TOLERATED DOSES OF PHENACETIN IN RELATION TO BODY WEIGHT AND ORGAN WEIGHTS

ELDON M. BOYD AND C. J. KRIJNEN

Department of Pharmacology, Queen's University, Kingston, Ontario, Canada

Received for publication February 5, 1969

In a study of the clinicopathologic syndrome of toxicity to phenacetin at the range of the acute, oral LD_{50} , Boyd in 1959 (1) used adult female rats and reported the $LD_{50} \pm SE$ to be 1.65 ± 0.35 g/kg body weight. In 1968, Boyd and Hottenroth (2) studied the toxicity of phenacetin at the range of the oral LD_{50} (100 days) and reported the acute oral $LD_{50} \pm SE$ in young male albino rats to be 4.14 ± 0.71 g/kg. Boyd and Hottenroth (2) also reported that female rats were more sensitive to the lethal effects of daily administration of phenacetin than were male rats. In a comparison of the acute oral LD_{50} in young male and female rats, sex of the animals was found to have no influence on the median lethal dose. To find a reason for these apparently contradictory results, it was decided to measure the clinicopathologic syndrome of toxicity to single oral doses of phenacetin, after methods reviewed by Boyd (3), in male and female albino rats from the age of weaning to that of middle-aged, 1 to 1.5 year old, adults. Over this interval of time, certain organs comprise changing percentages of body weight. Since values for the LD_{50} are normally expressed as g per kg body weight, it was decided to calculate LD_{50} values in addition as g/kg organ weights. Any organ in which variation in the LD_{50} so calculated was less than variation calculated as g/kg body weight, could be concluded to be more concerned with tolerance to lethal doses of phenacetin than is total body weight.

METHODS

The experiments were performed upon albino rats obtained from Canadian Breeding Laboratories of St. Constant, Quebec, Canada. On the day before administration of phenacetin, there were 50 to 55 animals in each of the following groups each expressed as mean $\pm$ standard deviation grams body weight: (a) males; 66 ± 9 , 120 ± 8 , 188 ± 18 , 300 ± 10 , 420 ± 32 and 549 ± 30 and (b) females; 67 ± 5 , 117 ± 9 , 207 ± 8 , 235 ± 19 , 246 ± 9 , 416 ± 27 and a group of pregnant females weighting 322 ± 72 g. The animals were placed in metabolism cages, one rat per cage, and given water but no food for 16 hours (overnight). Next morning each group was divided into six dosage subgroups, each subgroup given a dose of phenacetin estimated from pilot studies to produce a mortality rate just above 0 percent to just below 100 percent.

Phenacetin, U.S.P. was freshly prepared as a suspension in distilled water, with the aid of 0.2% gum tragacanth as a suspending agent, and given through an intragastric cannula in a constant volume of 20 ml/kg body weight. The animal was then returned

to its metabolism cage and given food (Rockland Mouse/Rat Diet Complete) and water *ad libitum*. Clinical signs of intoxication were recorded by cageside observation at intervals of 24 hours for 5 days. Gross pathology was noted on all animals which died and histopathology was assessed upon the organs listed in Table 2 in representative animals of each group.

The wet weight and water content of the organs listed in Table 4 were measured in 15 other animals of each body weight group at 48 hours after giving 20 ml/kg of distilled water containing no phenacetin. The details of method and statistical analysis have been reviewed by Boyd (3).

RESULTS AND DISCUSSION

Values for the LD_{50} calculated as g/kg body weight have been plotted against body weight in Fig. 1. It is apparent that the LD_{50} declines in both male and female rats as adult weight is reached.

Values for estimates of the intensity of clinical signs of intoxication are summarized

FIG. 1. The acute oral LD_{50} of phenacetin in relation to body weight of male and female albino rats.

TABLE I. An analysis of clinical signs during intoxication to phenacetin at the range of the oral LD_{50} .

Clinical sign	Mean $\pm$ S.D. (clinical units)	Sign in males more or less marked than in females at $P < 0.02$	Intensity of sign increased or decreased with increase in body weight at $P < 0.02$
Listlessness	2.5 ± 0.6	No	Increased
Decreased response to stimuli	2.5 ± 0.6	No	No
Dacryorrhea	1.0 ± 0.1	No	No
Ataxia	0.9 ± 0.3	No	No
Exophthalmos	0.9 ± 0.4	No	No
Restlessness	0.8 ± 0.3	No	No
Soiling	0.7 ± 0.1	Less	Increased
Diarrhea	0.5 ± 0.1	No	No
Piloerection	0.5 ± 0.2	More	Decreased
Abdominal distension	0.4 ± 0.2	Less	Decreased
Cyanosis	0.3 ± 0.1	More	Decreased
Epistaxis	0.3 ± 0.1	More	Decreased
Pallor	0.3 ± 0.2	No	No
Convulsions	0.1 ± 0.1	No	No
Hours to death	32 ± 11	No	No

TABLE 2. A summary of histopathological findings at death due to oral administration of phenacetin to male and female albino rats of increasing body weight.

