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A Two-Year Study with Cimetidine in the Rat: Assessment for Chronic Toxicity and Carcinogenicity

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A Two-Year Study with Cimetidine in the Rat: Assessment for Chronic Toxicity and Carcinogenicity. LESLIE, G. B., NOAKES, D. N., POLLITT, F. D., ROE, F. J. C., AND WALKER T. F. (1981). *Toxicol. Appl. Pharmacol.* **61,** 119–137. Cimetidine [*N*″-cyano-*N*-methyl-*N*′-{2-[(5-methylimidazol-4-yl)methylthio]ethyl}guanidine] was administered daily for 2 years by gavage to Wistar rats at dose levels of 950, 378, and 150 mg/kg/day. Two groups, one receiving distilled water daily and the other not treated, served as controls. Premature deaths occurred when cimetidine was accidentally introduced into the lungs or reached the lungs by seepage from the esophagus via the larynx during intragastric administration but cimetidine treatment did not otherwise affect survival, body weight gain, clinical condition, and hematological, or urinalysis parameters. Raised transaminase levels occurred occasionally during the second year of the study in top dose males and there was a significant increase in mean liver weight in top dose females killed terminally compared with controls. Histopathological observations of the livers of these animals indicated only nonspecific changes. Mean prostate and seminal vesicle weights were significantly lower in all groups receiving cimetidine than in controls and there was dose-related atrophy of the seminiferous tubules and atrophy of the male secondary sex organs. There were no other apparent effects of treatment on nontumor pathology. Overall tumor incidence, after the exclusion of Leydig-cell tumors, was not affected by cimetidine treatment. A significantly higher incidence of benign Leydig-cell tumors in the combined cimetidine-treated groups compared with the combined control groups was confined to rats killed during Weeks 105 and 106 and was not dose related. No meaningful treatment-related effects on incidence were observed for any other kind of neoplasm.

Cimetidine is a specific, competitive histamine H_2-receptor antagonist as defined by Black *et al.* (1972) and is an effective inhibitor of gastric acid secretion in animals and in man. Cimetidine has been shown to have low acute toxicity and repeated dose studies of up to 12 months duration in rats and dogs have revealed no major adverse effects (Leslie and Walker, 1977). Weak antiandrogenic effects, resulting in reduction in weight of the secondary sex organs in males, were observed in both rats and dogs but a formal test in male rats showed that potency and fertility were not affected. Cimetidine was not teratogenic in rabbits or rats and did not affect the fertility of female rats.

The present paper presents the results of a 2-year toxicity study in the rat with particular attention to incidences of neoplastic lesions.

METHODS

Compound. Cimetidine (*N*″-cyano-*N*-methyl-*N*′-{2-[(5-methylimidazol-4-yl)methylthio]ethyl}guanidine]

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was obtained from our own laboratories at Welwyn Garden City and from Bridge Chemicals Ltd., Tonbridge. Five batches of cimetidine, all of clinical grade purity, were used sequentially during the study. A molar stock solution of cimetidine base was made up once weekly by gentle heating with 1 N hydrochloric acid to give a solution of pH 6–7. This was diluted daily with distilled water to give fresh dosing solutions.

Animals and environment. The rats were of an ICI Wistar-derived strain, bred in our laboratories from caesarian-derived, barrier-maintained stock, specified pathogen-free, and inoculated with a known gut flora. They were 5½ weeks old when dosing commenced. The rats were housed in groups of five in polypropylene cages with stainless-steel grid tops and solid bases with white woodchip bedding. Porton Rat Diet (Labsure Ltd., Poole, Dorset) and tap water were available *ad lib.* Room temperature was maintained at 20°C (±2°C) with a relative humidity of 42% (±8%). The room was lit by fluorescent tubes, with a 12-hr, light-dark cycle.

Diet. The manufacturer's specifications for Porton Rat Diet appear in Table 1.

Dosing procedure. Male (426) and female (427) rats of four different batches (A–D) were allocated randomly to five groups as follows:

Group		No. of rats M	F
1	Cimetidine 950 mg/kg/day by gavage	100	99
2	Cimetidine 378 mg/kg/day by gavage	70	70
3	Cimetidine 150 mg/kg/day by gavage	65	65
4	Distilled water by gavage	84	85
5	Nondosed controls	107	108

Further details of allocation of rats of different batches to groups are shown in Table 2. As indicated in a footnote to that table the assessment of chronic toxicity was based on observations made on Batches A and B only.

The doses of cimetidine were equivalent to 190, 75, and 30 times those required for 50% inhibition of basal gastric acid secretion in the rat, as previously estimated in SK & F Laboratories. On a body weight basis these doses are equivalent to 57, 22.6, and 9 times the recommended daily dose for a 60-kg human (1 g in U.K.), respectively. For administration of cimetidine to rats at those dose levels up to 950 mg/kg/day, gavage was considered preferable to administration in the diet because cimetidine has an unpleasant taste.

Each rat was weighed daily and dosed by gavage. For the first 15 weeks every rat received 10 ml/kg body wt and from the 16th week 5 ml/kg body wt of the appropriate dose preparation. For the first 15 weeks cimetidine solutions of 9.5, 3.78, and 1.5% (w/v) were used for Groups 1, 2, and 3, respectively. These concentrations were doubled from the 16th week onward. The method of dosing described enabled us to achieve an accuracy on each occasion of ±2% of the stated dose.

Observations during the study. All rats were inspected twice daily on weekdays for overt clinical signs and given a thorough clinical examination once weekly. On Saturdays, Sundays, and public holidays, one inspection was made. Moribund animals were killed on the authority of one person (T.F.W.) and these and any rats found dead were given a standard postmortem examination. Full histopathological examinations were carried out, with the following exceptions: (1) 7 animals which died in the first 4 weeks of the study, (2) 1 animal from Group 3 which died on Week 24 and (3) 34 animals from Groups 2 and 3 which comprised the 6-month kill in these groups (see below). In case 1 no tissues were taken. In cases 2 and 3 a full set of tissues was preserved but only sections of the ovaries or testes, prostate or uterus, seminal vesicles, and adrenals were examined. In all three cases, detailed macroscopic observation at necropsy failed to reveal the presence of any lesions which indicated a need for microscopic assessment.

In a few instances, where autolysis was advanced, it was not possible to obtain readable sections from all tissues.

Body weights were recorded twice weekly during the first 4 weeks and weekly thereafter. Food consumption was measured after 4, 12, 24, 36, 48, 70, 82, 92, and 100 weeks in approximately 30 animals of each sex of each group (15 from Batch A, 15 from Batch B).

Planned interim kills of rats in groups 1–4 were carried out after 6 and 12 months (24 and 53 weeks) of dosing. These animals had been specified before the test commenced. An additional interim kill of animals from all groups was carried out at 10 months (43 weeks) to provide pathological material from control groups for comparison with that of treated animals which had died prematurely because of dosing accidents. All rats killed at these times received standard post mortem and histopathological examinations. Details of the interim kills are shown in Table 3. The terminal kill in all groups was during Weeks 105 and 106.

