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DOSE-RESPONSE CHANGES IN ORGAN WEIGHTS AND WATER CONTENTS FOLLOWING ADMINISTRATION OF TOXIC DOSES OF PARACETAMOL

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Our aim was to obtain information on what parameters should be included in estimates of the acute toxicity of a drug. Paracetamol was selected as the challenging agent following recent studies on the acute toxicity of paracetamol in this laboratory by Boyd & Bereczky (1966).

Measurement of the toxicity of new drugs is required by law in Canada (1965) and in the United States of America (1963) and legislation is under consideration in Great Britain (Dunlop, 1965). Legal requirements for estimates of acute toxicity usually include estimates of the LD50 but do not specify what other measurements must be made. For example, in Canada the Food and Drug Directorate (1965) requires " a description of the signs of toxicity " and the Expert Committee on Drug Toxicity (1964) of the Association of the British Pharmaceutical Industry has recommended a " quantitative study of acute effects " and " dose-response relations." The British Ministry of Health Committee on the Safety of Drugs has proposed " autopsy findings and any other particulars " (1963) and " details of laboratory and animal studies " (1965).

The basic question to be answered is what minima of parameters of acute toxicity are necessary to define the public health hazard involved. The opinion held in this department is that the syndrome of acute toxicity to a new drug should be viewed as the syndrome of a new disease to which man may be exposed. Studies of acute toxicity in animals should delineate the clinical and pathological features of the syndrome and thus provide a basis for the diagnosis, prognosis and treatment of the toxicity " disease " if and when it should appear in subsequent clinical pharmacology and therapeutic trials in man.

If this premise be accepted, it follows that cageside observations of animals in acute toxicity studies should parallel the general procedures of bedside observation in clinical medicine and that autopsy techniques should correspond to those used in clinical pathology. The protocol adopted must recognize the need for economy of time and effort and must be commensurate with the practical value of the information to be obtained. The technique now used in this laboratory has been described by Boyd and Bereczky (1966) and has followed earlier studies upon the significance of clinical

measurements (e.g., Boyd, Fulford & Harris, 1961 ; Boyd & Lloyd, 1965). Pathological observations include measurement of the fresh weight and water content of a series of body organs in surviving and non-surviving animals but without dose-response comparisons.

The project described here was designed to determine (*a*) if changes in organ weights and water contents are dose-dependent and (*b*) if appreciably more information could be obtained by adding such a dose-response modification to the technique. Changes in organ weights and water levels are usually most significant at the time of death. It has been our practice to do these measurements on any animal which can be autopsied within 1 hr of death to avoid postmortem shifts in organ weight and water content described by Boyd & Knight (1963). The dose of drug and interval to death are noted but not separately studied. Boyd & Bereczky (1966) have shown that interval to death is an important factor which would have to be standardized in any dose-response study. The average interval to death following lethal doses of paracetamol was 21 hr (Boyd & Bereczky, 1966). It was decided, therefore, to measure organ weights and water contents in albino rats surviving at 24 hr after oral administration of a range of doses of paracetamol.

METHODS

The animals used were male Wistar albino rats of 140 to 160 g body weight obtained from Canadian Breeding Laboratories and fed Purina laboratory chow and water *ad libitum*. A total of 245 animals was used in 12 drug-treated and one control group and divided so that the final

TABLE 1

ESTIMATING EQUATIONS AND CORRELATION COEFFICIENTS FOR REGRESSIONS OF CHANGES IN ORGAN WEIGHTS (Y) ON DOSE OF PARACETAMOL (X)

Organ weight was measured in g and expressed as mean percentage change from that in controls given no paracetamol. Dose of paracetamol is expressed as g/kg body weight