Organ	Histopathology	Effect of Sex	Effect of Age
Adrenal glands	Cortical sinusoids congested and lipoid granules prominent; occasional cortical necrosis.	No	No
Brain	Mild capillary congestion of the brain and meninges.	No	No
Gastrointestinal tract			
Cardiac stomach	Infiltrative ulcers in older rats.	No	Yes
Pyloric stomach	Irritant inflammation; necrotic ulcers in older rats.	No	Yes
Small bowel	Capillary-venous congestion of the lamina propria and occasionally sub-mucosa.	No	No
Cecum	Capillary-venous congestion of the lamina propria and occasionally sub-mucosa.	No	No
Colon	Mild congestion of the lamina propria.	No	No
Heart	Areas of capillary congestion in older rats.	No	Yes
Kidneys	Congestion of the loop region; cloudy swelling, fatty degeneration and necrosis of the convoluted tubules especially in older rats.	No	Yes
Liver	Sinusoidal congestion in younger rats; cloudy swelling, fatty degeneration and necrosis of the hepatic cells in older rats.	No	Yes
Lungs	Areas of edema and congestion.	No	No
Muscle (ventral abdominal wall)	Normal appearance	No	No
Ovaries	Occasionally congestion.	—	No
Pancreas	Normal appearance.		
Salivary (submaxillary) glands	Normal appearance.	No	No
Skin	Ischemic in older rats.	No	Yes
Spleen	Contraction of red pulp; phagocytized debris.	No	No
Testes	Mild interstitial edema in older rats.	—	Yes
Thymus gland	Congestion and loss of thymocytes.	No	No
Uterus	Normal appearance.	—	No

FIG. 2. A photomicrograph of the pyloric stomach of an adult female rat which died at 50 hours after oral administration of phenacetin in a dose of 3.0 g/kg showing a hemorrhagic, infiltrative ulcer.

FIG. 3. A photomicrograph of the liver of an adult female rat which died at 52 hours after oral administration of phenacetin in a dose of 2.5 g/kg showing diffuse fatty degeneration and pyknosis of some nuclei.

TABLE 3. The LD₅₀ calculated as g/kg organ weight.

Organ for calculation	LD ₅₀ in males higher or lower than in females	Change with increase in body weight		Mean LD ₅₀	Standard deviation	Pearson's coefficient of variation
		Males	Females			
Adrenal glands	Higher	None	None	12,600	3,960	31.4
Brain	No	Increase	Increase	333	120	36.0
Gastrointestinal tissues						
Cardiac stomach	No	None	None	2,290	425	18.6
Pyloric stomach	No	None	None	782	195	24.9
Small bowel	No	None	None	190	60	31.5
Cecum	No	Increase	Increase	698	196	28.1
Colon	No	Increase	Increase	497	136	27.3
Gastrointestinal contents						
Stomach	Lower	Increase	Increase	203	64	31.5
Small bowel	No	Increase	Increase	91	18	19.8
Cecum	No	Increase	Increase	146	28	19.2
Colon	No	None	None	208	49	23.6
Total luminal contents	No	None	None	33	6	18.2
Heart	No	None	None	906	142	15.7
Kidneys	No	None	None	346	55	15.9
Liver	No	None	None	54	10	18.5
Lungs	No	None	None	545	92	16.9
Muscle*	No	Decrease	Decrease	247	90	36.5
Ovaries	—	—	None	5,770	960	16.6
Salivary (submaxillary) glands	No	Increase	Increase	1,820	370	20.3
Skin	No	Decrease	Decrease	15.3	4.9	32.0
Spleen	Lower	None	None	1,010	171	16.9
Testes	—	None	—	313	68	21.7
Thymus gland	Higher	Increase	Increase	3,890	2,450	63.0
Residual carcass	No	None	None	5.6	2.0	35.7
Total body weight	Higher at maturity	Decrease	Decrease	2.7	0.7	25.9
Live body weight**	Higher at maturity	Decrease	Decrease	3.0	0.7	23.3

* Right half of the skeletal muscular layer of the ventral abdominal wall.

** Total body weight minus the weight of contents of the lumen of the gastrointestinal tract.

in Table 1. All of the signs appeared in male as well as in female animals and in old as well as in young animals. There were some significant ($P < 0.02$) variations in intensity as noted in Table 1.

The histopathological findings are summarized in Table 2 and exemplified in Figs. 2 and 3. There were no differences due to sex of the animal. Gastric ulcers, myocardial congestion, renal tubular necrosis, hepatic necrosis, ischemia of the skin and edema of testicular interstitial tissue were more marked in older animals.