Hematology and clinical chemistry. Blood samples were taken from the tails of 10 males and 10 females of each group (5 from Batch A, 5 from Batch B) at the following times after the start of the experiment: 7, 14, 27, 41, 53, 66, 83, and 103 weeks. The same rats were bled on each occasion except that substitutes from the same group and batch were used as replacements for rats which died. All blood samples were examined for hematological and clinical chemistry parameters (see below) except that clinical chemistry measurements were restricted to 5 males and 5 females from each group (i.e., Batch A animals only) in the case of the blood samples obtained at 7 weeks.

Hematological estimations comprised: hemoglobin (Hb) by Coulter hemoglobinometer (Coulter Electron-

TABLE 1

COMPOSITION OF PRD DIET

Manufacturer's specification			
Crude oil (%)	2.78	Choline chloride (mg)	200
Crude protein (%)	19.79	Folic acid (mg)	6
Crude fiber (%)	5.37	Nicotinic acid (mg)	20
Calcium (as Ca) (%)	0.72	Pantothenic acid (mg)	4
Phosphorus (as P) (%)	0.71	Amino acids added (%)	
Salt (as NaCl) (%)	1.03	Arginine	1.25
Metabolizable		Cystine	0.27
energy (kcal/kg)	2570	Glycine	0.97
Ash(%)*	4.8	Histidine	0.51
Moisture(%)*	10.3	Isoleucine	0.88
Trace elements		Leucine	1.58
added (ppm)		Lysine	1.07
Cobalt	0.4	Methionine	0.36
Copper	7	Phenylalanine	0.94
Iodine	1.3	Threonine	0.77
Iron (Fe)	30	Tryptophan	0.25
Magnesium	102	Tryosine	0.76
Manganese	25	Valine	1.04
Vitamins added to			
each kg of diet			
A (IU)	8000	Aflatoxin*	Not detected
B_1 (mg)	2		(<0.02 ppm)
B_2 (mg)	8	Nitrate (mg/kg)	3–15
B_{12} (μg)	12	Nitrite (mg/kg)	<0.1–1.2
D_3 (IU)	1000		
E (IU)	25		
K (mg)	10		

* By independent analysis.

ics Ltd., Hertfordshire, U.K.); total erythrocyte count (RBC), packed cell volume (PCV), mean corpuscular volume (MCV), and total leukocyte count (WBC) by Coulter Counter Model ZF; platelet count by Coulter Thrombocounter and differential white cell count.

Clinical biochemical estimation comprised: plasma

TABLE 2

ALLOCATION OF RATS FROM DIFFERENT BATCHES TO GROUPS

	Group 1 (950 mg/kg)		Group 2 (378 mg/kg)		Group 3 (150 mg/kg)		Group 4 (distilled water)		Group 5 (nondosed controls)	
Batch	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
A (September 1974)	45	44[a]	30	30	30	30	40	40	37	30
B (October 1974)	45	45	30	30	30	30	39[a]	40	20	28
C (February 1975)[b]	0	0	0	0	0	0	0	0	50	50
D (June 1975)[b]	10	10	10	10	5	5	5	5	0	0
Totals	100	99	70	70	65	65	84	85	107	108

[a] One rat in Group 1 lost at Week 63; one rat in Group 4 lost at Week 105.

[b] Batches C and D, which were added later to the study and ran the full 106 weeks, were examined for incidence of neoplasms but did not form part of the general toxicological assessment.

TABLE 3

SURVIVAL

	Group 1 (950 mg/kg) Batch				Group 2 (378 mg/kg) Batch				Group 3 (150 mg/kg) Batch				Group 4 (distilled water) Batch				Group 5 (nondosed controls) Batch			
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Male rats																				
No. animals allocated to group	45	45	0	10	30	30	0	10	30	30	0	5	40	39	0	5	37	20	50	0
No. killed at 6 months	0	10	—	0	0	9	—	0	0	8	—	0	0	10	—	0	0	0	0	—
No. killed at 10 months	1	0	—	0	1	0	—	0	2	0	—	0	5	0	—	0	8	0	0	—
No. killed at 12 months	8	0	—	0	8	0	—	0	7	0	—	0	9	0	—	0	0	0	0	—
No. dying during study																				
Weeks																				
0–26	2	6	—	2	1	4	—	4	1	3	—	1	0	1	—	0	0	0	0	—
27–54	7	3	—	2	4	3	—	2	3	6	—	0	0	1	—	0	0	0	2	—
55–78	2	6	—	1	4	4	—	2	1	1	—	0	2	0	—	0	3	1	4	—
79–104	8	7	—	1	4	5	—	1	5	2	—	1	8	13	—	1	9	9	13	—
0–104	19	22	—	6	13	16	—	9	10	12	—	2	10	15	—	1	12	10	19	—
No. killed terminally																				
Weeks 105–106	17	13	—	4	8	5	—	1	11	10	—	3	16	14	—	4	17	10	31	—

TABLE 3—*Continued*

	Group 1 (950 mg/kg) Batch				Group 2 (378 mg/kg) Batch				Group 3 (150 mg/kg) Batch				Group 4 (distilled water) Batch				Group 5 (nondosed controls) Batch			
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Female rats																				
No. animals allocated to group	44	45	0	10	30	30	0	10	30	30	0	5	40	40	0	5	30	28	50	0
No. killed at 6 months	0	9	—	0	0	8	—	0	0	9	—	0	0	10	—	0	0	0	0	—
No. killed at 10 months	1	0	—	0	1	0	—	0	3	0	—	0	5	0	—	0	8	0	0	—
No. killed at 12 months	7	0	—	0	8	0	—	0	9	0	—	0	9	0	—	0	0	0	0	—
No. dying during study																				
Weeks																				
0–26	3	4	—	1	2	4	—	2	0	1	—	0	0	1	—	1	0	0	0	—
27–54	6	7	—	3	4	8	—	2	3	2	—	0	1	0	—	0	1	1	0	—
55–78	3	0	—	0	2	1	—	2	1	2	—	0	1	3	—	1	1	1	3	—
79–104	11	7	—	3	4	3	—	1	2	5	—	2	12	9	—	0	4	5	13	—
0–104	23	18	—	7	12	16	—	7	6	10	—	2	14	13	—	2	6	7	16	—
No. killed terminally Weeks 105–106	13	18	—	3	9	6	—	3	12	11	—	3	12	17	—	3	16	21	34	—

glucose, plasma glutamate pyruvate transaminase, plasma glutamate oxaloacetic transaminase (only from week 27 onward), and plasma alkaline phosphatase, all by an Abbott ABA 100 Bichromatic Analyser (Abbott Diagnostics, Kent, U.K.).

