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# Dissolution and absorption of ICI 49,455

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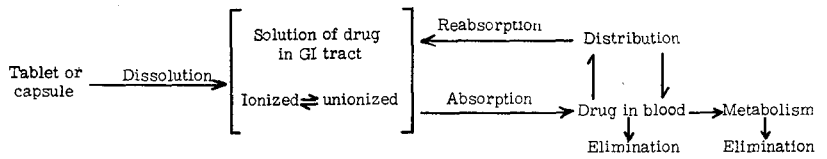
The dissolution rate of ICI 49,455 (1-isopropyl-1,2,3,4-tetrahydro-β-carboline) from tablet and capsule formulations cannot be related to *in vivo* absorption in dogs. The rate of partitioning of the drug into organic solvents and its absorption in goldfish and through human buccal membrane depend on the pH of the solution. Absorption increases with decrease in the degree of ionization. The dissociation constant and the elimination rate of the drug in man are such that high blood levels after oral administration are unlikely to occur.

Most drugs are absorbed from the gastrointestinal tract by a process of passive diffusion of the unionized moeity from solution across a lipid barrier. The amount of drug in the blood after oral administration is controlled by the factors in the following scheme.

SCHEME 1




In view of large variations in the blood levels of a potential analgesic, ICI 49,455 (1-isopropyl-1,2,3,4-tetrahydro-β-carboline) after its administration to volunteers as either plain or film-coated tablets, it was considered desirable to determine which factors in Scheme 1 were responsible.

Although most reports of dissolution rate-limited absorption relate to sparingly soluble drugs, Frostad (1961) and Levy (1964), respectively, have shown that the dissolution rate can control the absorption of the relatively water soluble sodium-*p*-aminosalicylate and acetylsalicylic acid. Levy, Leonards & Procknal (1965) have shown for aspirin formulations that *in vitro* dissolution can be correlated with rate of absorption. Rate of stirring and the pH of the dissolution medium must be carefully controlled (Levy, Leonards & Procknal, 1967).

The derivation of rate of absorption constants from blood level/time data is also the subject of much controversy (Loo & Riegelman, 1968). Intersubject variations in absorption, distribution, metabolism and excretion rates of drugs, all of which can produce variable blood levels of drug, are known to occur. Therefore, the factors affecting absorption of drug from solution should also be examined.

The work now described was an attempt to evaluate the relative contribution to the overall rate of absorption of dissolution rate of drug from formulations, the degree of ionization and the absorption of unionized drug.

For this purpose the *in vitro* dissolution rates of various formulations of the drug were determined using a modification of the method of Levy & Hayes (1960). The effect of pH on the partition rate of the drug between aqueous and organic solvent

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was examined using the method of Perrin (1967). *In vivo* absorption of the drug was examined using goldfish as described by Levy & Gucinski (1964), blood levels after oral administration of the drug to dogs, and the human buccal membrane method of Beckett & Triggs (1967).

## EXPERIMENTAL

ICI 49,455 as the hydrochloride salt has a $pK_a$ of 9·0; the equilibrium solubility at 37° in 0·1N hydrochloric acid is 1294 mg%.

The experimental formulations examined were (1) film-coated tablets, (2) uncoated tablets, (3) capsules and (4) an aqueous solution. Solid formulations contained 50 mg of the drug calculated as base.

*In vitro* dissolution rates were determined using the method of Levy & Hayes (1960) modified as follows. Formulations were enclosed within a rectangular basket (2 × 2 × 4 cm) constructed from 30 mesh stainless steel screen. The basket was centred on the base of a 400 ml water jacketed beaker, internal diameter 7 cm, containing 300 ml of simulated gastric juice, pH 1·4 (2·0 g sodium chloride, 7 ml conc. hydrochloric acid to 1 litre with water) at 37°. The dissolution medium was stirred by a single, angle bladed, glass Quickfit stirrer immersed 3 cm below the surface and driven by a geared, variable speed electric motor. Samples were removed periodically, filtered through a 0·45 $\mu$m Millipore filter and assayed spectrophotometrically at 278 nm by reference to a standard calibration curve. The percentage dissolution, corrected for cumulative sampling losses, was plotted as a function of time for each individual tablet. Dissolution times were derived from these plots and are presented as an average of six determinations for each formulation.

