A COMPARISON OF THE TOXICITY OF DECOMPOSED AND "NORMAL" PARALDEHYDE.

By Yeoh Teow Seng

(Department of Pharmacology, University of Singapore).

It was reported that a particular batch of paraldehyde received by the Singapore Government Medical Store and supplied to the General Hospital did not conform to the specifications in the British Pharmacopoea. This work was undertaken at the request of Dr. Gwee Ah Leng (Physician, General Hospital) to verify whether the toxicity of this paraldehyde is higher than a sample which conforms to the British Pharmacopoea specifications.

Accordingly a sample of paraldehyde from the General Hospital dispensary and a sample from the Department of Pharmacology (manufactured by Merck of Germany and received in April 1959) were tested for the specifications in the British Pharmacopoea (1958), according to the methods given in the latter. The results of these tests are shown in Table (1) below:

The results of the tests on the paraldehyde samples showed that the acetaldehyde content and acidity of the Hospital sample greatly exceeded the specifications set in the British Pharmacopoea while the sample from the Pharmacology department conformed to these specifications. This confirmed that the particular paraldehyde sample from the General Hospital is decomposed (from now on to be designated as "suspect" paraldehyde), while that from the Pharmacology department is not (from now on to be designated as "control" paraldehyde) and can therefore be used as a control for the toxicity test.

DESIGN OF EXPERIMENT

A preliminary toxicity test was done on mice to determine the approximate LD_{50} dose for both the control and suspect paraldehydes administered by the subcutaneous route. On the basis of this test a range of dose levels increasing by geometric progression (Factor 1.1) was worked out with the expected LD_{50} dose in the middle of this range. Owing to limitation of supply, only 5 mice were used for each dose level.

The day preceding the toxicity test, the mice were weighed before being fed and only those ranging from 23 to 27 grams, i.e. 25 ± 2 grams, were selected for the test.

The "control" and "suspect" paraldehydes were first diluted in the ratio of 1 : 12 with physiological saline. Serial dilutions for each dose level were than prepared in the ratio of 10 volumes diluted paraldehyde to 1 volume saline. In this way, the volume to be injected per unit weight of mice will be the same for each dose level.

The mice were picked up from the cage at random and weighed. The injections were given subcutaneously with a tuberculin syringe and spread over as wide an area as possible over the abdomen. The volume injected in each case was 0.95 c.c. for a 25 gram mouse. An adjustment of 0.02 c.c. was made for each gram difference from 25 grams. All the injections were accomplished within about 90 minutes. The results are summarised in Table (2).

TABLE 1. Results of tests on paraldehyde samples.

TEST	Br. Pharmacopoea specification	Paraldehyde (Hospital)	Paraldehyde (Pharmacol. Dept.)
Melting point	Not lower than 11°C.	4°C.	11.5°C.
Acidity: Expressed in volume of N/10 sodium hydroxide required to neutralise 5 ml. paraldehyde	Not more than 1.5 ml.	5.25 ml.	0.25 ml.
Acetaldehyde: Expressed in volume of N/2 NaOH	Not more than 0.8 ml.	4.85 ml.	0.15 ml.
Peroxidised Compounds: Expressed in volume of N/10 sodium thiosulphate	Not more than 2.0 ml.	0.6 ml.	0.2 ml.

TABLE 2. Mortality for "control" and "suspect" paraldehydes at dose levels increasing by geometric progression (factor 1.1.).

Dose in c.c. Paraldehyde per Kg. mouse	No. of mice	MORTALITY	
		"CONTROL" parald.	"SUSPECT" Parald.
1.653	5	0	0
1.818	5	0	1
2.0	5	2	2
2.20	5	3	2
2.420	5	5	3
2.662	5	5	5

ANALYSIS OF DATA.

(1) *The Reed-Muench method.* (Reed and Muench, 1938).

Tables (3A and 3B) were constructed to present the results obtained for the "control" and "suspect" paraldehydes. Columns (b) show the number of mice surviving at each of the dose levels [Column (a)] and columns (c) show the number that died.

