Inhibition of True Cholinesterase in TOCP Poisoning with Potentiation by 'Tween 80'

SINCE the work of Earl and Thompson[1,2] on hens, and the brief note by Mendel and Rudney[3] on rats, it has often been accepted[4,5] that in tri-ortho cresyl phosphate (TOCP) intoxication, pseudocholinesterase is markedly inhibited but that there is little or no decrease in true cholinesterase. A decrease in red blood cell cholinesterase has, however, been reported in rabbits[6] and a slight fall in man[7] after treatment with TOCP by mouth, and Taylor[8] has commented on parasympathomimetic effects in cats injected with this compound. I have investigated changes in cholinesterase in baboons after oral doses of TOCP. Monkeys have been found to be very resistant to TOCP by mouth[9,10] and it has been suggested the monkeys do not absorb it from the gut. In addition to pure TOCP therefore a 10 per cent emulsion in 10 per cent 'Tween 80' has also been tried in the present work at the suggestion of Dr J. D. Taylor of the Department of Pharmacology, University of Alberta. He has found this emulsion more active than the pure substance (personal communication from J. D. Taylor). The results to be described show that after administration of either preparation to the baboon, pseudocholinesterase is very readily inhibited, but that marked inhibition of true cholinesterase also occurs. In the reports of TOCP poisoning in man after accidental ingestion, diarrhoea and abdominal pain have often been noted and have sometimes been severe[11]. These effects would be expected to accompany inhibition of true cholinesterase.

The effects of pure TOCP were not uniform. Baboons *F* and *J* developed moderate diarrhoea within 24 h and this persisted for 48–72 h. Baboon *F* had slight limb weakness during this time, but neither it nor baboon *J* showed fasciculation and neither was seriously ill. Baboon *L*, which also showed the most marked changes in cholinesterase, was much more severely affected and was moribund when killed 7 days after poisoning. Diarrhoea was very severe, body weight falling by 20 per cent in the first 24 h and the skeletal muscles showed widespread coarse fasciculation and weakness. Fasciculation ceased after 4 days, but weakness persisted until death.

The effects of emulsified TOCP showed less variation, and were greater than those of the pure compound, despite the fact that the dose was reduced by 60 per cent (see Table 1). Baboons *H* and *K* both developed diarrhoea, severe muscle weakness and fasciculation. Baboon *G* suffered only slight anorexia after the first dose, given at the same time as atropine, but after the second was affected as severely as *H* and *K*. 'Tween 80' alone had no effect on concentrations of cholinesterase even in a large dose. The potentiating effect of 'Tween 80' could be caused by increased intestinal absorption of TOCP. Future toxicity testing of other aryl phosphates used in industry should perhaps take account of this.

The effect of atropine on baboon *I* was unexpected. Atropine was given at the same time as the emulsified TOCP, and 24 h later the animal was well except for a little anorexia. Table 1 shows that true cholinesterase fell much less sharply than in baboons *H* and *K*, and so the well-being of the animal was probably not caused only by blocking the parasympathomimetic effects of acetylcholine excess. Possibly atropine, given in large dose at the same time as the poison, delays or reduces intestinal absorption.

Table 1. VALUES FOR CHOLINESTERASE IN RED BLOOD CELLS, MUSCLE, AND PLASMA BEFORE AND AFTER TOCP AND 'TWEEN 80'

Baboon	Weight (kg)	Dose TOCP (ml./kg)	'Tween 80' (ml./kg)	Atropine (mg intramuscular)	Red blood cell cholinesterase + MCh (μl. CO_2/ml./min)			Muscle cholinesterase + MCh (μl. CO_2/g/min)			Plasma cholinesterase + BuCh (μl. CO_2/ml./min)		
					Normal	Poisoned	Decrease (%)	Normal	Poisoned	Decrease (%)	Normal	Poisoned	Decrease (%)
J	11·4	1	—	—	9·0	4·46	50·4	9·8	3·8	61·2	165	11·3	93·2
L	12·05	1	—	—	13·0	1·39	89·3	4·89	1·94	61·0	218	10·9	95
F	11·1	1	—	—	9·88* *SD* 1·89	5·43	45·0	—	—	—	198* *SD* 43	2·13	98·9
K	8·55	—	22	—	10·1	11·1	9·9 (incr.)	—	—	—	204	199	2·5
K	8·55	0·4†	3·6	—	11·3	1·04	90·8	8·53	1·60	81·2	193	2·67	98·6
H	11·5	0·4	3·6	—	9·5	1·13	88·1	6·87	1·07	84·4	175	5·0	97·1
G	10·55	0·1 0·3‡	0·9 2·7	5 —	7·12	3·25 0·325	54·4 95·4	— 5·94	— 0·24	— 96·0	193	6·93 9·4	96·4 95·1
I	11·2	0·4	3·6	5	9·17	4·33	52·8	—	—	—	241	17·9	92·6

* Normal values not available for this animal. Figures are the mean and standard deviation of the other normal values in the table.
† Given 5 weeks after the dose of 'Tween 80' above.
‡ Given 24 h after the first dose.

The weights of the animals, the dose given to each and the corresponding concentrations of cholinesterase are shown in Table 1. All were sexually mature females, of species *P. papio* and *P. anubis*. The preparations of TOCP provided by Drs H. F. Bondy and K. R. Payne of Coalite and Chemical Products, Ltd., were at least 99 per cent pure; they were made from ortho-cresol, in which phenol, about 0·5 per cent, was the only identifiable impurity. They were given by gastric tube under light phencyclidine anaesthesia. Cholinesterase was estimated with a conventional Warburg manometric apparatus[2] with butyrylcholine perchlorate (BuCh) and acetyl β-methylcholine chloride (MCh) (Koch-Light) as substrates for pseudo and true cholinesterase respectively. Red blood cells were separated from plasma by centrifuging heparinized blood, washed three times in 0·88 per cent sodium chloride, and finally lysed by adding distilled water up to the volume of the original blood sample. For estimation of muscle cholinesterase, *M. palmaris longus* was washed free of obvious blood in 0·88 per cent sodium chloride, lightly blotted, dissected free of connective tissue, and minced. The mince was then homogenized in 0·25 molar sodium bicarbonate to give 1 g of mince/4 ml. of homogenate. Blood and muscle were taken before and 21–27 h after poisoning, each animal thus providing its own control.

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