Organ	Estimating equation	Correlation coefficient	*P* (r = zero)
Adrenal glands	$Y = 0{\cdot}67 + 3{\cdot}15 \log X$	+0·325	0·30
Brain	$Y = -(0{\cdot}72 + 1{\cdot}52 \log X)$	−0·287	0·30
Gastrointestinal tract:			
Cardiac stomach	$Y = 3{\cdot}65 - 6{\cdot}03 \log X$	−0·425	0·20
Pyloric stomach	$Y = -(2{\cdot}59 + 12{\cdot}97 \log X)$	−0·915	<0·001
Small bowel	$Y = -(6{\cdot}23 + 9{\cdot}10 \log X)$	−0·569	0·05
Caecum	$Y = -(7{\cdot}77 + 12{\cdot}09 \log X)$	−0·592	0·02
Colon	$Y = -(4{\cdot}19 + 9{\cdot}02 \log X)$	−0·641	0·01
Heart	$Y = -(3{\cdot}62 + 2{\cdot}43 \log X)$	−0·271	0·30
Kidneys	$Y = -(3{\cdot}03 + 3{\cdot}51 \log X)$	−0·436	0·10
Liver	$Y = -(3{\cdot}20 + 20{\cdot}24 \log X)$	−0·873	<0·001
Lungs	$Y = -(1{\cdot}73 + 8{\cdot}16 \log X)$	−0·577	0·05
Muscle (vent. abd. wall)	$Y = -(6{\cdot}16 + 11{\cdot}98 \log X)$	−0·676	0·01
Salivary glands (submax.)	$Y = 0{\cdot}28 - 0{\cdot}02 \log X$	−0·001	1·00
Skin	$Y = -(4{\cdot}88 + 12{\cdot}63 \log X)$	−0·814	<0·001
Spleen	$Y = -(0{\cdot}89 + 34{\cdot}46 \log X)$	−0·848	<0·001
Testes	$Y = 4{\cdot}52 - 7{\cdot}06 \log X$	−0·405	0·20
Thymus gland	$Y = -(34{\cdot}08 + 10{\cdot}21 \log X)$	−0·242	0·40
Thyroid gland	$Y = -(2{\cdot}89 + 14{\cdot}01 \log X)$	−0·762	0·001
Residual carcass	$Y = -(3{\cdot}37 + 7{\cdot}27 \log X)$	−0·820	<0·001
Total body weight	$Y = -(3{\cdot}82 + 10{\cdot}27 \log X)$	−0·802	<0·001

mean initial body weight was exactly the same in each group (154 g) and the standard deviation was kept as low as possible (6 to 9 g). To accomplish this, one rat of each drug dosage group and two controls were autopsied over a period of 1 to 2 weeks and this unit experiment was repeated ten times. The animals were housed in metabolism cages with one rat per cage.

Food was withdrawn for 16 hr (overnight) before drug administration. Paracetamol (Eastman) was given by intragastric cannula as a freshly prepared aqueous suspension, stabilized by the addition of 0.2% gum tragacanth, in a volume of 20 ml./kg body weight. The doses used, with numbers of animals per dose in parentheses after each, were: 0.0 (31), 1.0 (14), 2.0 (13), 3.0 (17), 4.0 (18), 5.0 (18), 6.0 (25), 7.0 (15), 8.0 (22), 9.0 (17), 10.0 (18), 11.0 (16) and 12.0 (21) g/kg. Animals which were alive 24 hr later were killed with chloroform and immediately autopsied to a total of at least ten for each dose of paracetamol.

TABLE 2

ANALYSIS OF THE REGRESSIONS OF CHANGES IN ORGAN WATER CONTENTS (Y) ON DOSE OF PARACETAMOL (X)

Organ water content was measured as g water/100 g dry weight of tissue and expressed as mean percentage change from that in controls given no paracetamol. Dose of paracetamol is expressed as g/kg body weight

Organ	Estimated value of Y	Correlation coefficient	*P* (r = zero)	Y at X = 12
Adrenal glands	22·79 log X−0·65	+0·640	0·02	+24·0
Brain	1·13 log X−0·69	+0·406	0·2	+ 0·53
Gastrointestinal tract:				
Cardiac stomach	−(2·32+9·98 log X)	−0·737	0·005	−13·09
Pyloric stomach	−(1·26+5·21 log X)	−0·795	<0·001	− 6·88
Small bowel	3·35+6·31 log X	+0·418	0·2	+10·16
Caecum	1·64 log X−0·29	+0·311	0·3	+ 1·48
Colon	−(2·27+6·04 log X)	−0·668	0·01	− 8·79
Heart	−(1·66+4·05 log X)	−0·790	0·001	− 6·03
Kidneys	−(1·16+0·15 log X)	−0·022	1·0	− 1·32
Liver	15·15 log X−0·23	+0·865	<0·001	+16·12
Lungs	−(1·87+ 4·48 log X)	−0·859	<0·001	− 6·70
Muscle (vent. abd. wall)	−(2·61+ 2·00 log X)	−0·286	0·3	− 4·77
Salivary glands (submax.)	5·99+15·01 log X	+0·706	0·005	+22·19
Skin	−(2·17+ 2·05 log X)	−0·167	0·6	− 4·38
Spleen	0·13 log X−4·44	+0·021	1·0	− 4·30
Testes	−(1·80+2·35 log X)	−0·646	0·01	− 4·34
Thymus gland	1·45 log X− 3·24	+0·271	0·4	− 1·68
Thyroid gland	12·46 log X−28·45	+0·228	0·5	−15·01
Residual carcass	−(0·66+0·64 log X)	−0·136	0·7	− 1·35