The effect of pH on the rate of partitioning of ICI 49,455 was determined using the three phase model described by Perrin (1967). Buffer solutions containing approximately 2 mg % of the drug were placed in compartment A, connected to compartment B containing pH 7·4 phosphate buffer via compartment C containing cyclohexane. The rate of loss of drug from compartment A was measured by spectrophotometric assay of the residual concentration.

Goldfish (*Carassius aurantus*) approximately 5 cm long and 7 g in weight were shown to survive for 48 h without visible distress in 0·05M tris buffer from pH 6·0 to 9·5. The time of death was taken as the time when gill and mouth movements ceased and fish were unable to return to the normal swimming position when overturned by means of a glass rod. To eliminate subjective bias all determinations were made by the same individual and solutions were coded. The code was broken only after completion of the experiment. The average of the death times of six individual fish for each concentration or pH was recorded.

The absorption in dogs of the four formulations given by mouth was examined is a cross-over experiment. The dogs were dosed with ICI 49,455 (approx. 20 mg/kg) and blood samples taken over 7 h. Each dog received a standard meal before the drug to minimise the risk of vomiting.

The concentration of the drug in blood was determined by extracting alkaline samples with ethanolic heptane followed by back extraction into hydrochloric acid. The fluorescence of the acid layer was then measured using an activation wavelength of 280 nm (uncorrected) and an emission wavelength of 352 nm (uncorrected).

The effect of pH on the buccal absorption of the drug in volunteers was determined by the method of Beckett & Triggs (1967). The solutions used contained 0·25 mg

in 25 ml of Sorensens phosphate buffer pH 6·0 and 7·0, Borax HCl buffer pH 9·0, 0·05M, or Tris buffer pH 10·0, 0·05M. The unabsorbed drug, remaining in the expelled solution after 5 min contact with buccal mucosa, was assayed spectrophotometrically after solvent extraction. The results are expressed as the average of the results obtained from four volunteers.

## RESULTS AND DISCUSSION

The times for 80 and 50% dissolution of ICI 49,455 from the formulations were derived from the percentage dissolved/time curves and are plotted as a function of stirring speed in Fig. 1a and b respectively. Uncoated tablets and capsules dissolved

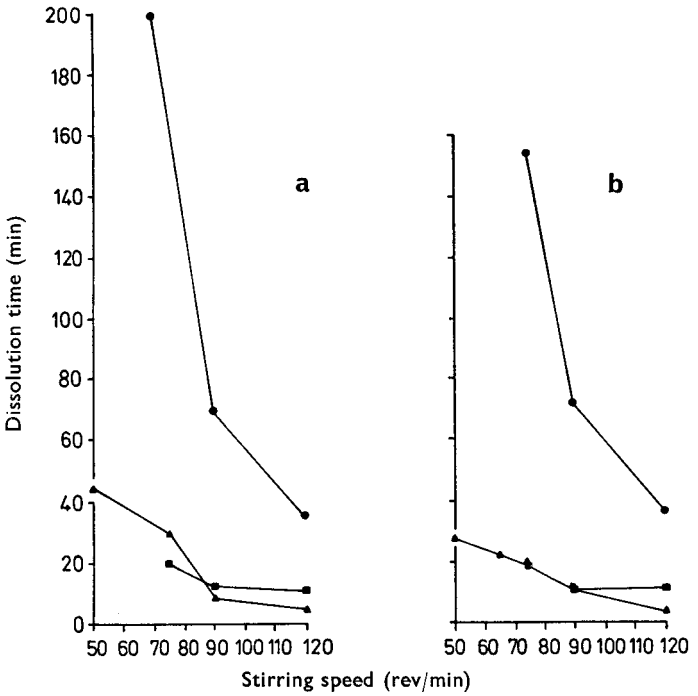


FIG. 1. Effect of stirring speed on the dissolution times of formulations of ICI 49,455. (*a*) 80% dissoln. (*b*) 50% dissoln. ●—● Film-coated tablets. ■—■ Capsules. ▲—▲ Plain tablets.

at approximately the same rate at the given stirring speed. Dissolution of film coated tablets showed a more marked dependence on stirring speed. The 50 and 80% dissolution times of film-coated tablets varied from approximately four times those of uncoated tablets at 120 rev/min to nine times at 75 rev/min. Such ninefold differences in dissolution rate might be expected to influence the rate of absorption and hence plasma level of a drug.