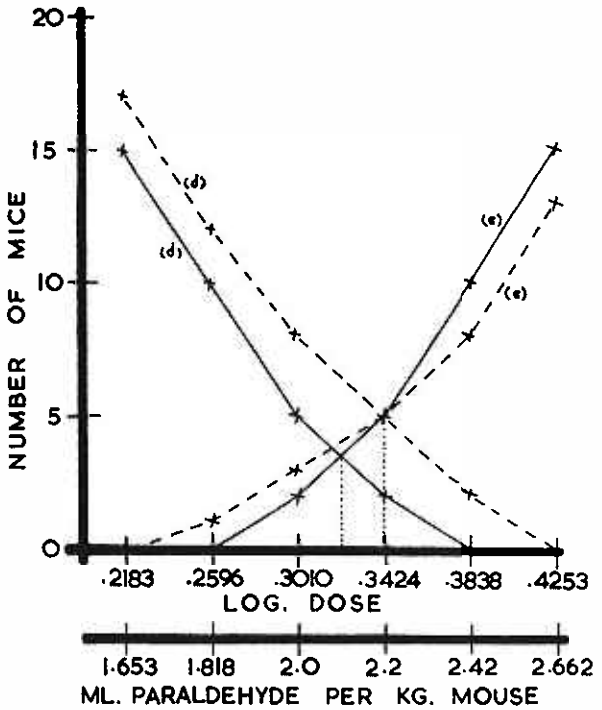

Fig. 1.

Calculation of LD_{50} of "control" and "suspect" paraldehydes by graphic method.

(d) = number of mice surviving at this and greater doses.

(e) = number of mice dying at this and smaller doses.

- - - = "Suspect" paraldehyde.

— = "Control" paraldehyde.

It is assumed that any mouse surviving a certain dose would have survived any smaller dose and any mouse dying at a certain dose would have died at any greater dose. Columns (d) which show the number of survivors at a particular and greater doses were derived by adding the figures in respective columns (b) from below upwards including the survivors at the particular dose level. Columns (e) which show the number of deaths at a particular and smaller doses were derived by adding the figures in respective columns (c) from above downwards including the deaths at the particular dose level. Columns (g) show the percentage mortality for each of the dose levels.

The LD_{50} dose may be obtained graphically from Fig. (1) and is the dosage value at which the number of animals dying at this and smaller dosage is equal to the number of animals surviving at this and larger doses; this is indicated by the point of intersection of the respective curves "d" and "e". A perpendicular from this intersection point to the abscissa gives the value of Log LD_{50}, since the dose values are plotted on a logarithmic scale. The proportionate distance of this log. value (LD_{50}) from the next lowest dose level on the abscissa can be calculated by applying the formula :

$$\frac{50\% - \%\text{ mortality at next lowest dose level}}{\%\text{ mortality at next highest dose level} - \%\text{ mortality at next lowest dose level.}}$$

For the "control" paraldehyde this proportionate distance was found to be half-way between the next lowest and the next highest dose levels. The arithmetic value of the LD_{50} will then be antilog. (log. 2 + 0.5 x log. 1.1) = 0.3217. The LD_{50} dose for "control" paraldehyde is therefore **2.097 ml. per kilogram** mouse.

For the "suspect" paraldehyde, the intersection point coincides with the dose level of **2.20 ml. per kilogram** mouse which is then the LD_{50} dose.

TABLE 3A. Mortality data obtained with "control" paraldehyde.

(a) ml. paraldehyde per kilogram mouse	(b) Survivors	(c) Dead	TOTAL (d) Survivors	TOTAL (e) Dead	(f) (d + e)	(g) Percentage Mortality (e) (d+e)
1.653	5	0	15	0	15	0
1.818	5	0	10	0	10	0
2.0	3	2	5	2	7	*29
2.20	2	3	2	5	7	71
2.420	0	5	0	10	10	100
2.662	0	5	0	15	15	100

* nearest whole figure

TABLE 3B. Mortality data obtained with "suspect" paraldehyde.