At autopsy were recorded gross pathology and the fresh weight and water content of the organs listed in Tables 1 and 2. Weight of organs was measured to 0.1 mg. The sample of skeletal muscle was the left half of the ventral abdominal wall muscle layer. The contents of the gastro-intestinal tract were removed by a standardized technique of washing and milking before weighing. Water levels were measured by drying a weighed aliquot of each organ or tissue to constant weight at 95° C in a Fisher forced draught constant temperature oven. The aliquot of skin for water analysis was taken from the dorsolumbar region. After removing the various organs listed in Tables 1 and 2, the residual carcass was weighed, cut into small pieces, homogenized in a Waring blender, and an aliquot weighed for water analysis. Water levels were calculated as g/100 g dry weight of tissue. Statistical methods were those of Croxton (1953).

RESULTS

Changes in body weight are illustrated in Fig. 1. Loss of body weight was correlated with log dose and the correlation coefficient (−0.802) was significantly different from

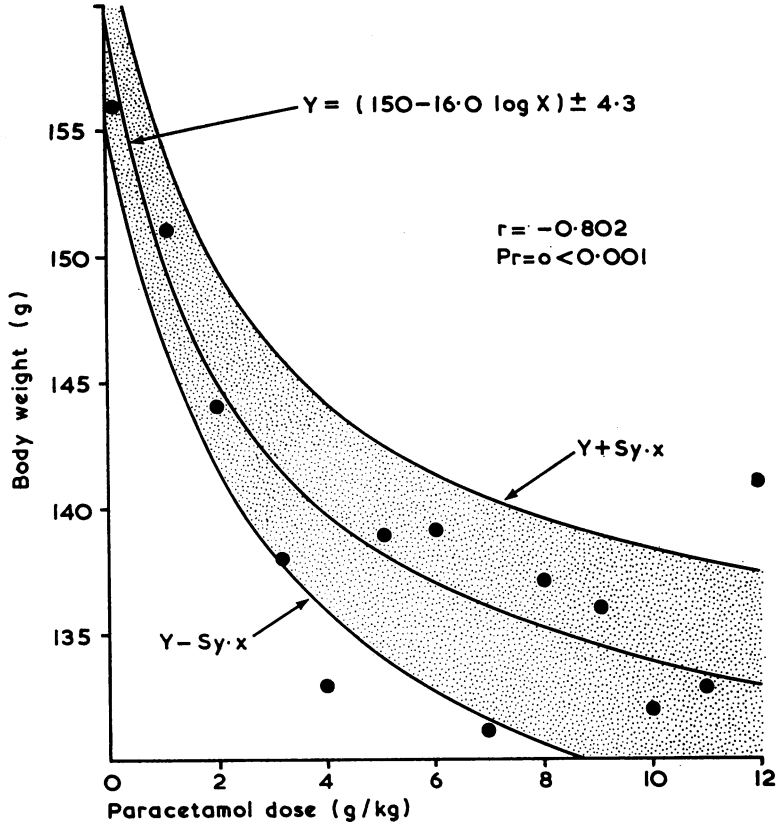

Fig. 1. The regression on dose of paracetamol of mean body weight measured 24 hr after giving the drug.

zero correlation at $P<0.001$. The bulk of the loss occurred when the dose of paracetamol had reached about 4 g/kg body weight or the LD50 of paracetamol (Boyd & Bereczky, 1966).

Differences in the weight of body organs were expressed as a percentage of the weight of the respective organ in the controls given distilled water only. The percentage differences were plotted against dose of paracetamol in the same manner as shown in Fig. 1. In all instances where significant correlation was found, the estimating equation, $Y=a+b \log X$, fitted the regression best. The regression lines are illustrated in Figs. 2 and 3 and the estimating equations, correlation coefficients, and probabilities (P) that the correlation coefficients equalled zero are listed in Table 1.

