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I.C.I. 63,197

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INDEX

I N D E X

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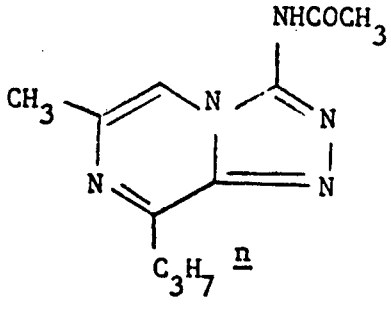
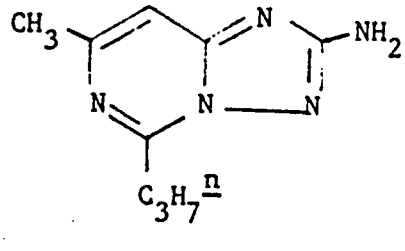
Tables

1. INTRODUCTION

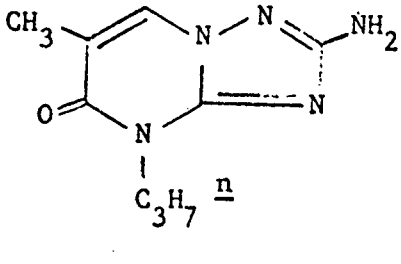
I.C.I. 63,197 is related to I.C.I. 30,966 and also to I.C.I. 58,301. I.C.I. 30,966 underwent clinical trial about nine years ago. Although it appeared to exert a bronchodilator effect in asthmatic patients, it was withdrawn from trials because it caused nausea. A submission to the Committee on Safety of Drugs has been made for I.C.I. 58,301 (CSD/29/61) which has a similar potency to I.C.I. 30,966 in preventing bronchospasm in guinea-pigs, but was tolerated by dogs and monkeys at higher doses without causing vomiting.



I.C.I. 58,301



I.C.I. 30,966



I.C.I. 63,197

I.C.I. 63,197 appears to be much more potent as an anti-bronchoconstrictor than either of the two compounds mentioned above, but, in addition to anti-bronchoconstrictor and anti-allergic activities, it shows a wide variety of activities in tests involving the central nervous system.

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Figures

2. C.N.S. PHARMACOLOGY

2.01 Introduction

Drugs used in the treatment of mental diseases affect experimental animals in various detectable ways. Each of these drugs produces a different profile of action in animals. There is no obvious relationship between the effects produced by the drugs on animals and the diseases for which they are prescribed. Some of the drugs produce only subjective changes in man, and it is difficult, if not impossible, to detect such changes in animals. It is, therefore, necessary to compare the activity of test compounds with that of standard psychotropic drugs in a series of animal tests. I.C.I. 63,197 has been compared with standard drugs in such tests in an attempt to predict its possible actions in man.

2.02 Effect on the agility of mice

Most tranquillisers are known to affect the ability of mice to balance on fixed or rotating rods.

2.021 Stationary rod

Groups of six mice were used in each experiment. One hour after dosing they were placed on a stationary horizontal metal rod just over 1 cm. in diameter, and some 40 cm. in length. The time at which they fell from the rod was measured if shorter than 30 seconds. The procedure was repeated three times more, except when the mouse walked the full length of the rod or it did not fall in 30 seconds. If this happened, it was not replaced on the rod. The "score" was obtained by subtracting from 30 the figures obtained, adding up these differences and dividing by 6 (the number of mice used). The score is biased in favour of the mice which walked the full length of the rod or did not fall within 30 seconds. A score of -30 means that the mice stayed on the rod for 30 seconds, or walked its whole length. A score of 120 means that they fell at once.

Table 1. Effect of I.C.I. 63,197 on the time that mice stay on a fixed rod

mg./kg.	100	30	10	3	1
I.C.I. 63,197					
Mean score	83	24	10.8	- 6.8	- 16
No. experiments	3	3	5	6	3
Standard error	± 8.6	± 5.2	± 7.0	± 7.0	± 10.0
Chlorpromazine					
Mean score	-	113	70.1	4.9	-
No. experiments		6	27	11	
Standard error		± 4.5	± 6.1	± 4.9	
Haloperidol					
Mean score	-	89.7	37	17	11
No. experiments		4	6	17	15
Standard error		±20	± 5.8	± 5.3	± 5.1
Chlordiazepoxide					
Mean score	-	47	15		
No. experiments		16	9		
Standard error		± 7.6	± 5.8		

The figures refer to the mean score obtained.

The score for 15 control experiments was -13 with a standard error of 3.2.

These results show that I.C.I. 63,197 at 100, 30 and 10 mg./kg. decreased the agility of mice as measured by the time that they balance on a stationary rod. The active dose is comparable to, but somewhat higher than that of haloperidol, chlorpromazine or chlordiazepoxide.

2.022 Rotating rod

Groups of twelve mice were used in each experiment. Half an hour after being dosed orally they were placed on a metal rod 2 cm. in diameter which rotated five times each minute. The time that the mice remained on the rod was measured with a maximum of thirty seconds.

Table 2. Effect of I.C.I. 63,197 on the time that mice stay on a rotating rod

mg./kg. I.C.I. 63,197	10	3	1
% inhibition	56	41	20
Standard error	± 14	± 5	± 11
No. of animals	24	48	48

The figures are the % inhibition relative to control calculated from the expression $\left(\frac{\text{control - treated}}{\text{control}}\right) \times 100$.

These results confirm those obtained using a fixed rod. They show that I.C.I. 63,197 at doses within the range of 1 - 10 mg./kg. decreases the ability of mice to stand on a rotating rod.

2.03 Effect on the motility of mice

Many of the drugs used as sedatives are known to decrease the motility of mice.

2.031 Chloride transfer

The apparatus consisted of two square toffee jars, placed mouth to mouth on their sides. One jar contained a layer of physiological saline 0.5 cm. deep, the other a similar depth of water (see Figure 1).

Groups of six mice were used in each experiment. Half an hour after dosing by stomach tube, they were placed in the jar containing saline. After a further hour, the amount of chloride that the mice had transferred to the jar with water was estimated chemically. This gives a measure of the number of times that the mice had passed from one container to the other before settling in the dry space provided by the mouths of the jars.

The results are set out in Table 3.

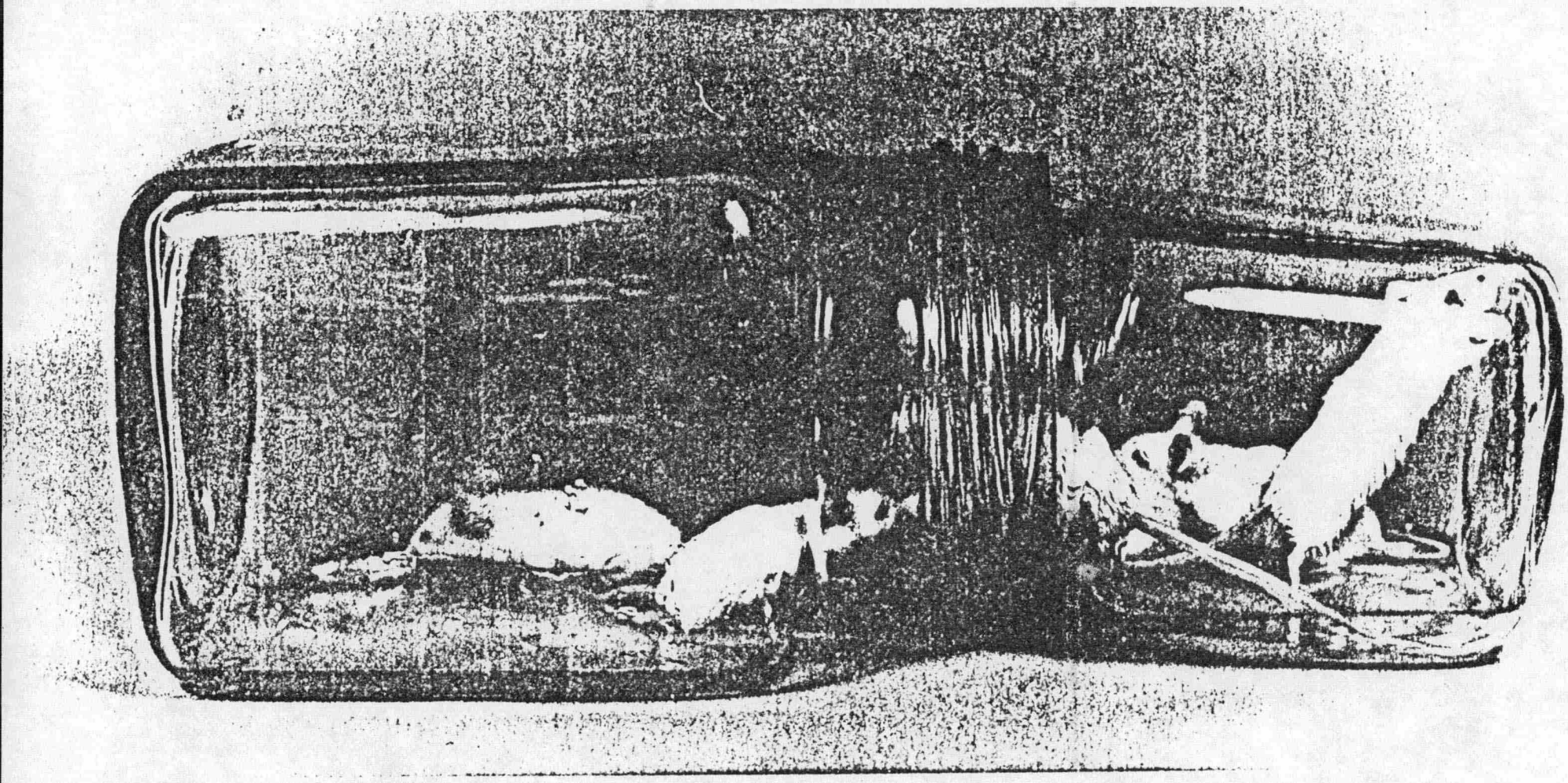


Figure 1 Effect of I.C.I. 63,197 on the motility of mice by the chloride transfer method.

Table 3. Effect of I.C.I. 63,197 on the motility of mice as measured by the chloride transfer method

mg./kg.	100	30	10	3	1	0.3	0.1
I.C.I. 63,197							
% inhibition	-	-	51.4	50.0	34.5	-	-
Standard error	-	-	±9.8	±7.5	±10.2	-	-
No. experiments	-	-	6	6	6	-	-
Chlordiazepoxide							
% inhibition	-	83.7	10.5	-3.7	-	-	-
Standard error	-	±10.4	±6.4	±12	-	-	-
No. experiments	-	9	16	18	-	-	-
Chlorpromazine							
% inhibition	-	-	67.0	12.2	13.1		
Standard error	-	-	±2.6	±9.3	±11.1		
No. experiments			26	18	6		
Haloperidol							
% inhibition	-	-	86.0	64.1	54.8	26.8	-22.3
Standard error			±2.4	±4.3	±5.3	±9.5	±15.1
No. experiments			8	20	12	8	8

The figures refer to % inhibition relative to control calculated from the expression $\left(\frac{\text{control - treated}}{\text{control}}\right) \times 100$.

These results show that I.C.I. 63,197 (1.0 - 10 mg./kg.), chlorpromazine (1.0 - 10 mg./kg.), haloperidol (0.3 - 10 mg./kg.) and chlordiazepoxide (3 - 30 mg./kg.) decrease the motility of mice.

2.032 Motility of mice in photobeam cages

Three mice were placed in each photobeam cage half an hour after dosing and the counters which record the interruption of the beam of light started. Most of the movement of the mice took place in the first half hour at the end of which the counters were read. The number of beam interruptions is expressed as a percentage of the number of interruptions which occurred with mice dosed with the dispersing fluid only.

Table 4. Effect of I.C.I. 63,197 on the number of interruptions in a photobeam cage produced by mice

I.C.I. 63,197 mg./kg. oral	1	0.3	0.1
% inhibition	66.2	45.5	25.0
Standard error	±3.05	±4.61	±4.6
No. of animals	24	24	18

The figures refer to the % inhibition relative to control calculated from the expression $\left(\frac{\text{control - treated}}{\text{control}}\right) \times 100$.

These results confirm that I.C.I. 63,197 decreases the motility of mice.

2.04 Effect on the temperature of mice

Many of the major tranquillisers lower body temperature.

Groups of six mice were used in each experiment. Their gastric temperature was measured. They were then dosed by stomach tube and one hour later their temperature again measured.

Table 5. Effect of I.C.I. 63,197 on the temperature of mice

mg./kg.	10	3
I.C.I. 63,197		
Temperature decrease	3.3	1.3
No. of experiments	6	3
Standard error	±0.33	±0.4
Chlorpromazine		
Temperature decrease	3.9	0.9
No. of experiments	20	28
Standard error	±0.34	±0.18
Haloperidol		
Temperature decrease	3.1	1.0
No. of experiments	26	16
Standard error	±0.26	±0.20

The figures refer to the average decrease of temperature expressed in °C.

The temperature of control mice varied by less than 0.1°C.

These experiments show that at comparable doses, I.C.I. 63,197 shares with chlorpromazine and haloperidol the property of decreasing the temperature of mice.

2.05 Effect on the temperature of reserpinised mice

Many drugs used in the treatment of depression counteract the temperature decrease produced in mice by reserpine.

Four groups of six mice were used in each experiment. Two groups were tested each day. The animals were given a subcutaneous injection of reserpine 2 mg./kg. late in the afternoon of the day prior to the test. They were kept in a constant temperature room at 20°C. Next morning the gastric temperatures of the mice were measured and then they were dosed by stomach tube. The temperatures of the mice were one or two °C. higher than that of the room. The temperatures of the mice were taken again four hours after dosing.

Table 6. Reversal of reserpine hypothermia by I.C.I. 63,197. Increase of the temperature of mice in °C. relative to control

mg./kg.	10	3	1	0.3	0.1
I.C.I. 63,197	5.7	6.2		4.3	3.1
Standard error	±0.5	±0.6		±0.45	±0.3
No. of animals	24	24		24	48
Imipramine	6.2 (39)	3.4 (47)	1.5 (31)	1.0 (4)	-
Desmethyl Imipramine	8.9 (2)	5.2 (11)	3.5 (20)	2.2 (8)	1.6 (4)
Amitriptyline	6.6 (4)	6.5 (7)	3.3 (11)	2.3 (5)	0.8 (2)

The figures represent the average increase in the temperature of the mice in °C. relative to that of controls which were dosed with the dispersing fluid only. Figures in parenthesis are the number of experiments.

This shows that the temperature of mice made hypothermic by reserpine rises when they are treated with I.C.I. 63,197 at doses above 0.1 mg./kg. This dose is lower than, but of the same order as that required of the tricyclic anti-depressant drugs to produce this effect.

2.06 Effect on monoamine oxidase (MAO)

Since the reversal of reserpine-induced hypothermia may be brought about by MAO inhibitors, I.C.I. 63,197 was tested for its effect on MAO.

Principle :

The method of Krajl (Biochem. Pharmacol., 1965, 14, 1683) was used. Kynuramine was the substrate and the intermediate aldehyde formed by MAO was rearranged in the presence of sodium hydroxide to the highly fluorescent 4-hydroxy-quinoline. Drug was added to controls and blanks after incubation in order to compensate for possible quenching or native fluorescence.

Method :

Incubation mixture :-

Phosphate buffer 0.5 M pH 7.4	100 µl.
Enzyme (5% w/v homogenate of rat liver in H_2O)	100 µl.
Water or inhibitor	200 µl.
Kynuramine dihydrobromide (200 µg./ml.)	100 µl.

Enzyme and inhibitor were pre-incubated for 5 min. before adding substrate. Incubation was continued for 10 min. at 37° with shaking in air and the reaction then stopped by adding 0.5 ml. 10% trichloracetic acid. After cooling in ice for 5 min. the suspension was centrifuged and 0.5 ml. of the supernatant fluid was mixed with 1.0 ml. N/1 NaOH. The fluorescence was read on an Aminco Bowman spectrophotofluorimeter at 315 nm activation, 380 nm fluorescence. Blanks were run using mebanazine at a final concentration of 200 µg./ml. to inhibit the enzyme completely.

Table 7. Effect on monoamine oxidase (MAO)

Drug	Final concentration (µg./ml.)	% inhibition
I.C.I. 63,197	250	Less than 5
Imipramine	250	75
Desmethylimipramine	250	63
Amitriptyline	250	77
Nortriptyline	250	76
Meprobamate	250	15
Amphetamine	250	73
Mebanazine	1 10	71 99

Conclusion

I.C.I. 63,197 is not a significant inhibitor of MAO.

2.07 Effect on the toxicity of d-amphetamine

The toxicity of amphetamine in mice is known to be increased by some stimulant drugs and decreased by some sedatives.

Mice were dosed orally with I.C.I. 63,197 or the dispersing fluid only. Half an hour afterwards, all mice received subcutaneous d-amphetamine. The number of mice surviving after 24 hours was counted, and the results are tabulated below.

Table 8. Effect on the toxicity of d-amphetamine

mg./kg. I.C.I. 63,197 oral	30	0	30	0
mg./kg. d-amphetamine subcutaneous	100	100	50	50
Number surviving/number in group	3/30	22/30	18/30	26/30
% survivors	10	73	60	82

At a dose of 30 mg./kg. I.C.I. 63,197 increases the toxicity of subcutaneous d-amphetamine at both 100 and 50 mg./kg. These results, therefore, suggest that I.C.I. 63,197 may have a stimulant action.

2.08 Effect on pentobarbitone hypnosis

Many sedative drugs are known to increase the time that mice take to recover their righting reflex after treatment with barbiturates.

Half an hour after oral administration of I.C.I. 63,197, mice were injected intraperitoneally with 50 mg./kg. sodium pentobarbitone. The time at which half the mice regained their righting reflex was noted and expressed as a percentage of the time taken to regain their righting reflexes by half of the control animals dosed orally with dispersing fluid only.

Table 9. Effect on pentobarbitone-induced hypnosis

mg./kg. I.C.I. 63,197 oral	30	10	3
% of control	267	156	80
No. of animals	60	100	80

From the above results, it is estimated that an oral dose of some 15 mg./kg. of I.C.I. 63,197 will double the time that mice take to regain their righting reflexes after an intraperitoneal injection of 50 mg./kg. pentobarbitone. In this test, I.C.I. 63,197 behaves as a sedative.

2.09 Effect on electroshock

Some drugs used as sedatives protect mice from electroshock.

Seizures were produced in mice by the application of an electric current from a constant current stimulator through clips attached to the ears. The animals received a single stimulus of 0.33 seconds duration and an intensity of 20 mA. The number of animals which failed to show a tonic extension is a measure of the protection afforded by the drug. One hour after dosing, all animals were protected by oral phenobarbitone 25 mg./kg. and chlordiazepoxide 100 mg./kg. No animals were protected by I.C.I. 63,197 at 100 and 30 mg./kg. More than 36 animals were used in each group. From this it is concluded that I.C.I. 63,197 does not protect mice from electroshock.

2.10 Effect on the electrocorticogram of conscious rats

Many of the drugs used in the treatment of mental diseases affect the electrical potentials of the brain.

Two gold plated electrodes were implanted over the cortex of rats anaesthetised with halothane. These electrodes were connected to an Elema-Schönander electroencephalograph which recorded the electrocorticogram. The electroencephalograph was connected to a device which counted the number of seconds during which four representative frequencies, 2.5, 7.5, 15 and 30 cycles per second (c.p.s.) occurred during the observation period.

When the animals had recovered from anaesthesia, the occurrence of these frequencies was counted continuously for 40 minutes and during this time, 10 second long tracings of the electrocorticogram were obtained every two minutes. The drugs were then administered intraperitoneally. After allowing 20 minutes for absorption, the incidence of the four frequencies was counted again and tracings of the electrocorticogram were obtained at two minute intervals for a period of 40 minutes as before. At least eight rats were used for each dose level.

Table 10. Effect of I.C.I. 63,197 on the electrical potentials of the cerebral cortex

	mg./kg. i.p.	No. of rats	mean change in number of resting patterns	Frequency analysis			
				2.5 c.p.s.	7.5 c.p.s.	15 c.p.s.	30 c.p.s.
I.C.I. 63,197	1	8	-6.6 *	-0.50	-2.5 *	-3.0 *	+0.5
Saline (control)		36	+0.7	+0.14	+0.32	+0.47	+0.39
Amphetamine	1	8	-8.4 *	-0.58	-3.10 *	-2.7 *	-0.01
Imipramine	10	16	+2.9 *	+0.04	-0.6	-1.8 *	-1.2 *
Chlorpromazine	10	20	+5.9 *	-1.3 *	+0.18	-2.4 *	-0.98 *
Phenobarbitone	50	20	+6.1 *	-0.96	+0.41	+0.61	+0.89
Chlordiazepoxide	10	16	+3.8 *	-0.62	+0.14	+1.6 *	+1.5 *

* significantly different from saline ($p < 0.05$)

The figures refer to the mean change in number of resting patterns before and after treatment. The figures referring to the frequency analysis were calculated from the expression : mean ($\sqrt[3]{}$ counts after dosing - $\sqrt[3]{}$ counts before dosing.)

These results show that the changes produced by I.C.I. 63,197 in the electrical potentials of rat cerebral cortex are more like those produced by amphetamine than any of the other drugs shown. Both produce a decrease in the number of resting patterns and decrease the incidence of frequencies at 7.5 and 15 cycles per second.

2.11 Squirm test in mice

A variety of substances injected into the peritoneal cavity of mice lead to sporadic characteristic squirming movements during the hour following injection. Previous dosing with analgesics and a variety of other substances decreases the number of squirms.

Mice were injected intraperitoneally with 0.4 ml./20 g. mouse of a 0.25% solution of acetic acid half an hour after they had been dosed by stomach tube with the substance under test. The number of squirms which the mice made in the 20 minutes following injection of acetic acid were counted.

Table 11. Squirm test in mice

mg./kg.	250	100	50	25	10	5	2.5	1.0	0.5
I.C.I. 63,197									
% Inhibition	-	-	-	-	-	87	71	54	23
Standard error	-	-	-	-	-	±4.4	±5.2	±6.0	±12.2
No. of animals	-	-	-	-	-	12	30	36	30
Pethidine									
% Inhibition	-	-	-	69	36	18	-	-	-
Standard error				±4.3	±4.4	±7.5			
No. of animals				1361	120	108			
Aspirin									
% Inhibition	78	44	36	40	-	-	-	-	-
Standard error	±6.6	±5.1	±5.3	±6.0					
No. of animals	72	192	180	108					

The % inhibition of the number of squirms made by the treated mice relative to the control animals was calculated from the expression :

$$\left(\frac{\text{control - treated}}{\text{control}}\right) \times 100.$$

A decrease in the number of squirms was observed when I.C.I. 63,197 was given at relatively low doses compared with those of aspirin and pethidine.

2.12 Hot plate test in mice

It is known that mice placed on a hot surface react in a characteristic way to the painful stimulus, and that the reaction time is prolonged if they have been treated with analgesics.

Individual mice were placed in glass containers with thin aluminium bottoms. The containers were then partially immersed in water maintained at 53°C. The time at which the animals licked one of their hind paws was noted. The containers were kept in the water no longer than thirty seconds.

The mice were challenged 30 and 15 minutes before and 15, 30, 45 and 60 minutes after dosing. The difference in seconds between the average reaction time after and before dosing is taken as a measure of the analgesic potency of the compound tested.

Table 12. Hot plate test in mice

Oral dose mg./kg.	100	50	25	10	3	1	0.3
I.C.I. 63,197							
Difference in seconds	-	-	-	18.6	4.99	2.29	0.89
Standard error	-	-	-	±0.35	±0.28	±0.30	±0.21
No. of animals	-	-	-	24	24	24	24
Pethidine							
Difference in seconds	11.0	6.4	3.4				
Standard error	±0.7	±1.0	±0.9				
No. of animals	190	200	170				

These experiments show that I.C.I. 63,197 in doses down to 1 mg./kg. behaves in these experiments like an analgesic.

2.13 Effect on food consumption

It is known that some neuroleptic drugs, particularly haloperidol, decrease the amount of food eaten by animals whilst they are under the influence of these drugs. The anorexiant action of the amphetamines is well known.

Mice deprived of food, but not of water, for 48 hours were housed singly. Groups of ten such mice were used in each experiment. A measured amount of food was offered to each animal at various intervals of time after being dosed by stomach tube. At the end of each hourly feeding period, the food was removed and measured.

Table 13. Effect on food consumption

	10	5	2.5	1.0	0.5	0.25	0.1	0.05
I.C.I. 63,197	-	-	-	84	81	46	30	-
Standard error	-	-	-	±7	±3.3	±3.2	±4.3	
No. of animals	-	-	-	20	50	60	60	
Chlorpromazine	85 (6)	38 (6)	24 (6)	-10 (3)	-	-	-	-
Haloperidol	-	-	-	80 (7)	68 (7)	46 (9)	-3 (5)	6 (3)
d-Amphetamine	-	-	89 (20)	57 (22)	44 (16)	42 (12)	22 (5)	-

The figures refer to the percentage inhibition of food intake relative to that of control animals dosed with the dispersing fluid only during the first hour after dosing. The figures in parenthesis refer to the number of experiments.

These experiments show that I.C.I. 63,197 decreases the food consumption of mice.

2.14 Conclusion

The triazolopyrimidone, I.C.I. 63,197, shows in experimental animals a variety of effects also shown at comparable doses by many of the drugs used in the treatment of mental disease. The profile of activities of I.C.I. 63,197 is not, however, the same as any of the known clinically useful drugs.

Some of these standard drugs, such as the benzdiazepines (e.g. chlordiazepoxide) and the barbiturates, protect mice from electroshock. I.C.I. 63,197 does not, but it shares with these two types of drug the properties of decreasing both the motility of mice and the time that these animals balance on a stationary or rotating rod. This decrease of agility and motility is shown by such drugs as the phenothiazines (e.g. chlorpromazine) and the propiophenones (e.g. haloperidol). These two drugs decrease the temperature of mice which is a property shared by I.C.I. 63,197. Under the conditions chosen, these two types of drug decrease the amount of food eaten by starved mice; at relatively low doses I.C.I. 63,197 has the same effect. The response of mice to intraperitoneal acetic acid (squirming movements) is decreased by I.C.I. 63,197 which also increased the reaction time of the animals when placed on a hot plate. These effects could be due to an analgesic action. Like many drugs used as sedatives, I.C.I. 63,197 increases the time required by mice to regain their righting reflexes after treatment with barbiturates.

The tricyclic antidepressants (e.g. imipramine) antagonise the hypothermic action of reserpine on mice. They do not affect their temperature, agility, motility or food intake. The hypothermic action of reserpine on mice is antagonised by I.C.I. 63,197. This effect shown at relatively low doses is not due to inhibition of the enzyme monoamine oxidase. The antidepressant drugs of the imipramine type potentiate the action of adrenalin and other catecholamines on the nictitating membrane of the cat; I.C.I. 63,197 does not. It is, therefore, unlikely that the increase of the temperature of reserpinised mice by I.C.I. 63,197 is due to a potentiation of catecholamines as is thought to be the case with imipramine. In mice, I.C.I. 63,197 slightly increases the toxicity of d-amphetamine.

Under the conditions chosen, I.C.I. 63,197 alters the electrocorticogram of conscious rats in the same direction as d-amphetamine and in a different direction to imipramine.

In contrast, I.C.I. 63,197 has, in animals, many of the properties of the drugs which depress the central nervous system. These results also show that these actions are not identical in all respects with those of any of the major types of drug used in the treatment of mental disease.

It is, therefore, not possible to state that I.C.I. 63,197 should be tried in any specific mental condition as a substitute for, or an improvement on any of the existing drugs. It is suggested that its possible actions should be evaluated in a variety of mental conditions.