The effect of pH on the rate of loss of drug from compartment A of the three-phase model is shown in Fig. 2. At pH 6 no transfer from compartment A into B and C was evident after 24 h. The results agree with those from the goldfish and buccal mucosa studies and show an increase in partition rate with increase in pH of solution.

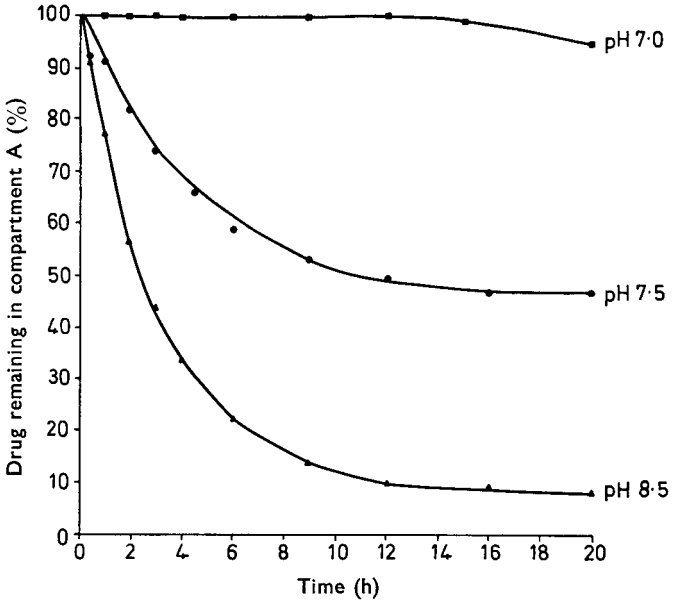


FIG. 2. Effect of pH on the loss of ICI 49,455 from compartment A of a three phase system.

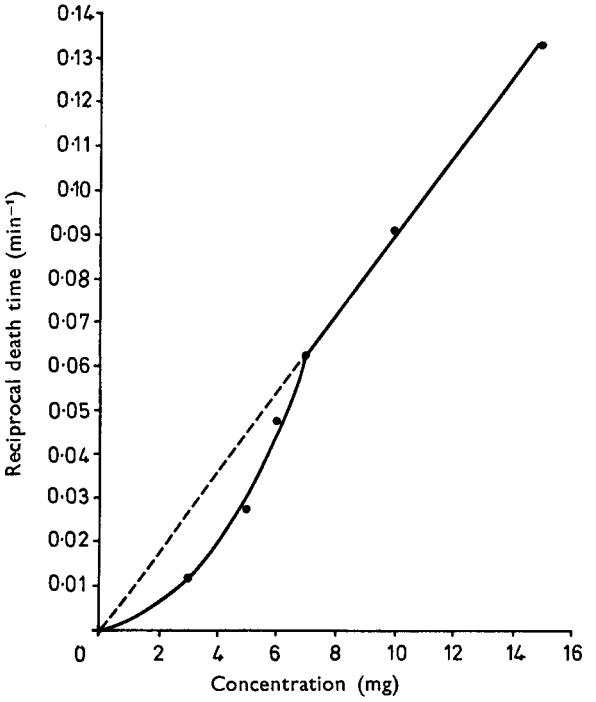


FIG. 3. Effect of concentration of ICI 49,455 on the death time of goldfish in pH 8·5 tris buffer.

The effect of concentration of ICI 49,455 on the death time of goldfish is shown in Fig. 3. According to the model proposed by Levy & Gucinski (1964) the time required for a pharmacological response (T) under constant experimental conditions is related to the drug concentration (C) in the following manner:

$$\frac{1}{T} = \frac{KC}{L} \quad .. \quad .. \quad .. \quad .. \quad (1)$$

Where K is the relative absorption rate constant and incorporates membrane surface area and L is the amount of drug required in the fish to elicit the pharmacological response. The assumptions made in the derivation of equation 1 have been discussed by Levy & Gucinski (1964).

The value of L need not be known for studies of the effect of pH on the absorption rate provided that the lethal dose is not modified by these environmental factors. Preconditioning of fish for 2 h in buffers or water before transfer to buffered solutions of the drug had no effect on the death time. Intraperitoneal injection of 1 mg of the drug in goldfish, preconditioned in water and transferred to buffer solutions or water after injection, showed that the buffer had no effect on the subsequent death time. Thin-layer chromatographic analysis of homogenized extracts of fish exposed to lethal and sublethal concentrations of the drug failed to reveal the presence of metabolites. The departure of Fig. 3 from linearity at low concentrations of drug suggests that the assumptions made by Levy & Gucinski (1964) are not applicable to the present drug.