The estimated mean loss of total body weight at 12 g/kg of paracetamol was 14.9% of the body weight of the controls. Corresponding figures were greater for skin, muscle, liver (Fig. 2), small bowel, pyloric stomach, thyroid gland, caecum, spleen, and thymus gland (Fig. 3) and lesser for salivary glands, brain, heart, kidneys, lungs, residual carcass (Fig. 2), adrenals, cardiac stomach, testes, and colon (Fig. 3).

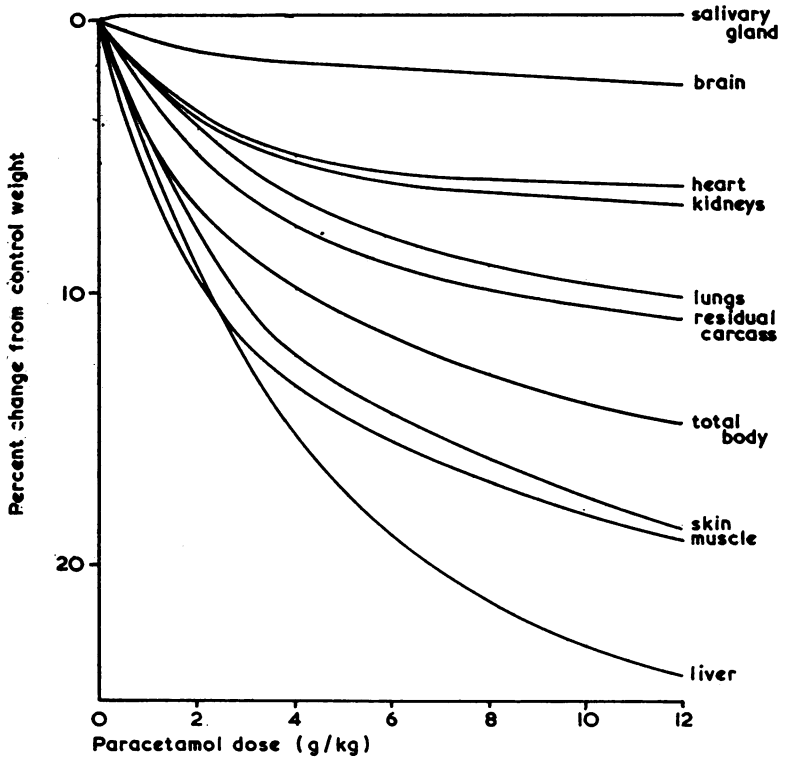

Fig. 2. The regression on dose of paracetamol of mean differences from controls in the weight of body organs, expressed as a percentage change from the mean weight in the controls given no paracetamol. "Residual carcass" was calculated after removing the indivdual organs listed in Table 1, and "total body" refers to total body weight. See Table 1 for estimating equations, correlation coefficients, and the statistical probability (*P*) of the regressions being significantly different from zero correlation.

Of the twenty correlation coefficients in Table 1, 12 were significantly different from zero correlation and eight were not significantly different. Administration of paracetamol produced no significant change in the weight of salivary glands, brain (Fig. 2), adrenal glands, cardiac stomach, and testes (Fig. 3). In heart, kidneys (Fig. 2), and thymus gland (Fig. 3), paracetamol produced a loss of weight which was not dose-dependent over the range of doses, number of doses, and number of animals per dose employed. In the remaining organs there was a dose-dependent loss of weight.

Changes in organ water content were analysed in a similar manner and the results are summarized in Table 2. Presentation of data in Table 2 is similar to that in Table 1 except that the value of Y at $X=12$ is added to clarify the regression.

It will be noted that there was significant positive correlation between water content and dose of paracetamol in the instances of adrenal glands, liver and salivary glands, and significant negative correlation for cardiac stomach, pyloric stomach, colon, heart, lungs and testes. In the remaining organs there was no significant correlation with log dose over the dosage range studied.