At the 12-month interim kill and at the terminal kill (Week 105–106), blood samples were taken from both the tail and from the inferior vena cava of all rats, and the following additional clinical chemistry parameters were measured: blood urea nitrogen (BUN), bilirubin, and total protein by Abbott ABA 100 Bichromatic Analyser; plasma sodium and potassium by IL 343 Photometer (Instrumentation Laboratories Ltd., Warrington, U.K.); chloride by an EEL 920 chloride meter (V.A. Howe & Co. Ltd., London, U.K.); plasma albumin and plasma acid phosphatase (male rats only) by Technicon Instruments Co. Ltd. (Hants., U.K.); cholesterol by Eskalab Alpha (Smith Kline Instruments Co. Ltd., Hertfordshire, U.K.). Full hematological and clinical chemistry determinations also were made for all rats killed before Week 105 due to ill health.

Urinalysis. Four-hour samples of urine were collected before the test began and after 6, 13, 26, 39, 52, 65, 82, and 103 weeks of dosing from the same rats as were used for the hematological investigations. The following parameters were measured: volume; specific gravity by Goldberg refractometer (American Optical Co.); presence of glucose by Clinistix (Ames) and the level by Abbott ABA autoanalyser, if required, and protein level by Chemlab autoanalyser (Chemlab Instrument Co. Ltd., London, U.K.). The spun sediment was examined microscopically and urine cell counts were made.

Ophthalmoscopy. The eyes of 10 male and 10 female rats from each group were examined by direct ophthalmoscopy after mydriasis with 1% Tropicamide (BP) at the following times after the start of the study: Batch A at 15, 27, and 53 weeks; Batch B at 11, 23, and 49 weeks. At the end of the study the eyes of all surviving rats were examined.

Sacrifice and postmortem examination. Rats were killed by ether overdose and bled via the posterior vena cava. Each animal was subjected to a thorough examination postmortem. The heart, liver, gonads, kidneys, adrenals, seminal vesicles, prostate or uterus, and brain were weighed. Samples of these organs, and of the tongue, eye, salivary gland, cervical lymph node, thyroids, lungs, thymus, aorta, sternal bone and marrow, bladder, stomach, duodenum, small and large intestines, pancreas, spleen, mesenteric lymph node, pituitary, skin, mammary gland, nerve, voluntary muscle, and any tissue of grossly abnormal appearance were fixed in formal sublimate solution (1 part saturated mercuric chloride: 4 parts 10% formalin). Sections of these tissues stained with hematoxylin and eosin were examined microscopically.

Statistical methods. In the case of body weights, food consumption, hematology and clinical chemistry parameters, and organ weights, group mean values were compared when considered appropriate by pooled Student's *t* tests. Statistical analysis of tumor incidence data was carried out according to the methods recommended by Peto (1974) and Peto *et al.* (1980). Tumors were classified as either incidental (discovered at postmortem or histopathological examination of an animal which died or was killed for another reason) or nonincidental (causing death or contributing to the cause of death).

RESULTS

Clinical Observations (Batches A and B Only)

Excessive salivation was commonly observed immediately after dosing with cimetidine in high dose rats (Group 1) and, occasionally, in medium dose rats (Group 2). Perineal soiling was also commonly seen in Group 1 rats, but in no other group. No unusual clinical signs were associated with treatment with cimetidine.

Food Consumption (Batches A and B Only)

Food consumption was similar in all groups. Mean daily food consumption for males and females decreased from 29 g and 22 g, respectively, in the early weeks of the study to 22 g and 18 g, respectively, in the latter weeks.

Body Weights (All Batches)

Body weight gain was not affected by treatment with cimetidine (Figs. 1 and 2).

Survival (All Batches)

Many animals in the cimetidine-treated groups died or were killed because of dyspnoea, presumed to be due to reflux of the esophageally administered dosing solution into the trachea or, in a few instances, direct administration into the trachea. The mode

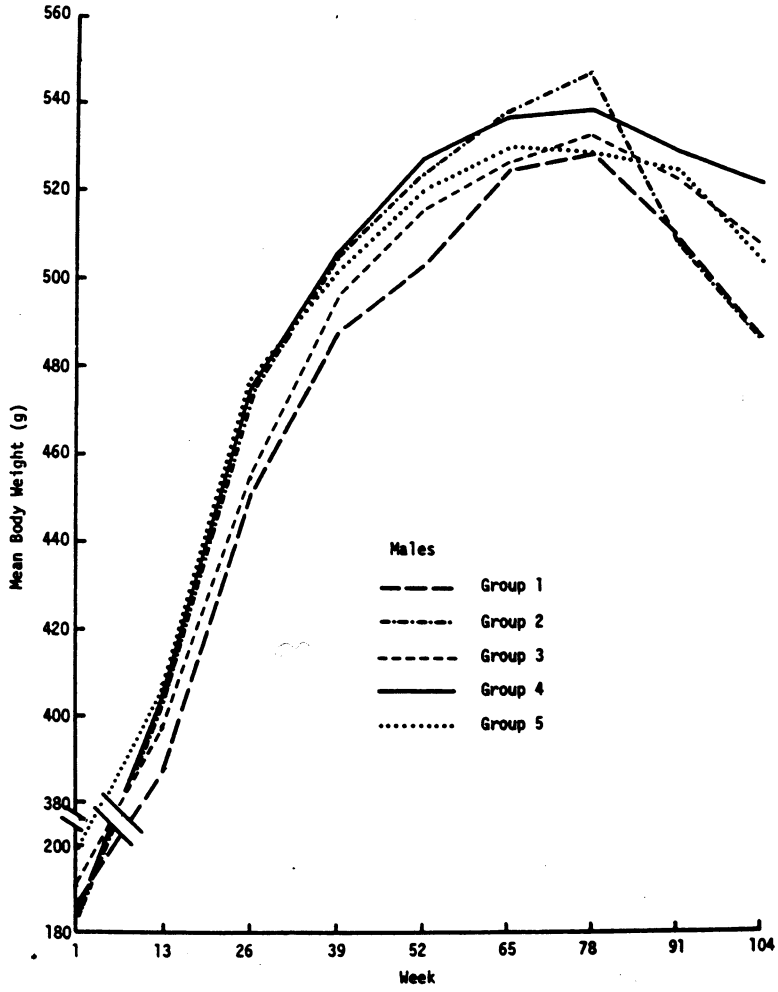

FIG. 1. Body weights of male rats. Each point represents the mean for survivors at that week.

of death (e.g., frothing at the mouth) and pathological changes in the lungs were consistent with this presumption.

As most deaths attributable to dosing accidents occurred at the higher dose levels: 64/199 in Group 1 (950 mg/kg), 47/140 in Group 2 (378 mg/kg), 18/130 in Group 3 (150 mg/kg), and only 2/169 in Group 4 (distilled water controls), a subsidiary experiment was carried out to investigate the toxicity of cimetidine solutions when deliberately administered by the intratracheal route.