In addition to these effects on the central nervous system, I.C.I. 63,197 shows anti-allergic and anti-bronchoconstrictor activities in experimental animals. Although these activities would not be contra-indicated in patients with mental disease, effects on the central nervous system would be undesirable in patients with asthma.

3. ANTI-BRONCHOCONSTRICTOR ACTIVITY

3.1 Histamine-induced bronchospasm in guinea-pigs

The apparatus used in this test consisted of a cylindrical 'Perspex' chamber, capable of holding up to four guinea-pigs, into which a solution of histamine was introduced as a fine spray. For the experiments described herein, a 1:80 solution of histamine acid phosphate, equivalent to 0.45% histamine base was sprayed into the chamber for 45 seconds. The times at which animals died were noted and ten minutes after commencement, air was drawn through the chamber for 2 minutes, and the number of surviving animals counted at 15 minutes.

Bronchodilator drugs, such as isoprenaline and aminophylline are active in this test (Tables 14 and 15). The results with various doses of I.C.I. 63,197 given orally to guinea-pigs at various times before exposure to histamine are shown in Table 16. Activity was demonstrable with a dose as low as 0.05 mg/kg. when given 1 hour before exposure. Oral doses of 1 mg/kg. were active within 5 minutes and for longer than 5 hours. The compound was also active by the subcutaneous, intraperitoneal and intravenous routes.

Table 14. Effect of isoprenaline on histamine bronchospasm in guinea-pigs

Dose (mg./kg.)	Route	Proportion of animals surviving when the drug is administered at the indicated time prior to exposure to histamine				
		5 min.	15 min.	30 min.	45 min.	60 min.
0.025	s.c.	2/4	5/10			
0.05	s.c.	4/4	8/10			
0.1	s.c.	7/8	31/38	4/4	2/4	0/4
0.25	s.c.	3/4	3/ 3			
0.5	s.c.		14/15			
1.0	s.c.	4/4				
0.1	i.p.	2/4	2/ 4			
0.25	i.p.	2/4	8/12	2/8		0/4
0.5	i.p.	1/3	6/ 8			
1.0	i.p.	2/2				

Untreated controls : 3/100

Table 15. Effect of aminophylline on histamine bronchospasm in guinea-pigs

Dose (mg./kg. oral)	Proportion of animals surviving when the drug is administered at the indicated time prior to exposure to histamine				
	5 min.	15 min.	30 min.	60 min.	2 hr.
100	0/4	1/12	5/12	6/12	3/8
200			3/ 4	2/ 4	2/4

Untreated controls : 0/36

Table 16. Effect of I.C.I. 63,197 on histamine bronchospasm in guinea-pigs

Dose (mg./kg. oral)	Proportion of animals surviving when the drug is administered at the indicated time prior to exposure to histamine						
	5 min.	15 min.	30 min.	1 hr.	2 hr.	3 hr.	5 hr.
0.025				1/ 4			
0.05	0/4	0/4	4/7	13/24	6/8	5/8	1/4
0.1	3/8	3/8	4/8	19/24	4/8	6/8	2/4
0.25	1/8	5/8	7/8	10/12	8/8	8/8	3/4
0.5	5/8	7/8	4/8	9/12	4/8	7/8	3/4
1.0	8/8	7/8	6/8	14/16	6/8	7/8	4/4

Untreated Controls : 13/123

3.2. Isolated Perfused Guinea-Pig Lung

Normal guinea-pigs were killed by a blow on the head and the heart and lungs removed. After removal of the pericardium, a cannula was tied into the pulmonary artery and 10-20 ml. of warmed Tyrode's solution injected to remove most of the blood, the effluent being allowed to escape through an incision in the auricle. A second cannula was tied into the trachea and the preparation mounted in the apparatus described by Bhattacharya and Delaunois (Arch. int. Pharmacodyn., 1955, 101, 495.)

This apparatus consists essentially of a cylinder made of 'Perspex' closed at the upper end by a lid containing holes through which pass tubes connected to the tracheal and arterial cannulae respectively. The lower end of the cylinder is attached to a small bellows pump delivering 7 ml. of air at a rate of 25 strokes/minute. A pressure transducer is attached to a side arm of the tube leading to the tracheal cannula. A small flap-valve allows the escape of air. Compression of air within the cylinder deflates the lungs and withdrawal of air inflates the lungs, the resulting pressure-changes being monitored by the transducer which activates a pen-recorder thus providing a record of the course of artificial respiration. During bronchospasm the lungs resist compression and there is therefore a rise in intra-tracheal pressure. An aspirator containing Tyrode's solution with 2% dextran is attached, via a warming coil, to the arterial cannula and the vascular system perfused at a rate of 2-3 ml./min., the effluent being allowed to escape through the flap-valve as it opens during the compression stroke of the pump.

Bronchoconstrictor agents were injected close to the arterial cannula. The degree of bronchospasm or bronchodilatation was estimated from the percentage change in the height of the recording.

I.C.I. 63,197 was capable of reducing the degree of spasm produced by histamine, acetylcholine, serotonin or bradykinin. Table 17 illustrates the effects obtained with acetylcholine. The lung was perfused with dextran-Tyrode until a stabilised height was produced on the recording and acetylcholine injected every 4 minutes until a reproducible degree of spasm (80-100%) was obtained. Perfusion was then changed to a solution of I.C.I. 63,197 in dextran-Tyrode and three more injections of acetylcholine given. The lung was then re-perfused with plain dextran-Tyrode and two more injections of acetylcholine given. Percentage inhibition of bronchospasm was calculated from the expression :-

$$\% \text{ inhibition} = \left(\frac{\% \text{ control spasm} - \% \text{ treated spasm}}{\% \text{ control spasm}}\right) \times 100$$

The degrees of inhibition of spasms obtained with various concentrations of I.C.I. 63,197 are shown in Table 17, significant activity was demonstrable at 0.06 µg./ml.

Similar results were obtained when histamine or bradykinin were used as spasmogens, although repeated injections of these substances were not always possible due to oedema.

Table 17. Effect of I.C.I. 63,197 on bronchospasm induced by acetylcholine in isolated perfused guinea-pig lungs

Period of perfusion	Concentrations of I.C.I. 63,197 in perfusion fluid (µg./ml.) Mean % inhibition of spasm (± S.E.)			
	0.5	0.25	0.125	0.0625
With compound				
4 min.	69.6 ± 6.2	47.0 ± 5.7	43.8 ± 11.9	30.4 ± 14.4
8 min.	68.8 ± 4.2	47.7 ± 5.3	53.9 ± 11.9	33.3 ± 10.3
12 min.	69.0 ± 6.5	48.5 ± 7.0	41.3 ± 9.8	37.5 ± 12.6
Subsequently with Tyrode				
4 min.	56.7 ± 8.3	24.7 ± 10.0	29.9 ± 8.5	16.0 ± 6.7
8 min.	29.4 ± 17.0	23.6 ± 10.0	29.9 ± 8.5	0.8 ± 2.5

3.3 I.C.I. 63,197 and β-adrenergic blockade

The activity of I.C.I. 63,197 in the histamine bronchospasm test was inhibited by the β-adrenergic blocking drug propranolol. The β-blocking drug practolol, which is more selective in blocking β receptors in the heart compared with the lungs, did not block the activity of I.C.I. 63,197.

Propranolol is known to potentiate histamine bronchospasm by virtue of its blockade of the reflex β-adrenergic stimulation following bronchoconstrictor stimuli (McCulloch, Proctor and Rand, Europ. J. Pharmacol., 1967, 2, 214.).

Table 18. The effect of β-adrenergic blocking drugs on the activity of I.C.I. 63,197 against histamine bronchospasm in guinea-pigs

Treatment			Proportion of animals surviving
I.C.I. 63,197 (mg./kg.)	Propranolol (mg./kg.)	Practolol (mg./kg.)	
1.0	-	-	6/8
1.0	0.5	-	0/8
1.0	0.1	-	2/8
1.0	0.05	-	6/8
-	0.5	-	0/8
1.0	-	4.0	6/8
-	-	4.0	0/8
-	-	-	0/8

The activity of I.C.I. 63,197 on the isolated perfused lung was not affected by the presence of propranolol (1 µg./ml.) in the perfusing fluid.

3.4 Potentiation of the bronchodilator activity of sympathomimetic amines by I.C.I. 63,197

Isolated guinea-pig lungs were prepared as described in Section 3.2. Repeated bronchospasms were induced by the injection of acetylcholine into the arterial cannula every four minutes; the amount of acetylcholine which was necessary in different experiments to cause a comparable degree of bronchospasm varied between 1 and 5 µg. The degree of bronchospasm was assessed by measuring the height of the tracing on the pen-recorder before and 1 minute after injection. When consistent degrees of bronchospasm (80-100%) had been established, inhibitory substances were injected into the arterial cannula four minutes before the acetylcholine, the injections of which were then repeated every four minutes, until the effect of the inhibitor had worn off.

Percentage inhibition of bronchospasm was calculated from the expression :

$$\% \text{ inhibition} = \left(\frac{\% \text{ control spasm} - \% \text{ treated spasm}}{\% \text{ control spasm}}\right) \times 100$$

The results of experiments with various sympathomimetic amines and I.C.I. 63,197 are shown in Table 19. It is apparent that a marked potentiation of the bronchodilator effect occurred when both compounds were given together.

Table 19. Potentiation of the bronchodilator activity of catecholamines by I.C.I. 63,197.

Treatment	Mean % inhibition of acetylcholine bronchospasm ± S.E.		
	Time after injection		
	4 min.	8 min.	12 min.
Adrenalin	28 ± 3.1	7 ± 6.5	13 ± 0.5
Adrenalin + I.C.I. 63,197	60 ± 9.1	31 ± 10.6	18 ± 7.5
Salbutamol	6.2 ± 3.1		
Salbutamol + I.C.I. 63,197	41 ± 10.9	16 ± 4.6	9 ± 0.5
Isoprenaline	19 ± 3.7	8 ± 1.4	7 ± 2.8
Isoprenaline + I.C.I. 63,197	45 ± 7.4	25 ± 0.7	2 ± 0.5
I.C.I. 63,197	0		

Adrenalin - 0.05 µg.

Salbutamol - 0.025 µg.

Isoprenaline - 0.05 µg.

I.C.I. 63,197 - 1 µg.

4. ANAPHYLACTIC SHOCK IN GUINEA-PIGS AND MICE

4.1 Anaphylactic shock in anaesthetised guinea-pigs

Evidence of an anti-anaphylactic action of I.C.I. 63,197 was obtained by a study of the course of anaphylactic shock in guinea-pigs, passively sensitised with homologous antibody, by the technique of Konzett and Rössler (Naunyn-Schmeiderbergs Arch. exp. Path. Pharmak., 1940, 195, 71.) as modified by Collier and James (Br. J. Pharmac. Chemother., 1967, 30, 283.).

Antibody was produced by the injection of crystallised ovalbumin, in Freund's complete adjuvant, into the foot pads of guinea-pigs. Guinea-pigs were passively sensitised by an intravenous injection of a solution of the antibody in saline. On the following day they were anaesthetised with pentobarbitone sodium (70 mg/kg. intraperitoneally) deeply enough to suppress spontaneous breathing. Cannulae were tied into the trachea and the jugular vein and the lungs inflated with a pump at 68 strokes/min. at a constant stroke volume between 6 and 8 ml., which was adjusted at the beginning of an experiment to give a minimal overflow volume in the Konzett-Rössler preparation. A recording of pressure changes in the trachea was made with the aid of a pressure transducer and a 'Devices' recorder. An increase in pressure is taken to indicate bronchospasm. During recording, the side arm of the intratracheal cannula was clamped automatically for 10 seconds during each 30 seconds to inflate the lungs more forcibly.

Anaphylactic shock was induced by intravenous injection of 1 mg. of twice recrystallised ovalbumin. I.C.I. 63,197 and/or mepyramine maleate were given intravenously 5 minutes before antigen. I.C.I. 63,197 was dissolved by the addition of minimal amounts of sodium hydroxide. Fresh solutions were prepared daily.

The intratracheal pressure was recorded for 10 minutes before and after the injection of antigen. Maximal spasm was measured by momentarily clamping the tube attached to the intratracheal cannula.

The degree of bronchospasm was calculated from the expression :

$$P = 100 \times \frac{(M-B)}{Y}$$

where P = % bronchospasm

M = Height of recording of maximal spasm

B = Height of recording before antigen injected

Y = Height of recording after antigen injected

The results are illustrated in Figures 2 and 3.

I.C.I. 63,197 at a dose of 0.5 mg/kg. intravenously did not influence the intense bronchospasm appearing immediately after injection of antigen, but, compared with the untreated controls, the animals recovered more rapidly and completely. In contrast, mepyramine maleate (2 mg/kg.) reduced the severity of bronchospasm occurring within the first two minutes after challenge. A combination of I.C.I. 63,197 with mepyramine had a more pronounced effect on all phases of anaphylaxis than did either treatment alone (Fig. 2).

Adrenalin (50 µg./kg.) given intravenously 5 minutes before antigen produced only a moderate degree of suppression of the anaphylactic response, but combined administration of adrenalin and I.C.I. 63,197 gave an effect greater than that produced by either agent alone (Fig. 3).

These observations are consistent with the hypothesis (supported by other work in our laboratories) that the immediate intense bronchospasm is due to a massive liberation of histamine and that the subsequent spasm is due to substances other than histamine. It is, therefore, postulated that I.C.I. 63,197 exerts its main effect on these "other substances".

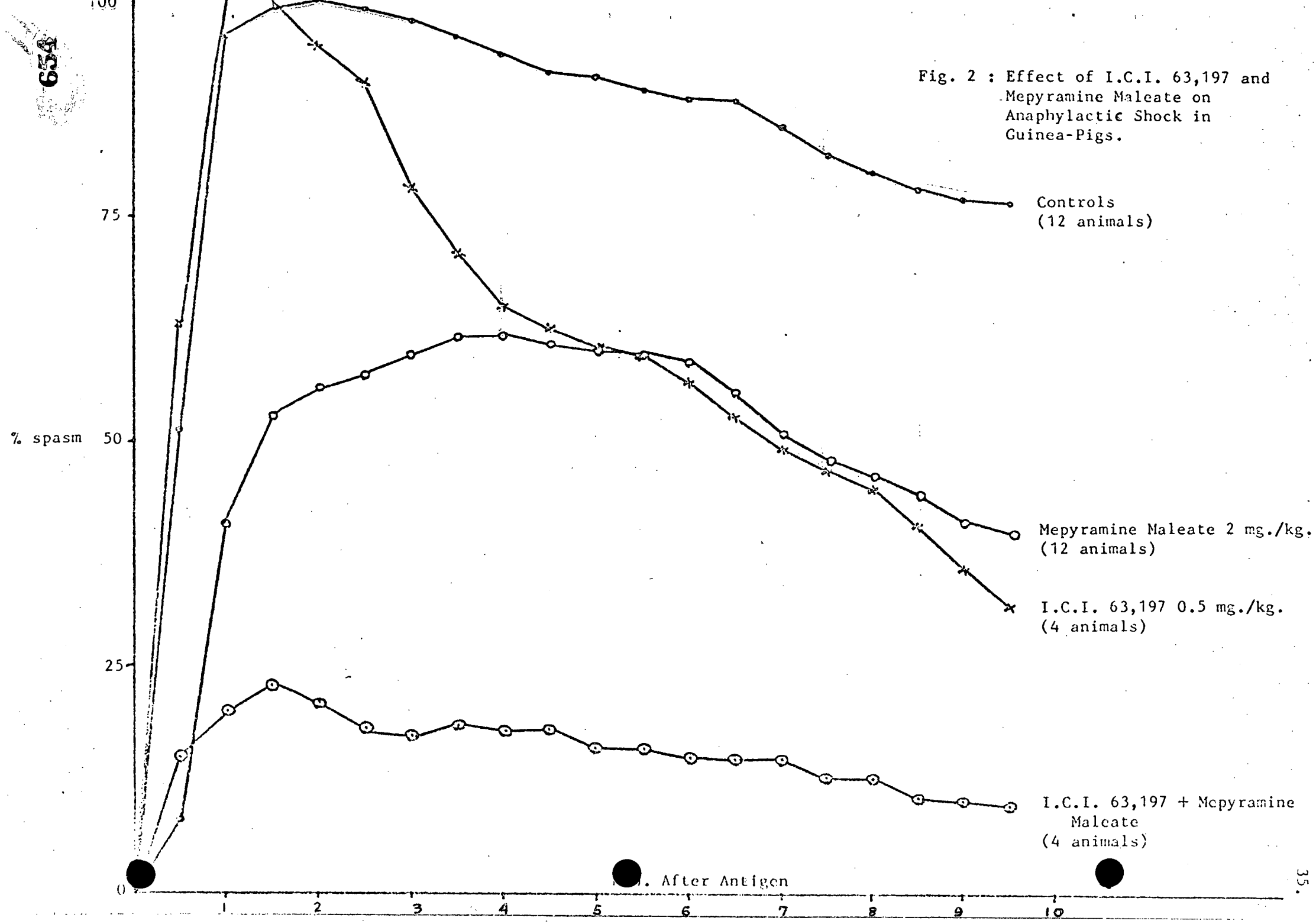


Fig. 2 : Effect of I.C.I. 63,197 and Mepyramine Maleate on Anaphylactic Shock in Guinea-Pigs.

Controls
(12 animals)

Mepyramine Maleate 2 mg./kg.
(12 animals)

I.C.I. 63,197 0.5 mg./kg.
(4 animals)

I.C.I. 63,197 + Mepyramine Maleate
(4 animals)

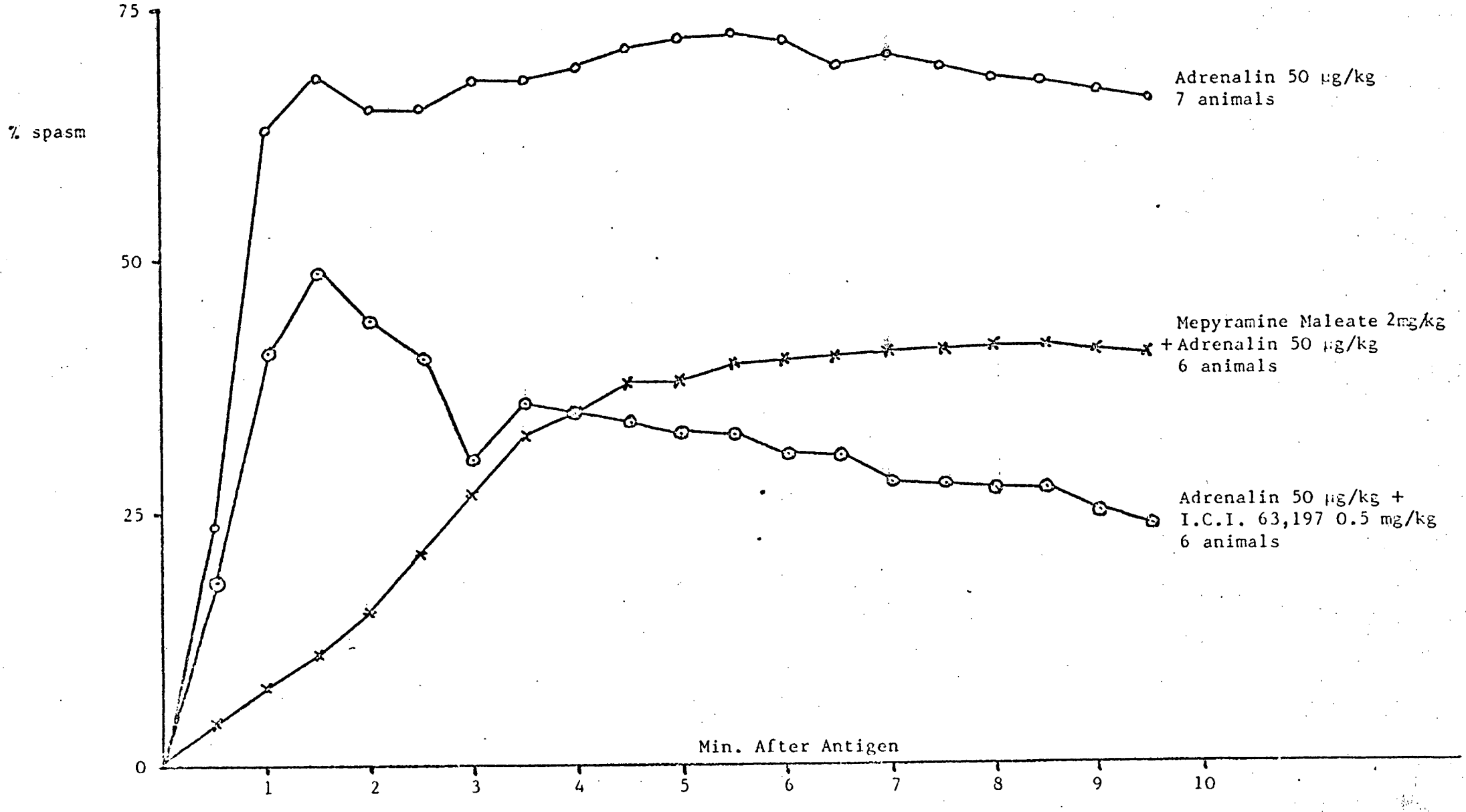


Fig. 3 : Effect of I.C.I. 63,197, Adrenalin and Mepyramine Maleate on Anaphylactic Shock in Guinea-Pigs

4.2 Effect of I.C.I. 63,197 on the anaphylactic release of histamine from isolated perfused lungs

Guinea-pigs were passively sensitised by an intravenous injection of the same guinea-pig anti-egg albumin serum used in the studies on anaphylaxis, but a ten-fold stronger concentration was used. On the following day the lungs were removed, mounted in the apparatus described in Section 4.1, and perfused with either dextran-Tyrode or a solution of I.C.I. 63,197 in dextran-Tyrode. After perfusion for 10 minutes or so, an injection of 50 µg. of egg albumin was given into the arterial cannula, the perfusate was collected during the next six minutes, the histamine content of the perfusate and of the lung determined fluorimetrically and the percentage of total histamine released by antigen calculated. The respiration of the lung was monitored during the experiment and the percentage spasm at 1 and 6 minutes after antigen calculated as described in Section 4.1.

Virtually complete inhibition of histamine release was obtained with a concentration of 5 µg./ml. of I.C.I. 63,197 and there was about 50% inhibition at a concentration of 1.25 µg./ml. (Table 20). The effect on the spasm was less marked than that obtained when acetylcholine was the spasmogen (c.f. Table 17).

Table 20. Effect of I.C.I. 63,197 on anaphylaxis and release of histamine in the passively-sensitised, isolated, perfused guinea-pig lung

Concentration of I.C.I. 63,197 in perfusion fluid (µg./ml.)	No. of lungs	Mean % spasm ± S.E.		Mean % histamine release ± S.E.
		1 min. after antigen	6 min. after antigen	
5	8	52.4 ± 10.8	17.3 ± 8.6	1.9 ± 0.4
2.5	13	70.7 ± 9.3	59.5 ± 10.7	18.5 ± 4.8
1.25	10	72.0 ± 9.1	70.1 ± 5.7	17.8 ± 2.8
0	31	95.0 ± 0.6	93.0 ± 1.6	30.5 ± 3.2

4.3 Effect of I.C.I. 63,197 on anaphylactic shock in mice

As a further model of allergic reactions, anaphylaxis in mice was selected as a systemic reaction not involving bronchospasm. Female mice were sensitised by an intraperitoneal injection of 0.5 ml. of a suspension of alum-precipitated egg albumin (20 mg.) and killed cells of Bordetella pertussis (2×10^9). Seven days after sensitisation, the mice were challenged by giving them an intravenous injection of 0.3 mg. of twice recrystallised egg albumin. One hour after this injection the severity of the reaction was assessed with the aid of the following scoring system:

0 = Normal animals

1 = Animal unwell, but still motile

2 = Animal collapsed, but moves when touched

3 = Animal unconscious

5 = Animal dead

The survival times were recorded up to 1 hour after injection, and at 24 hours the number of survivors was counted.

The results obtained with I.C.I. 63,197 at various dose levels are shown in Table 21. Protection was achieved with a dose of 5 mg./kg. given daily by mouth from two days before sensitisation to the day of challenge. The effect of I.C.I. 63,197 seemed to be exerted on the later phases of the reaction since, at a level of 10 mg./kg., activity was achieved with doses given on the day before and the day of challenge.

Table 21. Effect of I.C.I. 63,197 on anaphylactic shock in mice

Oral Dose (mg./kg.)	Days of dosing *	No. surviving after 24 hrs.		Mean Score **	
		Treated	Controls	Treated	Controls
10	-2 to 7	5/ 8	1/16	2.5	4.3
10	-4 to 7	4/ 4	2/ 8	2.8	3.8
10	6 to 7	6/ 8	6/16	2.4	3.1
5	-2 to 7	11/12	4/24	2.5	4.2
5	4 to 7	6/ 8	1/16	3.3	4.2
5	6 to 7	2/ 8	5/16	3.6	3.8
2.5	-2 to 7	6/ 8	2/16	2.6	4.0

* Animal sensitised on day 0.

** Mean Score : This represents the condition of the animals when inspected 1 hour after injection of antigen.

0 = normal

1 = mild shock - animal unwell, but still motile

2 = moderate shock - animal collapsed, but moves when touched

3 = animal unconscious

5 = animal dead

5. MEASUREMENT OF CYCLIC 3',5'-AMP PHOSPHODIESTERASE ACTIVITY

This enzyme is specific for the inactivation of cyclic AMP by conversion to 5' AMP. It is found in most tissues and plays a major role in regulating the cellular concentration of cyclic AMP which, in turn, is a mediator of the action of many hormones.

Principle

$$\text{Cyclic AMP} \xrightarrow{\text{Phosphodiesterase}} \text{5'-AMP} \xrightarrow[\text{venom}]{\text{snake}} \text{adenosine}$$

3',5'-cyclic AMP-^{3}H is used as substrate and the 5'-AMP formed is quantitatively converted to adenosine by an excess of snake venom nucleotidase present in the incubation mixture. The incubation is terminated by the addition of ion exchange resin which adsorbs unchanged cyclic AMP and leaves the adenosine in solution. Scintillation fluid is now added to the mixture and the sample is counted in a scintillation counter. The counts due to unchanged substrate are quenched by the ion exchange resin so that only the counts due to product are recorded.

Materials

The specific phosphodiesterase was extracted from beef heart by the method of Butcher and Sutherland (J. Biological Chemistry, 1962, 237, 1244) to the heat denaturation stage. Crude preparations have also been made from guinea-pig lung. The material now commercially available from Boehringer (Mannheim), Catalogue No. 15:53 EPAY, has also been found satisfactory. Tritiated 3',5'-cyclic AMP was obtained from Schwarz Bioresearch Inc., Orangeburg, New York, and Russell's Viper venom from Sigma Chemical Co.

Method

The stock solution of beef heart phosphodiesterase (3.1 mg. protein/ml.) was diluted x 20 with a solution of Russell's Viper venom (1 mg./ml.) and bovine serum albumin (2 mg./ml.) in 60 mM Tris-HCl buffer pH 7.8, containing 5 mM mercaptoethanol ("enzyme").

The stock solution of cyclic AMP-^{3}H (1.7×10^{-5}M, 2.35 Ci/mmole) was diluted x 100 with 120 mM Tris-HCl buffer pH 7.8, containing 120 mM magnesium chloride ("substrate").

I.C.I. 63,197 was dissolved in 10% aqueous dimethyl sulphoxide. The same solvent was added to controls.