The LD₅₀ expressed in g/kg total body weight was divided by the number of grams of an organ per kg body weight and multiplied by 1,000 to obtained a value for the LD₅₀ expressed as g/kg organ weight. When so calculated, three types of relationship to body

FIG. 4. The regression, on body weight, of values for the LD_{50} of phenacetin calculated as g/kg brain weight, kidney weight and weight of ventral abdominal wall (right half) muscle. The regressions for brain are shown a calculated line for Y (brain) and for muscle as Y (muscle). The regressions for kidney is shown as actual values (solid circles), the calculated line for Y (kidney) and the calculated area (shaded) of Y(kidney) plus and minus its standard error ($Sy.x$).

weight were encountered as exemplified in Fig. 4, e.g. positive correlation in brain, negative correlation in skeletal muscle and no correlation in kidney. Remaining values for the LD_{50} per kg organ weight are summarized in Table 3 as the mean $\pm$ S.D. in all groups and the relation to sex and body weight. The results in males were the same as those in females for most organs. The LD_{50} per kg of most organs was either not related to body weight or increased with increase in body weight with the notable exception of skeletal muscle and skin which decreased with increase in body weight.

This is explained in part by data on organ weight calculated as g/kg body weight and summarized in Table 4. It is obvious from Table 4 that as body weight increased most organs comprised a smaller part of body weight with the exception of skeletal muscle and skin (and residual carcass which contained a large amount of skeletal muscle).

Data on water content of body organs are summarized in Table 5. It appears from these data that if the LD_{50} had been calculated as g/kg dry weight of tissue the changes with age might have been more marked. Values for Pearson's coefficient of variation, which is the standard deviation expressed as a percentage of the mean, were lower for organ water content than for organ wet weight. Relationships due to calculation of LD_{50} values per kg organ dry weight would probably not be much different, therefore, than calculated per kg wet weight as in Table 3.

Values for Pearson's coefficient of variation of values for the LD_{50} per kg organ weight are assembled in the right hand column of Table 3. The coefficient for values of the LD_{50}

TABLE 4. Organ weight calculated as g/kg body weight.

Organ	Index in males more or less than in females	Change with increase in body weight*	Mean	Standard deviation	Pearson's coefficient of variation
Adrenal glands	Less	Decrease	0.234	0.078	33.3
Brain	No	Decrease	10.0	5.9	59.0
Gastrointestinal tissues					
Cardiac stomach	More	Decrease	1.22	0.32	26.2
Pyloric stomach	No	Decrease	3.64	1.00	27.5
Small bowel	More	Decrease	16.4	7.3	44.4
Cecum	More	Decrease	4.12	1.20	29.2
Colon	No	Decrease	5.28	2.04	38.5
Gastrointestinal contents					
Stomach	More	Decrease	15.0	6.7	44.7
Small bowel	More	Decrease	30.6	9.8	32.0
Cecum	More	Decrease	19.2	6.2	32.3
Colon	No	Decrease	13.3	3.1	23.3
Total luminal contents	No	Decrease	78.1	25.2	32.3
Heart	No	Decrease	3.05	0.52	17.0
Kidneys	More	Decrease	8.18	2.04	24.9
Liver	More	Decrease	47.0	9.1	19.3
Lungs	Less	Decrease	5.08	1.20	23.6
Muscle**	No	Increase	11.7	2.3	19.7
Ovaries	—	Increase	0.45	0.06	13.3
		→decrease			
Salivary (submaxillary) glands	No	Decrease	1.57	0.40	25.5
Skin	No	Increase	187	29	15.5
Spleen	More	Decrease	2.81	1.00	35.6
Testes	—	Increase	10.0	3.0	30.0
		→decrease			
Thymus gland	No	Decrease	1.12	0.58	51.8
Residual carcass	Less	Increase	500	39	7.8
(Total body weight-g)	—	—	(250)	(127)	(50.8)

* Results in males were similar to those in females.

** Right half of the skeletal muscular layer of the ventral abdominal wall.

calculated per kg total body weight was 25.9. The coefficient for certain organs such as adrenal glands, brain, small bowel, stomach contents, skeletal muscle, skin, thymus gland and residual carcass, was appreciably higher than 25.9 which suggested that the toxic action of phenacetin bore little relationship to the weight of these organs. The coefficient for other structures and organs such as cardiac stomach, the contents of the small bowel and cecum, heart, kidneys, liver, lungs, ovaries and spleen was appreciably less than 25.9 and the LD₅₀ per kg organ weight had little or no relation to body weight suggesting that the lethal action of phenacetin bore more relationship to the weight of these than to total body weight.

A relationship to liver weight had been anticipated since the lethal dose of many drugs is related more to weight of the liver than to body weight (1). A relationship to renal

TABLE 5. Organ water contents calculated as g water per 100 g dry weight of tissue.

