Drug Concentration in Goldfish at the Pharmacologic End-Point

Keyphrases ☐ Goldfish, overturn—relationship between bathing fluid and body drug concentrations ☐ Drug concentration, goldfish—overturn as a function of bathing fluid concentration ☐ Critical drug concentration—4-aminoantipyrine-induced overturn, goldfish ☐ 4-Aminoantipyrine—goldfish overturn as a function of bathing fluid concentration

Sir:

Recently, a two-compartment reversible model describing the occurrence of a pharmacologic effect in goldfish as a function of drug concentration in the bathing solution was examined (1). The mathematical relationships derived from this model and the experimental results demonstrate that a critical concentration is necessary in the fish's body before a pharmacologic response will occur. This concentration was assumed to be constant, regardless of the drug concentration in the bathing solution. The purpose of the present study was to determine the amount of drug in the fish's body at the time of occurrence of the pharmacologic effect as a function of drug concentration in the bathing solution.

Goldfish, weighing 2.5–3.7 g. (mean 3.1 g.), were obtained locally, and the time for 4-aminoantipyrine-induced overturn was determined (1). When overturn occurred (or in one group of experiments after 24 hr.), the fish were immediately transferred to 1 l. of distilled water for exactly 2 min. This procedure was necessary to remove any drug solution adhering to the fish's body and to expel any fluids retained in the fish's mouth. After this 2-min. rinse, the fish were frozen and assayed as previously described (2). Drug recovery determinations were carried out by homogenizing the fish with either 0.05 or 0.1 mg. of drug and assaying (2). Five fish were used at each level, and an average recovery of 98% was obtained.

The reciprocal time–drug concentration plot is shown in Fig. 1. As predicted by the proposed model (1), curvature occurs at the lower concentrations and dilute solutions of 4-aminoantipyrine (5 mg.% and less) do not cause overturn (Table I). No difference in 4-aminoantipyrine content could be detected in the goldfish at any concentration causing overturn. Goldfish exposed to 5 mg.% 4-aminoantipyrine solutions for 24 hr. without overturn had a significantly lower body content of drug than those fish that overturned.

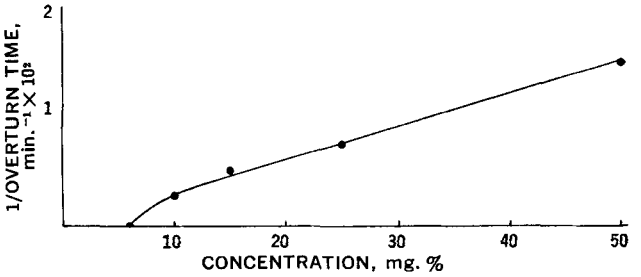

Figure 1—*4-Aminoantipyrine-induced overturn of goldfish as a function of concentration. (Data are from Table I.)*

The results of these experiments demonstrate that a critical concentration in the fish's body is necessary to induce a pharmacologic effect. This concentration appears to be independent of drug concentration in the bathing fluid over the range studied.

Table I—4-Aminoantipyrine Content in Goldfish at Overturn

Bathing Solution Concentration, mg. %	Number of Fish	Mean Overturn Time, min. ± *SD*[a]	Mean Drug Content, mg. Drug/g. Fish Tissue ± *SD*[b]
50	8	68.0 ± 1.8	0.071 ± 0.012
25	8	134.5 ± 11.1	0.066 ± 0.012
15	8	198.9 ± 25.1	0.068 ± 0.013
10	7	370.9 ± 30.3	0.065 ± 0.012
5	8	∞[c]	0.046 ± 0.005

[a] All significantly different from each other by Student's *t* test, $p < 0.05$. [b] 5 mg. % significantly different from all others by Student's *t* test, $p < 0.05$. No difference between 50, 25, 15, and 10 mg. %. [c] Greater than 24 hr.

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Formation of Coordinate Covalent Complex between Lithium and 8-Quinolinol

Keyphrases ☐ Lithium–8-quinolinol coordinate covalent complex—formation, fluorescence ☐ 8-Quinolinol–lithium coordinate covalent complex—formation, fluorescence ☐ Fluorometry—formation, analysis lithium–8-quinolinol complex

Sir:

Lithium ion reacts with 8-quinolinol to form a species whose fluorescence has been observed in water and in chloroform, and it has been suggested to represent the basis for a fluorometric determination of lithium (1). The fluorescence of the reaction product of lithium and 8-quinolinol has recently been under study in this laboratory. Our findings indicate that in water the lower limit of detection of lithium, by fluorometry, with 8-quinolinol ($\sim 5 \times 10^{-4}$ *M*) is not particularly impressive nor conducive to the employment of fluorometry as an analytical method of choice for lithium. However, the fact that a fluorescent product forms at all in the

reaction of Li^+ and 8-quinolinol is rather surprising and has some significance as far as the coordination chemistry of alkali metal ions in general and the neuropharmacology of lithium (2) in particular are concerned.