Male rats weighing 460–600 g were lightly anesthetized with ether and dosed once by the intratracheal route. Ten rats received 1.0 ml/kg distilled water, 10 received 1.0 ml/kg of 19% (w/v) cimetidine solution, taken from the Group 1 dosing solution. The cimetidine dosage was 190 mg/kg. All 10 rats receiving cimetidine suffered severe, prolonged dypsnoea and five died within 1 hr of treatment. The rats receiving distilled water suffered only slight, brief dypsnoea, and none died. This confirmed that cimetidine solution administered intratracheally

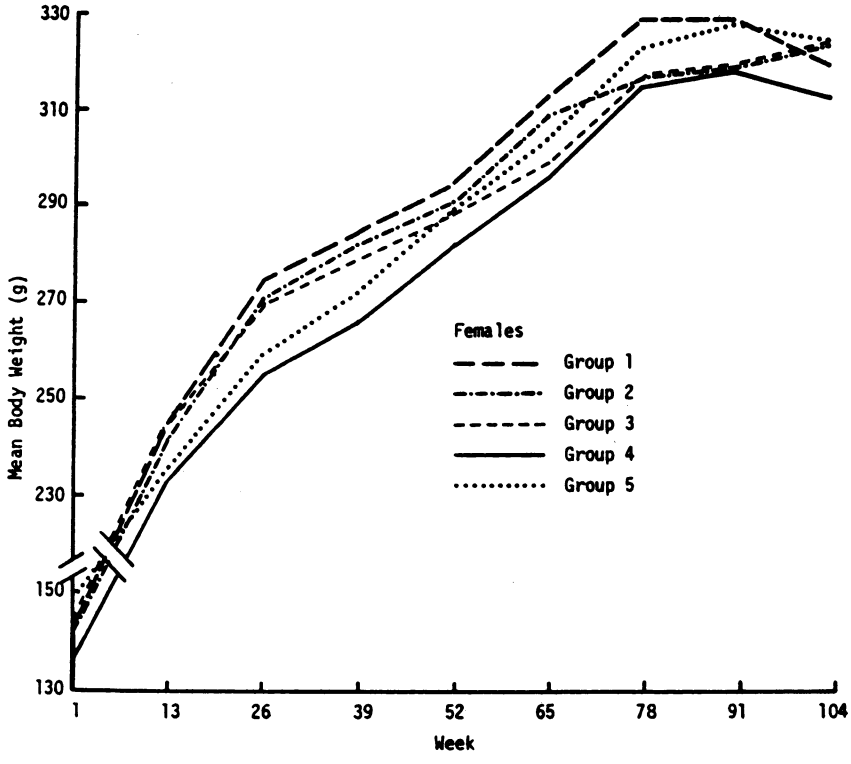

FIG. 2. Body weights of female rats. Each point represents the mean for survivors at that week.

was more acutely toxic than water administered by the same route or cimetidine administered intragastrically.

After correction for deaths attributable to dosing accidents, survival rates were not significantly different in treated and control groups (Figs. 3 and 4). Excluding dosing accidents, the death rate in all groups was relatively low until the last 6 months of the study, when it rose in all groups in a similar fashion. Within any group, the survival rates varied among batches (Table 3), but no consistent difference was seen in the survival rate of one batch compared with the other.

Hematology and Clinical Chemistry (Batches A and B)

Despite wide variations in hematological values, both within and between groups, comparison of mean group values at no time revealed any changes which could be attributed to treatment with cimetidine. Transaminase levels were higher in Group 1 males (950 mg/kg) than in control males during the second year of the study (Tables 4 and 5)[1] but these differences were only statistically significant at Weeks 66 and 103. No such changes were seen in transaminase levels among females. No other treatment-related changes were seen for any other clinical biochemical parameter.

[1] See NAPS document No. 03855 for 25 pages of supplementary material. Order from ASIS/NAPS c/o Microfiche Publications, P.O. Box 3513, Grand Central Station, New York, N.Y. 10017. Remit in advance in U.S. funds only for each NAPS Accession Number. Institutions may use purchase orders when ordering; however, there is a billing charge for this service. Make checks payable to Microfiche Publications. Photocopies are $5.00. Microfiche are $3.00. Outside of the U.S. and Canada, postage is $3.00 for a photocopy or $1.50 for a fiche.

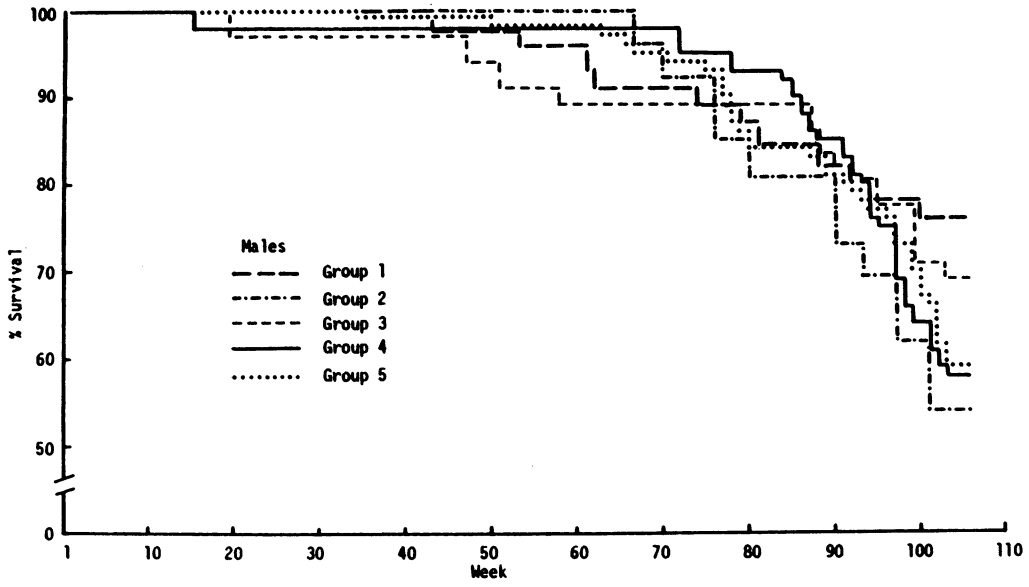

FIG. 3. Percentage survival of male rats up to Week 104. Rats whose deaths are attributed to dosing accidents have been excluded.

Urinalysis (Batches A and B)

No treatment-related effects were observed during the study.

Ophthalmoscopy (Batches A and B)

No abnormalities were seen which were related to treatment with cimetidine.

