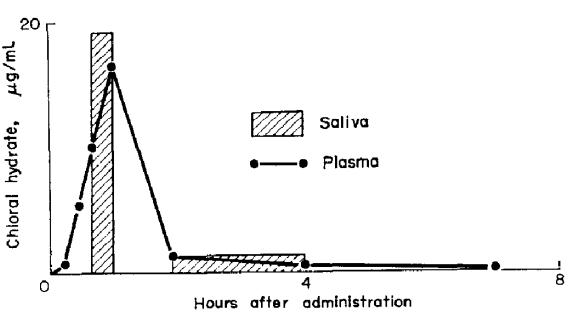

1 (c)

FIG. 1. Plasma and saliva concentrations of (a) trichlorethanol, (b) trichloracetic acid after oral administration of chloral hydrate (6 g/kg), and chloral hydrate.

DISCUSSION

Saliva samples from horses are used extensively for the control of 'doping', but there is little information from controlled experiments in this animal designed to evaluate this secretion as a suitable material to reflect the presence of a drug.

From investigations into the salivary secretion of drugs in ruminants Rasmussen,[8] claims that drugs pass the plasma/saliva barrier only in the unionized form. The quantity of drug diffusing from plasma to saliva therefore depends upon its degree of ionization in these body fluids, and thus upon their pH. Distribution ratios between saliva and plasma for drugs with pK values approximating to physiological pH values would thus be critically dependent upon saliva pH. Drugs not ionizing at these values would tend to equilibrate in a ratio of 1:1, whilst ionic forms of drugs would be retained in plasma. Other investigations[9, 10] do not conflict with this theory.

However, in our investigations, although the non-ionic chloral hydrate and trichlorethanol reached concentrations in saliva approximating to those in plasma, an additional mechanism must operate since some salivary secretion of trichloracetic acid (pK = 0·7) occurred.

It seems likely that drugs fully ionized at physiological pH values would behave in a manner similar to other electrolytes, and their levels be influenced by saliva flow rate:[3] this was not measured in these experiments.

Urochloralic acid is well established as the main product of chloral hydrate metabolism in man, dog, and rabbit[11, 12] More recent work by Butler[1, 13] and Marshall and Owens[2] showed that in both dog and man chloral hydrate was converted into trichlorethanol and trichloracetic acid and that there was a quantitative difference between the amounts of these respective metabolites produced by the two species. Studies on isolated tissues from rats and dogs[14] showed that reduction of chloral hydrate to trichlorethanol occurred in all tissues examined. Comparing the results of the experiments described here with those of Marshall and Owens,[2] the peak plasma concentration of trichlorethanol occurred soonest in man, a little later in the dog, and was slowest in the horse, taking place in this species one or two hours after the oral administration of chloral hydrate. This peak occurred at about the same time as the maximum concentration of blood sugar occurs after the oral administration of glucose to the horse[15] and possibly the absorption of chloral hydrate follows a similar pattern. In all three species trichloracetic acid was very slowly cleared from the plasma. This can most readily be explained in terms of binding with plasma proteins. Marshall and Owens[2] showed that 70–90 per cent of the trichloracetic acid was bound to protein and that the lower the concentration of trichloracetic acid, the greater the proportion bound. These authors also found that 16–35 per cent of the dose of chloral hydrate given to man could be accounted for in the urine as urochloralic acid; while Butler[14] quotes similar estimates for the dog as 40–60 per cent.

The experiments described here show that the horse is similar to the dog in that about 60 per cent of the chloral hydrate administered can be accounted for as urochloralic acid, and this is excreted within 48 hr, the greater part of the excretion taking place in the first 24 hr.

Although trichloracetic acid is present in plasma at high levels, it does not appear in the urine except in trace amounts; presumably this compound might be metabolized by the tissues in some way which involves dehalogenation. Considerable evidence exists for such a mechanism. For example, trichlorethylene is metabolized to

trichloracetic acid, trichlorethanol, and inorganic chloride,[16] carbromal is partially converted to ethyl butyryl urea[17] and carbon tetrachloride is partially converted to chloroform.[18] However, Bartonicek[19] has reported that after trichlorethylene administration some trichloracetic acid and trichlorethanol is excreted in the faeces. It is possible, therefore, that these compounds are to some extent excreted into the digestive tract of the horse.

Although these experiments confirmed the necessity for urine samples in the detection of 'doping', determination of the concentration of the drug in blood could give additional information by indicating the approximate time of administration. The present work showed that in the case of chloral hydrate, determination of the salivary concentration of trichlorethanol reflected the concentration of this metabolite in blood. This secretion, therefore, could be substituted for blood for forensic purposes in the detection of 'doping' by chloral and possibly other drugs.

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