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# Dose versus survival time curves in the evaluation of 'prompt' and 'delayed' acute toxicities

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The acute toxicity of arecoline, diisopropylfluorophosphate, nicotine and pilocarpine alone or in association with atropine has been evaluated from percentage lethality and from the linear correlation of (doses/(survival time)) vs doses. The experimental points obtained with arecoline and diisopropylfluorophosphate, alone or in association with atropine 50 mg $kg^{-1}$, are apparently ordered according to two straight lines. That at lower doses gave the LD50 values of the compounds studied. The values found are comparable to those found with percentage lethality. The straight line found at high doses may indicate that over certain doses the drugs kill by a different mechanism. It is concluded that the evaluation of the survival time may be a reliable method in identifying and in evaluating quantitatively the two forms of toxicity.

The results obtained in the evaluation of acute toxicity from the hyperbola dose vs survival time (Molinengo 1979) are, in our experience, always in agreement with those obtained with the method which uses the percentage lethality at increasing doses. A particular situation was found with arecoline: the experimental points are apparently ordered according to two hyperbolae, causing some uncertainty in the evaluation of the LD50.

To confirm and extend this observation, we used both methods to measure the acute toxicity of arecoline hydrochloride alone and in association with atropine.

The acute toxicity of nicotine sulphate, pilocarpine hydrochloride and diisopropylfluorophosphate (DFP) was also evaluated alone and in association with atropine, to see if the particular doses vs survival time curve, found with arecoline, is a characteristic of drugs of cholinergic systems.

## *Methods*

Drugs were administered intraperitoneally to female, albino mice (25 g). Atropine sulphate (50 mg $kg^{-1}$) was given 30 min before the administration of the selected drug.

To evaluate the LD50 values by the method of percentage lethality, six doses of each drug alone, or in association with 50 mg $kg^{-1}$ of atropine, were used (n = 10/dose) and percentage lethality assessed after 48 h. To evaluate the LD50 values by the method based on survival time curves, the time after which each animal died at increasing doses was evaluated with an approximation of 10 s. All experiments were performed in the afternoon at a room temperature of 20 ± 2 °C.

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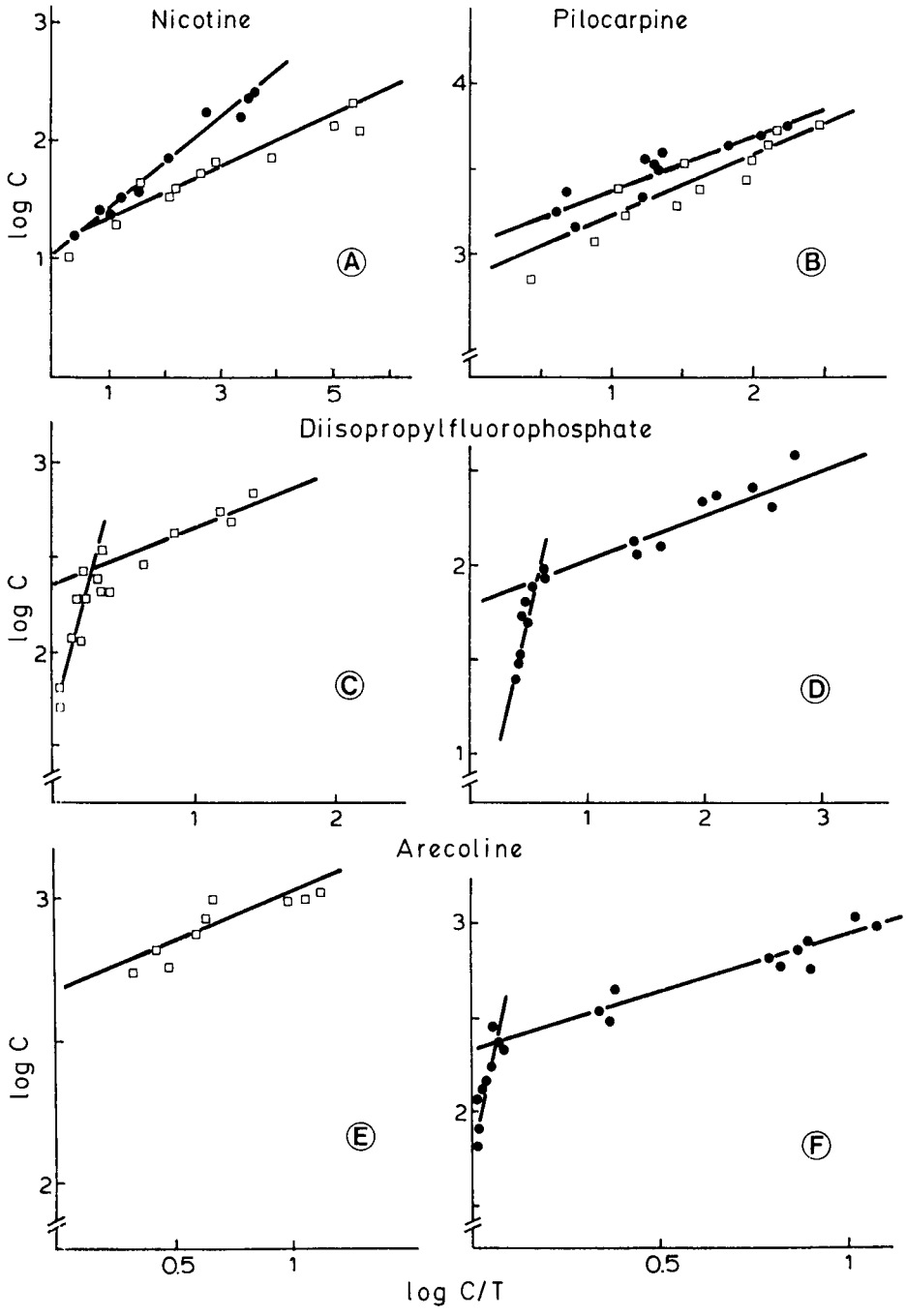


FIG. 1. Linear regressions (log C/T) vs log C (C doses in mg $kg^{-1}$); T survival time in min. (●) points obtained with drugs alone; (□) points obtained with drugs in association with atropine sulphate (5 mg $kg^{-1}$). The statistical and analytical constants of the linear regressions are given in the text.