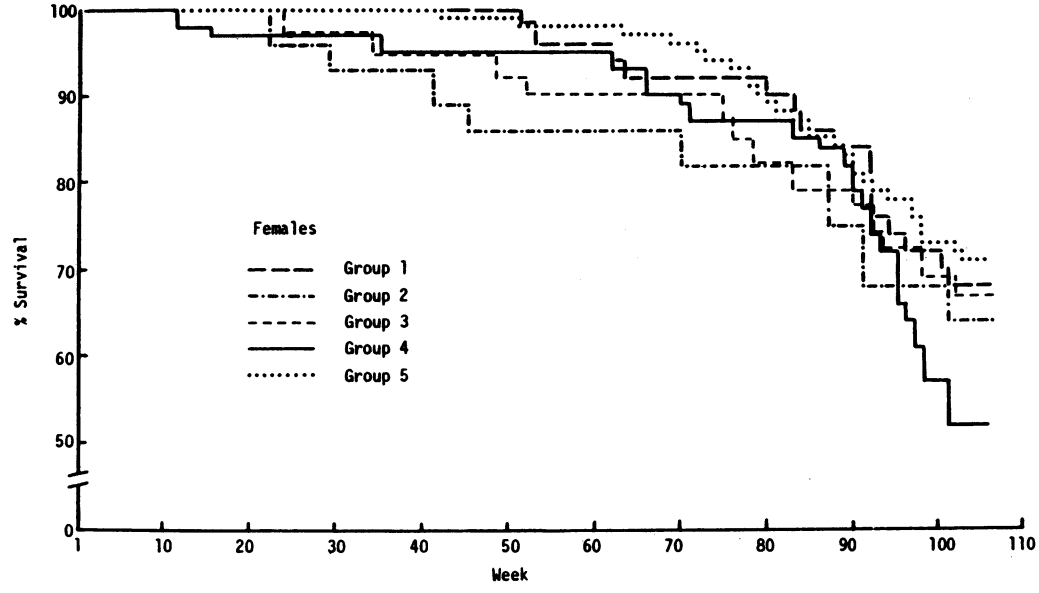

FIG. 4. Percentage survival of female rats up to Week 104. rats whose deaths are attributed to dosing accidents have been excluded.

Postmortem Observations (All Batches)

Postmortem evidence of lung dosing—patchy, hemorrhagic lungs, and the presence of fluid in the trachea—was commonly seen in rats from the two higher dose groups which died or were killed *in extremis*, especially during the first year of the study. Otherwise no gross abnormalities were seen that are not commonly encountered in aged rats and there was no excess of particular abnormalities in treated as compared with control groups.

Organ Weights (Batches A and B)

Among the animals killed at the end of the study, a significant increase was seen in the mean liver weight in Group 1 females (950 mg/kg) compared with either Group 4 (distilled water controls) or Group 5 females (nondosed controls) (Table 6). Among males, the mean liver weights in Group 1 were raised compared with those in the control groups (Table 7), but not significantly so.

During the second year of the study, seminal vesicles and prostates were noted to be smaller than usual in 21/55 male rats in Group 1, 5/30 in Group 2, 2/31 in Group 3, 2/54 in Group 4,and 0/49 in Group 5. Among rats killed at the end of the study, the mean group prostate and seminal vesicle weights were significantly lower in all three dose groups than those of the two control groups (Table 7). The decrease in weights was clearly dose related. Testis weights were significantly lower in the top dose group (Group 1) compared with the nondosed controls (Group 5), but not compared with the distilled water controls (Group 4) (Table 7). Apart from the findings listed above, no significant differences were seen in the weights of any other organs and no further postmortem changes were seen which were considered to be associated with cimetidine treatment.

Histopathological Findings

(A) *Nonneoplastic pathology (Batches A and B only).* The findings other than those for neoplasms are summarized in Table 8[1] for decedents and for animals killed at 6 and 12 months and in Table 9[1] for animals killed terminally.

In general the findings were unremarkable. In the glandular stomach, a few animals of both sexes in all groups exhibited focal distention of gastric glands, edema, or inflammation. The incidence was not related to dose. No treatment-related changes were observed in the forestomach, pancreas, or spleen.

In the liver, top dose males exhibited higher incidences of centrilobular vacuolation of hepatocytes, probably because of fatty degeneration, of centrilobular hepatocellular enlargement, minimal to moderate bile duct hyperplasia, and hepatocyte necrosis. A slightly higher incidence of centrilobular hepatocellular enlargement was seen in top dose females killed terminally. There was no treatment-related effect on these parameters nor on any other parameter for liver pathology in animals of either sex exposed to the middle or low dose levels of the drug.

As expected in rats aged over 2 years, animals in all four groups killed terminally exhibited a high incidence of involution of the thymus, manifest as cortical hypoplasia. There was no evidence that the treatment influenced the incidence or severity of this change or any other feature of thymic pathology. Similarly no treatment-related effects were observed in the mesenteric lymph node or bone marrow.

In the endocrine system, an approximately equally low incidence of hypertrophy of parathyroid gland tissue was seen in all groups. The pathological findings in the thyroid included the presence of branchial cleft remnants, cysts, and distended follicles. Only two rats in the whole study exhibited hyperplastic lesions (one Group 1 female de-

TABLE 6

ORGANS WEIGHTS—FEMALE SURVIVORS

	n	Body weight (g)	Heart	Liver	Brain	Ovaries	Kidneys	Adrenals	Uterus
Group 1 (950 mg/kg)	31	316 ± 25	1.03 ± 0.12	15.24 ± 2.45[a]	2.22 ± 0.12	0.15± 0.03	2.49 ± 0.35	0.10 ± 0.03	0.86 ± 0.29
Group 2 (378 mg/kg)	15	311 ± 47	0.98 ± 0.12	13.05 ± 1.70	2.24 ± 0.19	0.13 ± 0.05	2.38 ± 0.25	0.09 ± 0.03	1.04 ± 0.55
Group 3 (150 mg/kg)	23	313 ± 37	1.01 ± 0.11	13.11 ± 1.91	2.21 ± 0.09	0.14 ± 0.04	2.29 ± 0.31	0.08 ± 0.02	1.08 ± 0.47
Group 4 (distilled water)	29	308 ± 36	0.99 ± 0.11	11.90 ± 2.41	2.17 ± 0.11	0.14 ± 0.03	2.22 ± 0.23	0.10 ± 0.02	1.18 ± 0.97
Group 5 (non-dosed controls)	37	318 ± 29	0.97 ± 0.10	12.30 ± 1.82	2.17 ± 0.09	0.15 ± 0.03	2.20 ± 0.25	0.09 ± 0.02	0.93 ± 0.46

Note. The results are expressed in g, Mean ± SD.

[a] Significantly greater than either Group 4 or Group 5 ($p < 0.001$).

