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Acute and Subacute Toxicity of Tyramine, Spermidine, Spermine, Putrescine and Cadaverine in Rats

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Abstract—The acute and subacute toxicity of five biogenic amines—tyramine, spermidine, spermine, putrescine and cadaverine—were examined in Wistar rats. Tyramine and cadaverine had a low acute oral toxicity of more than 2000 mg/kg body weight. Putrescine had an acute oral toxicity of 2000 mg/kg body weight and spermidine and spermine each of 600 mg/kg body weight. All amines investigated caused a dose-related decrease in blood pressure after intravenous administration, except for tyramine, where an increase was found. In 6-wk studies the biogenic amines were administered in the diet to groups of 10 male and 10 female rats. Tyramine and cadaverine were given at levels of 0, 200, 2000 or 10,000 ppm, spermine and putrescine at levels of 0, 200, 2000 or 5000 ppm and spermidine at levels of 0, 20, 200 or 500/1000 ppm in the first study and at levels of 0 or 10,000 ppm in a second study. Spermine was the most toxic. The high dose level showed a great number of changes, such as emaciation, aggressiveness, convulsions and paralysis of the hind legs. Growth, food intake and water intake were considerably decreased. Slight anaemia (males) and changes in plasma clinical chemistry occurred. The relative weights of the thyroid, adrenals, spleen and heart were increased and that of the liver decreased. Impaired kidney function, together with renal histopathological changes and changes in plasma electrolytes and urea, occurred with spermine. Histopathological examinations also revealed decreased glycogen content in the liver, reduction of spermatogenesis, severe depletion of splenic white pulp, acute involution of the thymus and moderate myocardial degeneration in the heart. Myocardial degeneration was also seen in one mid-dose male. Adverse effects were also observed in the top dose groups of all other amines. Decreased body weights associated with diminished food intake were generally seen. Slight increases in packed cell volume, haemoglobin concentration and thrombocytes occurred with cadaverine. With spermidine, decreased plasma creatinine, calcium and inorganic phosphate were observed and decreased potassium levels with cadaverine. The no-observed-adverse-effect level was 2000 ppm (180 mg/kg body weight/day) for tyramine, cadaverine and putrescine, 1000 ppm (83 mg/kg body weight/day) for spermidine and 200 ppm (19 mg/kg body weight/day) for spermine. © *1997 Elsevier Science Ltd*

Abbreviations: ALAT = alanine aminotransferase; ALP = alkaline phosphatase; ASAT = aspartate aminotransferase; MAO = monoamine oxidase; MCH = mean corpuscular haemoglobin; MCHC = mean corpuscular haemoglobin concentration; MCV = mean corpuscular volume; NOAEL = no-observed-adverse-effect level; RES = reticuloendothelial cells.

INTRODUCTION

Many foods of vegetable or animal origin contain a great number of mono-, di- and polyamines, the so-called biogenic amines. These amines are organic bases with a low molecular weight with an aliphatic, aromatic or heterocyclic structure. Some of these amines are formed naturally by metabolic processes, whereas others are formed by enzymatic decarboxylation from amino acids by microbial activity (Maga, 1978; Smith, 1980–81). The level in foods varies widely, from very low to thousands of parts per million (Askar and Treptow, 1986; Bos *et al.*, 1986; Halász *et al.*, 1994; Maga, 1978; Smith, 1980–81, Ten Brink *et al.*, 1990). In the last decades, increasing attention has been paid to these amines, because it became apparent that they may exert biological activities of a diverse nature and play an important role in many physiological functions. For instance, certain biogenic amines are important in the regulation of DNA, RNA and protein synthesis, and probably also in the stabilization of membranes (Gahl and Pitot, 1981; Smith, 1980–81; Tabor and Rosenthal, 1956; Tabor and Tabor, 1964).