Organ	Level in males higher or lower than in females	Change with increase in body weight*	Mean	Standard deviation	Pearson's coefficient of variation
Adrenal glands	Higher	Decrease	208	22	10.6
Brain	No	Decrease	359	21	5.9
Gastrointestinal tissues					
Cardiac stomach	Higher	Decrease	297	29	9.8
Pyloric stomach	Higher	Decrease	316	36	11.4
Small bowel	Higher	Decrease	371	20	5.4
Cecum	Higher	Decrease	363	79	21.7
Colon	Higher	Decrease	368	33	9.0
Heart	No	Decrease	340	10	2.9
Kidneys	No	Decrease	341	14	4.1
Liver	No	Decrease	252	9	3.6
Lungs	No	Decrease	379	10	2.6
Muscle**	Higher	Decrease	292	43	14.7
Ovaries	—	Decrease	241	20	8.3
Salivary (submaxillary) glands	No	Decrease	294	13	4.4
Skin	Higher	Decrease	185	37	20.0
Spleen	No	Decrease	331	17	5.1
Testes	—	None	643	16	2.5
Thymus gland	Higher	Decrease	310	92	29.7
Residual carcass	Higher	Decrease	221	29	13.1

* Changes in males were similar to those in females.

** Right half of the skeletal muscular layer of the ventral abdominal wall.

weight might be expected in view of the renal elimination of phenacetin and its metabolites. Since phenacetin produces death by inhibition of respiration, a relationship to weight of the heart and lungs is reasonable. The relationship to ovarian weight may reflect apparent differences due to sex of animals in the toxicity of phenacetin although if so one might have expected a similar relation to testicular weight. The relationship to weight of spleen may be connected with the stress reaction produced by lethal doses of phenacetin but again if so one might expect a relation to weight of the adrenal and thymus glands which was not found. The relationship to weight of cardiac stomach and intestinal contents was not anticipated. It may be connected with evidence reviewed by Boyd (3) that the toxicity of many drugs is increased with increase in the volume of distilled water in which they are dissolved or suspended for intragastric administration. The greater the volume of water administered into the stomach with a drug under study, the greater would become the weight of the intestinal contents—at least until the water was absorbed—and, by evidence herein found, the greater would become the toxic action of the drug. The weight of intestinal contents may also affect the rate of absorption of phenacetin.

The results of this study are of significance in connection with various publications. For example, Kato *et al.* (4) reported that the effects of certain drugs in male Sprague-Dawley rats differed from those in females and that the difference disappeared when the liver was inhibited by SKF 525A. Kato *et al.* (5) found that the ability of the liver of

female rats to metabolize certain drugs was greatest at age 30 days and gradually declined to age 150 days. Kato and Takanaka (6, 7) explained that the decline in drug metabolism in old rats was due to a decrease in the activity of hepatic drug-detoxifying enzymes in these old animals.

SUMMARY

The acute oral LD₅₀ of phenacetin calculated as g/kg body weight was identical in male and female rats but decreased as adult weight was reached in both sexes. Most of the clinical and pathological signs of toxicity were identical at all ages in both sexes. The apparent increase in the toxicity of phenacetin in adult animals was due mainly to an increase in the weight of muscle and skin per kg body weight. The LD₅₀ calculated as g/kg organ weight remained fairly constant in certain organs and did not vary with increase in body weight, suggesting that the toxicity of phenacetin is related to the weight of these key organs. The key organs were liver, kidneys, heart, lungs, ovaries (but not testes), spleen (but not adrenal and thymus glands), cardiac stomach, and curiously, weight of intestinal contents.

Acknowledgment: This project was assisted financially by the Food and Drug Directorate, Department of National Health and Welfare, Ottawa, Canada, and by Merck Frosst Laboratories of Montreal, Canada.

REFERENCES

- 1) BOYD, E.M.: *Toxic. appl. Pharmac.* **1**, 240 (1959)
- 2) BOYD, E.M. AND HOTTENROTH, S.M.H.: *Toxic. appl. Pharmac.* **12**, 80 (1968)
- 3) BOYD, E.M.: *Canad. med. Assoc. J.* **98**, 278 (1968)
- 4) KATO, R., CHIESARA, E. AND FRONTINO, G.: *Biochem. Pharmac.* **11**, 221 (1962)
- 5) KATO, R., VASSANELLI, P., FRONTINO, G. AND CHIESARA, E.: *Biochem. Pharmac.* **13**, 1037 (1964)
- 6) KATO, R. AND TAKANAKA, A.: *Jap. J. Pharmac.* **18**, 381 (1968)
- 7) KATO, R. AND TAKANAKA, A.: *Jap. J. Pharmac.* **18**, 389 (1968)