TABLE 7

ORGAN WEIGHTS—MALE SURVIORS

	n	Body weight (g)	Heart	Liver	Brain	Testes	Kidneys	Adrenals	Seminal vesicles	Prostate
Group 1 (950 mg/kg)	30	485 ± 48	1.40 ± 0.19	21.30 ± 5.67	2.44 ± 0.19	4.45 ± 1.51[a]	3.56 ± 0.99	0.08 ± 0.02	0.25 ± 0.10[b]	0.29 ± 0.19[b]
Group 2 (378 mg/kg)	13	481 ± 46	1.33 ± 0.17	20.47 ± 3.36	2.44 ± 0.15	4.50 ± 1.36	3.42 ± 0.72	0.08 ± 0.02	0.51 ± 0.22[b]	0.52 ± 0.15[c]
Group 3 (150 mg/kg)	21	504 ± 46	1.35 ± 0.14	18.58 ± 2.93	2.43 ± 0.11	5.25 ± 1.47	3.31 ± 0.35	0.08 ± 0.01	0.66 ± 0.32[b]	0.61 ± 0.34[d]
Group 4 (distilled water)	30	518 ± 50	1.40 ± 0.15	19.19 ± 2.30	2.44 ± 0.07	5.02 ± 0.98	3.41 ± 0.51	0.08 ± 0.01	1.13 ± 0.35	1.01 ± 0.36
Group 5 (nondosed controls)	27	510 ± 49	1.41 ± 0.13	19.30 ± 2.51	2.44 ± 0.14	5.13 ± 0.72	3.39 ± 0.39	0.08 ± 0.02	1.16 ± 0.51	0.83 ± 0.34

Note. The results are expressed in g, Mean ± SD.

[a] Significantly lower than Group 5 ($p < 0.05$) but not Group 4.

[b] Significantly lower than both Group 4 and Group 5 ($p < 0.001$).

[c] Significantly lower than both Group 4 ($p < 0.001$) and Group 5 ($p < 0.01$).

[d] Significantly lower than both Group 4 ($p < 0.001$) and Group 5 ($p < 0.05$).

cedent had focal parafollicular hyperplasia and one Group 4 male decedent had focal follicular cell hyperplasia). The lack of any obvious treatment-related increase in hyperplastic lesions of the thyroid suggests that the slightly higher incidence of benign thyroid tumors in Groups 1 and 2 animals (see next section) was a chance finding. A variety of nonspecific changes was observed in the adrenals of all groups, but taking the findings for decedents and animals killed terminally together, there is clearly no evidence of any treatment-related effect. Cystic changes, hypertrophy, and chromophobe cell hyperplasia of the pituitary gland were no more frequent in treated groups than in the controls.

The kidneys of rats of both sexes in all groups showed changes indicative of slight to marked chronic tubular nephrosis, a condition which occurs very commonly in untreated aged rats of many strains. The features of the condition include round cell infiltration, the presence of eosinophilic tubular casts, multiple foci of tubular hyperplasia, and, in a few instances, areas of interstitial fibrosis. The data displayed in Tables 8 and 9 indicate that treatment was not associated with any adverse effect on the incidence or severity of these or any other renal changes.

The lungs of most rats exhibited minimal to slight pneumonitis and deposits of round cells around main airways. These changes are a more or less universal finding in aged rats. The fact that they were rarely more than slight in severity reflects the fact that the experiments were conducted under carefully maintained specified pathogen-free conditions. In animals killed terminally, it is clear from Table 9 that treatment was not associated with any increased incidence or severity of the condition. As has been made clear above, there were several early deaths in Groups 1, 2, and 3 because of the entry of drug solution into the lungs during gavage. A dose-related increased incidence of pulmonary congestion and edema attributable to this is evident in the data for decedents summarized in Table 8. It is noteworthy that there is no treatment-related increase in pulmonary congestion in rats killed terminally (see Table 9). A variety of other pulmonary lesions was recorded but in no case was the incidence associated with treatment.

No treatment-related changes were seen in the heart. No pathology changes whatsoever were seen in the brain in any group. In the eye, focal, extensive, or complete retinal atrophy was seen in 5/89 Group 1 males (4/59 decedents and 1/30 terminal kill) and 14/87 Group 1 females (5/56 decedents and 9/31 terminal kill) compared with 8/79 Group 4 males and 15/78 Group 4 females. These figures are consistent with no treatment-related effects on this parameter. In our opinion, the fact that in rats killed terminally the retinal atrophy was graded as complete in 6/31 Group 1 females compared with only 2/29 Group 4 females is a chance finding.

There was no evidence of any effect of treatment on voluntary muscle, skin, the ovaries, or the uterus. Mammary gland hyperplasia and galactocoele formation were less frequent in treated than control females.

In males killed terminally (Table 9), there was evidence of the known, weak antiandrogenic effects of cimetidine (Leslie and Walker, 1977) in terms of a treatment-related increase in atrophy of the seminiferous tubules of the testis (Group 1, 16/30 rats affected; Group 2, 4/13; Group 3, 6/21; Group 4, 6/30; Group 5, 4/27) and atrophy of the seminal vesicles (Group 1, 17/30; Group 2, 3/13; Group 3, 5/21; Group 4, 1/30; Group 5, 0/27), prostate (Group 1, 17/29; Group 2, 2/13; Group 3, 4/21; Group 4, 1/30; Group 5, 0/27) and epididymides (Group 1, 9/27; Group 2, 1/13; Group 3, 0/18; Group 4, 1/30; Group 5, 0/27). Although an increased incidence of Leydig-cell tumors was seen in cimetidine-treated rats (see next section) there was no parallel increase in the incidence of Leydig-cell hy-

perplasia (Group 1, 3/30; Group 2, 1/13; Group 3, 2/21; Group 4, 1/30; Group 5, 3/27).

(B) *Overall tumor incidence (all Batches).* No neoplasms were seen in rats sacrificed at 6 months. Among 16 rats from Group 5 (nondosed controls) sacrificed at the 10-month interim kill two tumors were seen: an adenoma of the lung and a chromophobe adenoma of the pituitary. No tumors were seen among 19 rats from Groups 1 to 4 killed at this time.

Among 65 rats from Groups 1 to 4 sacrificed at the 12-month interim kill, eight tumors were seen: a mesothelioma in a Group 1 male; a papillary carcinoma of the mammary gland in a Group 4 female; chromophobe adenomas of the pituitary in a Group 1 male, a Group 3 female, and a Group 4 female; a hemangioma of the axillary lymph node in a Group 3 male; a phaeochromocytoma in a Group 3 female; and a mammary fibroadenoma in another Group 3 female.

No neoplasms were seen in any rat dying before Week 16. The results for all decedents from Week 16 onward are listed in Table 10.[1] Survival differences among groups need to be taken into account in the interpretation of the data depicted in this table. There was a considerably lower overall tumor rate, both for male and female rats, in the cimetidine-treated groups than in the control groups, as would be expected in view of the higher number of premature deaths in the cimetidine-treated groups.

The incidence of tumors among rats killed terminally is shown in Table 11.[1] Although the mean number of neoplasms per survivor was lower both in the males (0.62) and the females (0.48) of Group 5 (nondosed controls) than in any of the four dosed groups, including the distilled water controls (range, 0.88–1.29 in males, 0.79–1.03 in females), tumor incidence for each sex was similar among the three cimetidine-treated groups and the distilled water controls. A slightly higher overall incidence of tumors in male survivors in the cimetidine-treated groups compared with Group 4 is explained by the higher incidence of Leydig-cell tumors among these animals (Table 12).