(a) ml. paraldehyde per kilogram mouse	(b) Survivors	(c) Dead	TOTAL (d) Survivors	TOTAL (e) Dead	(f) (d + e)	(g) Percentage Mortality (e) (d+e)
1.653	5	0	17	0	17	0
1.818	4	1	12	1	13	*8
2.0	3	2	8	3	11	*27
2.20	3	2	5	5	10	50
2.420	2	3	2	8	10	80
2.662	0	5	0	13	13	100

* nearest whole figure

(2) *Method of Thompson and Weil (1952).*

The mortality data obtained in the toxicity test (Table 2) was used further to estimate the LD_{50} dose by employing the method of moving-average interpolation (Thompson and Weil, 1952). Reference was also made to the tables (Weil, 1952) for calculation of the LD_{50} dose and its confidence interval. To avoid lengthy reproduction of the tables, formulae and calculation only the result is given.

For the "control" paraldehyde the estimate of LD_{50} was found to be 2.097 ml. per kilogram mouse with a confidence interval (95 times in 100) of 2.084 to 2.112 ml. per kilogram mouse. For the "suspect" paraldehyde the LD_{50} was 2.236 ml. per kilogram mouse with a confidence interval of 1.967 to 2.54 ml.

It can be seen that the estimates of LD_{50} dose obtained by the moving-average interpolation method and the Reed-Muench method agree very closely with each other.

DISCUSSION

The pharmacology and toxicology of paraldehyde are not completely understood. Paraldehyde is a polymer of 3 molecules of acetaldehyde and is unstable. It decomposes to acetaldehyde, acetic acid and probably other compounds when exposed to light and air. The scanty reports in the literature of the complications arising from its use may be grouped as follows:—

(a) Corrosive burns of mucosal surfaces due to contact with "decomposed" paraldehyde (B.M.J. 1954), after oral or rectal administration.

(b) Sloughing of skin, sterile abscesses of the buttocks and sciatic nerve damage, resulting from intra-muscular administration into the gluteal region (Hayward and Boshell, 1957).

The complications mentioned in (a) and (b) above are explained by the high acetic acid content (may be as high as 40 per cent as reported in B.M.J., 1954) that can be present in decomposed paraldehyde.

(c) Metabolic acidosis (Waterhouse and Stern 1957; Elkinton et. al. 1957). This is explained by Hayward and Boshell (1957) on the hypothesis that paraldehyde is depolymerised to acetaldehyde and then oxidised to acetic acid. The acetic acid thus formed is metabolised to carbon dioxide and water by the citric acid

cycle. If the requirements for the normal disposition of acetate are not present, the acetate are not present, the acetate may pool in the blood and contribute to a metabolic acidosis. In this respect the intraction of paraldehyde and tetraethylthiuram disulphide ("Antabuse") is interesting, since the latter has been shown in vitro to inhibit the activity of the enzyme aldehyde oxidase, which catalyses the conversion of acetaldehyde to acetic acid. Three cases of transient confusional psychosis in patients receiving concurrent antabuse and paraldehyde therapy have been reported (Christie G. L. 1956).

(d) Central nervous effects simulating severe intoxication with ethyl alcohol, with slurring of speech, ataxic gait, intense headache, nausea and prolonged drowsiness. These effects are believed by Hemphill and Heller (1944) as not being due to an overdosage with "pure" paraldehyde but due to some unknown products(s) of decomposition.

(e) Large doses cause prolonged unconsciousness with respiratory difficulty, cyanosis and pulmonary oedema. These effects may be assumed to be due to an extension of the hypnotic properties of paraldehyde.

The extent contributed by each of the abovementioned effects toward the over-all toxicity of the drug cannot be estimated The LD_{50} dose then can only be related to the over-all effects exerted by paraldehyde. With this qualification in mind, it is concluded on the basis of this toxicity test, that the toxicity of this particular sample of "decomposed" paraldehyde (as supplied by the General Hospital dispensary) is not increased, when compared to paraldehyde conforming to British Pharmacopoea specifications, when tested on mice.

SUMMARY

(1) Simultaneous toxicity tests were done on mice to determine the LD_{50} dose of "decomposed" paraldehyde and paraldehyde conforming to British Pharmacopoea specifications. The toxicity of the former was found not to exceed that of the latter.

(2) A brief survey of the complications likely to occur with the use of decomposed paraldehyde is given.

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