*Results*

The percentage lethality was transformed into angular coordinates and the LD50 values obtained from the linear regression log doses vs angular coordinates. The LD50 values so obtained are reported with their standard errors in Table 1. According to the method previously described, the hyperbolae doses vs survival time were reduced to straight lines plotting (doses (C))/(survival time (T)) vs doses (C). A graphical representation of these plots is impossible owing to the huge variation of the quotient (C/T) (for arecoline it varies from 400 to 0·15). Therefore the doses were transformed into log doses and the graphs of Fig. 1 were obtained.

Inspection of these seems to indicate that with arecoline (Fig. 1F) and with DFP alone (Fig. 1D) or in association with atropine (Fig. 1C) the data are ordered according to two straight lines.

The slopes (b) of the two regressions obtained at low doses and at high doses of arecoline are b = 20·16 ± 0·21 (s.e.) (correlation coefficient (r) = 0·82 probability of a casual result (*P*) = 1–0·1%) and b = 0·62 ± 0·12 (s.e.) (r = 0·92 $P < 0·1\%$) respectively.

Similarly, with DFP the slopes of the two straight lines are b = 0·25 ± 0·20 (r = 0·84 *P* = 1–0·1%) at high doses and b = 2·01 ± 0·12 (r = 0·95 $P < 0·1\%$) at low doses. In association with 50 mg kg$^{-1}$ the slopes of the straight lines found with DFP are: b = 0·26 ± 0·16 (r = 0·93 *P* = 1–0·1%) and b = 1·63 ± 0·18 (r = 0·86 *P* = 1–0·1%). These results offer statistical evidence of differences between the correlations obtained at low or at high doses of DFP and of arecoline. In these cases two y-intercepts are obtained. The following values were found: arecoline 64·58 ± 18·80 (s.e.) and 230 ± 38 (s.e.); DFP alone 54 ± 10 and 4·77 ± 1·86; DFP in association with 50 mg kg$^{-1}$ of atropine 236 ± 19 and 39·52 ± 11·62.

In the range of doses examined here, nicotine, pilocarpine and arecoline in association with atropine gave one straight line (Fig. 1A, B, E). The y-intercept of the corresponding regressions are in Table 1.

Table 1. LD50 values obtained from the linear regression log doses vs angular coordinates.

| Drug | LD50 ± s.e. (mg kg$^{-1}$) from the percentage lethality* | LD50 ± s.e. (mg kg$^{-1}$) from the survival time curve† |
|---|---|---|
| Pilocarpine hydrochloride | 990·00 ± 104·00 | 1197·00 ± 186·00 |
| Diisopropylfluorophosphate | 6·75 ± 1·10 | 4·77 ± 1·86 |
| Arecoline hydrochloride | 86·87 ± 12·00 | 64·58 ± 18·80 |
| Nicotine sulphate | 10·20 ± 1·23 | 17·90 ± 3·32 |
| In association with 50 mg kg$^{-1}$ of atropine sulphate | | |
| Pilocarpine hydrochloride | 1081·00 ± 107·00 | 955·00 ± 157·60 |
| Diisopropylfluorophosphate | 29·20 ± 1·08 | 39·52 ± 11·62 |
| Arecoline hydrochloride | 565·00 ± 111·00 | 470·85 ± 43·60 |
| Nicotine sulphate | 12·20 ± 1·21 | 18·20 ± 4·50 |

* n = 50. † n = mg$^{-1}$

*Discussion*

In previous work (Molinengo 1979), evidence has been offered that the y-intercept of the regressions (C/T) vs C is the LD50 of a drug and, having found two regressions with diisopropylfluorophosphate and with arecoline, we might surprisingly suppose that for these drugs there are two LD50 values. But the LD50 of a drug is defined as the lowest dose which kills 50% of the animals. Therefore only the y-intercept of the straight line obtained at the lowest dose may be considered the LD50 of the drug.

This consideration is substantiated by the results given in Table 1, from which it is seen that there is an acceptable agreement between the LD50 values obtained from the percentage lethality at increasing doses and the LD50 values obtained from the straight lines (C/T) vs C found in the lower range of doses. The second straight line found with arecoline and diisopropylfluorophosphate at high doses may indicate that over certain doses the drugs kill the animal by a different mechanism.

This interpretation is supported by the observation that the straight line found at lower doses of arecoline is not evident when atropine (50 mg kg$^{-1}$) is associated with arecoline. This suggests that only the mechanism by which low doses of arecoline kill the mice is completely antagonized by atropine.

The hypothesis that certain drugs may kill by a different mechanism according to the range of doses is not new. For example, the prompt lethality of hemicholinium-3 observed at high doses is due to the neuromuscular blocking action which is antagonized by neostigmine and edrophonium whereas the slow lethality at low doses is modified to only a limited degree (Bowman & Marshall 1972).

Similarly Fischetti (1957) suggested that death caused by high and low doses of diisopropylfluorophosphate is determined by a different mechanism.

In conclusion, our results show that when a drug displays a prompt toxicity at high doses and a slow toxicity at low doses, the plot of (C/T) vs C is a reliable method for identifying and evaluating quantitatively the two forms of toxicity.

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