50 µl. enzyme solution and 50 µl. drug or solvent were pre-incubated at 30° for 5 min. in a Tri-Carb scintillation vial. 50 µl. substrate was then added and the incubation continued for 2 min. The reaction was terminated by adding 0.8 ml. of a 50% suspension of Dowex 1 x 2, minus 400 mesh chloride form, in water. After a period of 10 min., to allow equilibration, 8.5 ml. scintillation fluid was added and the sample counted.

Blanks were run, omitting the phosphodiesterase from the enzyme solution and the net counts per minute after subtracting blanks were taken as proportional to the initial velocity of the reaction for the purposes of kinetic calculations. By varying the inhibitor and substrate concentrations velocity data were obtained from which the inhibitor constant Ki was calculated by plotting 1/v against (I) by the method of Dixon (Biochem. J. 1953, 55, 170).

The validity of the above method has been checked by analysis of the phosphate liberated by the snake venom from the 5'-AMP formed in the reaction.

Results

Enzyme source	Ki (M) x 10^4		
	I.C.I. 63,197	Theophylline	Caffeine
Beef heart	2.5	3.4	
Guinea-pig lung	0.34		
Boehringer (beef heart)	1.25	2.4	2.6

The Ki is the dissociation constant of the enzyme-inhibitor complex and hence is inversely related to the affinity of the inhibitor for the enzyme. The concentration required to produce 50% inhibition (I_{50}) is more dependent on the conditions of the assay and the type of inhibition, but under the above conditions, I.C.I. 63,197 has an I_{50} of about 50 µg./ml. and its inhibition is believed to be of the non-competitive type.

Inhibition by theophylline and caffeine is of the competitive type, hence under conditions of relatively high substrate concentration such as may be obtained in some tissues, their inhibition may compare even less favourably with I.C.I. 63,197 than the above figures would suggest. It follows also from this difference of inhibitor type that it is not meaningful to compare I_{50} values for I.C.I. 63,197 and theophylline.

6. GENERAL PHARMACOLOGY

6.1 Measurement of cardiac function and peripheral blood flow

6.11 Anaesthetised dogs

Dogs were anaesthetised by intravenous injection of pentobarbitone (30 mg./kg.) followed by chloralose (60 mg./kg.). A cuffed endotracheal tube was inserted and the animals artificially respired with room air. An electro-magnetic flow probe was placed around the ascending aorta to measure rate of blood flow. Arterial blood pressure was recorded via a cannula in the left common carotid artery and attached to a pressure transducer. Heart rate and electro-cardiogram were obtained by means of fine needle electrodes inserted under the skin. Heart rate was displayed on a cardiotachometer. Continuous records of blood pressure aortic flow and E.C.G. were recorded on a Devices M.4 recorder.

In separate experiments blood flow to the hind limb of dogs was measured by placing a flow probe around the external iliac artery. Drugs were injected into the cephalic vein and iliac artery.

Results

The experiments on cardiac function and peripheral blood flow are preliminary, but it appears that single intravenous injections of I.C.I. 63,197 in the dose range 10 to 500 µg./kg. produce an increase in heart rate and aortic blood flow. Doses higher than 500 µg./kg. reduce mean arterial blood pressure.

There has been no indication that I.C.I. 63,197 potentiates the β-adrenergic stimulatory effects of isoprenaline (intra-arterially or intravenously) on peripheral blood flow.

6.12 Conscious dogs

Observations were made on dogs trained to stand quietly. Heart rate was measured by cardiotachometer. Arterial blood pressure was recorded via a polythene catheter which had been implanted at least one week previously into the descending aorta. Respiration rates were counted by visual observation.

Results

Two male dogs were used and their blood pressures, heart rates and respiration rates measured. They were then given a single oral dose of I.C.I. 63,197 (0.1 mg./kg. or 0.2 mg./kg.) and the measurements repeated at 30 minute intervals for 2 hours. The experiment was then repeated on days 2 and 3 with increasing doses of I.C.I. 63,197.

The results are shown in Table 22. There was no significant change in heart rate or blood pressure. Dog 625 G showed a transient increase in respiratory rate 30 minutes after a dose of 0.4 mg./kg., but there was no increase after 0.8 mg./kg. on the following day.

No further drug was given for 5 days, and then for the following 14 days both dogs received daily doses of 0.2 mg./kg. by mouth before being fed. They vomited occasionally.

On day 23, a dose of 1 mg./kg. was given. There was a marked increase in heart rate and respiratory rate and, in one dog, an increase in arterial blood pressure. On the following day dog 485 G was given propranolol (1 mg./kg. i.v.) and the other, 625 G, given saline intravenously. They were then both dosed orally with I.C.I. 63,197 at 1 mg./kg. There was no increase in heart rate or blood pressure in the dog which had been pre-treated with propranolol. The heart rate of the control animal increased as on the previous day. The increase in respiratory rate appeared to have been reduced by propranolol (Table 23).

Table 22. . Effects of I.C.I. 63,197 on the cardiovascular and respiratory systems of conscious dogs

Th[illegible]dogs were given one single dose on three succes[illegible]e days.

I.C.I. 63,197 (mg./kg.oral)	Day No.	Parameter	Time of observation					I.C.I. 63,197 (mg./kg.oral)	Time of observation				
			Control	+30	60	90	120		Control	+30	60	90	120
			Dog ♂ 458 G						Dog ♂ 625 G				
0.1	1	Mean Arterial B.P. mm Hg	141	134	129	131	139	0.2	128	126	112	130	125
		Heart Rate beats/min.	107	118	108	105	123		107	103	98	118	118
		Respiration rate/min.	48	44	46	48	46		36	30	30	32	30
0.2	2	Mean Arterial B.P. mm Hg	122	128	129	140	139	0.4	126	121	119	124	111
		Heart Rate beats/min.	77	92	108	105	107		102	115	132	128	135
		Respiration rate/min.	34	36	42	42	56		30	157	40	48	99
0.4	3	Mean Arterial B.P. mm Hg	128	111	108	120	-	0.8	98	97	79	77	
		Heart Rate beats/min.	123	108	110	122	-		112	98	112	101	
		Respiration rate/min.	30	36	40	44	-		30	32	36	32	

Table 23. Effects of I.C.I. 63,197 on the cardiovascular and respiratory systems of conscious dogs.

Dogs maintained on daily oral dosé of 0.2 mg./kg. for 14 days prior to treatment described below.

I.C.I. 63,197 (mg./kg.oral)	Day No.	Parameter	Dog ♂ 458 G: Time of observation					Dog ♂ 625 G: Time of observation				
			Control	+30	60	90	120	Control	+30	60	90	120
1.0	23	Mean Arterial B.P. mm Hg	109	127	145	156	142	120	116	117	117	122
		Heart Rate beats/min.	92	125	180	187	160	95	101	177	195	170
		Respiration rate/min.	38	56	216	236	198	24	42	200	229	180
			Propranolol (1.0 mg./kg./i.v.)					Saline (i.v.)				
1.0	24	Mean Arterial B.P. mm Hg	107	110	110	119	114	80	104	84	104	96
		Heart Rate beats/min.	107	97	120	120	110	102	165	210	217	192
		Respiration rate/min.	34	81	190	60	48	30	96	185	170	160

6.13 Test for cardiovascular reactivity

Cats were anaesthetised with chloralose (80 mg./kg. i.v.). Arterial blood pressure and heart rate were recorded and the changes in these parameters produced by a test sequence consisting of :

(i) carotid artery occlusion

(ii) peripheral vagal stimulation

(iii) intravenous injection of adrenalin

(iv) intravenous injection of acetylcholine

(v) intravenous injection of isoprenaline.

The effects on these changes produced by various doses of I.C.I. 63,197 were then measured.

Results

The effects on heart rate and mean arterial blood pressure of a series of five tests, as described above, were measured in animals given intravenous injections of saline or I.C.I. 63,197 in the dose range 0.025 to 1 mg./kg. No significant differences were observed. The injection of I.C.I. 63,197 caused a transient, slight fall in basal blood pressure (Figs. 4 and 5).

FIG. 4

MEAN FEMORAL BLOOD PRESSURE mm Hg IN THE ANAESTHETISED CAT

(Cat ♂ 3.0 kg)

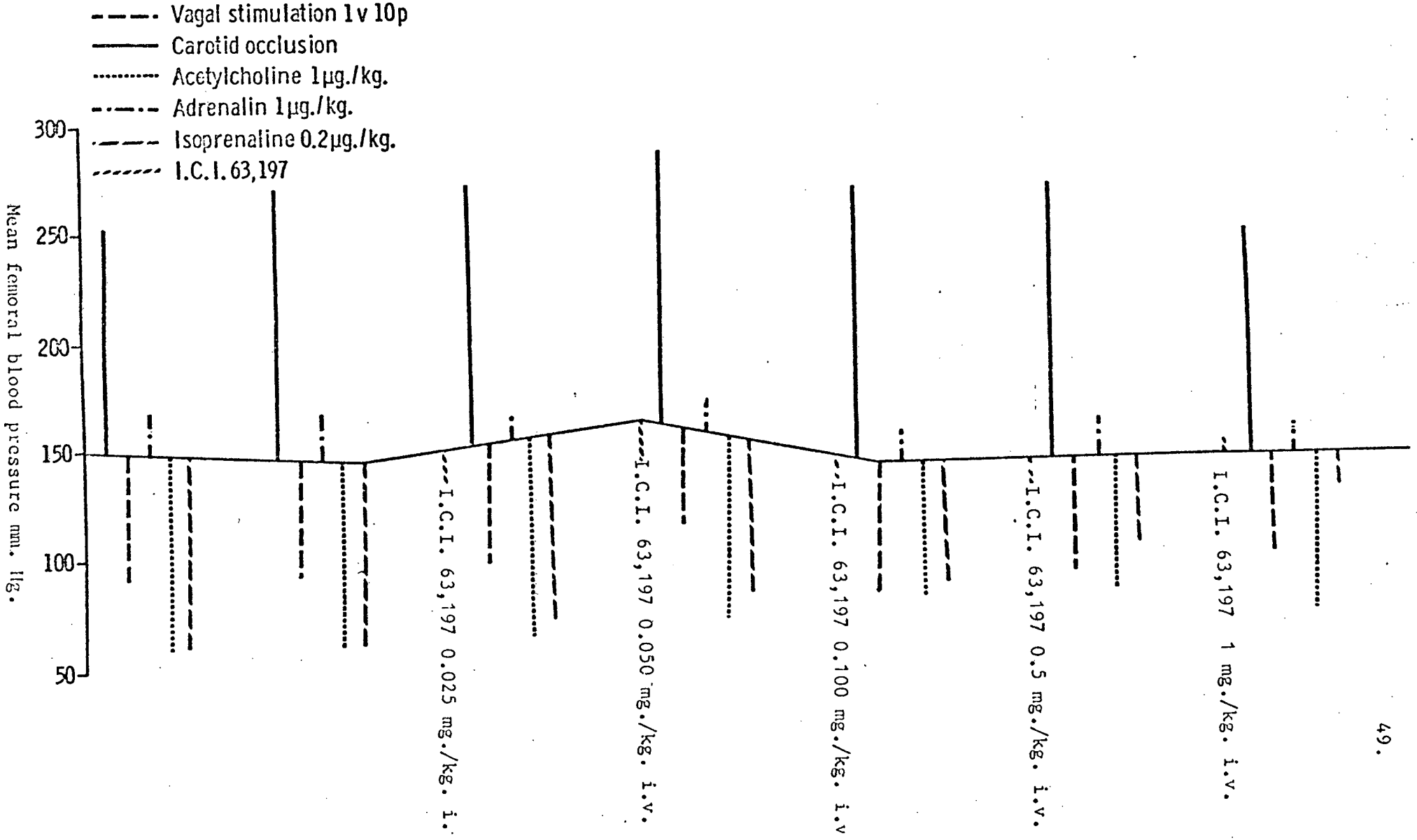
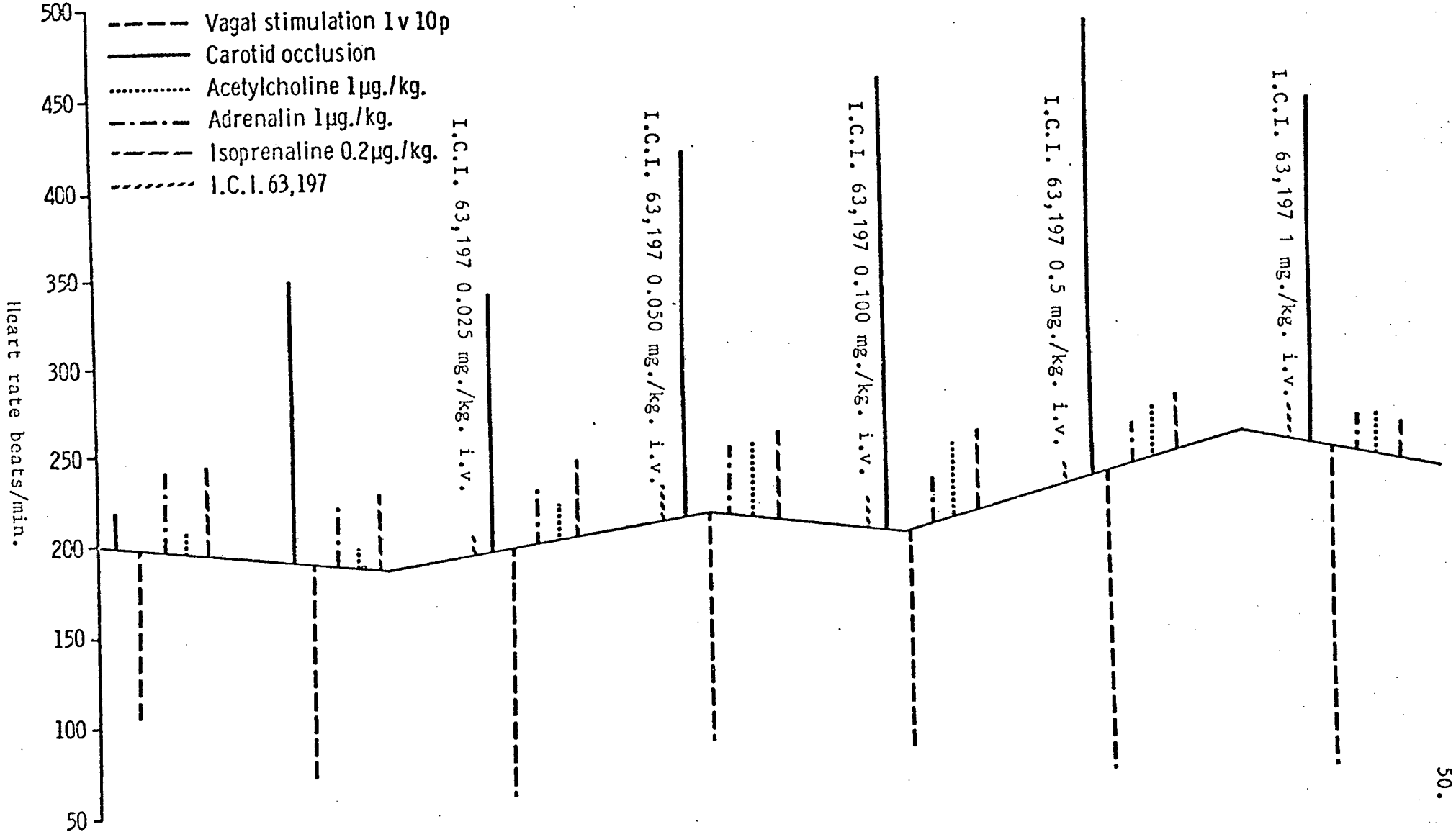


FIG. 5

HEART RATE (BEATS/MIN.) IN THE ANAESTHETISED CAT

(Cat ♂ 3.0 kg)



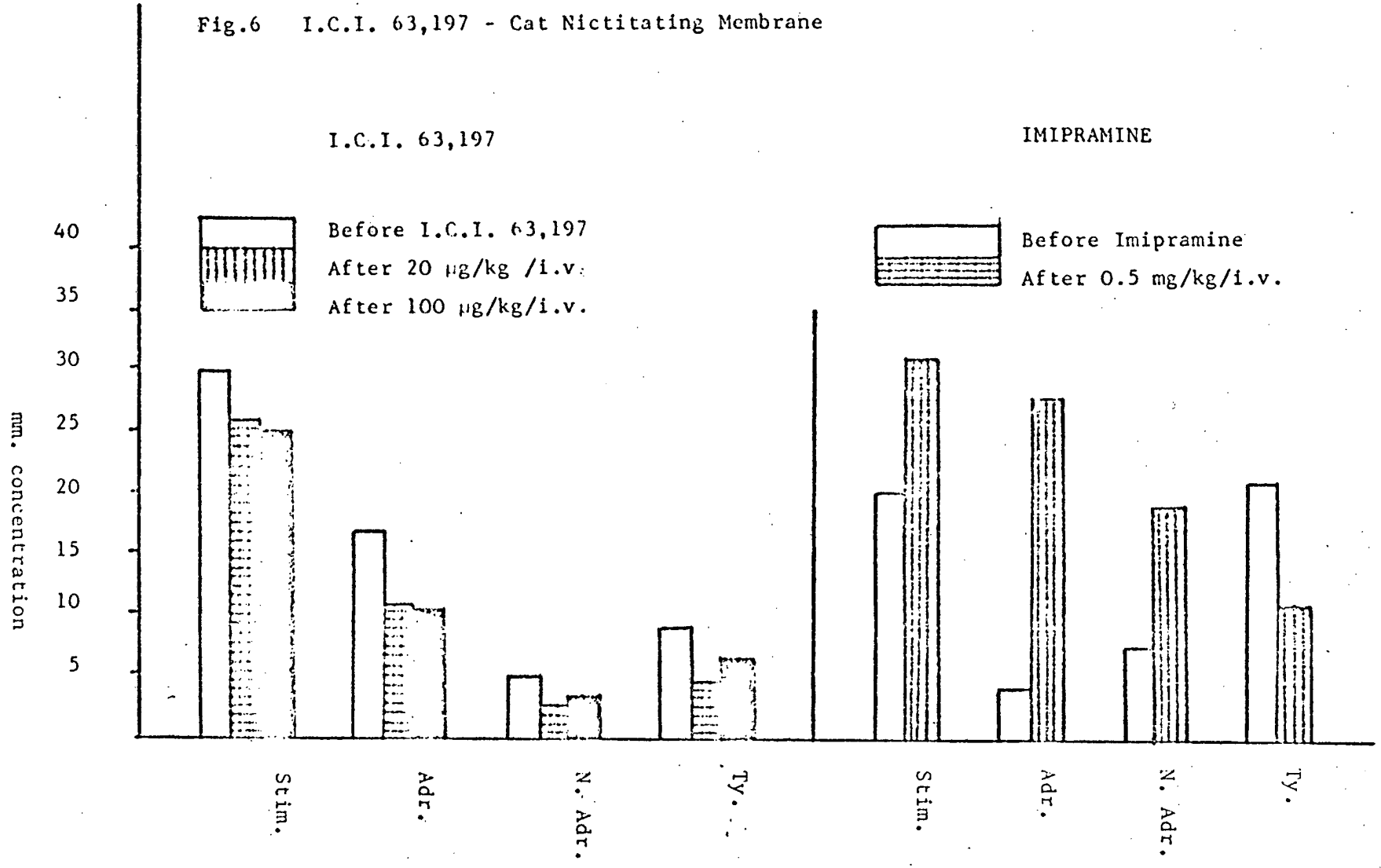
6.14 Cat nictitating membrane

Sub-maximal contractions of the cat nictitating membrane were induced by pre-ganglionic stimulation of the cervical sympathetic nerve and also by intra-arterial injection of adrenalin, nor-adrenalin and tyramine into the carotid artery.

Results

I.C.I. 63,197 at doses up to 0.1 mg./kg. i.v. very slightly reduced the contractions induced by electrical stimulation or intra-arterial injections of adrenalin, nor-adrenalin or tyramine. In contrast, imipramine (0.5 mg./kg. i.v.) potentiated the contractions induced by stimulation, adrenalin and nor-adrenalin whilst reducing the contractions caused by tyramine (Fig. 6).

Fig.6 I.C.I. 63,197 - Cat Nictitating Membrane



6.2 In vitro studies

At a concentration of 1 µg./ml., I.C.I. 63,197 did not inhibit contractions of the isolated guinea-pig ileum caused by histamine or acetylcholine.

6.3 Conclusion

The above results show that, at the doses used, I.C.I. 63,197 has little effect on the cardiovascular or respiratory systems. Unlike imipramine, it does not potentiate the action of catecholamines on the cat nictitating membrane. This is in contrast to the potentiation of the bronchodilatory effects of catecholamines.

7. SERUM CONCENTRATIONS AFTER ORAL ADMINISTRATION TO DOGS AND RATS

Serum concentrations of I.C.I. 63,197 have been measured in dogs and rats after oral administration. The animals investigated were those used in the three-month toxicity trials (TPD/64 and TPR/194), and, in the case of rats, extra animals dosed in an identical manner to those in the main trial.

7.1 Assay method for I.C.I. 63,197 in serum

I.C.I. 63,197 was quantitatively extracted from serum at physiological pH into chloroform, an aliquot of which was evaporated to dryness. The residue containing the compound was taken up in water for spectrophotofluorimetric analysis.

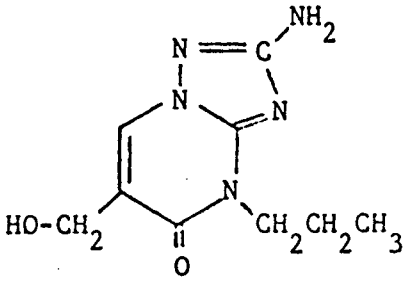
The details are as follows :-

Into a glass-stoppered test tube were placed 1 ml. serum, 1 ml. water (± standard amounts of I.C.I. 63,197) and 12 ml. chloroform. The compound was extracted by shaking the tube for 5 - 10 minutes. The tubes were centrifuged to separate the solvent layer and a 9 ml. aliquot of the chloroform transferred to a clean glass-stoppered tube. The chloroform was evaporated to dryness under a stream of air, with the water bath temperature at approximately 40 - 50°C. The residue was dissolved in 2 ml. water for fluorimetric analysis.

Fluorescence was measured in an Aminco-Bowman Spectrophoto-fluorimeter with activation of fluorescence set at 301 nm and emission at 430 nm (wavelengths uncorrected). I.C.I. 63,197 is intensely fluorescent, the minimum detectable level being in the order of 0.001 µg./ml. water. Recovery of I.C.I. 63,197 from rat and dog serum was quantitative (i.e. 100%) when added at concentrations up to 1.0 µg./ml. In the range 0.01 to 1.0 µg./ml. serum, the error in replication was not greater than 6%.

This method allowed detection of I.C.I. 63,197 down to levels of about 0.005 µg./ml. serum.

A major metabolite of I.C.I. 63,197 in mouse, rat, guinea-pig, dog and monkey urine is the hydroxy-methyl compound :-



I.C.I. 68,916

This metabolite has a fluorescent spectrum almost identical to that of I.C.I. 63,197 and can be extracted from serum under identical conditions. Using ^{14}C-labelled I.C.I. 63,197, this hydroxylated metabolite has been detected in rat serum, but, of the total radioactivity in the sample, only 4% was present in this form compared with 57% of unchanged I.C.I. 63,197 and some 30% of non-extracted activity (see Metabolism - Section 8.) Similar evidence is not available for dog, but the I.C.I. 63,197 serum concentration data presented below include any I.C.I. 68,916 which may be present.

7.2 Serum concentrations in the dog (Trial TPD/64)

Two male and two female dogs from each of the three dose groups were investigated after 41, 55 and 90 days treatment with I.C.I. 63,197. The doses were given once daily in tablet form.

The results are given in Tables 24 and 25 and the average concentrations are illustrated in Fig. 7.

There was no significant difference in the serum concentrations in males and females, nor was there a difference in the levels observed at 41 - 55 days and 90 days.

There was a linear relationship between dose and peak serum concentration as shown in Fig. 7 (slope: peak concentration of 0.26 µg./ml. per 1 mg./kg. dose). The areas under the average serum concentration-time curves (Fig. 7) in the 0-6 hr. period were 0.245, 0.562 and 1.778 µg.hr./ml. for the 0.15, 0.5 and 1.5 mg./kg. doses respectively; again there was a linear dose-response relationship (slope: an area of 1.18 µg.hr./ml. per 1 mg./kg. dose).

Accurate determination of the biological half-life was not possible from the data obtained, but in 4 cases (Dogs 11637♂, 11650♀, 11657♀ and 11658♀), values of 2.1 hr., 2.6 hr., 2.6 hr. and 5.3 hr. were recorded.