8-Quinolinol in dilute acid, neutral, and dilute basic aqueous solutions (those of concern here and in biological systems) is nonfluorescent. However, the excited cation and excited anion of 8-quinolinol show green fluorescence in concentrated acid (3) and concentrated base (4) solutions, even though the ground-state cation predominates below pH 5 and the ground-state anion is present above pH 10. In the pH range of 5–10, the predominant ground-state species derived from 8-quinolinol is the neutral phenolic species. The observation of fluorescence from 8-quinolinol only in concentrated acid or base solutions has been shown to be due to the attainment of prototropic equilibrium in the lowest excited singlet state between the cation and the zwitterion derived from 8-quinolinol (pK* −7) (3) and between the zwitterion and the anion (pK* 16) (4). Consequently, excitation of 8-quinolinol in neutral, dilute acid, or dilute base solutions results in the formation of the nonfluorescent excited zwitterion, no matter which ground-state species is excited. Accordingly, in the presence of alkali metal ions such as Na^+, K^+, Rb^+, and Cs^+ which, by virtue of their low charges and large ionic radii are very weakly polarizing and do not tend to form complexes with soft Lewis bases such as 8-quinolinol, the ligand exists in near neutral solutions predominantly as the free neutral species, and no fluorescence is observed from these solutions. However, solutions containing 8-quinolinol and Li_2CO_3 show the same fluorescence as those of commercial samples of lithium–8-quinolinolate in water ($\bar{\nu}_{max.}$ 2.08 $\mu m.^{-1}$).

Chelates of 8-quinolinol with polyvalent nontransition metal ions (*e.g.*, Mg^{+2} and Zn^{+2}) are well known for their green fluorescence (5). The fluorescences of these compounds are explicable in terms of the qualitative similarity in the electronic configuration of the chelated 8-quinolinolate to that of the protonated 8-quinolinol (the metal ion polarizes both the phenolic group and the heterocyclic nitrogen atom positively). Since ligand-exchange kinetics are generally slow compared with the kinetics of prototropic reactions, the excited chelate fluoresces, even in near neutral aqueous solutions, before it can dissociate in the lowest excited singlet state. Moreover, the electronic absorption spectra of the nontransition metal chelates of 8-quinolinol show low frequency absorption maxima at frequencies below 3 $\mu m.^{-1}$ in neutral aqueous and chloroform solutions, while the ^1La band of 8-quinolinol (the neutral species) appears at 3.20 $\mu m.^{-1}$ in both of these solvents. The ^1La band of the protonated 8-quinolinol appears at 2.79 $\mu m.^{-1}$.

The sodium, potassium, rubidium, and cesium salts of 8-quinolinol in water, buffered to pH 7.00, all show their long wavelength absorption maxima at 3.20 $\mu m.^{-1}$. (Actually the term "salts" is used loosely here; at pH 7.00, the 8-quinolinol is almost exclusively present as the neutral free ligand.) However, the lithium salt shows its long wavelength maximum at 2.87 $\mu m.^{-1}$. Consequently, it is proposed that while Na^+, K^+, Rb^+, and Cs^+ all form predominantly ionic salts with the 8-quinolinolate anion, in which the anion is free to hydrolyze in water, the compound formed between lithium and 8-quinolinolate anion is largely covalent, thereby accounting for its fluorescence and the large red shift of the 8-quinolinol absorption spectrum in neutral aqueous solutions. In support of this conclusion, continuous variation plots of the Li^+ to 8-quinolinol mole ratio *versus* fluorescence intensity and absorbance of the long wavelength absorption band of the complex, respectively, were carried out at pH 12.0 (to assure complete dissociation of the free ligand to the anion). These studies indicate that the complex has a 1:1 stoichiometry. Presumably, the remaining two coordination sites of the sp^3 hybridized Li^+ ion are hydrated.

The formation of a coordinate covalent complex involving Li^+ can be rationalized on the basis of the small ionic radius of lithium, imparting it with polarizing ability greater than that of a typical alkali metal ion. The small radii of first row elements have been employed to explain frequent deviations from "normal" periodic behavior. These results may be of some interest in analytical and inorganic chemistry. However, the implications are farther reaching in the area of neuropharmacology. It has been known for some time that lithium carbonate and other lithium salts have been effective in the pacification of manic psychotic behavior (2). The mechanism of sedation is not understood, although it has been proposed that Li^+ alters the metabolism of norepinephrine and catecholamine (6). If Li^+ was to occur exclusively as an ionic species *in vivo*, it could only cross the lipoprotein neural cell membrane by active transport processes, since a charged species, being lipophobic, could not cross by diffusional means. If, however, Li^+ was present to some extent as an uncharged chelate species (*e.g.*, with an amino acid) lipophilicity, as evidenced by the extractability of lithium–8-quinolinolate into chloroform, and thus diffusional membrane permeability come into the realm of possibility. Whether lithium acts at the outer neural surface, crosses the neural membrane by active transport or by diffusional transport, is conceivably of importance in interpreting the nature of its sedative action.

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