The curve doses vs survival time in the evaluation of acute toxicity

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The method universally used to estimate acute toxicity is based on the evaluation of percentages of dead animals at various doses. Beccari (1949) proposed a new method; the estimation of acute toxicity is made by measuring the time after which an animal dies (survival time) and the time after which recovery takes place in animals treated with doses which did not kill them (recovery time). The curve doses vs survival time is an hyperbola and the curve doses vs recovery time is a straight line. The point at which the two curves intersect is, according to Beccari (1949), the LD50 of the drug. This procedure presents difficulties which might explain its limited application in pharmacology.

The difficulties are: (i) it is easy to decide when an animal is dead, it is difficult and often entirely subjective to decide when an animal has recovered from the toxic effects of the drug. (ii) Accurate and well standardized statistical treatments have been developed for the traditional method based on percentages of deaths. This is not the case for Beccari's method with which the LD50 is obtained but one has no indication about its variance, confidence limits etc. In order to overcome these difficulties the relation $T = C\,b/(C-a)$ has been selected for the hyperbola of the survival time (T) vs doses (C).

This equation may be easily transformed in the linear equation: $C = a + b\,(C/T)$. A plot of C/T vs C must give a straight line and the constants a and b of the regression line are easily obtained. But the constants a and b are the asymptotes of the hyperbola and a is the smallest dose of a drug that kills and b is the shortest time of survival.

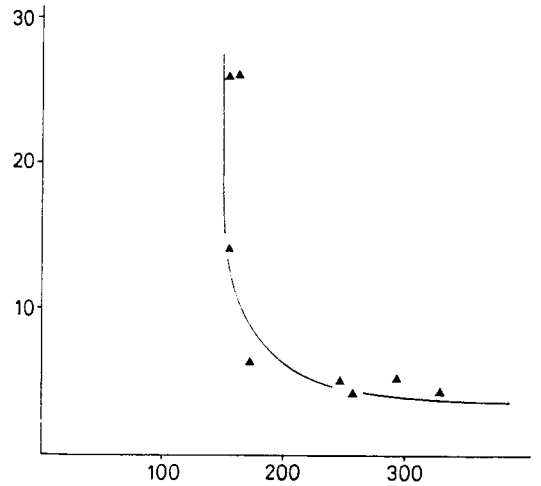

Fig. 1. Survival time curve of pentobarbitone. Abscissa: pentobarbitone (mg kg^{-1}). Ordinate: survival time (min). The asymptotes a and b of the hyperbola are the constants of the linear regression $C = a + b \cdot (C/T)$

Each point of the hyperbola is the mean of the frequency distribution curve of animals dying at the given time. If we accept that these frequency distribution curves are symmetrical, then the points of the hyperbola are doses which kill 50% of the animals in the considered time. Therefore the asymptote a is the smallest dose of drug which kills 50% of the population in an infinite time—a definition of the LD50.

In this way the LD50 is obtained from the survival time only and it is not necessary to have the recovery time curve with its difficulties and limitation.

The variance of the LD50 may be obtained from the data of the linear correlation $Y = a + bX$ in which $Y = C$ and $X = C/T$.

The standard error (e_y) of the regression line is:

$$e_y = \sqrt{\frac{1}{n-2}\cdot\left(Sy^2 - \frac{(Sxy)^2}{Sx^2}\right)\cdot\left(\frac{1}{n} + \frac{(x-\bar{x})^2}{Sx^2}\right)}$$

and for the coefficient a ($x = 0$) we have:

$$e_a = \sqrt{\frac{1}{n-2}\cdot\left(Sy^2 - \frac{(Sxy)^2}{Sx^2}\right)\cdot\left(\frac{1}{n} + \frac{\bar{x}^2}{Sx^2}\right)}$$

in which $Sx^2 = S(x-\bar{x})^2$; $Sy = S(y-\bar{y})^2$; $Sxy = S(x-\bar{x})\,(y-y)$ and n = number of experiments. With the standard error of a (LD50) many other statistical tests can be performed. In order to test the validity of this method the LD50 of 10 different drugs was evaluated. Drugs were administered to female

Table 1. LD50 values obtained by the method described compared with values from the literature obtained from the traditional method.