Table 24. I.C.I. 63,197 Dog Serum Levels. Toxicity Trial TPD/64

Dose (mg./kg.)	Dog No.	Duration (days)	µg./ml. serum. Hrs. after dose					
			½	1	2	4	6	24
0.15	♂ 11637	41	0.024	0.028	0.030	0.062	0.030	0
	♂ 11638	41	0.068	0.038	0.048	0.058	0.020	0
	♀ 11641	55	0	0	0.012	0.018	0.048	0.043
	♀ 11642	55	<0.005	0.098	0.042	0.082	0.058	0.047
	♂ 11637	90	0.130	0.118	0.086	0.050	0.019	0
	♂ 11638	90	0.030	0.037	0.065	0.077	<0.005	0
	♀ 11641	90	0.019	0.023	0.038	0.040	0.050	0.020
	♀ 11642	90	0.018	0.016	0.015	0.015	0.012	0.014
0.50	♂ 11645	41	0.024	0.041	0.043	0.068	0.062	0.025
	♂ 11646	41	0	0.016	0.132	0.107	0.063	0
	♀ 11649	55	0.094	0.174	0.174	0.209	0.098	0.058
	♀ 11650	55	0.106	0.217	0.142	0.122	0.058	0.074
	♂ 11645	90	0.010	0.011	0.026	0.022	0.178	0.021
	♂ 11646	90	0.132	0.142	0.128	0.137	0.186	0.048
	♀ 11649	90	0.021	0.019	0.050	0.033	0.027	0.018
	♀ 11650	90	0.043	0.118	0.176	0.135	0.084	<0.005
1.50	♂ 11653	41	0.036	0.036	0.042	0.340	0.274	0.058
	♂ 11654	41	<0.005	0.033	0.053	0.193	0.127	0.026
	♀ 11657	55	0.217	0.269	0.459	0.376	0.122	0.016
	♀ 11658	55	0.066	0.495	0.637	0.482	0.400	0.034
	♂ 11653	90	0.244	0.324	0.360	0.440	0.295	0.057
	♂ 11654	90	0.052	0.074	0.181	0.440	0.251	0.036
	♀ 11657	90	0.200	0.612	0.660	0.403	0.229	0.052
	♀ 11658	90	0.051	0.060	0.273	0.461	0.396	0.060

Table 25. Analysis of Dog Serum Concentrations *

Dose (mg./kg.)	N**	µg./ml. serum ± S.E. Hrs. after dose					
		½	1	2	4	6	24
0.15	8	0.036 ± 0.015	0.045 ± 0.015	0.042 ± 0.009	0.050 ± 0.009	0.030 ± 0.007	0.015 ± 0.007
0.50	8	0.054 ± 0.018	0.092 ± 0.029	0.109 ± 0.021	0.104 ± 0.022	0.094 ± 0.020	0.030 ± 0.010
1.50	8	0.108 ± 0.034	0.238 ± 0.080	0.333 ± 0.085	0.392 ± 0.033	0.262 ± 0.037	0.042 ± 0.006

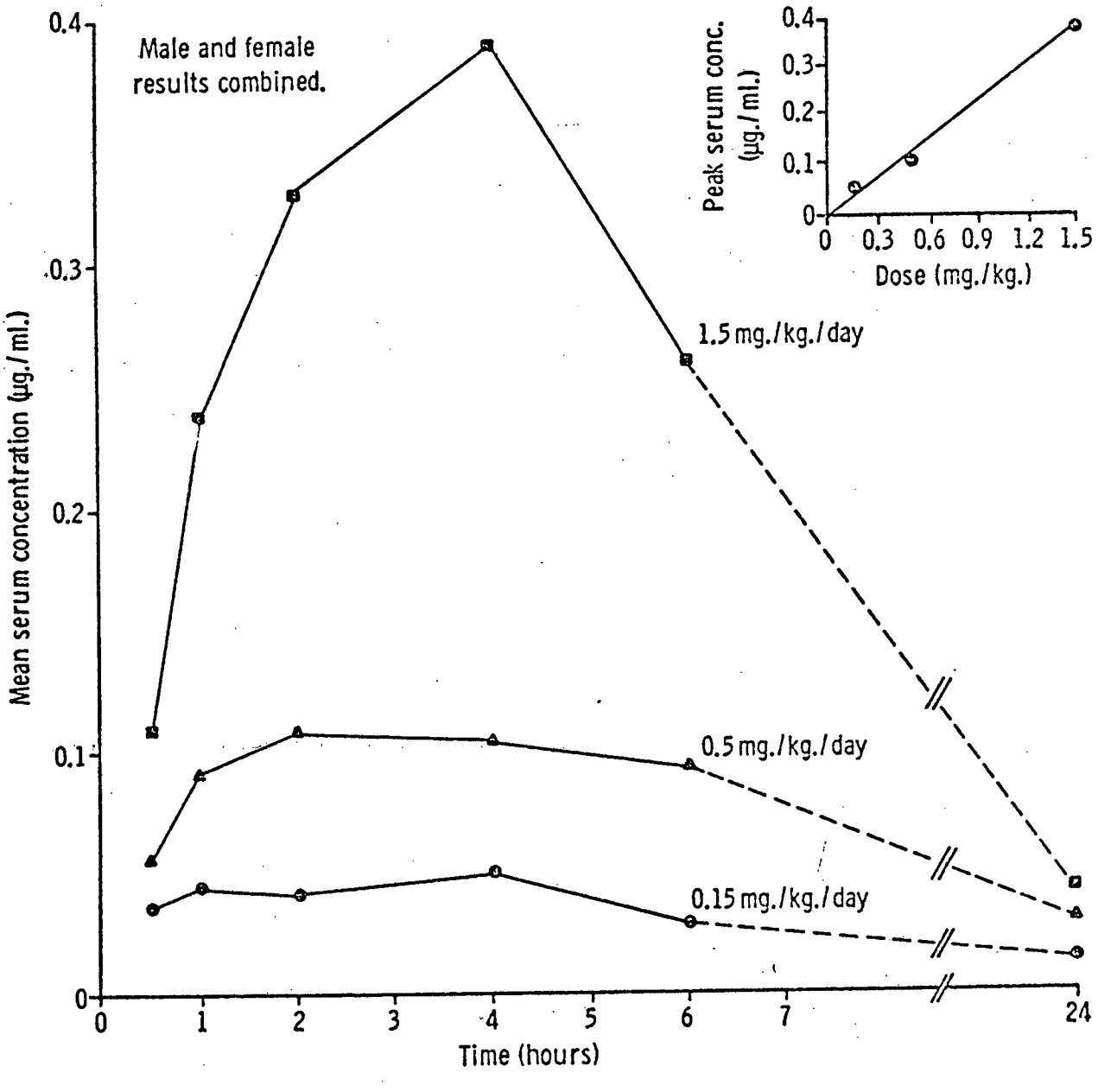
* Males and females combined; data on days 41, 55 and 90 combined

** Number of dogs.

Figure 7

Average Serum Concentrations After Prolonged Dosing.

I.C.I. 63,197 Dogs (Trial TPD/64)



7.3 Serum concentrations in the rat (Trial TPR/194)

Serum concentrations were measured in male and female rats treated for 52 and 92 days in the toxicity trial. In addition, extra rats were given single doses or 14 doses (once daily) for serum level estimations. In all cases, I.C.I. 63,197 was administered as a suspension by stomach tube.

The results are shown in Table 26. Despite the variable nature of the results, no consistently significant effect of repeated dosing was observed either in increasing or decreasing the levels seen after a single dose, nor was there a difference in the levels of males and females. All the results for a given dose level, therefore, were combined as shown in Table 26 and illustrated in Fig. 8.

The data obtained approximated to a linear relationship between dose in the range 0.25 - 1.25 mg./kg. and (a) peak serum concentrations (slope: peak concentration of 0.11 µg./ml. per 1 mg./kg. dose) and (b) area under the curve from 0 - 7 hr. (slope: area of 0.5 µg.hr./ml. per 1 mg./kg. dose), but on increasing the dose to 5 mg./kg., neither the peak level nor the area under the curve increased accordingly.

Determination of the biological half-life of I.C.I. 63,197 in rat was not possible from the available data.

The serum concentrations in rats were considerably lower than those in dogs at equivalent doses. Rat serum concentrations are compared with those in guinea-pig and mouse in the section on metabolism.

Table 26. I.C.I. 63,197 : Rat Serum Levels After One or More Doses. Trial TPR/194

Dose (mg./kg.)	Sex	No. doses	µg./ml. serum. Hrs. after dose								
			½	1	2	3	4	5	6	7	24
0.25	♂	1	0.027	0.013	0.020	<0.005	0.021	0.007	-	0	0
	♂	1	0.018	0.026	0.012	0.013	0.013	0.018	-	0	0
	♀	1	0.029	0.014	0.020	0.018	0.010	0.019	-	0.009	0
	♀	1	0.041	0.012	0.020	0.010	0.019	0.008	-	0.006	0
	♂	14	0.028	0.008	0	<0.005	0.008	0	-	0.006	0
	♂	14	0.030	<0.005	0.008	0.011	0.007	0	-	0	0
	♀	14	0	0.019	0.007	<0.005	0.006	0.006	-	0	0
	♀	14	0.034	0.019	0.028	0.016	0.006	0.006	-	0.005	0
	♂	52	0.092	0.070	0.046	-	0.046	-	0.025	-	-
	♀	52	0.057	0.025	0.070	-	0.017	-	0.043	-	-
	♂	92	0.012	0.014	0.005	-	<0.005	-	<0.005	-	-
	♀	92	0.008	0.011	0.024	-	<0.005	-	0.006	-	-
0.25 Mean ± S.E. (♂ + ♀) (1 - 92)			0.031 ±0.007	0.019 ±0.005	0.022 ±0.006	0.008 ±0.003	0.013 ±0.004	0.008 ±0.003	0.018 ±0.010	<0.005 -	0 -

Continued ... ...

... ... continued

Table 26. I.C.I. 63,197 : Rat Serum Levels After One or More Doses. Trial TPR/194

Dose (mg./kg.)	Sex	No. doses	µg./ml. serum. Hrs. after dose								
			½	1	2	3	4	5	6	7	24
1.25	♂	1	0.124	0.119	0.127	0.098	0.090	0.108	-	0.072	0.069
	♂	1	0.098	0.135	0.090	0.085	0.098	0.080	-	0.085	0.059
	♀	1	0.129	0.148	0.103	0.090	0.103	0.137	-	0.090	0.064
	♀	1	0.111	0.150	0.127	0.085	0.077	0.080	-	0.072	0.059
1.25 Mean ± S.E. (♂ + ♀) (1)			0.115 ±0.007	0.138 ±0.007	0.112 ±0.009	0.089 ±0.003	0.092 ±0.006	0.101 ±0.014	-	0.080 ±0.005	0.063 ±0.002

Continued ... ...

... ... continued

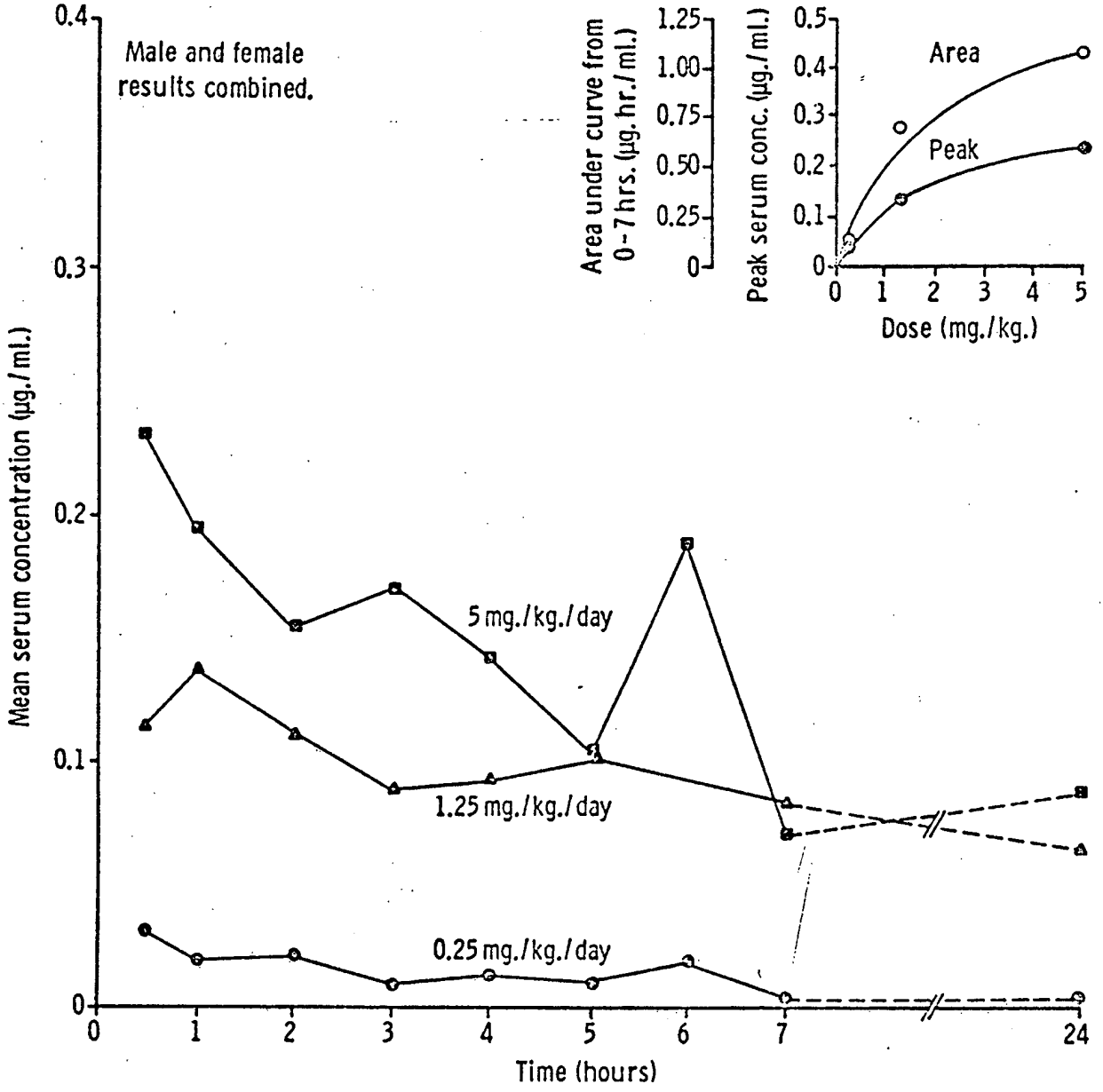
Table 26. I.C.I. 63,197 : Rat Serum Levels After One or More Doses. Trial TPR/194

Dose (mg./kg.)	Sex	No. doses	µg./ml. serum. Hrs. after dose								
			½	1	2	3	4	5	6	7	24
5.0	♂	1	0.245	0.183	0.206	0.300	0.144	0.144	-	0.065	0.107
	♂	1	0.284	0.363	0.190	0.300	0.261	0.102	-	0.063	-
	♀	1	0.269	0.331	0.180	0.222	0.183	0.149	-	0.089	0.146
	♀	1	0.331	0.245	0.253	0.238	0.081	0.206	-	0.138	0.138
	♂	14	0.326	0.100	0.050	0.068	0.110	0.050	-	0.023	0.043
	♂	14	0.155	0.053	0.063	0.135	0.120	0.043	-	0.037	0.065
	♀	14	0.110	0.047	0.058	0.053	0.057	0.053	-	0.072	0.043
	♀	14	0.210	0.185	0.094	0.043	0.080	0.072	-	0.077	0.048
	♂	52	0.310	0.294	0.214	-	0.214	-	0.246	-	-
	♀	52	0.262	0.254	0.278	-	0.025	-	0.254	-	-
	♂	92	0.176	0.135	0.127	-	0.270	-	0.118	-	-
	♀	92	0.118	0.167	0.131	-	0.159	-	0.131	-	-
5.0 Mean ± S.E. (♂ + ♀) ($\overline{1}$ - 92)			0.233 ±0.023	0.196 ±0.030	0.154 ±0.022	0.170 ±0.038	0.142 ±0.023	0.102 ±0.021	0.187 ±0.036	0.070 ±0.012	0.084 ±0.017

Figure 8

Average Serum Concentrations After One or More Doses.

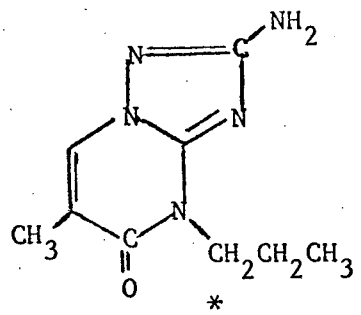
I.C.I. 63,197 Rats (Trial TPR/194)



8. THE METABOLISM OF I.C.I. 63,197

8.1 Introduction

I.C.I. 63,197 has been prepared in a labelled form with ^{14}C as shown below :

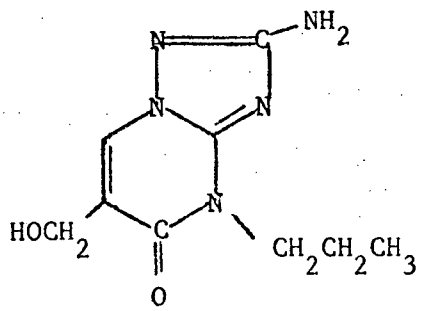


^{14}C I.C.I. 63,197

The labelled compound used in the experiments described below was shown to contain 98.2 - 98.8% I.C.I. 63,197, specific activity : 6.79 µCi/mg.

^{14}C I.C.I. 63,197 was dosed orally to mice, rats, guinea-pigs, dogs, and rhesus monkeys. The excretion and distribution of labelled material have been studied. I.C.I. 63,197 is well absorbed after oral administration and in all species except rats, at least 70% of the dosed radioactivity is passed in urine within 48 hours. Rat differs from the other species in passing a large proportion (43%) of an oral dose in faeces; it has been shown that biliary excretion is of major importance in this species.

I.C.I. 63,197 is extensively metabolised in all species studied; one single non-conjugated major metabolite, I.C.I. 68,916, occurs in the urine of all the species, and has been identified and isolated in sufficient quantity for some of its properties to be evaluated.



I.C.I. 68,916

The sera from rat, guinea-pig, and mouse contain only small amounts (4 - 7% of the total radioactivity in serum) of this metabolite, the one major component present being I.C.I. 63,197.

The tissue distribution curves in guinea-pigs show that maximum levels of radioactivity in serum and brain occur at about 1 hour after oral administration. The serum and tissue levels were found to be steady over the period $\frac{1}{4}$ to 4 hours after dosing.

48 hours after oral administration of ^{14}C I.C.I. 63,197 to a guinea-pig, the serum level was found to be 1% of the maximum level at 1 hour and only very low levels of radioactivity were detected in liver, kidney, bile, lung and heart.

8.2 Excretion

^{14}C I.C.I. 63,197 was dosed orally to small animals by catheter as an aqueous solution and to dogs and monkeys in capsules. Urine and faeces were collected separately and assayed for radioactivity. In Table 27, the total radioactivity found in urine and faeces is represented as a cumulative percentage of the dose.

Table 27. Excretion of radioactivity following oral administration of ^{14}C I.C.I. 63,197

Species :	Rat ♂[a]		Guinea-Pig ♂[b]		Mouse ♂[c]		Mouse ♂[d]		Dog ♂	Rhesus Monkey ♀
Dose mg/kg.	2.1		1.7		12.9		8.4		0.25	0.04
Time (hr.)	U	F	U	F	U	F	U	F	U (only)	U (only)
4										37.7
5										51.3
6										58.5
7	23.3	2.6	11.8	0.6	15.3		21.8			65.7
24	55.0	21.5	63.5	1.8	66.3	9.1	67.7		100.6	
31	57.3		69.0				70.6			98.6
48	59.4	41.6	73.6	11.8	69.6	10.6	74.4		103.9	100.4
55			74.1				75.0		104.0	100.6
72	59.7	42.9	74.8	14.3	70.0	11.0	76.9	5.6	104.0	
96	59.8	43.2	75.0	15.0						
Total (U + F)[e]	104.6		90.0		90.2		90.9		104.0	100.6

a : 1.6% dose in exhaled air in first 24hr.

b : no radioactivity detected in exhaled air

c : 9.2% dose in exhaled air in first 24hr.

d : 8.4% dose in exhaled air in first 24hr.

e : includes % dose in exhaled air where appropriate

U = Urine

F = Faeces

8.3 Urinary Metabolites

Urine samples from animals dosed orally with ^{14}C I.C.I. 63,197 were examined by thin-layer chromatography (T.L.C.) in chloroform/methanol (9 : 1) on Merck Silica GF plates. Urine samples from different species were found to contain the amounts of unchanged I.C.I. 63,197 and its metabolite, I.C.I. 68,916, indicated in Table 28.

Table 28. Proportions of I.C.I. 63,197 and its metabolite I.C.I. 68,916 in urine

Species	Dose mg/Kg	Sample (hr)	% Dose in sample	% ICI 63,197[a]	% ICI 68,916[a]
Rat ♂	2.1	7-24	32	9.6	14.8
Guinea-Pig ♂	1.7	7-24	52	2.9	36.8
Dog ♂	0.25	0-24	100	1.0	33.0
Rhesus Monkey ♀	0.04	0-4	38	3.0	38.1
Mouse ♂	13.5	0-7	44	2.3	35.2

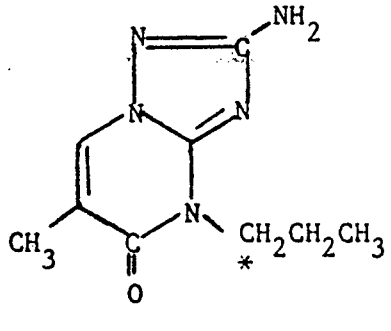
a : of the total radioactivity in the urine sample indicated.

Species differences

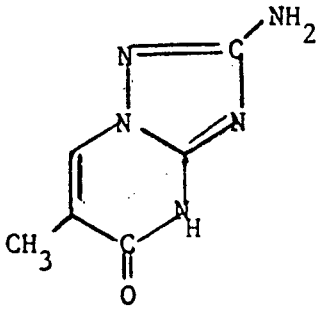
Urine samples from all species showed only two major fractions when examined by T.L.C.: I.C.I. 68,916 and polar (origin spot) material. The latter probably contains conjugates, but these have not yet been identified.

The rat produces a minor urinary metabolite (5% of the material in urine) whose molecular weight corresponds to that of I.C.I. 63,197 with an additional oxygen atom and an additional acetyl group. Its fluorescence spectrum ($Ex^{n}\lambda_{max}$ 285 nm; $Em^{n}\lambda_{max}$ 389 nm) is identical with that of N-acetyl I.C.I. 63,197 (I.C.I. 63,342). This minor metabolite has not been observed in urine from other species. But trace amounts of a labelled compound having the same T.L.C. properties have been found in chloroform extracts of mouse and guinea-pig serum.

The mouse excretes a significant proportion (9.2, 8.4 and 4.7% in 3 experiments) of an oral dose (8 - 14 mg./kg.) in exhaled air and in this respect differs from rat (1.6%) and guinea-pig (none detected). This could indicate dealkylation, but the dealkylated compound I.C.I. 65,329 has not been detected in urine from animals dosed orally with I.C.I. 63,197.



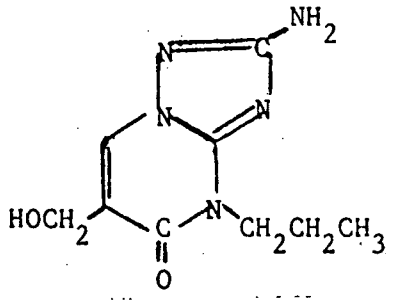
^{14}C I.C.I. 63,197



I.C.I. 65,329

As rat, mouse, guinea-pig, dog and rhesus monkey all produce I.C.I. 68,916 as the single major non-conjugated urinary metabolite, these species have at least one major metabolic pathway in common. However, lack of information concerning the polar metabolites precludes further comparison at the present time.

8.4 The properties of I.C.I. 68,916, urinary metabolite of I.C.I. 63,197



I.C.I. 68,916

I.C.I. 68,916, m.p. 183 - 184°, $Ex^{n}\lambda_{max}$ 302 nm, $Em^{n}\lambda_{max}$ 430 nm, has been isolated from a chloroform extract of mouse urine. It has not been synthesised.

The structure of the metabolite was deduced from its mass spectrum and N.M.R. spectrum. The parent ion in the mass spectrum has $^{m}/_{e}$ = 223, showing that the molecular formula differs from that of I.C.I. 63,197 by one oxygen atom. The N.M.R. spectrum shows that all the protons of the propyl side chain and the ring proton are intact. However, the signal due to the methyl group in I.C.I. 63,197 (8.04 τ) is replaced by a two-proton signal at 5.76 τ, the chemical shift being consistent with substitution at the carbon by an oxygen function.

^{14}C I.C.I. 68,916 has been dosed orally to a male guinea-pig. The urine (60% of the dose) contained I.C.I. 68,916 (49%) and conjugated material (34%).

The fluorescence spectrum of I.C.I. 68,916 is identical with that of I.C.I. 63,197. It is extracted at pH7 into chloroform and if present in blood from animals dosed with I.C.I. 63,197, it will be assayed as I.C.I. 63,197 (by the method described in Section 7.1). It has been shown that sera from guinea-pigs, mice and rats contain I.C.I. 68,916, but in all cases the amount present is small ($\leq$ 7%).

I.C.I. 68,916 has the same order of activity as I.C.I. 63,197 in the reserpine hypothermia test (in mice) and one quarter the activity of I.C.I. 63,197 as an anti-bronchoconstrictor agent in the isolated perfused guinea-pig lung experiment. It is active, but less active than I.C.I. 63,197 as an anorexiant.

The emetic properties of I.C.I. 68,916 have been evaluated in the marmoset since insufficient material was available for testing in the dog. Marmosets have not been dosed orally with I.C.I. 63,197 at 0.6 mg./kg. without concomitant vomiting. A male marmoset received 0.1, 0.6 and 2.5 mg./kg. I.C.I. 68,916 (doses administered on every second day) without adverse reaction. The same animal, a day after receiving 2.5 mg./kg., salivated and was slightly sick after receiving 5.0 mg./kg. I.C.I. 68,916. From this one experiment the emetic dose of I.C.I. 68,916 would appear to be about ten times higher than that of I.C.I. 63,197 in the marmoset.

8.5 Faecal and Biliary Excretion

Rat is the only species studied in which a significant proportion (43%) of an oral dose is excreted in faeces. Homogenisation of rat faeces in methanol afforded an extract containing 13% of the faecal radioactivity. The extract was shown by T.L.C. to contain a component having the same T.L.C. properties as I.C.I. 63,197 (23%) and unresolved polar compounds.

^{14}C I.C.I. 63,197 was dosed orally (1.3 mg./kg.) to a male rat whose bile duct had been cannulated. In the first 6 hrs. 20% of the dose was excreted in bile and by 44 hrs. 58% of the dose. At the same dose level a male guinea-pig passed only 2.5% in bile in a 5½hr. experiment: the bile was found to contain I.C.I. 63,197 (15%) I.C.I. 68,916 (6%) and polar material (79%).

8.6 Tissue Distribution of radioactive material in guinea-pigs

Male guinea-pigs were dosed orally (1.0 mg./kg.) with an aqueous solution of ^{14}C I.C.I. 63,197 and killed at different times after dosing. The results of these experiments are tabulated overleaf. The specific activity of the dosed I.C.I. 63,197 = 15,060 dpm/µg.

From these results it is apparent that serum and tissue levels are comparatively steady over the period ½ to 4 hours after oral administration, the maximum levels occurring at about 1hr. The half-life of radioactivity in serum is of the order of 4 hours. Higher levels of radioactivity occur in bile, liver, and kidney, but it should be noted that even at 6hr. the comparatively high level in bile corresponds to only ca 8 µg/ml. The brain level has dropped by 6hrs. and was not detectable at 24hr; only very low levels of radioactivity were present at 48hr. in all tissues examined.

Table 29. Distribution of labelled material in guinea-pigs following ^{14}C I.C.I. 63,197 1.0 mg./kg.

Time (hr)	Serum	Blood	Bile	Liver	Lung	Heart	Spleen	Kidney	Muscle	Fat	Brain
1/4	16.4[a]	11.5	19.5	15.7	3.8	6.9	2.6	7.2	3.8	1.8	3.8
	100[b]	70	119	96	23	42	16	44	23	11	23
1/2	16.0	11.7	24.2	13.0	5.7	3.9	3.0	6.6	4.3	2.2	3.6
	100	73	151	81	36	24	19	41	27	14	22
3/4	16.6	12.9	33.4	14.9	7.9	7.6	3.2	7.6	5.5	2.6	4.8
	100	78	202	90	48	46	19	46	33	16	29
1	22.4	17.9	46.8	18.9	5.6	12.3	3.1	10.6	7.8	3.8	7.2
	100	80	209	84	25	55	14	47	35	17	32
1½	21.1	14.4	47.8	16.4	6.0	11.2	3.1	11.1	8.2	3.2	4.0
	100	68	226	77	29	53	14	53	39	15	19
2	21.4	14.7	57.7	16.5	6.4	8.5	2.9	11.2	11.2	2.9	4.8
	100	69	269	77	30	40	13	52	52	13	22
3	17.2	clotted	69.8	15.4	5.1	4.9	3.1	12.3	10.3	3.4	4.9
	100		406	90	30	29	18	72	60	20	28
4	17.5	11.6	78.2	14.2	6.5	7.7	2.9	10.7	5.4	2.9	4.2
	100	66	446	81	37	44	16	61	31	16	24
6	11.6	8.6	122.5	10.0	4.5	3.5	2.8	11.0	5.0	2.2	2.9
	100	74	1058	86	39	30	24	95	43	19	25
24	0.41	0.30	3.6	0.86	0.31	0.10	0.09	0.35	0.15	0.20	ND
	100	71	863	207	75	24	22	85	35	47	
48	0.23	0.15	0.60	0.28	0.20	0.08	ND	0.23	ND	ND	ND
	100	60	256	119	85	32		98			

a : throughout : dpm/ml. or g x 10^{-3} b : throughout : % serum level ND : not detected

8.7 Whole-body autoradiography

Mice were dosed either intravenously or orally with 0.5 mg. (ca. 25 mg./kg.) ^{14}C I.C.I. 65,197 (specific activity 6.8 µCi/mg.) and sacrificed at the times shown below :-

	Time in hours						
Oral		1/4		1			
i.v.	1/12	1/4	1/2	1	3	5	24

Radioactivity was distributed throughout all the tissues; it was almost completely cleared from the animal at 24 hours.