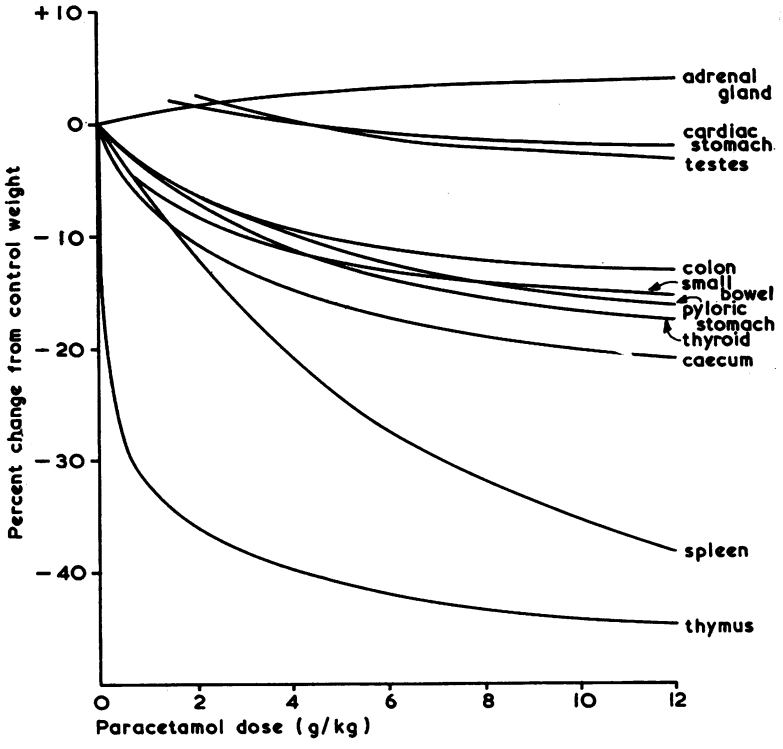

Fig. 3. The regression on dose of paracetamol of mean differences from controls in the weight of body organs expressed as a percentage change from the mean weight in the controls given no paracetamol. See Table 1 for estimating equations, correlation coefficients, and the statistical probability (*P*) of the regressions being significantly different from zero correlation.

Three additional dose-response observations were made. Residual paracetamol was noted at 24 hr in the stomach of 10% of rats at a dose of 3 g/kg and this incidence gradually rose to 100% at doses of 7 g/kg and over. Ulcers appeared in pyloric stomach at a dose of 6 g/kg and over and at 12 g/kg were haemorrhagic. Areas of necrosis of the liver appeared as light-coloured spots in 10% of rats at doses up to 4 g/kg and increased to 40 to 60% following larger doses. The kidneys were occasionally pale and spotted but this was not significantly related to dose of drug.

DISCUSSION

Boyd & Bereczky (1966) determined the weight and water content of body organs in ten rats at 24 hr after giving an LD50 of paracetamol and in 20 controls given no paracetamol. Their data are summarized in Table 3 and compared with the results of the present dose-response study.

In those organs in which Boyd & Bereczky (1966) found a significant decrease in weight, a significant negative correlation with dose was recorded in all except heart, kidneys, and thymus gland. Failure, in this study, to demonstrate significant dose-

TABLE 3

A COMPARISON OF CHANGES FOUND BY BOYD & BERECZKY (1966), FROM ADMINISTRATION OF A MEDIAN LETHAL DOSE OF PARACETAMOL, WITH DOSE-RESPONSE CORRELATION AT 24 HR

Lack of statistical significance at the 5% level in "t" tests of mean differences in the LD50 studies of Boyd & Bereczky (1966) and in the correlation coefficients found herein is indicated by the word " none." Statistical significance at $P=0{\cdot}05$ or less is indicated by " decrease " or " increase " for the studies of Boyd & Bereczky (1966) and by " negative " or " positive " for the dose-response correlations

	Weight			Water content		
Organ	At LD50	Dose correlation	Agreement	At LD50	Dose correlation	Agreement
Adrenal glands	None	None	Yes	None	Positive	No
Brain	None	None	Yes	None	None	Yes
Gastrointestinal tract:						
Cardiac stomach	None	None	Yes	Decrease	Negative	Yes
Pyloric stomach	Decrease	Negative	Yes	Decrease	Negative	Yes
Small bowel	Decrease	Negative	Yes	Increase	None	No
Caecum	Decrease	Negative	Yes	None	None	Yes
Colon	Decrease	Negative	Yes	None	Negative	No
Heart	Decrease	None	No	Decrease	Negative	Yes
Kidneys	Decrease	None	No	None	None	Yes
Liver	Decrease	Negative	Yes	Increase	Positive	Yes
Lungs	None	Negative	No	Decrease	Negative	Yes
Muscle (vent. abd. wall)	Decrease	Negative	Yes	None	None	Yes
Salivary glands (submax.)	None	None	Yes	Increase	Positive	Yes
Skin	Decrease	Negative	Yes	None	None	Yes
Spleen	Decrease	Negative	Yes	Decrease	None	No
Testes	None	None	Yes	Decrease	Negative	Yes
Thymus gland	Decrease	None	No	None	None	Yes
Thyroid gland	Decrease	Negative	Yes	Decrease	None	No
Residual carcass	Decrease	Negative	Yes	None	None	Yes
Total body weight	Decrease	Negative	Yes			