(C) *Specific tumor types.* A low incidence of pancreatic tumors in treated groups but not in controls (Islet-cell tumors in two males of Group 1 killed at 104 and 106 weeks, respectively, exocrine adenomas in two males of Group 1 killed at 101 and 106 weeks, respectively, and a mixed endocrine/exocrine adenoma in a female of Group 3 killed at 103 weeks) is considered to have been a chance finding (see Discussion).

The data for four types of tumor—pituitary tumors in both sexes, mammary tumors in females, Leydig-cell tumors in males, and follicular neoplasms of the thyroid gland—were combined, respectively, for decedents and terminal kill animals and analyzed statistically.

Leydig-cell tumors. The proportions of rats with Leydig-cell tumors of the testis are shown in Table 12. The data were analyzed using the method for incidental tumors, with the modification that the χ^2 statistic contains a contribution from rats without tumors.

A significantly higher incidence of Leydig-cell tumors was seen in the combined cimetidine-treated groups compared with the combined control groups, both for rats killed or dying at any time during the study (Weeks 1-106) and for rats killed during Weeks 105 and 106. The incidence of Leydig-cell tumors in the combined cimetidine-treated groups was not significantly different from that in the combined control groups among rats dying during Weeks 79 and 104. The difference in tumor incidence was therefore confined to animals killed during Weeks 105 and 106. Within the cimetidine-treated groups there was no dose-related trend in incidence of Leydig-cell tumors (Table 12).

Tumors of the pituitary gland. The incidence of pituitary tumors among survivors

TABLE 12

LEYDIG-CELL TUMOR INCIDENCE IN RATS KILLED OR DYING DURING DIFFERENT PERIODS

	Group 1 (950 mg/kg)	Group 2 (378 mg/kg)	Group 3 (150 mg/kg)	Group 4 (distilled water)	Group 5 (nondosed controls)
Total[a]	23/98[b]	14/68[c]	15/65[d]	13/84	22/107
Percentage incidence of tumors	23.5	20.6	23.1	15.5	20.6
0–26 Weeks	0/18	0/16	0/13	0/11	0/0
27–54 Weeks	0/21	0/18	0/18	0/15	0/10
55–78 Weeks	0/9	4/10	0/2	0/2	2/8
79–104 Weeks[e]	6/16	4/10	3/8	6/22	9/31
105–106 Weeks[f]	17/34[g]	6/14[h]	12/24[i]	7/34	11/58

Note. Number of rats with a tumor/number of rats killed or dying with adequate pathological examination.

[a] Significant difference between combined cimetidine-treated groups and combined control groups, $\chi^2(1) = 11.79$, $p < 0.001$.

[b] Significantly different from combined control groups, $\chi^2(1) = 6.33$, $p < 0.025$.

[c] Not significantly different from combined control groups, $\chi^2(1) = 3.40$, $p > 0.05$.

[d] Significantly different from combined control groups, $\chi^2(1) = 5.81$, $p < 0.025$.

[e] No significant difference between combined cimetidine-treated groups and combined control groups, $\chi^2(1) = 0.54$, $p > 0.05$.

[f] Significant difference between combined cimetidine-treated groups and combined control groups, $\chi^2(1) = 14.27$, $p < 0.001$.

[g] Significantly different from combined control groups, $\chi^2(1) = 10.01$, $p < 0.005$.

[h] Not significantly different from combined control groups, $\chi^2(1) = 2.55$, $p > 0.1$.

[i] Significantly different from combined control groups, $\chi^2(1) = 7.67$, $p < 0.01$.

was lower in the females of Group 1 (high dose, 7/34) and Group 5 (nondosed, 12/71) than in other groups (Group 2, 8/18; Group 3, 9/26; Group 4, 12/32) (Table 11). However, none of the differences between the individual groups was statistically significant nor was the difference between the combined cimetidine-treated groups and the combined control groups significant.

For both male and female rats, the patterns of incidence for incidental and nonincidental tumors (see Statistical Methods for definition) were scrutinized but no treatment-related effect was seen.

Tumors of the thyroid gland. A total of 18 rats in the study were found to have thyroid tumors at necropsy. Six cases of parafollicular adenoma appeared to be distributed randomly between treated and control groups. One nondosed control male was killed because of a large follicular cell carcinoma during the 103rd week of the study and a nondosed female was found to have a follicular cell carcinoma when it was killed at the end of the study. By contrast, no rat in the three cimetidine-treated groups developed a malignant tumor of the thyroid gland. However, the number of rats with benign or malignant tumors of follicular cell origin was slightly higher in Groups 1 and 2 than in other groups (Group 1, 4/98 males and 1/96 females; Group 2, 1/59 males and 2/62 females; Group 3, 0/57 males and 1/55 females; Group 4, 1/84 males and 0/85 females; Group 5, 1/107 males and 1/108 females). Only two of the benign neoplasms, both incidental, occurred in decedents (a Group 1 male killed in Week 82 and a Group 2 male dying in Week 73). All other follicular adenomas were observed in rats killed at the end of the study. For this reason, and as survival rates between Weeks 55 and 106

did not vary significantly among groups, a statistical analysis was performed on the proportions of rats with follicular cell tumors killed or dying during the second year of the study. Ignoring the nature of the tumors, i.e., whether they were benign or malignant, the incidence in the pooled cimetidine-treated groups, if males and females are combined, is greater than in the pooled control groups, and in Group 1 the incidence in males alone is significantly higher than in the other group males ($p < 0.05$, exact test). However, a carcinoma is clearly a more serious and advanced lesion than an adenoma. For this reason we do not believe that the slight excess of benign tumors in Group 1 has any biological significance.

Tumors of the mammary gland. Analyses were carried out separately for all rats with histopathologically confirmed mammary tumors, and for rats with malignant tumors only, using the method for nonincidental tumors and taking the week that the tumor was first observed as the first occurrence. Table 13 shows the number of tumors observed (O) and the number expected (E) under the null hypothesis that tumor rate is independent of treatment.

For both malignant mammary tumors and for all mammary tumors, incidence rates were significantly higher in the distilled water group than in the other groups. Further analysis of the data for all mammary tumors showed that the excess in the distilled water group was not apparent before Week 83 nor after Week 103 but was very marked between those weeks ($O_1 + O_2 + O_3 + O_5 = 8$, $E_1 + E_2 + E_3 + = 19.67$; $O_4 = 16$, $E_4 = 4.33$; $\chi^2(1) = 38.3$, $p < 0.001$). During this period, of 49 tumorless survivors in Group 4, 16 (32.7%) developed a tumor by Week 103. In the other four groups combined the proportion was only 8 out of 185 (4.3%).