At 15 min. the orally dosed animal had very high activity in the stomach contents with some labelling of urine and bile, but no activity was detected in either liver or kidney at this time. At 1 hour after an oral dose, much activity still remained in the stomach, but some absorption had occurred; activity was detected in all tissues with high concentrations in renal medulla, urine and bile. (Fig. 9)

At 5 min. after an i.v. dose, all tissues, including brain, intestinal wall and urine, were labelled. A small amount of activity was present in the contents of the pyloric region of the stomach. Levels of activity in all tissues, except bile, duodenal contents and urine, decreased progressively over the first hour (Fig. 10). At one hour the nasal secretions were also labelled (Fig. 11). At 3 and 5 hours all tissues were still labelled with high concentrations in bile, duodenal contents, bladder contents and nasal secretions. Very little activity was present in fully formed faecal pellets. After 24 hours, very little activity remained in the animal but the bladder wall was labelled.

Since only a small proportion of dosed radioactivity is recovered in mouse faeces after oral administration, and since after the i.v. dose there is evidence of extensive biliary excretion, re-absorption from the gut is indicated in this species.

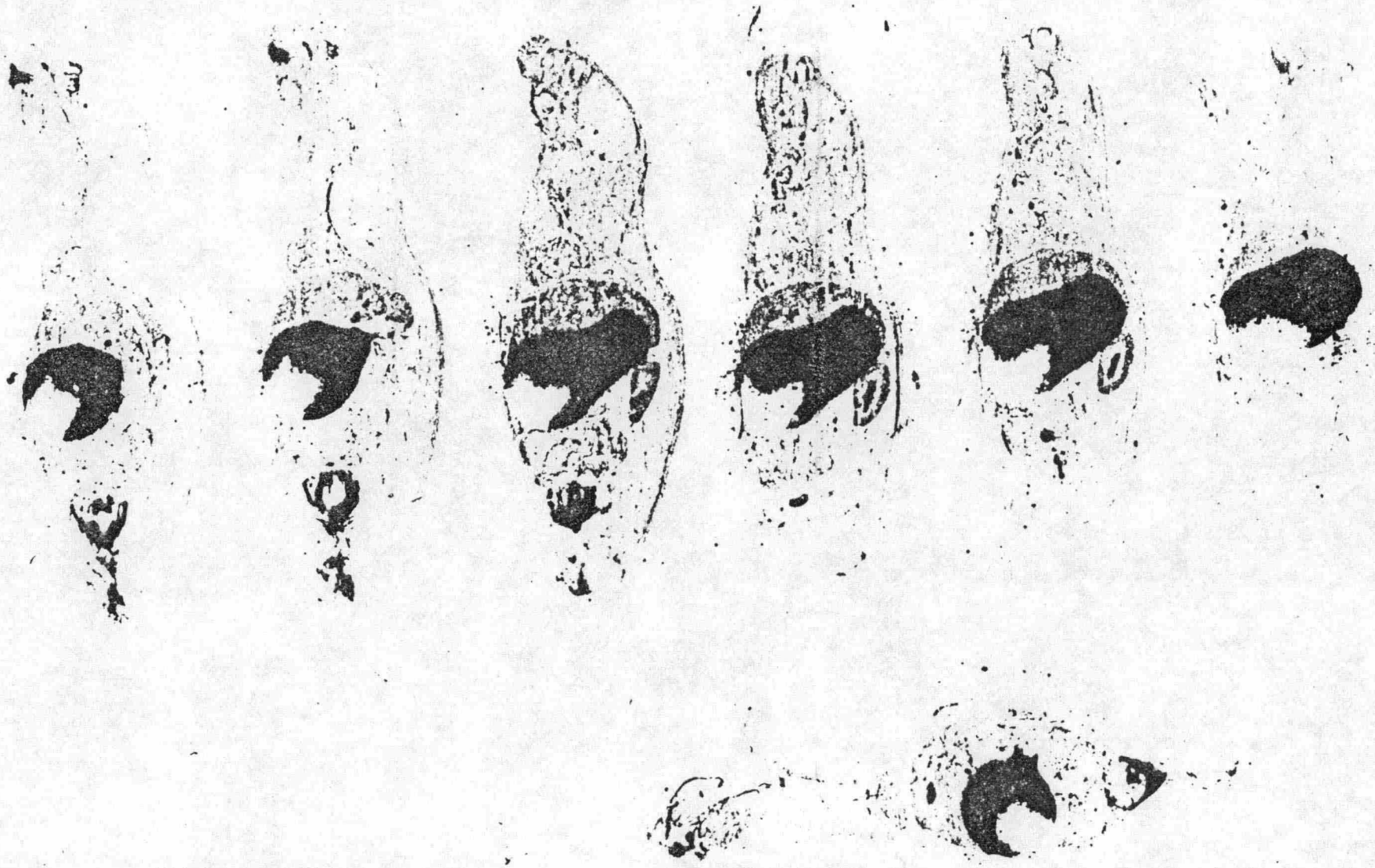


Figure 9 Whole-body autoradiography of mice - 1 hour after oral administration of I.C.I. 63,197.

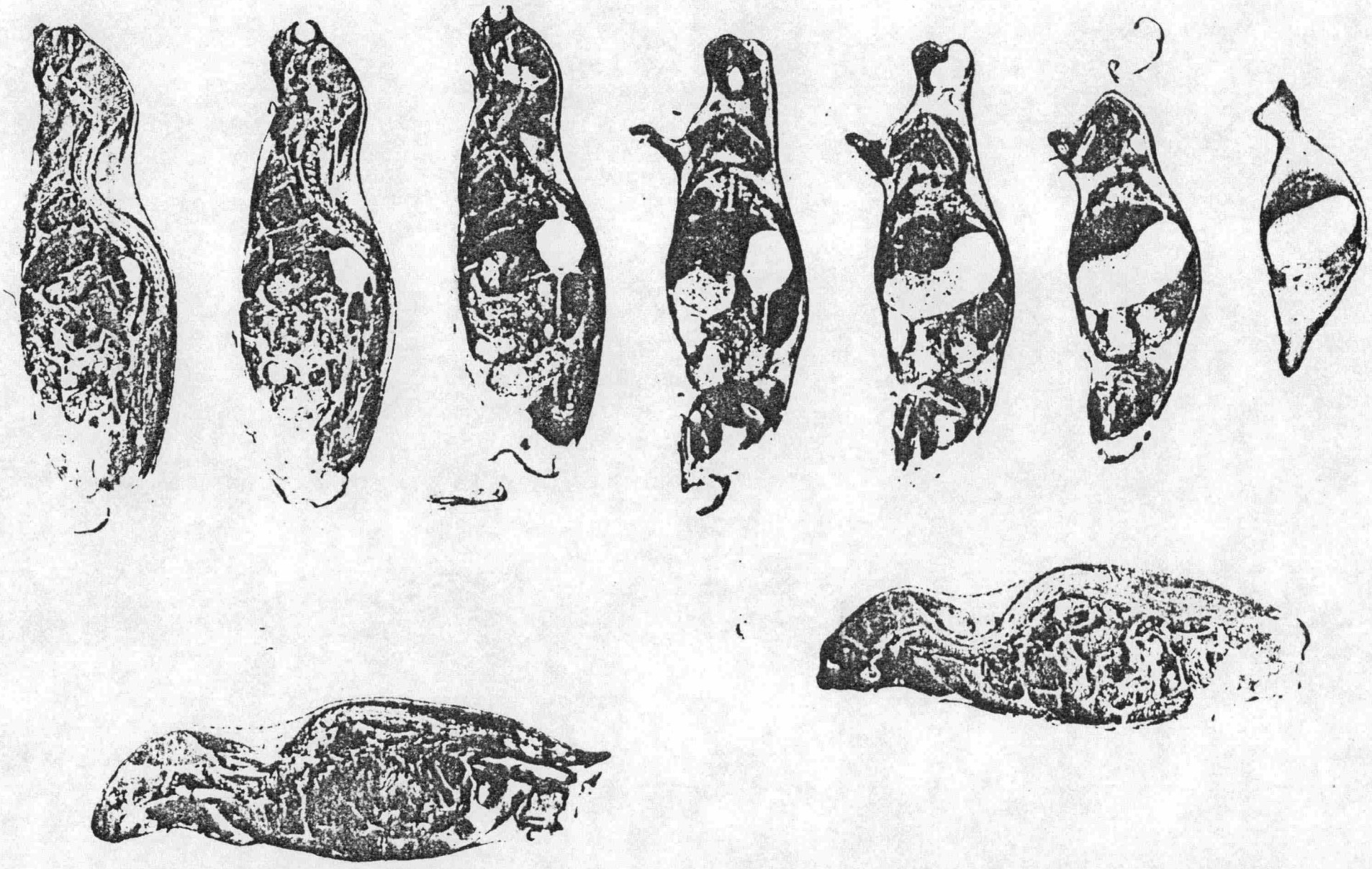


Figure 10 Whole-body autoradiography of mice - 5 minutes after i.v. administration of I.C.I. 63,197.

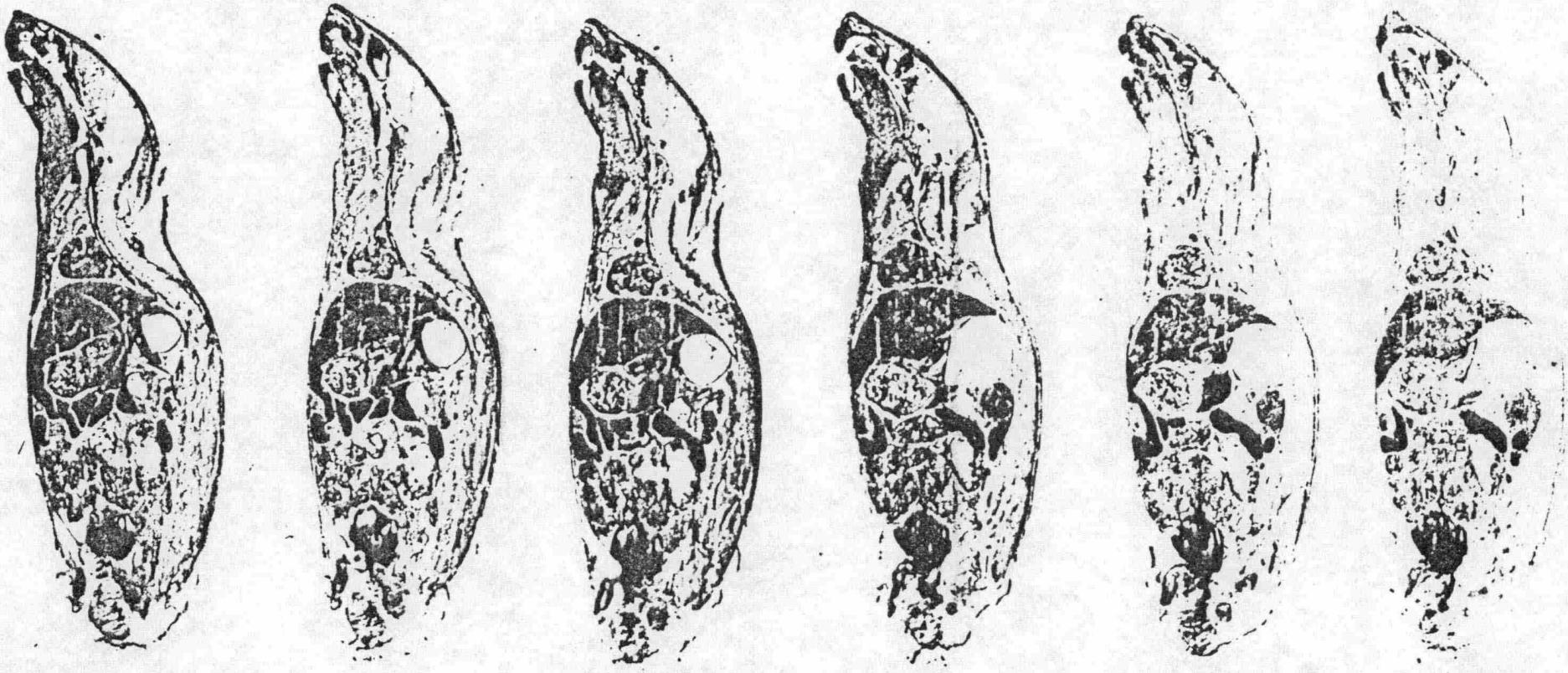


Figure 11 Whole-body autoradiography of mice - 1 hour after i.v. administration of I.C.I. 63,197.

8.8 Comparative serum levels of I.C.I. 63,197 and its metabolites

Eight male mice, five male rats, and five male guinea pigs were each dosed orally (1.0 mg./kg.) with ^{14}C I.C.I. 63,197. They were all killed 1 hour after dosing and blood removed. The sera from the mice were pooled and the sera from the other ten animals examined separately. The serum samples were counted to determine the total radioactivity levels and aliquots extracted with chloroform. The extracts were counted and also examined by T.L.C. in order that the amounts of each labelled component present could be determined.

Results in Table 30 indicate that :

(i) the total (radioactive) serum level in guinea-pig is about five to six times higher than those in rat and mouse.

(ii) 96% of the material in guinea-pig serum is extractable into chloroform at physiological pH, whereas 70% is extractable from rat serum and only 55% from mouse serum.

(iii) A major component in the serum in all three species is I.C.I. 63,197 (guinea pig : 71%, rat : 57%, mouse 34%.)

(iv) I.C.I. 68,916 is a minor component in the serum of all three species (guinea pig : 5%, rat : 4%, mouse : 7%).

Table 30. Comparative serum levels and serum constituents in guinea-pig, rat and mouse

Species (all ♂)	Serum level dpm/ml[a]	% Extracted into chloroform[b]	% Composition of chloroform extract determined by TLC (A) and calculated % in serum (B)									
			I.C.I. 63,197		I.C.I. 68,916		unidentified minor metabolites					
							X		Y		Z	
			A	B	A	B	A	B	A	B	A	B
Mouse[c]	2852	55	62	34	13	7	2	1	7	4	8	4
Rat 1	3663	61	84	51	6	4	2	1	ND		ND	
2	3522	82	78	64	6	5	4	3	ND		4	3
3	3936	77	83	64	5	4	4	3	ND		1	1
4	3829	63	84	53	4	3	4	3	ND		1	1
5	3619	67	79	53	3	2	6	4	ND		3	2
Rat (Average)[d]	3714 ± 75	70 ± 4		57±3		4		3		0		1
Guinea-pig 1	19362	91	72	66	5	5	9	8	1	1	5	5
2	18333	94	78	73	4	4	8	8	ND		2	2
3	18769	100	77	77	6	6	6	6	ND		3	3
4	19700	94	76	72	4	4	8	8	2	2	3	3
5	16014	99	66	65	6	6	9	9	5	5	6	6
Guinea-pig (Average)[d]	18436 ± 648	96 ± 2		71±2		5		8		2		4

a : specific activity 15060 dpm/µg. (as I.C.I. 63,197)

b : at physiological pH

c : values for serum pooled from 8 animals

d : ± S.E.

ND : Not detected (less than 1%)

The serum levels of I.C.I. 63,197 and I.C.I. 68,916 were calculated from the data in Table 30.

Duplicate serum samples from the guinea-pigs and the rats, and the pooled sera from the mice were assayed by the method based on the fluorescence of I.C.I. 63,197 (see Section 7.1). This method will assay both I.C.I. 63,197 and I.C.I. 68,916 as I.C.I. 63,197.

The results from the serum levels determined by the two methods are tabulated below: it will be seen that there is excellent agreement in the two sets of results.

Table 31. Comparison of serum levels of I.C.I. 63,197 + I.C.I. 68,916 determined by fluorescence and T.L.C.

Species (all ♂)		Serum concentration of I.C.I. 63,197 + I.C.I. 68,916	
		by fluorescence µg./ml.	by T.L.C. µg./ml.
Guinea-pig	1	1.12	0.91
	2	1.07	0.94
	3	1.06	1.04
	4	1.04	0.99
	5	0.88	0.76
Rat	1	0.10	0.13
	2	0.12	0.16
	3	0.15	0.18
	4	0.12	0.14
	5	0.12	0.13
Mice (8 animals)		0.12	0.08

8.9 Summary and Conclusion

I.C.I. 63,197 is well absorbed after oral administration to mouse, rat, guinea-pig, dog and rhesus monkey; in all species, except rat, at least 70% of the dosed radioactivity is passed in urine in 48 hours. Rat differs from other species in passing a large proportion (43%) of an oral dose in faeces. It has been shown that biliary excretion is of major importance in this species and whole body autoradiography indicates that biliary excretion and reabsorption occurs in mice.

I.C.I. 63,197 is extensively metabolised in all the species studied. The urine from all the species contains I.C.I. 68,916, a metabolite in which the methyl group of I.C.I. 63,197 has been hydroxylated. In guinea-pigs, it has been shown that serum levels and tissue levels of total radioactivity are notably steady over the period $\frac{1}{4}$ to 4 hours after oral administration with maximum levels at about 1 hour. The maximum serum level of I.C.I. 63,197 is higher in guinea-pig (0.87 µg./ml.) than in rat (0.17 µg./ml.) and mouse (0.06 µg./ml.) after an oral dose of 1 mg./kg.; I.C.I. 68,916 is a minor component in the serum of all three species - guinea-pig : 5%, rat : 4%, mouse : 7% of the total radioactivity in serum.

Measurement of the concentrations of I.C.I. 63,197 in the serum of rats and dogs after prolonged dosing showed :

(i) no difference in the levels between sexes

(ii) a linear dose - peak serum level response and a linear dose - area under the curve response in dogs throughout the range of doses tested (i.e. 0.15 - 1.5 mg./kg./day) with slopes of 0.26 µg./ml. per 1 mg./kg. dose and 1.18 µg.hr./ml. per 1 mg./kg. dose respectively. Similar effects were noted in rats in the dose range up to 1.25 mg./kg., with slopes of 0.11 µg./ml. and 0.52 µg.hr./ml. per 1 mg./kg. dose, i.e. about half the response seen in the dog.

(iii) a biological half-life of <3 hrs. in dogs.

There was no evidence to suggest that the serum concentrations significantly increased or decreased after prolonged administration.

REFERENCE 15

701

IMPERIAL CHEMICAL INDUSTRIES LIMITED

PHARMACEUTICALS DIVISION

Title: A Summary of Clinical Results of the Phosphodiesterase Inhibitor ICI 63,197 in a Variety of Disease States.

Author: P.F.C. Bayliss

Submitted by: P.F.C. Bayliss

Copies made: 24

Issuing Dept.: Clinical Research

Date: 23rd July, 1973.

I N D E X

INTRODUCTION

ICI 63,197 was initially selected to go to clinical trial upon the basis of its anti-bronchoconstrictor effect in animals. Further work revealed that it had a variety of effects in animals suggestive of a possible central nervous system action. Clinical trials were begun in a variety of disease states in addition to studies in normal volunteers. At an early stage it became obvious that the agent had a variety of unpleasant side effects (nausea, vomiting, dizziness, flushing) at low doses (1 - 4 mg.) which made clinical trials difficult to conduct. At a later stage the agent appeared to induce angina pectoris in two patients with no previous history of the complaint. Because of this, and the fact that no beneficial effect had been seen in pilot studies, it was decided that no further work should be done with the agent and that existing trials with the agent should be wound up.

This report summarises the results seen in various areas of medicine. The appendix contains a brief description of each trial carried out, together with what results we possess. It will be appreciated that because of the severe side effects, lack of beneficial effect and difficulty in predicting a target disease state in which ICI 63,197 might be effective it was only possible to study small numbers of patients, most trials being stopped or abandoned rather than reaching completion.

SUMMARY OF RESULTS

1. Respiratory system

No evidence of protection against histamine induced bronchospasm (aerosol or i/v histamine) could be shown. No potentiation of the broncho-dilator effects of isoprenaline or salbutamol were shown.

2. Cardiovascular system

No consistent effect was seen upon the blood pressure of either normotensive or hypertensive subjects. No consistent effect on pulse rate was seen. No evidence of potentiation of the effects of isoprenaline on heart rate were shown. Angina pectoris seems to have been induced in 2 subjects.

3. Psychiatric disorders

No beneficial effect was seen in patients with anxiety, depression or schizophrenia. In depression there was a suggestion that there was a worsening of the depressed mood.

4. The endocrine system

ICI 63,197 did not produce any effect in thyroid or adreno-cortical function. In one female subject there was a surge in LH levels. No consistent effect was produced upon a standard intravenous glucose tolerance test. There was a suggestion that ICI 63,197 suppressed the rise in insulin levels following an oral glucose load.

5. Obesity

No effect on body weight was shown.

6. Pharmacokinetics

The halflife of ICI 63,197 was between $1\frac{1}{2}$ and $3\frac{1}{2}$ hours.

7. Side effects

Nausea, vomiting and dizziness were commonly seen with ICI 63,197 at 1 mg. unit doses and above. Angina pectoris appeared on chronic dosing of 2 mg. TDS in 2 patients after 4 and 6 weeks respectively. Capillary fragility with a positive Hess's test was seen in one subject.

APPENDIX

Summaries of all clinical trials

CLINICAL PHARMACOLOGICAL STUDY OF ICI 63,197 IN NORMAL VOLUNTEERS

(PROFESSOR J. CROOKS, DUNDEE)

PROTOCOL

Fit, healthy University students were selected for the study. Informed consent was obtained from each volunteer. Subjects were not on any other medication at the time of the test.

Each subject took on one occasion a dose of ICI 63,197 of between 0.25 and 8 mg., when the following parameters were measured:-

a) Blood level of ICI 63,197 at 1, 2, 3, 4, 5, 6, and 8 hours after the dose.

b) Pulse rate and blood pressure at -10 minutes, 0 and 1, 2, 3, 4, 5, 6, and 8 hours and 3 days after the dose.

c) Plasma L.H., P.B.I. and cortisol at -10 minutes and 1 hour after a dose.

d) A blood sample was taken at -10 minutes and 4 hours and 3 days after the dose for Hb, WBC, diff. ESR, alkaline phosphatase, bilirubin, SGOT, 5 NT, urea, sodium, potassium chloride, albumin and globulin.

e) A urine sample was tested at -10 minutes and 4 hours after the dose for protein (albustix) and sugar (clinistix).

f) Two 24 hour urine collections, one immediately before and one immediately after dosing.

A note was made of any adverse effects encountered.

RESULTS

Details of subjects studied

No.	Initials	Age (yrs)	Sex	Weight (kg)	Dose ICI 63,197 (mg)
1	ML	23	F	50.5	0.25
2	IL	22	M	77.5	0.5
3	HMcD	21	M	65.5	1
4	PL	22	M	74.0	1
5	MM	20	F	56.5	2
6	IMcL	24	F	56.0	2
7	N	22	F	55.0	2
8	PR	21	M	79.0	3
9	C	23	M	72.0	3
10	APC	21	M	82.5	4
11	CB	23	M	80.0	4
12	CC	21	M	80.0	8

Blood levels of ICI 63,197

These are shown below (μg/ml.):-

No.	Dose of ICI 63,197 (mg)	Time (hrs.)							
		1	2	3	4	5	6	7	8
1	0.25	0.016	0.006	0.004	0.005				ND
2	0.5	0.008	0.017	0.005	0.005				ND
3	1.0	0.005	0.019	0.004	0.006		0.005		0.004
4	1.0	0.017	0.016	0.009	0.006		0.008		
5	2.0	0.018	0.034	0.024	0.018		0.007		
6	2.0	0.034	0.065	0.044	0.039		0.015		0.006
7	2.0	0.062	0.068		0.056	0.044	0.037	0.031	0.025
8	3.0	0.044	0.031			0.006		0	0
9	3.0	0.050	0.056		0.044	0.031		0.018	0.025
10	4.0	0.081	0.041	0.034	0.060		0.01		0.014
11	4.0	0.045	0.056	0.044	0.033		0.016		0.009
12	8.0	0.047	0.085	0.068	0.041		0.029		0.042

ND = not detected, i.e. < 0.004 μg/ml.

The half life varies from $1\frac{1}{2}$ - $3\frac{1}{2}$ hours in this series.

Effect on pulse rate and blood pressure

Pulse rate (beats/min.)

		Time (hrs.)								
No.	Dose(mg.)	-10 mins	0	1	2	3	4	6	8	3 days
1	0.25	95	80	80	80	80	80	80	80	80
2	0.5	90	90	88	68	80	80	80	80	80
3	1.0	75	75	90	72	80	80	80	80	72
4	1.0	92	84	80	80	84	80	80	80	80
5	2.0	90	96	98	96	96	96	94	96	90
6	2.0	92	84	82	76	80	80	82	80	80
7	2.0	90	88	84	88	84	80	80	88	84
8	3.0	88	72	56	56	-	64	72	72	72
9	3.0	80	76	68	68	-	84	76	80	72
10	4.0	76	78	76	78	78	78	60	60	66
11	4.0	88	84	74	66	68	66	66	60	66
12	8.0	72	86	66	68	66	68	60	64	66

Blood pressure (mmHg.)

		Time (hrs.)								
No.	Dose(mg.)	-10 mins	0	1	2	3	4	6	8	3 days
1	0.25	120/70	120/70	110/70	110/60	100/60	100/60	110/60	110/60	100/60
2	0.5	130/70	130/70	130/70	110/70	110/60	110/60	100/60	90/60	110/70
3	1.0	110/70	110/70	110/70	100/70	100/60	100/60	100/60	100/60	110/75
4	1.0	110/70	-	120/70	100/70	100/60	110/60	110/60	120/70	110/60
5	2.0	110/70	100/60	100/60	110/60	90/60	100/60	100/60	100/60	110/70
6	2.0	110/60	-	110/60	110/60	100/60	110/60	110/70	110/70	110/65
7	2.0	140/80	130/75	105/75	110/80	110/70	120/80	120/80	120/80	140/80
8	3.0	100/70	120/70	105/70	100/70	100/70	105/70	115/75	100/60	110/70
9	3.0	140/80	120/70	100/70	110/80	115/80	110/70	120/70	130/80	140/80
10	4.0	110/60	100/60	100/60	90/60	80/50	100/60	100/80	100/60	110/60
11	4.0	140/70	-	110/70	110/70	100/50	115/80	170/70	120/70	130/70
12	8.0	150/60	150/60	100/60	120/80	120/80	120/80	120/80	130/80	150/80

Effect on plasma, L.H., protein bound iodine and cortisol

L.H. levels (m.IU/ml.)

			Time (mins.)						
No.	Dose(mg.)	Sex	-10	10	15	30	60	120	150
1	0.25	F	9.0				41.0	8.0	
2	0.5	M	11.0				8.1	5.0	
3	1.0	M	8.3				5.2	8.0	
4	1.0	M	13.0					4.7	
7	2.0	F		47.0		20.0	17.0		12.5
8	3.0	M		16.5	10.0	18.0	23.0	11.5	
9	3.0	M		16.0	10.0		33.0.		17.5

Protein bound iodine

		Time (mins.)	
No.	Dose(mg.)	-10	60
1	0.25	7.4	6.9
2	0.5	5.9	6.0
3	1.0	7.4	7.0
4	1.0	4.9	5.2
5	2.0	9.4	9.3
6	2.0	5.1	5.2
7	2.0	4.2	3.7
8	3.0	4.0	5.0
9	3.0	6.2	5.8
10	4.0	5.4	5.7
11	4.0	5.5	5.4
12	8.0	5.4	5.4

Cortisol level

		Time (mins.)	
No.	Dose(mg.)	-10	60
1	0.25	17.1	12.0
2	0.5	23.8	10.9
3	1.0	16.0	17.6
4	1.0	20.2	11.2
5	2.0	26.3	29.8
6	2.0	18.9	11.7
7	2.0	22.9	26.9
8	3.0	28.0	22.7
9	3.0	13.4	12.9
10	4.0	23.8	34.3
11	4.0	17.0	17.0
12	8.0	26.8	25.8

Effect on 24 hour cyclic Amp levels (μ moles/24 hours)

No.	Dose(mg.)	24 hrs. before dose	24 hrs. after dose
1	0.25	3.71	4.33
2	0.5	2.69	3.95
3	1.0	4.35	4.9
4	1.0	3.13	2.71
5	2.0	2.26	-
6	2.0	2.37	-
7	2.0	3.18	1.68
8	3.0	5.36	3.44
9	3.0	4.96	2.88
10	4.0	3.79	5.25
11	4.0	5.15	1.19
12	8.0	1.4	4.21

Blood tests

Hb, WBC, diff.ESR, alkaline phosphatase, bilirubin, SGOT, 5 NT, urea, sodium, potassium, chloride, albumin and globulin levels at 4 hours and 3 days did not differ significantly from the pre-dose figures.