response correlation in these tissues appeared to be due to exclusion of doses below 1 g/kg. This is suggested by the estimating equations listed in Table 1 and particularly by the regression line for the thymus gland shown in Fig. 3. Boyd & Bereczky (1966) found, in addition, no significant decrease in the weight of lungs, while a significant negative regression was recorded herein (see Tables 1 and 3). Significance of the regression for change in weight of lungs on log dose was, however, only at the 5% level, which indicates that it could have become insignificant at a higher value for N (number of doses). Or the result could indicate that a lung weight-reducing factor does not appear until huge doses of paracetamol are given.

With these qualifications and limitations, it may be concluded that a significant change in organ weight found by "t" testing at the LD50 dose is associated with a significant correlation with dose of paracetamol. Should this relationship hold for all drugs, there would be little advantage in adding a dose-response study as the extra work involved is considerable. For any one drug, however, one cannot conclude that such a relationship exists unless the dose-response study is made. For example, while the interval to death is usually inversely related to the killing dose of a drug, it is not so related in the case of death due to bowel obstruction from intragastric barium sulphate (Boyd & Abel, 1966).

Significant changes in organ water contents reported by Boyd & Bereczky (1966) were found associated with dose-response relationships except in the instances of small bowel,

spleen and thyroid gland (Table 3). From the estimating equations listed in Table 2, it is apparent that the changes recorded by Boyd & Bereczky (1966) occurred from the lower range of doses of paracetamol used herein with little or no further change as dose was increased. Had the dose range been extended below 1 g/kg and had larger doses been excluded, significant dose-response correlations probably would have been found.

Boyd & Bereczky (1966) found no significant change in the water content of adrenal glands and colon (Table 3) whereas significant positive and negative dose-response correlations were found (Table 2). These changes appear to have been missed because the larger doses of paracetamol were excluded in the studies of Boyd & Bereczky (1966). These two changes were the only ones revealed by the dose-response study which added significantly to the data of Boyd & Bereczky (1966)—apart from the demonstration that significant mean changes were dose-dependent. These two changes represent some 5% of the total observations.

The general conclusion from these studies on paracetamol is that a dose-response study of organ weights and water contents yields little new information except that changes recorded at the range of the LD50 are dose-dependent. Unless there are specific reasons for a dose-response study, essentially the same information can be obtained, probably for most drugs, by " t " testing of mean differences in organ weights and water contents at or near death in ten to 20 animals given doses of the drug in the range of the LD50 and in controls given no drug. The latter is a much simpler procedure.

SUMMARY

1. The objectives of this project were to determine (*a*) if the changes in fresh weight and water content of body organs previously found at 24 hr after giving paracetamol in the range of the oral LD50 were dose-dependent, and (*b*) if significant further information could be obtained by such a dose-response study.

2. The weight and water contents of eighteen organs and residual carcass of albino rats were accordingly measured at 24 hr after giving paracetamol by intragastric cannula in doses ranging from 1 to 12 g/kg body weight. Differences from controls given no paracetamol were plotted against dose of drug.

3. Eleven out of fourteen significant changes in organ weights and seven out of ten significant changes in organ water contents were found dose-dependent. It appeared that failure to demonstrate dose-response relationships in six measurements was due to the limits of dose selected.

4. A dose-response relationship was revealed in the weight of one organ (lungs) and in the water content of two organs (adrenal glands and colon) and this had not been disclosed by the previous " t " tests of mean differences at the LD50 dose.

5. The results suggest that most differences found significant by " t " testing at the LD50 dose range will probably be dose-dependent and that little additional information is likely to be derived from the considerable additional work of doing a dose-response series.

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