There were no significant differences in tumor rates between the three cimetidine-dosed groups and the nondosed controls and, hence, no evidence that cimetidine affected the risk of development of mammary tumors.

DISCUSSION

Previous studies with cimetidine had indicated that it was a powerful inhibitor of gastric acid secretion (Brimblecombe *et al,* 1975) and that weak antiandrogenic effects were to be expected in response to treatment at each of the dose levels chosen (Leslie and Walker, 1977), the mechanism possibly being that cimetidine, itself a weak androgen, blocks the cytoplasmic receptor sites for di-

TABLE 13

FEMALES RATS WITH MAMMARY TUMORS: NUMBERS OBSERVED (O) AND NUMBERS EXPECTED (E)

	All mammary tumors[a]		Malignant mammary tumors[b]	
	O	E	O	E
Group 1 (950 mg/kg)	13	13.84	3	4.78
Group 2 (378 mg/kg)	3	7.43	2	2.41
Group 3 (150 mg/kg)	10	10.49	5	3.41
Group 4 (distilled water)	23	12.77[c]	9	4.62[d]
Group 5 (nondosed controls)	22	26.47	5	8.78

[a] Significant difference between all five groups, $\chi^2 = 11.67$, $p < 0.05$.
[b] No significant difference between groups.
[c] Significant difference between distilled-water controls and other four groups, $\chi^2 = 10.00$, $p < 0.01$.
[d] Significant difference between distilled-water controls and other four groups, $\chi^2 = 5.14$, $p < 0.05$.

hydrotestosterone (Winters *et al.*, 1979).[2] Also, Crean *et al.* (1978) reported evidence of hypertrophy of antral and fundal gastric mucosa in rats. It was, therefore, of particular interest in the present study to look for changes in the gastrointestinal tract, gonads, and secondary sex organs. Administration by gavage, chosen because of the unpleasant taste of cimetidine, presented problems because aqueous solutions of cimetidine are poorly tolerated if they enter the lungs. A few animals were lost because of accidental instillation into the trachea but more died prematurely because of seepage of cimetidine solution from the esophagus into the lungs via the larynx during or immediately after its introduction into the lower esophagus or stomach. Apart from these premature deaths, cimetidine treatment was associated with few effects and with none which adversely affected survival.

No significant adverse effects were observed on food consumption, body weight, clinical signs, behavior, ophthalmoscopy, or in biochemical, hematological, or urinalysis parameters in response to cimetidine. Increases in mean liver weight and occasional elevations of transaminase levels in rats receiving cimetidine 950 mg/kg/day were associated with a higher incidence of centrilobular hepatocellular vacuolation and enlargement, bile duct hyperplasia and hepatocyte necrosis in males and, in females, a slightly higher incidence of centrilobular hepatocellular enlargement. The latter change fell into Squire and Levitt's (1975) category "foci or areas of cellular alteration."

With the possible exceptions of mammary tumor incidence in females, Leydig-cell tumors in males, and follicular tumors of the thyroid gland, no significant treatment-related effects on tumor incidence were observed after survival differences had been allowed for. It is considered that the occurrence of two Islet-cell adenomas and two cases of exocrine adenoma of the pancreas in high-dose males, and one case of mixed Islet-cell and exocrine adenoma in a low-dose female, despite the absence of tumors of either of these kinds in either control group, was a chance finding. Tumors of both these kinds are of common occurrence in laboratory rats, but the factors which affect the incidence of the one do not as a rule influence that of the other.

A significantly ($p < 0.001$) higher incidence of mammary tumors was encountered in females of the distilled water control group which died between Weeks 83 and 103 but not before the 83rd week and not after the 103rd week. The observation is considered to be spurious since there was no parallel excess incidence in untreated females during this period and no dose-related deficiency of mammary tumors in females in the cimetidine-treated groups. Follicular adenomas of the thyroid gland were encountered more frequently in high-dose males (4/98) than in any other group. This was not considered to indicate an effect of treatment since all four tumors were benign, whereas one untreated control male developed a malignant tumor of follicular origin, and there was no pattern suggestive of treatment in females. In any study of the present kind where large numbers of comparisons are made for tumors of different types, statistically significant differences are bound to appear by chance. Where excess incidences affect low-dose or control groups, it is the usual practice to accept them as spurious as we have done with the excess of mammary tumors in the distilled-water control group (see above).

Cimetidine (475 mg/kg b.i.d.) has been shown to decrease the growth rates of the prostate and seminal vesicles of castrated adult rats treated exogenously with testosterone when both were given over a 7-day period.[3] It was not surprising, therefore, in

[2] H. Saunders, and P. Sivelle, personal communications.

[3] P. Sivelle, personal communication.

the present study, to find that the group mean prostate and seminal vesicle weights in rats killed terminally were significantly lower in all treated groups than in the controls. The known, weak antiandrogenic effect of cimetidine (Leslie and Walker, 1977) was also expressed as a treatment-related increase in atrophy of the seminiferous tubules and atrophy of the seminal vesicles, prostate, and epididymides in rats killed terminally. In spite of its weak antiandrogenic activity, cimetidine has no effect on the mating performance or fertility of male rats treated for at least 70 days before mating at any of the three dose levels in the present study. No effects were seen on either the morphology or reproductive capability of the male offspring of female rats treated with cimetidine up to 950 mg/kg/day during pregnancy and lactation (Leslie and Walker, 1977).

There is no clear way of distinguishing between Leydig-cell hyperplasia and neoplasia other than by size of lesion. In the present study, we regarded foci of hyperplastic Leydig cells of 1 mm diameter or more as neoplasms. Irrespective of their size such lesions are nonfatal. In other words, their presence is normally first detected in animals that have died from other causes. Although exposure to high doses of cimetidine was, overall, associated with an increased incidence of Leydig-cell tumors so defined, the results are somewhat puzzling in that the excess of tumors was not associated with any excess of lesions classified as Leydig-cell hyperplasia, was confined to animals which survived to week 105 or 106 of the study and showed no dose-related trend. Since 15–20% of control animals (Groups 4 and 5) killed at the end of the experiment exhibited testicular atrophy and 20% had Leydig-cell tumors, it is questionable whether rats of the strain used were suitable for predicting the effect of antiandrogenic activity in the testis of other species, including man.

When Leydig-cell tumors are excluded, the pattern of mean numbers of neoplasms per survivor was consistent with there being no effect of cimetidine on the incidence of any kind of neoplasm, benign or malignant. The size of the difference in overall tumor incidence between the dosed and nondosed controls illustrates once again the extent to which nonspecific factors, such as dosing with distilled water by gavage, may influence tumor incidence (Riley, 1975).

Although the mean pharmacological action of cimetidine is to reduce the secretion of acid in the stomach, no evidence of any pathological change attributable to treatment was observed either in the stomach or elsewhere in the gastrointestinal tract in the present study, despite the hypertrophy of antral and fundal mucosa reported earlier (Crean *et al.*, 1978).

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