Urine tests

Neither protein nor sugar were detected in the urine at 4 hours or 3 days.

Possible side effects

These are shown below:-

No.	Dose (mg.)	Possible side effects
1	0.25	Nil.
2	0.5	Mild nausea and light headedness.
3	1.0	Nausea at 1 hour.
4	1.0	Severe dizziness at 15 minutes. Felt as if he had taken "pep pills" from 1 - 4 hours.
5	2.0	Mild nausea.
6	2.0	Nil.
7	2.0	Dizziness and sweating at 30 minutes followed by some nausea.
8	3.0	Dizziness and nausea marked ¾ - 2 hours.
9	3.0	At 30 minutes dizzy, pale, sweating. Nausea marked.
10	4.0	Nausea and flushing at 15 minutes. Vomited at 30 minutes. Light headedness for 2 - 3 hours.
11	4.0	Dizziness, flushing of face, sweating from ½ - 2 hours.
12	8.0	At 30 minutes sweaty, flushed and light headed. Vomited at 2 hours.

CONCLUSIONS

No clearly defined results emerged from this study, although certain suggestive ones were seen. The following points may be made:-

(1) The half life of ICI 63,197 in the human, following a single oral dose is between 1½ and 3½ hours.

(2) No clear effect was seen on pulse rate, although a slight fall was seen in some subjects. Similarly, no clear effect was seen on blood pressure, although in some subjects a fall was seen in the 2 - 4 hour period.

(3) One female subject (on the lowest dose) showed a surge of L.H. at 1 hour that was back to normal by 2 hours. No effect was seen on P.B.I. No regular effect was seen on cortisol levels although in some subjects there was a fall at 1 hour.

(4) The 24 hour urinary excretion of cyclic AMP rose in subjects after ICI 63,197, although in others it fell.

(5) The agent was poorly tolerated at doses above 1 - 2 mg. Nausea, vomiting, dizziness, sweating and flushing were complained of.

EFFECT OF ICI 63,197 UPON THE ENDOCRINE SYSTEM IN NORMAL SUBJECTS

(DR. D. DAVIES, MANCHESTER)

PROTOCOL

Fit, healthy University students who are on no drugs (including the contraceptive "pill") were chosen for this study. They gave informed consent to participation.

Subjects were studied in the fasting state.. Blood samples were taken immediately before and at ½, 1, 1½, 2, 3, 4, 5, and 6 hours after a single oral dose of 2 mg. ICI 63,197. Blood samples were assayed for:-

a) Growth hormone

b) Insulin

c) Cortisol

d) Thyroxine iodine

e) Glucose

f) L.H. and F.S.H.

g) Blood level of ICI 63,197 (at 0, 1 and 2 hours)

A note was made of any adverse reactions complained of. A cup of coffee was taken by the volunteers between ½ and 1 hours, a light meal between 1½ and 2 hours and a cup of tea between 4 and 5 hours.

RESULTS

Details of subjects studied

No.	Initials	Age (yrs)	Sex	Menstrual cycle	Day of cycle
1	PWC	19	M		
2	ID	23	M		
3	JC	21	F	6/28-35	9
4	AD	18	M		
5	JA	21	F	1/28	7
6	AS	21	F	5/28-30	21
7	PC-Y	21	M		
8	MPY	21	M		

Blood levels of ICI 63,197 (ng/ml)

	Time (hrs.)					
No.	½	1	1½	2	4	5
1	ND	ND	ND			
2	ND	ND	ND		ND	ND
3	0.04	0.07		0.05	0.03	
4	0.03	0.03		ND	0.05	
5	0.03	0.03		0.025	0.035	
6	ND	0.02		0.03	ND	
7	ND	0.02		0.03	0.03	
8	0.02	0.03		0.02	ND	

ND = not detected, i.e. 0.004 µg/ml.

Effect on Human Growth hormone

	Time (hrs.)								
No.	0	½	1	1½	2	3	4	5	6
1	33	27	6.9	3.6					
2	1.4	3.6	15	16.5	2.1	1.5			
3	5.7	7.6	5.1	2.6	2.0	1.5	6.4	2.7	1.7
4	1.3	3.7	2.9	1.8	1.3	1.6	1.6	2.4	1.4
5	3.9	2.0	1.6	1.3	1.4	1.3	1.8	1.7	2.0
6	8.8	7.8	2.5	1.5	1.3	1.1	1.3	1.5	1.1
7	2.7	9.4	6.0	10.5	3.7	2.0	2.7	6.0	22
8	1.6	2.6	6.0	4.0	1.7	1.4	1.4	1.4	1.3

Effect on Insulin levels

	Time (hrs.)								
No.	0	½	1	1½	2	3	4	5	6
1	13	13	18	13					
2	10	7	10	10	7	40			
3	60	30	26	74	24	170	25	42	20
4	18	30	19	14	26	50	36	39	32
5	16	17	21	20	75	75	48	70	42
6	22	22	23	18	115	88	52	58	58
7	36	24	21	30	37	38	27	20	24
8	17	18	18	24	18	64	43	24	31

Effect on cortisol level

	Time (hrs.)								
No.	0	½	1	1½	2	3	4	5	6
1	18.5	15	12						
2	11.5	32.5	26.5		12.5				
3	15.5	14	11		9.5				
4	16.5	12	15		13.5				
5	13.5	13.5	21		15.5		7.0		
6	16.5	13.5	11.5		12.0		16.0		
7	18.0	12.5	24.0		24.0		18.5		
8	16	13.5	12.0		18.0		12.0		

Effect on Thyroxine iodine levels

	Time (hrs.)								
No.	0	½	1	1½	2	3	4	5	6
1	4.3		3.8						
2	4.5		4.6		4.5				
3									
4									
5	3.7		4.0		3.9		3.9		
6	3.6		3.6		3.4		3.3		
7	4.5		5.5		5.0		5.2		
8	5.1		5.5		5.4		5.6		

Effect on blood glucose

	Time (hrs.)								
No.	0	½	1	1½	2	3	4	5	6
1	60	70	60	75					
2	70	60	55	55	60	65			
3	60	65	70	75	50	95	60	70	70
4	65	60	70	60	70	85	75	75	70
5	70	65	65	70	85	75	80	85	80
6	80	60	60	55	85	85	80	70	55
7	70	70	55	60	80	80	65	60	75
8	70	60	55	65	65	60	65	65	90

Effect on L.H. (milli.I.U/ml.)

	Time (hrs.)								
No.	0	½	1	1½	2	3	4	5	6
1	6	5	5	5					
2	3	6	4	3	5				
3	5	5	5	5	6	5	6	4	
4	7	6	7	7	7		7	9	7
5	3	5	5	4	6	6	5	9	6
6	2	2	1	5	3	2	3	2	7
7	ND	1	5	4	3	2	2	1	2
8	1	1	1	3	3	1	1	6	2

ND = not detected i.e. less than 1 m. I.U./ml.

Effect on F.S.H. (milli.I.U./ml.).

	Time (hrs.)								
No.	0	½	1	1½	2	3	4	5	6
1	1	1	1	ND					
2	ND	ND	ND	ND	ND			ND	
3	6	5	6	5	5	5	5	5	
4	1	1	1	1	ND		ND	ND	ND
5	4	4	3	2	3	3	2	3	3
6	5	7	5	7	7	6	5	5	5
7	4	4	7	4	5	4	4	4	5
8	6	4	6	6	6	0	6	5	8

ND = not detected i.e. less than 1 m.I.U./ml.

Possible side effects

These are shown below:-

No.	Possible side effects
1	Nausea and flushing 10 mins. after tablet. Gone by 1½ hours but fainted at 2 hours.
2	Flushing and slight nausea noted at 10 minutes.
3	Flushing and slight nausea noted at 1½ - 3½ hours.
4	Nausea present ½ - 3 hours.
5	Nausea 30 minutes. Vomited at 45 minutes.
6	Marked nausea 1 - 1½ hours.
7	Flushed, sweating and restless at 1 hour. Nausea throughout.
8	Sweating at 1 hour.

CONCLUSIONS

(1) ICI 63,197 did not have any significant effect upon any parameters measured, in the setting of the study.

(2) Every subject experienced some type of adverse reaction, especially nausea and flushing.

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EFFECT OF ICI 63,197 UPON ORAL & INTRAVENOUS GLUCOSE TOLERANCE TESTS

DR. D. DAVIES, MANCHESTER.

PROTOCOL

Two fit, healthy young volunteers were chosen. They gave informed consent to taking part in the study. They underwent a glucose tolerance test (GTT) on 4 occasions each. Twice the GTT was an oral one, once with a placebo tablet and once with a single dose of 2 mg. ICI 63,197, and on the other two occasions the GTT was an intravenous one, again both with placebo and 2 mg. ICI 63,197. Tests were carried out at 7 day intervals.

Blood samples were taken 60 mins., 30 mins. and immediately before the glucose load was given and for the intravenous test 2, 5, 10, 20, 30, 40 and 60 minutes after, and for the oral test 30, 60, 90, 120 and 150 minutes after.

Blood samples were assayed for:-

(a) Glucose

(b) Insulin

(c) Free fatty acid levels.

Possible side effects were noted.

RESULTS

Two male subjects were studied.

724

Oral GTT

Effect of Glucose levels

		Time (mins.)							
No.	Regime	-60	-30	0	30	60	90	120	150
1	Placebo	80	75	70	100	130	80	45	45
1	Active	75	80	65	110	95	75	70	75
2	Placebo	35	35	65	100	70	60	40	45
2	Active	60	55	55	65	85	70	60	60

Effect on Insulin levels

		Time (mins.)							
No.	Regime	-60	-30	0	30	60	90	120	150
1	Placebo	16	16	13	76	125	35	18	14
1	Active	14	18	16	56	44	16	14	16
2	Placebo	18.5	15.5	14	84	92	54	52	18
2	Active	12	12	14.5	12	47	52	32	22.5

Effect on FFA levels (mEq/l)

		Time (mins.)							
No.	Regime	-60	-30	0	30	60	90	120	150
1	Placebo	2.24	2.24	2.12	2.67	1.00	1.05	1.24	1.90
1	Active	1.17	1.03	1.08	1.01	0.61	1.08	2.34	2.09
2	Placebo	0.80	0.69	0.93	1.42	0.74	0.77	0.83	1.09
2	Active	1.14	1.59	1.64	2.27	3.18	0.89	0.73	0.93

Intravenous GTT

Effect on Glucose levels

		Time (mins.)									
No.	Regime	-60	-30	0	2	5	10	20	30	40	60
1	Placebo	55	55	65	165	165	155	140	110	95	70
1	Active	75	75	70	215	175	170	145	115	100	75
2	Placebo	65	60	60	130	140	145	120	90	75	60
2	Active	65	70	70	315	250	230	200	175	155	115

Effect on Insulin levels

		Time (mins.)									
No.	Regime	-60	-30	0	2	5	10	20	30	40	60
1	Placebo	19	18	20	53	45	40	34	34.5	34.5	27
1	Active	17	20	17	52	62	56	62	52	52	25.5
2	Placebo	13	9.4	9.4	31	70	44	33	33	31	20
2	Active	7.8	9.4	9.4	7.8	9.4	14	33	41	51	44

Effect on FFA levels (mEq/l)

		Time (mins.)									
No.	Regime	-60	-30	0	2	5	10	20	30	40	60
1	Placebo	0.99	1.28	1.39	1.31	1.28	1.31	0.83	0.54	0.51	0.48
1	Active	1.84	1.82	1.82	2.0	1.86	1.91	1.68	1.05	1.09	0.9
2	Placebo	0.81	0.88	0.93	1.41	1.29	1.31	1.29	1.52	1.14	1.29
2	Active	1.55	1.20	1.22	1.73	1.83	1.97	2.25	1.69	1.50	0.78

Possible side effects

No side effects were seen.

CONCLUSIONS

Although only 2 subjects were used, ICI 63,197 seemed to suppress the insulin rise following the glucose load in both subjects in the oral test.

EFFECT OF ICI 63,197 IN HISTAMINE INDUCED BRONCHOSPASM IN ASTHMATIC PATIENTS

(DR. J.W. KERR, GLASGOW)

PROTOCOL

Allergic asthmatic patients with reversible airways obstruction were chosen for the study. Pregnant women and patients under 18 or over 45 years were excluded.

Patients were studied on the occasions when they took either ICI 63,197 2 mg. orally, or an identical placebo tablet, in a double blind randomised fashion. 2 hours later, bronchospasm was induced with an intravenous injection of histamine, and the forced expiratory volume in seconds (FEV_1) and the vital capacity were measured before and at 5, 10, 15, 20 and 25 minutes thereafter.

A note of possible side effects were made.

RESULTS

Details of patients studied

No.	Initials	Age (yrs)	Sex	Diagnosis
1	AS	60	M	"pollen asthma"
2	DI	22	M	hay fever and asthma
3	ED	22	F	asthma
4	IN	41	M	asthma

Effect on FEV_1 (litres)

No.	Time after i/v histamine (mins.)											
	Placebo						ICI 63,197					
	0	5	10	15	20	25	0	5	10	15	20	25
1	1.90	1.70	1.70	1.75	1.80	1.90	2.0	1.80	1.70	1.80	2.00	2.00
2	4.15	4.45	4.20	4.00	4.65	4.70	4.50	4.50	4.50	4.30	4.40	4.40
3	0.65	0.70	0.65	0.65	0.65	0.65	0.75	0.55	0.50	0.50	0.50	0.65
4	1.80	1.45	1.30	1.30	1.90	1.90	1.65	0.8	0.75	0.8	0.8	0.8

Effect on vital capacity (litres)

No.	Time after i/v histamine (mins.)											
	Placebo						ICI 63,197					
	0	5	10	15	20	25	0	5	10	15	20	25
1	3.70	3.50	3.55	3.70	3.80	3.80	3.60	3.50	3.50	3.50	3.60	3.60
2	5.60	5.40	5.15	5.20	5.65	5.60	5.50	5.30	5.30	5.30	5.35	5.40
3	1.50	1.45	1.50	1.50	1.50	1.50	1.50	1.30	1.16	1.15	1.15	1.25
4	3.40	3.05	2.75	2.60	2.60	2.60	2.10	1.90	1.90	2.05	2.05	2.10

Possible side effects

Two patients complained of nausea following ingestion of ICI 63,197 and one was sick. No side effects were noted during the placebo periods.

CONCLUSIONS

No evidence was obtained from these 4 patients to show that ICI 63,197 gave any protection from the falls in FEV_1 and VC induced by intravenous histamine.

EFFECT OF ICI 63,197 WITH AND WITHOUT SALBUTAMOL UPON FORCED EXPIRATORY VOLUME IN ASTHMATICS. (DR. K.N.V. PALMER, ABERDEEN).

PROTOCOL

Asthmatic patients between the ages of 18 and 45, who had reversible airways obstruction, were included in the study. Pregnant women were excluded. The aim of the study was to look for possible potentiation of the effect of salbutamol upon bronchospasm induced by an aerosol of histamine.

Patients were seen on 4 occasions, when one of the following were given:-

a) 2 mg. salbutamol

b) 2 mg. ICI 63,197

c) 2 mg. salbutamol + 2 mg. ICI 63,197

d) placebo.

Drugs were given in a double blind manner in random sequence. Two hours after taking the tablet a dose of histamine by aerosol was given. This dose had been previously determined as one that would produce a measurable bronchospasm.

Before and at 1, 5, 15 and 30 minutes after the histamine the forced expiratory volume in 1 second (FEV_1), pulse rate and blood pressure were measured.

RESULTS

Only 2 patients were included in the study as in the second severe side effects occurred.

Details of the patients studied

No.	Initials	Age (yrs)	Sex	Weight (kg)
1	RR	32	M	71
2	DW	34	M	70

Effect upon FEV_1 (litres)

Patient No. 1

	Histamine challenge (time in mins.)				
Regime	0	1	5	15	30
placebo	2.75	1.7	1.75	2.4	2.65
ICI 63,197	2.95	3.25	3.75	3.70	3.90
salbutamol	2,8	4.0	4.05	4.05	4.10
combination	2.55	2.10	2.10	2.65	2.95

Patient No. 2

	Histamine challenge (time in mins.)				
Regime	0	1	5	15	30
placebo	2.45	1.25	1.25	1.30	1.32
ICI 63,197	3.15	2.2	2.25	2.72	3.0

Effect on pulse rate (beats/min.)

Patient No. 1

	Histamine challenge (time in mins.)				
Regime	0	1	5	15	30
placebo	88	80	76	72	70
ICI 63,197	64	60	60	66	64
salbutamol	68	72	72	72	72
combination	76	56	58	60	60

Patient No. 2

	Histamine challenge (time in mins.)				
Regime	0	1	5	15	30
placebo	76	93	88	80	72
ICI 63,197	64	76	84	80	76

Effect on blood pressure

Patient No. 1

	Histamine challenge (time in mins.)				
Regime	0	1	5	15	30
placebo	115/80	115/80	110/75	105/65	110/65
ICI 63,197	110/55	110/60	110/60	110/60	110/60
salbutamol	110/55	110/60	110/60	110/60	110/60
combination	120/70	120/70	120/70	120/75	120/80

Patient No. 2

	Histamine challenge (time in mins.)				
Regime	0	1	5	15	30
placebo	110/75	125/75	110/70	105/65	100/65
ICI 63,197	110/70	120/80	120/80	110/75	110/70

Possible side effects

Patient No. 1 had no side effects during the study. Patient No. 2 took ICI 63,197 at the first sitting. 20 minutes after ingestion the patient complained of severe nausea, followed by copious vomiting. The patient was markedly agitated and restless and became pale, cold and clammy. These features slowly wore off over the next 2 hours. Because of these symptoms this patient was not given ICI 63,197 on a second occasion and the triallist was unwilling to expose further patients.

CONCLUSIONS

In both patients there was some evidence that the effect of histamine upon FEV_1 was reduced by ICI 63,197. In the one patient taking salbutamol there was clear protection against histamine bronchospasm but when combined with

ICI 63,197 this effect of salbutamol disappeared. The data is insufficient to draw any conclusions.

Severe nausea and vomiting occurred in one patient.

EFFECT OF ICI 63,197 ON THE EFFECTS OF INHALED ISOPRENALINE (DR.H.BEUMER, UTRECHT)

PROTOCOL

Patients with mild/moderate emphysema who were known to respond to inhaled isoprenaline by bronchodilatation were chosen for the study. They were tested on two occasions, upon which they took either a single dose of ICI 63,197 or an identical placebo in random order, double blind. Measurements were done on each occasion as follows:-

Time (mins.)	
0	ICI 63,197 or placebo tablet given.
60	Pulse rate, nitrogen washout and functional reserve capacity measured.
90	Pulse rate, nitrogen washout and functional reserve capacity measured.
120	As at 90 mins.

RESULTS

12 patients were studied.

Effect on pulse rate

* = time after isoprenaline inhalation

	Placebo			ICI 63,197		
No.	Control	30 mins.*	60 mins.*	Control	30 mins.*	60 mins.*
1	80	84	84	72	70	72
2	76	84	80	80	84	84
3	84	80	76	84	88	80
4	60	52	60	68	64	60
5	72	72	68	80	72	80
6	68	64	72	86	60	68
7	92	92	88	100	88	80
8	80	84	80	96	96	96
9	84	76	76	84	84	84
10	80	72	76	80	72	72
11	96	100	88	96	88	88
12	120	120	120	124	124	118

Effect on Nitrogen washout

* = time after isoprenaline inhalation

	Placebo			ICI 63,197		
No.	Control	30 mins.*	60 mins.*	Control	30 mins.*	60 mins.*
1	64.0	64.0	64.0	64.0	64.0	64.0
2	64.0	64.0	64.0	63.9	64.0	64.0
3	64.0	64.0	63.9	64.0	64.0	64.0
4	48.2	47.4	55.1	54.0	64.0	55.2
5	64.0	64.0	64.0	64.0	64.0	64.0
6	64.0	57.6	55.9	64.0	58.9	55.4
7	27.5	26.7	25.7	28.0	29.3	25.3
8	64.0	64.0	64.0	64.0	64.0	64.0
9	64.0	64.0	64.0	64.0	63.9	64.0
10	64.0	64.0	64.0	64.0	64.0	64.0
11	64.0	64.0	64.0	64.0	64.0	64.0
12	64.0	64.0	64.0	64.0	64.0	64.0

Effect on functional reserve capacity

	Placebo			ICI 63,197		
No.	Control	30 mins.	60 mins.	Control	30 mins.	60 mins.
1	6.57	6.61	6.60	5.77	6.22	5.95
2	4.57	5.34	5.30	5.05	4.83	4.27
3	5.22	5.09	5.51	5.78	5.04	5.56
4	4.36	4.40	4.54	5.12	5.27	4.65
5	6.55	6.24	6.60	6.39	6.16	6.20
6	4.70	4.26	4.41	4.82	4.17	4.20
7	2.60	2.48	2.57	2.38	3.30	2.72
8	6.69	5.69	6.43	6.30	6.25	6.48
9	4.99	4.58	4.62	5.17	5.09	5.13
10	5.24	4.57	4.97	4.93	4.66	4.97
11	6.69	6.88	6.81	6.65	6.84	6.71
12	4.16	4.43	4.43	4.19	4.25	4.39

CONCLUSIONS

No effect of ICI 63,197 was demonstrated over and above placebo upon pulse rate, functional reserve capacity and nitrogen washout after the inhalation of isoprenaline. However, it must be noted that no effect of isoprenaline per se was detected. This is probably due to the fact that the first measurement at 30 minutes after inhalation was too late.

EFFECT OF ICI 63,197 IN ENDOGENOUS DEPRESSION (DR. D. ECCLESTON, EDINBURGH)

PROTOCOL

Patients with classical endogenous depression who were not on any other medication were chosen for the study. Patients under the age of 18 or over 60 were excluded. Patients were given 2 mg. ICI 63,197 TDS for 21 days and were to leave the trial at that time if no beneficial effect had been seen.

Depression was rated (Hargreaves scale) daily, as were possible side effects.

RESULTS

4 patients completed 21 days treatment each. The trial was then abandoned, due to side effects and a worsening of depression in all 4 patients, apparently due to ICI 63,197.

Detailed results are not available, but the triallist reported the following:-

(1) All 4 patients became markedly worse over the 21 days of treatment. This worsening was considered more than would be expected from the natural history of their respective illnesses. They were all given ECT on stopping ICI 63,197.

(2) In one patient the effect of ECT was considered to be greater than expected from the patient's clinical state.

(3) All 4 patients complained of nausea in the first 3 - 5 days of the trial, although this wore off with no intervention.

(4) One patient developed a tendency to bruise with a positive Hess's test. The agent was withdrawn whereupon the capillary fragility disappeared over the next 3 - 4 days.

ICI 63,197 IN SCHIZOPHRENIA (DR. R.V. MAGNUS, BIRMINGHAM)

PROTOCOL

Chronic inpatient schizophrenics were chosen for the study upon the basis that they had sufficient symptomatology to show a significant change. Patients under 18 or over 50 years of age were excluded, as were pregnant women and people with physical illness.

Patients were given 1 placebo tablet TDS for 6 days, followed by 1 mg. ICI 63,197 (1 x 1 mg. tablet) TDS for 7 days, followed by 2 mg. ICI 63,197 (1 x 2 mg. tablet) TDS for 7 days if the agent was tolerated. All tablets were identical.

On entry to the trial, the following were recorded:-

(a) age, sex, weight

(b) diagnosis

(c) rating of schizophrenia

(d) blood sample for Hb, WBC, diff. ESR, urea, bilirubin, alkaline phosphatase and SGOT.

On days 6, 13 and 20, the following were recorded:-

(a) rating of schizophrenia

(b) clinical assessment

(c) possible side effects

(d) pulse rate and blood pressure

(e) blood sample for the tests mentioned above.

RESULTS

Details of patients studied

No.	Initials	Age (yrs)	Sex	Weight (kg)	Diagnosis
1	CJT	19	M	140	Schizo-affective disorder
2	MW	19	M	156	High grade sub-normal with chronic schizophrenia
3	FM	49	M	112	Chronic paranoid schizophrenia
4	WJ	54	M	150	Chronic schizophrenia
5	WH	52	M	-	Paranoid schizophrenia
6	SP	28	F	112	Chronic hebephrenic schizophrenia

Effect on clinical state

No patient showed any significant change in their schizophrenic state. Their rating scores are shown below:-

No.	Day 0	Day 6[1]	Day 13[2]	Day 20[3]
1	15	13	12	12
2	13	13	13	10
3	9	9	9	12
4	16	17	11	11
5	14	14	14	12
6	21	21	20	25

1 = end of 6 day placebo period

2 = end of 7 days at 1 mg. ICI 63,197 TDS

3 = end of 7 days at 2 mg. ICI 63,197 TDS

Possible side effects

These are shown below:-

No.	Possible side effects
1	Nil
2	Slight nausea at 2 mg. TDS
3	Marked nausea at 2 mg. TDS
4	Nil
5	Nil
6	Nil

Effect on pulse and B.P.

These are shown below:-

Pulse (beats/min.)

No.	Day 0	Day 6	Day 13	Day 20
1	80	72	80	72
2	80	72	72	72
3	80	80	72	80
4	80	72	72	72
5	72	72	80	80
6	80	-	100	100

B.P. (mmHg)

No.	Day 0	Day 6	Day 13	Day 20
1	120/70	120/70	130/70	140/75
2	130/75	120/70	120/70	130/80
3	130/90	120/70	120/70	130/70
4	130/80	120/70	130/70	130/80
5	135/70	120/70	140/80	130/70
6	130/70	-	130/70	130/80

Effect on blood tests

No significant effect was seen upon Hb, WBC, diff. ESR, urea, bilirubin, alkaline phosphatase or SGOT.

CONCLUSIONS

(1) ICI 63,197 had no effect upon the schizophrenic state.

(2) ICI 63,197 had no significant effect upon pulse rate, blood pressure on the blood tests used.

(3) 2 out of 6 patients complained of nausea at 2 mg. ICI 63,197 TDS.

ICI 63,197 IN ANXIETY STATES (DR. R.V. MAGNUS, BIRMINGHAM)

PROTOCOL

Inpatients with established anxiety states were selected for the study. Those under 18 or over 50 years of age were excluded, as were pregnant women and people with overt physical illness.

Patients were given 1 placebo tablet TDS for 5 days, followed by 1 mg, ICI 63,197 (1 x 1 mg. tablet) TDS for 7 days, followed by 2 mg. ICI 63,197 (1 x 2 mg. tablet) TDS for 7 days, if the patient could tolerate it. All tablets were identical.

On entry to the trial the following were recorded:-

(1) age, sex, weight

(2) diagnosis

(3) severity of anxiety

(4) anxiety rating using the Taylor Manifest Anxiety Scale

(5) blood sample for Hb, WBC, diff.ESR, urea, bilirubin, alkaline phosphatase and SGOT.