Drugs and route	n	LD50 in mg kg^{-1}	
Pentobarbitone sodium i.p.	8	136·60 ± 9·90	128·76 ± 2·75 (Spector 1956)
Chlorpromazine hydrochloride i.p.	11	248·45 ± 21·10	225–250 (Gordon 1967)
Atropine sulphate i.p.	10	181·80 ± 31·90	250 (Spector 1956)
Quinidine sulphate i.p.	10	121·19 ± 16·66	189·96 ± 39·50 (Spector 1956)
Imipramine hydrochloride i.p.	11	91·87 ± 12·12	115 (Del Mastro & Muttini 1966)
Procaine hydrochloride i.p.	11	126·29 ± 18·26	123·80 ± 7·10 (Spector 1956)
Picrotoxin i.p.	8	6·07 ± 1·43	4·5 (Spector 1956)
Neostigmine methylsulphate s.c.	7	0·44 ± 0·09	0·42 ± 0·07 (Spector 1956)
3-Methyl-4-furazan carbohydrazide oral	6	230·00 ± 40·00	162–204 (Fundarò & Cassone (1975)
Ethanol oral	6	12580 ± 3970	9488 (Spector 1956)

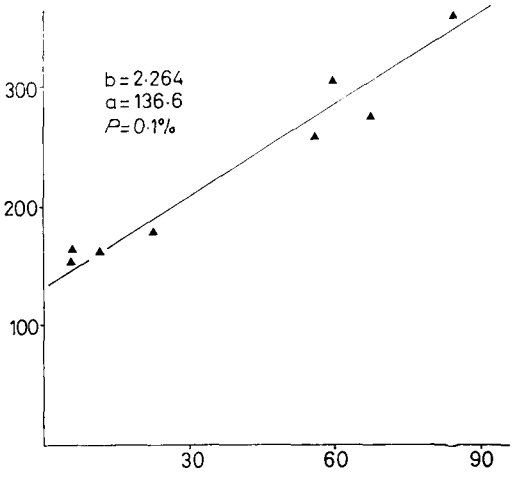

FIG. 2. Plot of C/T (abscissa) versus C (ordinate). T = survival time; C = doses of pentobarbitone (mg kg^{-1}). The analytical constants of the linear correlation (C = a + b C/T) and the probability of casual result are: a = 136·6, b = 2·264 and $P<0·1\%$.

albino mice (~25 g) intraperitoneally or subcutaneously or by stomach tube. The times of death were evaluated with an approximation of 10″.

A sample set of data is reported in the Figures. In Fig. 1 the curve concentration vs survival time of pentobarbitone sodium is given and in Fig. 2 the corresponding linear correlation C/T vs C is given.

The doses (C) were not transformed into log doses according to Clark (1937) and also by Beccari (1949). In any case the method is entirely empirical and from a practical point of view the log transformation of doses seems to be unimportant. For example, the LD50 of pentobarbitone sodium obtained with the log transformation is 134·80 mg kg^{-1} instead of 136·60 mg kg^{-1}; similar results were obtained with the other drugs examined here.

The LD50 values obtained with the method described are compared in Table 1 with data found in the literature and obtained by the traditional method based on percentages of dead animals at various doses.

These results indicate that the method here proposed to evaluate acute toxicity gives reliable results and allows a considerable reduction of the number of animals killed.

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Neuropharmacological studies on the neuroleptic potential of domperidone (R33812)

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Neuroleptic agents are potent antagonists of emesis in both animals and man. This action is thought to involve blockade of dopamine sensitive receptors in the chemoreceptor trigger zone, but is not generally specific in that the same drugs can inhibit the function of other cerebral dopamine systems (Janssen 1970). It was therefore of interest that agents from a series of benzimidazoline derivatives, including domperidone and halopemide, were recently reported to possess potent anti-emetic activity whilst failing to cause certain biochemical or behavioural changes indicative of effect on other cerebral dopamine systems, even though they had the ability to potently displace neuroleptics in in vitro receptor binding assays (Leysen et al 1978; Niemegeers, Laduron, personal communications). Therefore, the present studies were designed to assess the ability of domperidone to exert neuroleptic-like blockade of central dopamine receptor mechanisms.

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The ability of domperidone to modify locomotor hyperactivity responses was assessed both following peripheral and intracerebral administration to male C.F.E. rats. Hyperactivity was measured using individual Perspex cages each equipped with one photocell unit placed off-centre, and interruptions of the light beam were recorded electromechanically in counts per 5 min (see Costall & Naylor 1976, for experimental details). In one series of experiments hyperactivity was induced by 50 μg dopamine injected in 1 μl bilaterally into the nucleus accumbens and by 25 μg dopamine injected in 2 μl bilaterally into the striatum following a nialamide pretreatment, 100 mg kg^{-1}, 2 h (see Costall & Naylor 1976, for stereotaxic techniques and experimental details). The effects of control agents, haloperidol, sulpiride and metoclopramide were assessed at the same time as domperidone. The i.p. administration of haloperidol, 0·025–0·8 mg kg^{-1}, and sulpiride, 2·5–20 mg kg^{-1}, was shown to reduce or abolish the hyperactivity induced from the