The effect of pH on the death time of fish immersed in 10 mg% solutions of the drug was examined using Levy & Gucinski's (1964) modified equation:

$$\frac{1}{T} = \frac{K\,F.C}{L} \quad .. \quad .. \quad .. \quad .. \quad (2)$$

where F is the fraction of unionized drug.

The results should yield a straight line plot of Fx 1/T passing through the origin (where F = 0) if the drug is absorbed only in the unionized form. Such a plot could not be obtained. Nevertheless, the drug was found to be lethal at pH 9·0 in 1 min whereas the same concentration of drug at pH 6 failed to kill within 24 h. Thus unionized drug is rapidly absorbed but a decrease in pH of the medium with a consequent increase in the degree of ionization reduces the absorption rate.

Table 1. ICI 49,455 μg/ml *of blood after oral administration of* 20 mg/kg *as* (1) *film coated tablets* (2) *uncoated tablets* (3) *capsules* (4) *an aqueous solution*

| Dog No. | Formulation | ICI 49,455 μg/ml of blood | | | | Time after dose (h) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | ¼ | ½ | 1 | 1½ | 2 | 3 | 5 | 7 |
| I | 1 | — | 0·08 | 0·40 | 1·04 | 1·12 | 0·81 | 0·26 | 0·1 |
| | 2 | — | 1·04 | 1·13 | 1·01 | 0·62 | 0·42 | 0·15 | 0·18 |
| | 3 | — | 0·41 | 1·31 | 1·48 | 1·14 | 0·96 | 0·38 | 0·16 |
| | 4 | 0·26 | 0·88 | 0·84 | — | 1·12 | 0·53 | 0·25 | 0·14 |
| II | 1 | — | 0·83 | 1·98 | 1·55 | 1·15 | 0·74 | 0·31 | 0·07 |
| | 2 | — | 0·10 | 1·54 | 1·70 | 1·37 | 1·15 | 0·34 | 0·10 |
| | 3 | — | 1·62 | 1·57 | 1·10 | 0·94 | 0·56 | 0·18 | 0·08 |
| | 4 | 1·14 | 1·86 | 1·79 | — | 1·61 | 0·78 | 0·28 | 0·05 |
| III | 1 | — | 0·86 | 1·06 | 1·12 | 0·95 | 0·69 | 0·48 | 0 16 |
| | 2 | — | 0·71 | 1·14 | 1·13 | 1·13 | 1·11 | 0·46 | 0·22 |
| | 3 | — | 1·80 | 2·19 | 1·80 | 1·37 | 0·85 | 0·32 | 0·125 |
| | 4 | 0·33 | 0·62 | 0·75 | — | 0·93 | 0·72 | 0·30 | 0·10 |

The blood level/time data obtained from oral dosage in dogs are shown in Table 1. No significant advantage can be claimed for any of the formulations. There is no evidence to suggest that ICI 49,455 was more rapidly or efficiently absorbed from

solution than the solid formulations. The peak blood levels occurred 30 to 120 min after dosage and on a dose/kg body weight basis are similar to those achieved in man.

Dissolution is thus not the limiting factor in the absorption of the drug in dogs; hence the variations in blood levels obtained in human trials are unlikely to be caused by the variable dissolution rates of formulations.

The effect of pH on the absorption of the drug from the buccal cavity gave a linear response, 15% of the dose was absorbed at pH 6 whereas 75% was absorbed at pH 10. Although the method used included a rinse technique it is possible that binding of drug to the buccal mucosa or swallowing may occur, producing apparently high levels of absorption. The results show that the rate of absorption increases with increase in pH and the fraction of unionized drug.

Variable absorption of the drug cannot be related to *in vitro* dissolution rates of formulations, although large variations in dissolution rates were detected. The variations in blood levels are probably caused by variations in pH at the site of absorption and by inter-subject variations in elimination rate. Although unionized drug rapidly penetrates biological membranes, the ionic environment encountered in the upper part of the gastrointestinal tract is such that the drug will be almost completely ionized. The rapid absorption, required to balance the high elimination rate and produce adequate plasma levels of the drug, is not achieved. Formulations designed to increase the dissolution rate of drug will therefore have no effect on the absorption rate of the drug.

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