At the 5th, 12th and 19th days the following were recorded:-

(1) Taylor Manifest Anxiety score

(2) clinical assessment

(3) pulse rate and blood pressure

(4) possible side effects

(5) blood sample for tests above

RESULTS

Details of patients studied

No.	Initials	Age (yrs)	Sex	Weight (kg)	Diagnosis	Rating of anxiety
1	VM	18	M	154	Anxiety state	Severe
2	AC	32	F	112	Anxiety state	Moderate
3	MN	24	F	116	Anxiety state	Moderate
4	RT	44	F	120	Anxiety state with obsession	Severe
5	KH	22	M	130	Anxiety state	Moderate

Effect on anxiety

Clinically, patients No. 1 and 5 became somewhat worse on ICI 63,197, patients No. 2 and 3 showed n o change while patient No. 4 showed an improvement. The Taylor scores are shown below:-

No.	Day 0	Day 5[1]	Day 12[2]	Day 19[3]
1	31	31	39	39
2	19	22	23	23
3	29	29	29	25
4	36	35	29	16
5	27	22	36	25

1 = end of placebo period

2 = end of 1 mg. TDS for 7 days

3 = end of 2 mg. TDS for 7 days

Possible side effects

These are shown below:-

No.	Possible side effect
1	Vomited x 1 on 2 mg. TDS. Then settled.
2	Nil
3	Nil
4	Nausea on 2 mg. TDS. Maxolon 10 mg. TDS given with good effect
5	Nausea on 2 mg. TDS. Maxolon 10 mg. TDS given with good effect

Effect on pulse and blood pressure

Pulse rate and B.P. throughout the study are shown below:-

Pulse rate (beats/min.)

No.	Day 0	Day 5	Day 12	Day 19
1	80	80	88	80
2	100	80	80	80
3	80	80	72	72
4	88	-	80	72
5	88	72	88	80

B.P. (mmHg)

No.	Day 0	Day 5	Day 12	Day 19
1	140/80	130/70	130/70	130/70
2	130/70	130/70	130/70	130/70
3	120/70	120/70	110/70	110/70
4	140/70	130/70	130/70	120/70
5	130/80	120/70	130/70	130/70

Blood tests

No significant changes were seen in the values of Hb, WBC, diff. ESR, bilirubin, alkaline phosphatase, urea, and SGOT during this study.

CONCLUSIONS

(1) ICI 63,197 did not significantly affect anxiety

(2) ICI 63,197 did not significantly affect pulse rate, blood pressure or the blood tests used.

(3) ICI 63,197 produced nausea or vomiting in 3 out of 5 patients, all at 2 mg. TDS.

ICI 63,197 IN HYPERTENSION (DR. F.J. ZACHARIAS, BEBINGTON)

PROTOCOL

Patients with a sustained diastolic hypertension in the range 100 - 115 mmHg were chosen for the study. They were to be on no other drugs Patients under 18 or over 60 years of age, and pregnant women were excluded.

Patients took ICI 63,197 2 mg. QDS for 4 weeks in the first instance, and this could be continued as clinically indicated.

On entry to the trial, the following were recorded:-

(a) age, sex, weight

(b) diagnosis

(c) blood pressure (standing and lying)

(d) blood sample for Hb, WBC, diff.ESR, bilirubin, alkaline phosphatase SGOT and urea.

At weekly intervals through the trial the following were recorded:-

(a) blood pressure

(b) body weight

(c) possible side effects

(d) the blood tests mentioned above.

RESULTS

Details of patients studied

No.	Initials	Age (yrs)	Sex	Weight (kg)	Diagnosis
1	CCS	45	M	85.0	Hypertension
2	RJT	-	M	67.7	Essential hypertension with asthma
3	SN	64	M	77.3	Essential hypertension

Effect on blood pressure

The standing B.P.s (mmHg) during treatment with ICI 63,197 are shown below. There was no significant difference between standing and lying B.P.

	Week of treatment												
No.	0	1	2	3	4	5	6	7	8	9	10	11	12
1	180/110	150/100	145/95	165/100	145/100	150/95							
2	210/115	180/110	175/110	195/115	180/115	180/120	165/105	220/115					
3	125/115	180/115	180/105	165/110	170/110	175/110	165/105	150/105	170/110	165/105	170/110	175/110	180/115

Effect upon body weight

No significant change was seen in body weight during treatment with ICI 63,197.

Possible side effects

These are shown below:-

No.	Possible side effects
1	Nil
2	Severe indigestion, dizziness, flushing. Eventually refused to continue.
3	Nausea, severe heartburn, flushing. Agent finally stopped due to side effects.

Effect on blood tests

No significant change was seen in Hb, WBC, diff.ESR, bilirubin, alkaline phosphatase, SGOT or urea.

CONCLUSIONS

(1) ICI 63,197 did not significantly affect the B.P. of these hypertensive subjects.

(2) There was no effect on body weight on the blood tests used.

(3) 2 of the 3 had severe gastrointestinal side effects with dizziness and flushing.

EFFECT OF ICI 63,197 IN OBESE SUBJECTS

DR. D. DAVIES, MANCHESTER.

PROTOCOL

Patients in the age range 30 - 55 years with a body weight at least 30% over the ideal for their age, sex, height and build, were selected for the study. The trial was a double blind cross-over study of ICI 63,197 2 mg. TDS against identical placebo tablets given TDS, 6 weeks each. Patients were reviewed at fortnightly intervals when body weight, skin fold thickness, pulse rate and blood pressure were noted. A record of possible side effects was kept. Blood samples were taken at each visit for haemoglobin, white cell count, differential count, ESR, platelet count, bilirubin, alkaline phosphatase, SGOT, SGPT, Albumin, Globulin and urea. Urine was also checked for the possible presence of sugar, protein or blood.

RESULTS

Four patients had entered the study before it was discontinued, due to the appearance of angina pectoris in two patients while on the active preparation.

Details of patients studied

No.	Initials	Age (yrs)	Sex	Weight (kg)
1	SMcK	19	M	142.4
2	MT	50	F	166.0
3	AT	38	F	101.8
4	BA	33	F	83.0

750

Effect on body weight

No.	Baseline	Placebo			Active		
		Week 2	Week 4	Week 6	Week 2	Week 4	Week 6
1	142.4	134.0	134.8	134.2	140.6	138.0	138.0
2	166.0			163.7	161.0	161.0	160.6
3	101.8				103.8	106.2*	
4	83.0				82.0	82.8	83.3*

* trial stopped due to appearance of angina

Effect on skin fold thickness (cms.)

No.	Baseline	Placebo			Active		
		Week 2	Week 4	Week 6	Week 2	Week 4	Week 6
1	R3.1 L3.3	R2.7 L3.1	R2.7 L3.0		R2.9 L3.1		R2.8 L3.1
2	2.2	2.2		2.0	1.9	2.1	2.1
3	R3.0 L3.1				R3.2 L3.1*		
4	R3.0 L2.8				R3.0 L2.9	R2.7 L2.7	R2.7 L2.7*

* trial stopped due to appearance of angina
R = right arm
L = left arm

Effect on pulse rate (beats/min.)

No.	Baseline	Placebo			Active		
		Week 2	Week 4	Week 6	Week 2	Week 4	Week 6
1	84	72			56	72	60
2	80	88		96	80	72	80
3	80				88	80*	
4	72				64	72	72*

* trial stopped due to appearance of angina

Effect on blood pressure (mmHg)

No.	Baseline	Placebo			Active		
		Week 2	Week 4	Week 6	Week 2	Week 4	Week 6
1	120/80	115/80	130/70		120/80	130/75	115/80
2	190/100	200/110		120/80	170/100	150/100	190/110
3	145/85				120/80	110/80	
4	110/70				110/70	110/70	120/75

Effect on blood tests

No abnormality was detected in Hb, WBC, diff. ESR, platelets, bilirubin, alkaline phosphatase, SGOT, SGPT, albumin, globulin or urine during the study.

Effect on urine tests

No abnormal urine findings were detected during the study.

Possible side effects

Three patients (Nos. 1, 2 and 4) complained of marked dyspepsia, flatulence and nausea while taking the active agent. In two patients (Nos. 3 and 4) classical angina of effort appeared for the first time ever while on the active regime. For this reason, the trial was abandoned.

Neither patient who took the placebo run had any side effects while on that regime.

CONCLUSIONS

As far as a possible anti-obesity effect is concerned this trial is inadequate to draw any conclusions. No effect was seen on pulse rate or blood pressure and blood and urine tests remained normal. Upper gastrointestinal

side effects were marked with the agent. The appearance of angina pectoris in two patients who had never had this symptom and in whom the cardiovascular system was clinically normal was apparently due to the compound. This view is strengthened by the fact that since withdrawal no more attacks have been recorded even on severe exercise. The mechanism whereby this was produced must be speculative but it could be due to the potentiation of endogenous catecholamines by ICI 63,197 on exercise, or perhaps the mobilisation of free fatty acid which could increase myocardial oxygen requirements.

REFERENCE 16

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REPORT NO: CTL/R/390 (R)

THE CONCENTRATION OF PP 796 REQUIRED TO PRODUCE EMESIS IN EXPERIMENTAL ANIMALS AND AN ESTIMATION OF THE EMETIC DOSE IN MAN

M S Rose

October, 1976

SUMMARY

From the limited evidence of clinical trials and data from experimental animals, it is concluded that PP 796 should be added to paraquat formulations at a level of 5 mg in 10 ml (0.05%). It is estimated that the majority of those ingesting 10 ml of this formulation will vomit within an hour.

The ICI development compound ICI 63197 produced by ICI Pharmaceuticals Division is a phosphodiesterase inhibitor (Farrell, 1970, Vol II) which has been shown to have a potent emetic action (Bayliss, 1973). This compound has been reclassified by ICI Plant Protection Division as PP 796.

When PP 796 is included in a paraquat formulation in amounts that will cause emesis within 1 hour in dogs and monkeys, the toxicity of the formulation to these species is reduced (Rose, 1976). In order to reduce the toxicity of the paraquat formulation to man, therefore, it will be necessary to add sufficient PP 796 to cause emesis, in a volume of paraquat concentrate that would normally be lethal if ingested. A volume of 10 ml of the 20% w/v paraquat concentrate is considered to be the smallest amount containing a possible lethal amount of paraquat to man (Fletcher, 1974). The question that remains to be answered therefore, is what amount of PP 796 should be added to this volume of formulation?

An emetic response in dogs, monkeys and pigs has been obtained with PP 796 over the dose range 0.1-1.0 mg/kg body weight (Table 1). On this basis a dose of 2 mg/kg was chosen as one that would clearly ensure vomiting in dogs and monkeys, and this dose was, therefore, used for studying the effect of emesis on paraquat toxicity in these species (Rose, 1976).

Clinical studies (Bayliss, 1973) have indicated that man is more sensitive to the emetic effects of PP 796 than the experimental animals studied, emesis being seen with doses in the range 0.03-0.11 mg of PP 796/kg body weight (equivalent to total doses in the range 2-8 mg). In the first human study involving 12 healthy volunteers (average body weight 70 kg), 1 was given 0.25 mg, 1 was given 0.5 mg, 2 were given 1.0 mg, 3 were given 2 mg, 2 were given 3 mg, 2 were given 4 mg and 1 was given 8 mg. Of these, the volunteer given 8 mg vomited as did one of those given 4 mg. Nausea was a marked effect reported by almost all of the volunteers. It can be seen that when the blood levels of PP 796 in the 2 volunteers given 4 mg are compared, the one that vomited absorbed the compound more quickly than the other (Table 2). This suggests that, as with dogs, the rate of absorption might be

critical in determining whether vomiting will occur. After this first volunteer study, one conclusion reached was that "The agent was poorly tolerated at doses above 1-2 mg. Nausea, vomiting, dizziness, sweating and flushing were complained of". As a consequence of this, all further studies were carried out with a maximum dose of 2 mg. Of those who took 2 mg, approximately 10% vomited and 60% complained of nausea.

From the limited data available in man, therefore, it can be argued that a dose of 5 mg should certainly cause nausea and ought to induce vomiting in the majority of those ingesting it (Table 1). It should be noted that the clinical studies were carried out using PP 796 in tablet form. This will have led to an inevitable delay in absorption (Farrell, 1970, Vol I). When present in paraquat formulations PP 796 will be in solution and may, therefore, be more readily absorbed. An additional factor that should also be considered is the irritancy of the paraquat concentrate, which causes nausea and vomiting (albeit after a delay of many hours).

In conclusion, the addition of PP 796 to formulated paraquat at the rate of 0.05% (5 mg emetic to 10 ml formulation) should be sufficient to ensure that most people ingesting 10 ml will vomit. Inspection of the statistics of paraquat poisoning incidents reported to ICI shows that most cases involve ingestion of quantities in excess of 20 ml, many suicides involving 50 ml or more. Under these circumstances, and considering (1) the irritant nature of the formulation, and (2) the fact that PP 796 will be in a soluble, dispersed form, it seems highly likely that vomiting will occur within an hour, with a consequent reduction in the amount of paraquat available for absorption.

MSR/JFH/10 Feb 77

TABLE 1

The emetic action of PP 796

	Dose	Nos. Vomiting	% Vomiting response	Total dose (mg)
Dog*	0.5 mg/kg	3/8	35	
	1.5 mg/kg	6/8	75	
Pig**	0.25 mg/kg	0/8	0	
	0.5 mg/kg	3/8	35	
	1.0 mg/kg	5/8	63	
Monkey &** Marmoset	0.05 mg/kg	0/5	0	
	0.1 mg/kg	5/24	21	
	0.2 mg/kg	8/19	42	
	0.3 mg/kg	2/15	13	
	0.4 mg/kg	5/15	33	
	0.5 mg/kg	4/5	80	
	1.0 mg/kg	2/2	100	
Man+	0.015 mg/kg	0/2	0	1
	0.03 mg/kg	4/37	11	2
	0.06 mg/kg	1/2	50	4
	0.11 mg/kg	1/1	100	8

* Data from Farrell (1970) Vol II

** Data from Todd (1977)

\+ Data from Bayliss (1973)

TABLE 2

+Comparison of blood concentrations of PP 796 in 2 volunteers given 4 mgs in tablet form

	micrograms PP 796/ml		
Hours after dosing	1	2	3
Volunteer No 10*	0.081	0.041	0.034
Volunteer No 11	0.045	0.056	0.044

* Vomited after 30 minutes

\+ Data from Bayliss (1973)

References

Bayliss, P.F.C. (1973) A summary of clinical results of the phosphodiesterase inhibitor ICI 63197 in a variety of disease states. Report No: PH20992B

Case, D.E. and Dunlop, D. (1977) The intravenous administration of ICI 63197 to beagle dogs. Report No: PH23517C

Farrell, F.G. (1970) ICI 63197 Submission of evidence to the Committee on Safety of Drugs (Vol. I. Chemistry and Pharmacy; Vol. II. Pharmacology and Biochemistry; Vol. III. Toxicity and Proposed Clinical Trials). Report No: PH18987C

Fletcher, K. (1974) in Forensic Toxicology, ed. by B Ballantyne, published by John Wright and Sons Limited, Bristol

Rose, M.S. (1976) The effect of administration of an emetic (PP796) on paraquat toxicity in dog and monkey. Report No: CTL/R/391

Todd, A.H. (1977) The emetic effects of ICI 63197 in pigs, monkeys and marmosets. Report No: PH23516C

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IMPERIAL CHEMICAL INDUSTRIES LIMITED
PHARMACEUTICALS DIVISION

TITLE :- The emetic effects of ICI 63,197 in pigs, monkeys and marmosets

AUTHOR(S) :- Todd A H

DATE :- 28 Jan 1977

IMPERIAL CHEMICAL INDUSTRIES LIMITED

PHARMACEUTICALS DIVISION

Title : The emetic effects of ICI 63,197 in pigs, monkeys and marmosets.

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Introduction

This report brings together information contained in project team reports CPR 114/16 and CPR 095/14 on the emetic properties of ICI 63,197 in pigs, monkeys and marmosets. The work was carried out in the period 1969 - 1972, by Dr. A.W. Broome and Mr. P.A. Melvin.

ICI 63,197 : EMETIC PROPERTIES IN PIGS, MONKEYS AND MARMOSETS

1. PIGS

Groups of eight 40 - 50 lb. pigs were fed a standard pig-fattening ration, containing concentrations of ICI 63,197 ranging from 4 to 40 g/long ton. This was calculated so that ingestion of 500 g. diet would provide dose levels of ICI 63,197 of 0.1 - 1.0 mg/kg to 20 kg pigs. The pigs were provided with 500 g of the medicated diet twice daily for 2 days, and observed for quantity of food eaten and emesis. The results are given in the following table.

Dosage level		Number of Pigs	Response after 2 feeds		
Concentration in diet	mg/kg/pig		Refused diet	Emesis	Slow eating
40 g/ton	1.0	8	8	5	-
20 g/ton	0.5	8	8	3	-
10 g/ton	0.25	8	6	0	2
4 g/ton	0.1	8	0	0	8

2. MONKEYS AND MARMOSETS

Seven Rhesus Monkeys and two marmosets were dosed with ICI 63,197 on varying numbers of occasions, and observed for clinical signs.

The compound was administered by stomach tube as a suspension in an inert dispersing agent*, diluted with water as necessary, except where otherwise indicated.

The results are given in detail on the following pages. A summary of the emetic response to various oral doses of ICI 63,197 is given in the following table:-

Dose (mg/kg oral)	Number of administrations	Number of emeses	% emeses
0.025	6	2	33
0.05	5	0	0
0.1	24	5	21
0.2	19	8	42
0.3	15	2	13
0.4	15	5	33
0.5	5	4	80
0.6	2	2	100
1.0	2	2	100

*'Lissapol' NX (Nonylphenolethylene oxide condensate) 0.1%

'Lissapol' C (Sodium salt of sulphated cetyl/oleyl alcohol mixture) 0.1%

'Dispersol' OG (Polyglyceryl ricinoleate) 0.1%

Distilled water to 100.0%

Rhesus Monkey 561, ♂ , weight 14.0 kg.

6.1.69	Dosed 1 mg/kg.	Sick at 10 mins.
17.3.69	Dosed 0.1 mg/kg.	Sick at 10 mins and 20 mins.
19.3.69	Dosed 0.025 mg/kg.	Slightly sick at 10 mins.
20.3.69	Dosed 0.025 mg/kg.	Slightly sick at 10 mins and 25 mins.

Rhesus Monkey 613, ♂ , Weight 9.5 kg.

6.1.69	Dosed 1 mg/kg. Squirming round cage on stomach. Sick from 10 - 30 mins. Tranquilised for about 2 hours.
17.3.69	Dosed 0.1 mg/kg. Sick at 10 mins. Tranquilised for 1 hour.
19.3.69	Dosed 0.025 mg/kg. Unaffected.
20.3.69	As above.
21.3.69	Dosed 0.05 mg/kg. Mild reaction, tranquilised. Dosed 0.1 mg/kg in afternoon. No symptoms.
24.3.69	Dosed 0.1 mg/kg. Tranquilised but not sick.
25.3.69	Dosed 0.1 mg/kg. Tranquilised.
26.3.69	Dosed 0.1 mg/kg. Slightly tranquilised. Fed before dosing, from today.
27.3.69	Dosed 0.2 mg/kg. Slightly tranquilised.
28.3.69	Dosed 0.3 mg/kg. Slightly tranquilised.
29.3.69	As above.
30.3.69	As above.
31.3.69	Dosed 0.4 mg/kg. Tranquilised.
1.4.69	Dosed 0.5 mg/kg. Tranquilised. Not fed before dosing from today.
2.4.69	Dosed 0.5 mg/kg. Very sick at 20 mins.
3.4.69	Dosed 0.4 mg/kg. Sick at 20 mins.
4.4.69	Dosed 0.3 mg/kg. in distilled water. Unaffected.
5.4.69	Dosed 0.3 mg/kg. in distilled water. Unaffected.
6.4.69	Dosed 0.4 mg/kg in distilled water. Unaffected.
7.4.69	Dosed 0.4 mg/kg in distilled water. Sick after 25 mins.

Rhesus Monkey 640, ♀ , Weight 4.4. kg.

20.3.69	Dosed 0.025 mg/kg. Unaffected.
21.3.69	Dosed 0.05 mg/kg. Unaffected. Dosed 0.1 mg/kg. in afternoon. Unaffected.
24.3.69	Dosed 0.1 mg/kg. Sick after 15 mins.
25.3.69	Dosed 0.1 mg/kg. Sick after $1\frac{1}{4}$ hours.
26.3.69	Dosed 0.1 mg/kg. Unaffected. Fed before dosing.
27.3.69	Dosed 0.2 mg/kg. Unaffected.
28.3.69	Dosed 0.3 mg/kg. Sick by 5 hours.
1.4.69	Not fed before dosing. Dosed i.v. slowly at 0.05 mg/kg (in dispersing agent) and after 4 mins. given a further 0.05 mg/kg. Heaving and sick from 15 - 25 mins. Sick again at 3 and $3\frac{1}{2}$ hours.

Rhesus Monkey 614, ♂ , Weight 6.8 kg.

20.3.69	Dosed 0.025 mg/kg. Unaffected.
21.3.69	Dosed 0.05 mg/kg. Unaffected. Dosed 0.1 mg/kg. in afternoon. Unaffected.
24.3.69	Dosed 0.1 mg/kg. Not observed to be sick, but fluid in cage after 2½ hours.
25.3.69	Dosed 0.1 mg/kg. Slightly tranquilised.
26.3.69	Dosed 0.1 mg/kg. Slightly tranquilised. Fed before dosing from today.
27.3.69	Dosed 0.2 mg/kg. Hardly affected.
28.3.69	Dosed 0.3 mg/kg. No symptoms but suddenly sick at 5 hours.
29.3.69	Dosed 0.3 mg/kg. No symptoms.
30.3.69	Dosed 0.3 mg/kg. Unaffected.
31.3.69	Dosed 0.4 mg/kg. Unaffected.
1.4.69	Dosed 0.5 mg/kg. Sick at 1 hour. 1 hour later dosed again at 0.5 mg/kg. Sick 2 hours later.
2.4.69	Dosed 0.4 mg/kg. Unaffected.
3.4.69	Dosed 0.4 mg/kg. Unaffected.
4.4.69	Dosed 0.4 mg/kg. in distilled water. Unaffected.
5.4.69	Dosed 0.5 mg/kg. in distilled water. Sick after 35 mins. and again in the afternoon.
6.4.69	Dosed 0.4 mg/kg. in distilled water. Sick after 74 mins.
7.4.69	Dosed 0.4 mg/kg. in distilled water. Unaffected.

Rhesus Monkey A, ♀ , Weight 4.2 kg.

1.4.69 Dosed 0.1 mg/kg. Very sick after 15 mins. Slightly tranquilised for 1½ hours.

Rhesus Monkey B, ♀ , Weight 4.3 kg.

1.4.69 Dosed 0.1 mg/kg. Unaffected.

2.4.69 Dosed 0.1 mg/kg. Unaffected.

3.4.69 Dosed 0.2 mg/kg. Tranquilised. Sick at 25 mins.

4.4.69 Dosed 0.1 mg/kg. Unaffected.

5.4.69 Dosed 0.1 mg/kg. Unaffected.

6.4.69 Dosed 0.2 mg/kg. Unaffected.

7.4.69 Dosed 0.3 mg/kg. Unaffected.

Rhesus Monkey C, weight 5.5 kg. (sex not recorded).

26.3.69	Dosed 0.05 mg/kg.	Unaffected.
27.3.69	Dosed 0.1 mg/kg.	Unaffected.
28.3.69	Dosed 0.2 mg/kg.	Sick by 5 hours.
29.3.69	Dosed 0.2 mg/kg.	Unaffected.
30.3.69	Dosed 0.2 mg/kg.	Sick about 5 hours.
31.3.69	Dosed 0.2 mg/kg.	Sick at 3 hours.
1.4.69	Dosed 0.2 mg/kg. Not fed before dosing.	Sick at 3/4 hour.
	$1\frac{1}{4}$hrs later dosed again at 0.2 mg/kg.	Sick 1 hour 20 mins later.

Rhesus Monkey D, weight 5.2 kg. (sex not recorded)

26.3.69	Dosed 0.05 mg/kg.	Unaffected.
27.3.69	Dosed 0.1 mg/kg.	Unaffected.
28.3.69	Dosed 0.2 mg/kg.	Unaffected.
29.3.69	Dosed 0.2 mg/kg.	Unaffected.
30.3.69	Dosed 0.2 mg/kg.	Sick about 5 hours.
31.3.69	Dosed 0.2 mg/kg.	Unaffected.
1.4.69	As above. Not fed before dosing.	
2.4.69	Dosed 0.3 mg/kg.	Unaffected.
3.4.69	As above.	
4.4.69	As above.	
5.4.69	Dosed 0.4 mg/kg.	Unaffected.
6.4.69	Dosed 0.4 mg/kg.	Sick 28 mins.
7.4.69	Dosed 0.4 mg/kg.	Sick $2\frac{1}{2}$ hours.

Marmoset A, ♂ , weight 250 g.

Marmoset B, ♀ , weight 400 g.

3.4.69	Dosed 0.1 mg/kg. in distilled water.	Unaffected.
4.4.69	Dosed 0.2 mg/kg. in distilled water. ♀ not exactly sick but frothing.	♂ sick 15 mins.
5.4.69	Dosed 0.1 mg/kg. in distilled water.	Unaffected.
6.4.69	Dosed 0.2 mg/kg in distilled water.	Salivating but not sick.
7.4.69	Dosed 0.3 mg/kg. in distilled water.	Unaffected.
8.4.69	Dosed 0.4 mg/kg. in distilled water.	Unaffected.
9.4.69	Dosed 0.6 mg/kg. in distilled water.	Both sick at 15 mins.

REFERENCE 18

Ref 10772

Plant Protection Division

REPORT SERIES
MED/76/3C

COMPANY SECRET
CATEGORY C

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MEDICAL DEPARTMENT

PARAQUAT EMETIC FORMULATION: AN ESTIMATE OF THE LIKELY SPRAY CONCENTRATIONS OF PP796 UNDER NORMAL WORKING CONDITIONS.

Author: J K Howard

Date of Issue: November 1976

SUMMARY

It is concluded, on the basis of known concentrations of paraquat in air under normal spraying conditions, that, if PP796 is added to the 20% concentrate at a rate of 5 mg/10 ml, the likelihood of a spray worker absorbing sufficient to produce symptoms of nausea and/or vomiting are so remote that they may be totally discounted.

2. Spray strength 2% paraquat - normal working conditions

The situation is slightly different in these circumstances. At this level of spray dilution the amount of PP796 in the spray solution is 5 mg/100 ml. If it is assumed that a factor of 4 can be introduced into the above calculations for the 0.5% dilution, then the level of paraquat in the air to which the operator is exposed will be in the order of 40-50 ug/m^3 and the level of PP796 in the order of 0.1 ug/m^3. Assuming the same minute ventilation, and 50% of droplets to be of respirable size, the amount of PP796 reaching the alveoli is unlikely to exceed 0.01 ug/hour. Once again this is a dose level which is so small that the chances of reaching an 'emetic dose' under such conditions may be discounted. It should be noted, however, that this figure has been arrived at by extrapolation from the experimental data obtained from 0.5% spray dilutions and no actual measurements have been made at a spray strength of 2% paraquat.

3. Spray strength 0.5% paraquat: Working in spray mist

Hogarty (1975) has shown that a person working in a spray mist of 0.5% paraquat may be exposed to concentrations in the order of 10 mg/m^3 of air. This represents a thousandfold increase from normal working conditions at this dilution and the worker would be in an atmosphere containing the equivalent of 2ml of spray solution/m^3, containing in the order of 25 ug of PP796. Assuming a respiratory minute ventilation again of 30 litres/minute and assuming also that at least 50% of the droplets in the inspired air will be of respirable size, then the level of PP796 reaching the alveoli is likely to be in the region of 22.5 ug/hour. It is again very difficult to envisage an 'emetic dose' ever being absorbed by inhalation even under these extreme conditions.

It is, however, extremely unlikely that an operator could work for any length of time under these conditions as skin and eye irritation would rapidly prove too uncomfortable for work to continue. Furthermore, it is likely that oral ingestion of both paraquat and PP796 would be more significant under these conditions than inhalation, as particles would impinge on the face, agglomerate and trickle into the mouth. This would particularly apply to the larger size particles which make up 99% of the spray volume and would not have had time to fall out by gravity. It is not possible to make an exact estimate of the amount of spray that would be ingested, but it is likely that the effect of the spray would drive the operator out of the mist before sufficient of either compound had been absorbed to produce systemic toxicity.

It should also be pointed out that working in the spray mist cannot be considered as normal working conditions. The level of paraquat in the atmosphere to which the worker would be exposed is far higher than the threshold limit value of 500 ug/m^3 and under no circumstances could these conditions be viewed as acceptable for work.

Conclusion

The foregoing discussion is based on the levels of paraquat in the air to which spray operators are exposed under normal working conditions. Under these conditions it is calculated that the concentration of PP796, when added to the paraquat concentrate at a rate of 5 mg/10 ml, would never reach significant levels in the atmosphere in the operator's breathing area at standard spray dilutions up to 2%. The corollary of this is that the addition of PP796 to paraquat concentrate at the recommended rate will not prove to be a hazard for the user under recommended working conditions.

Imperial Chemical Industries Limited

Brixham Laboratory

FT 14/76

BL/B/1783

PP 796

Determination of the Acute Toxicity of PP 796 to Rainbow Trout (Salmo gairdnerii)

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PP 796

DETERMINATION OF THE ACUTE TOXICITY OF PP 796 TO RAINBOW TROUT (SALMO GAIRDNERII)

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Contents

1. Introduction

At the request of Plant Protection Division of Imperial Chemical Industries Limited, the acute toxicity of PP 796 to Rainbow Trout (Salmo gairdnerii) was determined in freshwater.

The 24, 48 and 96 hour LC_{50} values were measured using a continuous flow system.

2. Summary and Conclusions

2.1 The acute toxicity to Rainbow Trout of compound PP 796 was determined in freshwater at 13°C.

2.2 As PP 796 has an extremely low solubility in water and solvents, Dimethyl Sulphoxide (DMSO) was used to prepare the stock concentrates. A level of less than 100 mg/l of DMSO was used in the fish exposure vessels.

2.3 24, 48 and 96 hour LC_{50} values were determined by direct reading of the geometric mean survival period/concentration graph shown in Figure 1.

Times of death of individual fish are shown in Table 1.

2.4 The following levels were measured:

24 hour LC_{50} = 88.0 mg/l as PP 796

48 hour LC_{50} = 56.0 mg/l as PP 796

96 hour LC_{50} = 40.0 mg/l as PP 796

No deaths occurred at an exposure concentration of 33.0 mg/l during the 96 hour test period, but as the fish exhibited toxic symptoms, this was not a no effect level.

At a concentration of 15.0 mg/l, the fish did not exhibit toxic symptoms and this may be regarded as a no effect level, under the test conditions.

2.5 Measurements were undertaken throughout the 96 hour period for pH, dissolved oxygen, temperature and water hardness. The results obtained are shown in Tables 2-4.

2.6 Analytical measurements were made of the levels of PP 796 in each fish exposure concentration. The results obtained are shown in Table 5.

3. Materials and Methods

3.1 Test Species

Rainbow Trout (Salmo gairdnerii) a sensitive freshwater species were selected as the test animals. The fish were obtained from the Samaki Trout Farm, Wiltshire.

Batches of fish were kept in stock tanks for a period of 4 weeks at a temperature of 10°C prior to testing. The fish were acclimatised for a period of 2 days in the test vessels at a temperature of 13°C ± 0.5°C, before commencement of the test.

The fish ranged in weight from 11.2 to 19.0 gm. with a mean weight of 14.84 gm.

The range in length was 95 mm to 110 mm with a mean length of 101.7 mm.

3.2 Test Material

The compound tested was 2-amino-4, 5-dihydro-6-methyl-4-propyl-s-triazolo(1,5-a) pyrimidin-5-one.

The compound was coded PP 796 batch number identification PH/3511/47 ICI 63197 and it was supplied by Plant Protection Division of Imperial Chemical Industries Ltd.,

The sample of PP 796 was a finely divided pale yellowish buff powder with a purity of 91%.

4. Test Conditions

4.1 Apparatus

The apparatus used in this study was a continuous flow through system (Reference 1).

The concentrated stock solutions were fed to the system by a series of peristaltic pumps and a further series of these pumps was used to supply freshwater. The concentrated solutions of PP 796 and freshwater were fed to glass splash heads where mixing occurred before the test solution passed into the fish test vessels.

The dilution water was supplied from a 20,000 gallon reservoir and the total hardness measured daily was found to be in the range 51 to 65 mg/l as $CaCO_3$ over the test period as shown in Table 4.

Twenty litre glass vessels were used to hold the test fish and the test solutions were renewed at a rate of 200 ml/minute. A complete exchange of the test solution occurred within 3 hours.

The dilution ratio of the stock concentrates to freshwater was 1:100 in this study.

Ten Rainbow Trout were tested in each concentration and a control was run simultaneously with each set of experiments.

The water temperature was maintained at $13^{o}C \pm 0.5^{o}C$ throughout the test.

4.2 Exposure Concentrations

Series	I	: 33, 22, 15	mg/l as PP 796
	II	: 330, 220, 150	mg/l as PP 796
	III	: 100, 68	mg/l as PP 796
	IV	: 47	mg/l as PP 796

The amount of dimethyl sulphoxide (DMSO) used in the preparation of the stock solutions was kept to the minimum and never exceeded a level of 100 mg/l in any test solution.

At this concentration DMSO is very unlikely to have any effect whatsoever on the test organisms.

The reported 96 hour LC_{50} value of DMSO to Rainbow Trout is of the order of 33,000 mg/l. WILLFORD (Reference 2).

4.3 Physical Parameters Monitored

Dissolved oxygen levels in each test vessel were measured twice daily using a Yellow Springs Incorporated Model 51A dissolved oxygen meter.

The lowest recorded oxygen level was 87% of the air saturated value. The values obtained are shown in Table 2. The pH of the test solutions were measured twice daily using an Electronic Instruments Model 23A pH meter, and the values recorded ranged from 7.55 to 7.70 pH units as shown in Table 3.

Temperature readings were taken twice daily and no deviation was recorded from the nominal values of $13^{o}C \pm 0.5^{o}C$.

5. Results

The individual times of death of the fish were recorded in minutes and these are tabulated in Table 1. An approximate estimate of the time for 50% of the test population to die (ie ET_{50}) is the geometric mean survival period (GMSP), which was computed with the formula:

$$GMSP = \exp \left\{ \sum_{i=1}^{n} \sqrt[n_i]{(\log_e t_1)^{n1} \ (\log_e t_2)^{n2} \ \ldots\ldots (\log_e t_n)^{n_n}} \right\}$$

where N_i is the number of fish which die at time t_i and $\sum_{i=1}^{n} n_i$ is the total number of fish used in the test.

For this test $\sum_{i=1}^{n} n_i = 10$.

A sample plot of the number of dead fish against time usually gives a skewed distribution. The above formula normalises the distribution by taking the natural logarithms of the times of death and employs the assumption that, for a nearly normal distribution, the geometric mean approximates to the actual median.

The GMSP values are reported in Table 1.

A graph was then constructed of the Geometric Mean Survival Period values against concentrations using logarithmic scales. (See figure 1).

The 24, 48 and 96 hour LC_{50} values were then read directly from this graph.

24 hour LC_{50} = 88.0 mg/l PP 796
48 hour LC_{50} = 56.0 mg/l PP 796
96 hour LC_{50} = 40.0 mg/l PP 796

No deaths occurred during the 96 hour test period at exposure concentrations of 33.0, 22.0 and 15.0 mg/l of PP 796.

A "no effect" level, the concentration at which no symptoms of distress were observed throughout the 96 hour test period, was found to be 15 mg/l of PP 796.

Observations of distress during the test are recorded in Table 5.

6. Analytical Method

6.1 Procedure for the determination of low concentrations of PP 796 in freshwater.

Introduction

PP 796 is a finely divided crystalline powder with a colour ranging from white to buff. The chemical name of the compound is shown under 3.2 - Test Material.

The substance fluoresces when irradiated with UV light and this property has been used as the basis for a procedure for its determination in dilute aqueous solution at concentrations down to 0.1 mg/l and below.

The presence of DMSO (dimethyl sulphoxide), used to facilitate the preparation of standards, has no significant effect on the relative emission at concentrations at least 200 times greater than that of the PP 796.

Analytical Method

Equipment and reagents

Perkin Elmer MPF-3 Scanning Spectrofluorimeter equipped with high pressure xenon source.

1cm x 1cm quartz fluorimetric cuvettes

Dimethyl sulphoxide

Procedure

Set up the MPF-3 under the following conditions:-

Mode = Ratio

λ (excitation) = 305 nm
λ (emission) = 420 nm
Excitation and emission slit widths = 8 nm.

If the sample contains suspended matter, allow this to settle, then introduce a suitable portion of the clear liquid into a clean 1cm x 1cm quartz cuvette and measure the fluorescent emission under the conditions specified above. Correct this value by subtraction of the emission due to an appropriate control.

Construct a calibration curve by plotting relative emission as a function of PP 796 concentration for a suitable range of standards (not exceeding 2 mg/l) subjected to the same procedure as the samples, and use this curve to read off the levels of PP 796 in the unknowns.

A typical calibration curve is presented in Figure 2.

Preparation of standards

Solution A =

Dissolve 0.0100g. of PP 796 in 1 ml of DMSO, dilute to 100 ml with distilled water and mix well. Solution A contains 100 mg. PP 796/1.

Solution B =

Dilute 10.0 ml of Solution A to 100 ml with distilled water and mix well. Solution B contains 10 mg. PP 796/1.

Dilute appropriate volumes of solution B quantitatively with distilled water to produce a suitable range of working standards (not exceeding 2 mg. PP 796/1). These solutions should be freshly prepared before use.

Notes

(1) Clean working methods are especially important in fluorimetry. In particular the optical cells should be soaked in chromic/sulphuric acid and thoroughly rinsed with distilled water before use.

(2) Standard (B) (10 mg. PP 796/1) and Standard (a) (100 mg PP 796/1) may be stored (preferably in a refrigerator) for at least 4 days without a significant change in emission.

6.2 Analytical Results

The analytical results obtained for the levels of PP 796 in the fish exposure vessels are reported in Table 6.

The percentage of the nominals based on the mean values obtained ranged from 51.1% for the 220 mg/l concentration to 92.98% for the 47 mg/l concentration.

The 24 hour to 96 hour LC_{50} values ranged from 40 to 80 mg/l as PP 796, and in this range the percentage recovery was 71 to 93%.

References

1. Maddock B.G. and Williams B.R.H.
A Continuous Flow Apparatus for Assessing the Toxicity of Substances to Aquatic Animals.
ICI Brixham Laboratory Report BL/A/1579.
May 1974.

2. Willford W.A.
Toxicity of Dimethyl Sulphoxide (DMSO) to fish
Investigations in Fish Control No. 20.
United States Department of the Interior Resource Publication 37
Washington DC
April 1967.

TABLE 1

FISH DEATH TIMES AND GEOMETRIC MEAN SURVIVAL PERIODS

Material Tested - PP 796

Run No 1

Concn. in mg/l	Times in Mins. from '0'	Deaths	Survivors	G M S P in Mins.	Comments
15	5760	0	10		Apart from darkening this was a no effect level.
22	5760	0	10		
33	5760	0	10		

Run No 2

330	120	1	9		
	140	1	8		
	240	2	6	256.8	
	265	1	5		
	300	1	4		
	365	3	1		
	385	1	0		
220	240	1	9		
	300	1	8		
	365	1	7	504.8	
	540	4	3		
	810	2	1		
	840	1	0		
150	365	1	9		
	540	2	7	708.4	
	810	3	4		
	840	1	3		
	855	1	2		
	915	2	0		

Species - Rainbow Trout

Fish per Concn. 10

TABLE 1 Contd.....

FISH DEATH TIMES AND GEOMETRIC MEAN SURVIVAL PERIODS

Material Tested - PP 796 Run No 3

Concn. in mg/l	Times in Mins. from '0'	Deaths	Survivors	G M S P in Mins.
100,	750	1	9	
	960	1	8	
	1140	1	7	1329.7
	1260	1	6	
	1500	2	4	
	1620	2	2	
	1710	1	1	
	1740	1	0	
68	1260	1	9	
	1620	1	8	
	1740	1	7	1937.5
	2040	3	4	
	2190	1	3	
	2220	1	2	
	2280	2	0	

Run No 4

47	3980	1	9	
	4250	2	7	
	4600	1	6	4803
	4920	2	4	
	5300	2	2	
	5380	1	1	
	5440	1	0	

Species - Rainbow Trout Fish per Concn. 10

TABLE 2

OXYGEN RESULTS % SATURATION* MATERIAL TESTED PP 796 RAINBOW TROUT RUN NO 1											
		TIMES OF TESTING IN MINS FROM START AND O_2% SATURATION*									
UNIT NO	CONCN. IN mg/l	60	720	1260	2760	3480	4200	5040	5580	5760	MEAN
1A	15	90	92	94	95	94	95	96	95	96	94.1
2A	22	95	93	92	94	94	94	95	94	94	93.9
3A	33	93	93	93	94	95	96	96	95	97	94.6
6A	CONTROL	92	94	94	95	95	95	94	95	96	94.4

* Please note that results are given as a percentage of the air saturated values at the temperature of the test (13.0°C ± 0.5°C), and are correct to ± 3% of the true value.

OXYGEN RESULTS % SATURATION * MATERIAL TESTED PP 796 RAINBOW TROUT RUN NO 2						
		TIMES OF TESTING IN MINS FROM START AND O_2% SATURATION *				
UNIT NO	CONCN. IN mg/l	60	300	540	915	MEAN
3C	330	97	99	-	-	98.0
2C	220	98	98	96	-	97.3
1C	150	96	98	97	98	97.25
4C	CONTROL	97	97	97	98	97.25

* Please note that results are given as a percentage of the air saturated values at the temperature of the test (13.0°C ± 0.5°C), and are correct to ± 3% of the true value.

OXYGEN RESULTS % SATURATION * MATERIAL TESTED PP 796 RAINBOW TROUT RUN NO 3							
		TIMES OF TESTING IN MINS FROM START AND O_2% SATURATION *					
UNIT NO	CONCN. IN mg/l	60	600	1146	1680	2040	MEAN
4A	100	97	95	95	98	-	96.25
5A	68	97	97	97	97	97	97.0
6A	CONTROL	96	98	97	97	97	97.0

* Please note that results are given as a percentage of the air saturated values at the temperature of the test (13.0°C ± 0.5°C), and are correct to ± 3% of the true value.

TABLE 2 Contd.....

OXYGEN RESULTS % SATURATION * MATERIAL TESTED PP 796 RAINBOW TROUT RUN NO 4											
UNIT NO	CONCN. IN mg/l	TIMES OF TESTING IN MINS FROM START AND O_2% SATURATION *									
		40	460	1180	2020	2620	3160	4000	4600	5300	5480	MEAN
1A	47.0	94	95	96	95	96	96	94	95	94	95	95.0
6A	CONTROL	95	95	94	96	97	96	95	95	93	95	95.1

* Please note that results are given as a percentage of the air saturated values at the temperature of the test (13.0°C ± 0.5°C), and are correct to ± 3% of the true value.

TABLE 3

pH RESULTS MATERIAL TESTED PP 796 RAINBOW TROUT RUN NO 1										
UNIT NO	CONCN. IN mg/l	TIMES IN MINS FROM START AND RESULTS OF pH TESTS*								
		60	720	1260	1860	2760	3480	4200	5040	5580	5760
1A	15	7.60	7.50	7.50	7.60	7.50	7.50	7.60	7.55	7.60	7.60
2A	22	7.60	7.60	7.50	7.55	7.60	7.50	7.60	7.60	7.65	7.65
3A	33	7.50	7.50	7.55	7.60	7.60	7.50	7.65	7.55	7.60	7.65
6A	CONTROL	7.55	7.50	7.60	7.60	7.50	7.50	7.60	7.60	7.60	7.60

* Please note that pH results are correct to $\pm$ 0.2 pH units at the temperature of testing (13.0°C $\pm$ 0.5°C).

pH RESULTS MATERIAL TESTED PP 796 RAINBOW TROUT RUN NO 2					
UNIT NO	CONCN. IN mg/l	TIMES IN MINS FROM START AND RESULTS OF pH TESTS*			
		60	300	540	915
1C	150	7.65	7.70	7.70	7.70
2C	220	7.60	7.65	7.75	-
3C	330	7.60	7.65	-	-
4C	CONTROL	7.70	7.65	7.70	7.60

* Please note that pH results are correct to $\pm$ 0.2 pH units at the temperature of testing (13.0°C $\pm$ 0.5°C).

pH RESULTS MATERIAL TESTED PP 796 RAINBOW TROUT RUN NO 3						
UNIT NO	CONCN. IN mg/l	TIMES IN MINS FROM START AND RESULTS OF pH TESTS*				
		60	600	1140	1680	2040
4A	100	7.65	7.60	7.65	7.65	-
5A	68	7.60	7.65	7.60	7.70	7.60
6A	CONTROL	7.65	7.65	7.60	7.65	7.60

* Please note that pH results are correct to $\pm$ 0.2 pH units at the temperature of testing (13.0°C $\pm$ 0.5°C).

TABLE 3 Contd.....

pH RESULTS MATERIAL TESTED PP 796 RAINBOW TROUT RUN No 4										
UNIT NO	CONCN. IN mg/l	TIMES IN MINS FROM START AND RESULTS OF pH TESTS*								
		40	460	1180	2020	2620	3160	4000	4600	5300	5480
1A	47	7.65	7.60	7.65	7.60	7.60	7.70	7.65	7.70	7.60	7.65
6A	CONTROL	7.60	7.60	7.65	7.65	7.65	7.65	7.70	7.70	7.70	7.65

* Please note that pH results are correct to $\pm$ 0.2 pH units at the temperature of testing (13.0°C $\pm$ 0.5°C).

TABLE 4

HARDNESS DETERMINATIONS RESULTS MATERIAL TESTED PP 796 RAINBOW TROUT RUN NOS 1 and 2		
DAY NO	DATE	TOTAL HARDNESS AS mg/l $CaCO_3$
1	28.11.76	ND
2	29.11.76	53
3	30.11.76	52
4	1.12.76	51
5	2.12.76	51
6	5.12.76	52
7	6.12.76	65

Mean = 54.0 mg/l as $CaCO_3$

Hardness Values are expressed as ppm $CaCO_3$ and include both the magnesium and calcium hardness.
Determinations carried out by titration, using EDTA with solochrome indicator at pH = 10.0.

HARDNESS DETERMINATIONS RESULTS MATERIAL TESTED PP 796 RAINBOW TROUT RUN NOS 3 and 4		
DAY NO	DATE	TOTAL HARDNESS AS mg/l $CaCO_3$
1	12. 1.77	63
2	13. 1.77	65
3	14. 1.77	64
4	15. 1.77	61
5	16. 1.77	ND
6	17. 1.77	61
7	18. 1.77	61
8	19. 1.77	61

Mean = 62.29 mg/l as $CaCO_3$

Hardness Values are expressed as ppm $CaCO_3$ and include both the magnesium and calcium hardness.
Determinations carried out by titration, using EDTA with solochrome indicator at pH = 10.0.

TABLE 5

SYMPTOM SHEET
SPECIES : RAINBOW TROUT
MATERIAL TESTED : PP 796

TIME IN MINS.	CONCENTRATIONS 68 mg/l	100 mg/l	150 mg/l	220 mg/l	330 mg/l
0 - 5	Initial agitation soon subsided	Initial agitation soon subsided	Some hyperactivity	Some hyperactivity	Initial agitation did not subside and considerable hyperactivity.
10					Aggressive behaviour noted in some fish
40					Tendency to collect under lid of vessel
50				Tendency to collect under lid of vessel	
			Fish darker in colour.	Fish darker in colour	Fish darker in colour.
60	Slight distress only	Some signs of distress but not severe.			All fish very distressed and have a head up and tail down attitude whilst at same time revolving about a longitudinal axis.
80				Head up tail down attitude with revolution about a longitudinal axis.	
90				All fish disoriented	All fish very disoriented.
120					1st Death

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TABLE 5 Contd.....

SYMPTOM SHEET
SPECIES : RAINBOW TROUT
MATERIAL TESTED : PP 796

TIME IN MINS.	CONCENTRATIONS 68 mg/l	100 mg/l	150 mg/l	220 mg/l	330 mg/l
140				Intermittent keeling noted.	Remaining fish keeled.
150			Some spasmodic 'coughing' noted, fish tending to swim on sides.	Fish swimming on sides at times.	
240	Disorientation noted	Disorientation now severe		1st Death. All fish more or less permanently keeled.	All fish more or less permanently keeled.
365			1st Death		
385					ALL DEAD
600	Disorientation now severe	Disorientation very severe now			
750		1st Death			
840				ALL DEAD	
915			ALL DEAD		
960	Tendency for fish to have head up tail down attitude.				
1260	1st Death				
1740		ALL DEAD			
2280	ALL DEAD				

TABLE 5 Contd.....

SYMPTOM SHEET
SPECIES : RAINBOW TROUT
MATERIAL TESTED : PP 796

TIME IN MINS.	CONCENTRATIONS 15 mg/l	22 mg/l	33 mg/l	47 mg/l
0-5	Apart from slight darkening this was a no effect level. No symptoms were recorded at 15 mg/l.	Initial agitation soon subsided	Initial agitation soon subsided	Initial agitation soon subsided.
120		Slight darkening in colour	Slight darkening in colour.	Slight darkening in colour.
1120				Some increase in amount of darkening.
2560				Tendency to head up tail down attitude.
3160				Head up tail down attitude now more marked and there appears to be some disorientation.
3460				Disorientation confirmed. Tendency to collect under lid noted.
3980				1st Death. Remaining fish very disoriented.
4250				All fish more or less permanently keeled.
5440				ALL DEAD
5760	NO DEATHS This is no effect level.	NO DEATHS	NO DEATHS	

TABLE 6

ANALYTICAL RESULTS PP 796 mg/l
SPECIES : RAINBOW TROUT

NOMINAL EXPOSURE CONCENTRATION	EXPOSURE TIME IN MINUTES	CONCENTRATION DETERMINED	MEAN RESULTS	NOMINAL PERCENT
15	840	11.6		
	2280	11.4	10.25	68.3
	3720	11.0		
	5760	7.0		
22	840	17.5		
	2280	20.0	19.2	87.3
	3720	20.0		
33	840	25.0		
	2280	26.5	26.2	79.4
	3720	27.0		
47	0	20.0		
	720	47.0		
	1440	48.0		
	2880	51.0	43.7	92.98
	4320	49.0		
	5760	47.0		
68	1440	58.5		
	2880	68.0	63.2	92.94
100	1440	79.0		
	2880	63.0	71.0	71.0
150	30	120.0		
	720	122.5	121.2	80.8
220	30	115.0		
	720	110.0	112.5	51.13
330	30	290.0		
	720	295.0	292.5	88.64

CHART WELL Graph Data Ref. 5633 Log 3 Cycles x 3 Cycles

FIGURE 1

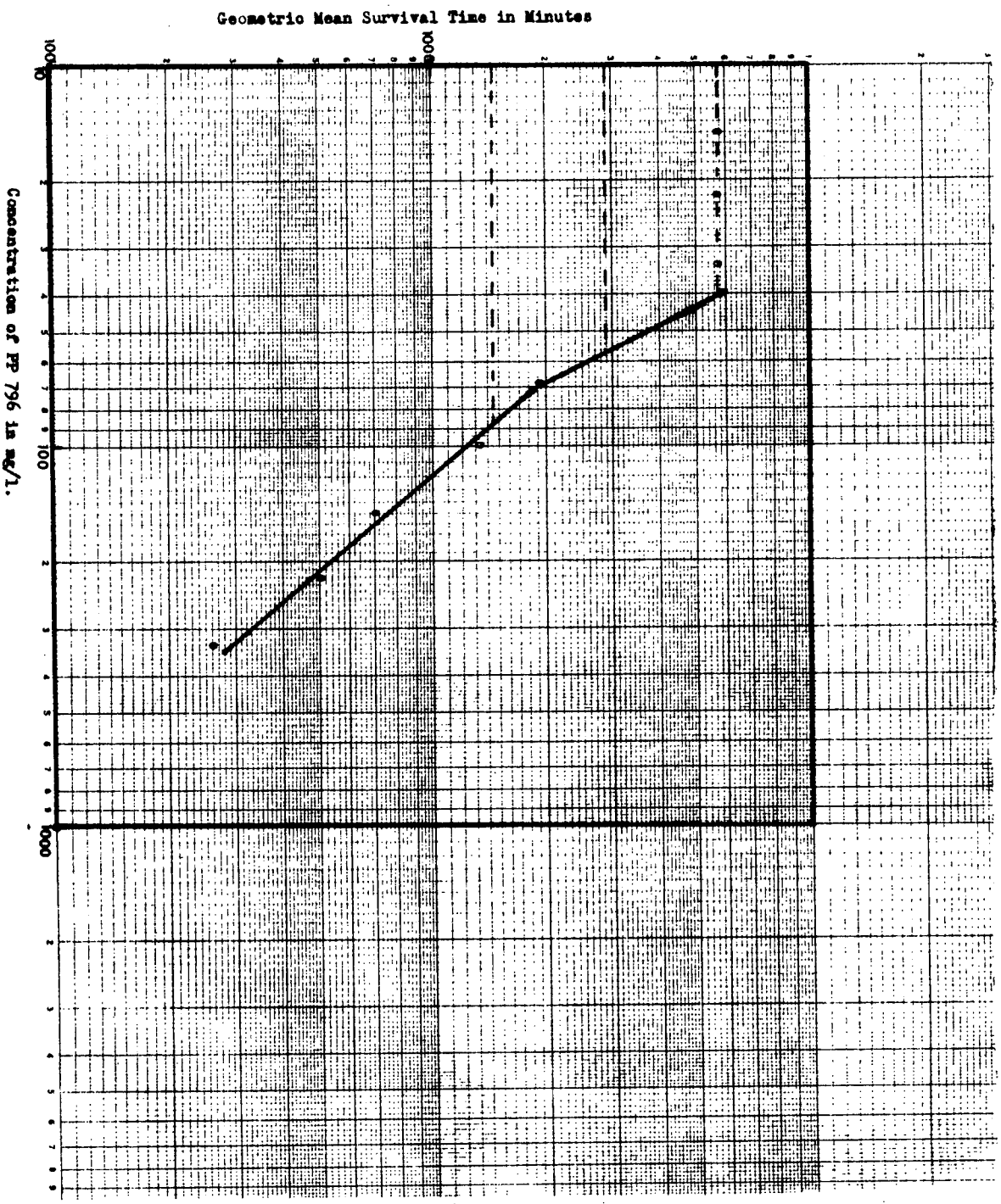

FIG 2

TYPICAL CALIBRATION FOR PP 796 IN AQUEOUS DMSO

(AQUEOUS DMSO CONCENTRATION NO GREATER THAN 200 ppm)