There is a large body of data on the pharmacological effects of biogenic amines in humans, especially with respect to histamine and tyramine. However, despite the potentially high biological activity of many biogenic amines and their occurrence in many foods, sometimes at a rather high level, no toxicity data are available that allow the establishment of a no-observed-adverse-effect level (NOAEL) in

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laboratory animals. Therefore, it is also not possible to calculate an acceptable daily intake (ADI) for man. For this reason, a programme was initiated to investigate the oral toxicity of five of these amines which are quantitatively most important in foods, namely tyramine, cadaverine, putrescine, spermine and spermidine. In addition, acute iv studies were conducted to examine the influence of the five biogenic amines on blood pressure. The results of acute and subacute studies with these amines in Wistar rats are reported here.

MATERIALS AND METHODS

Test substances

P-(2-aminoethyl)-phenol-hydrochloride (tyramine) (mol. wt 173.64 g/mol, Ref. No. 8373, purity 99%) was obtained from E. Merck AG (Darmstadt, Germany). *N*-(3-Aminopropyl)-1,4-butanediamine-trichloride (spermidine) (mol. wt 254.63 g/mol, Ref. No. 85580, purity > 99%), *N*,*N*′-bis(3-aminopropyl)-1,4-butanediamine-tetrahydrochloride (spermine) (mol. wt 348.19 g/mol, Ref. No. 85610, purity > 99%), 1,4-diaminobutane-dihydrochloride (putrescine) (mol. wt 161.08 g/mol, Ref. No. 32810, purity > 99%), and 1,5-diaminopentane-dihydrochloride (cadaverine) (mol. wt 175.10 g/mol, Ref. No. 33220, purity > 99%) were all obtained from Fluka AG (Buchs, Switzerland).

Animals and maintenance

Wistar-derived SPF-bred rats (Cpb:WU; Wistar random or Bor:WISW) were obtained from TNO Central Institute for the Breeding of Laboratory Animals, Zeist, The Netherlands or from F. Winkelmann Institute for the Breeding of Laboratory Animals GmbH & Co. KG, Borchen, Germany. They were housed conventionally under barrier conditions, in suspended stainless-steel cages fitted with wire-mesh floor and front, two rats per cage in the acute studies and five rats per cage in the subacute studies. The room temperature was maintained at 22 ± 3°C and the relative humidity at 55 ± 15%. Artificial light was provided continuously, for 12 hr/day from 07.30 hr until 19.30 hr. The number of air changes was about 10/hr.

Diets

The rats used in the acute studies were fed the Institute's basal diet, which is a cereal-based open formula diet (Rutten and de Groot, 1992). For the subacute studies the Institute's basal diet or a purified diet was used. The percentage composition of the purified diet was as follows: casein 20; DL-methionine 0.2; wheat starch 64.8; cellulose (solka floc) 5; mineral mixture (Jones Foster) 4; vitamin B preparation 0.2; vitamin ADEK preparation 0.4; choline chloride (50%) 0.4; soyabean oil 5. In the first and second study with spermidine, the Institute's basal diet was used. Because the level of some biogenic amines in the basal diet (on analysis, in mg/kg/diet: tyramine 92, spermidine 43, spermine 19, putrescine 102 and cadaverine 174) was considerably higher than in purified diet (on analysis, in mg/kg/diet: spermidine 0.5 and putrescine 0.1), the subacute studies with tyramine, spermine, putrescine and cadaverine were carried out with purified diet. The biogenic amines were added to the diet at various levels. Homogeneity of the test diets was achieved by mixing for 2 min in a mechanical blender (Stephan cutter). Fresh batches of 6 kg of each diet were prepared twice during the study and stored in a deep-freezer at about −20°C pending use. The diets were refreshed twice a week.

Blood pressure measurements after iv injection

Male rats (Cpb:WU; Wistar random) weighing 350–450 g were anaesthetized with urethane (1–1.5 g/kg body weight, ip). After a stabilization period of 30 min, blood pressure was recorded continuously from a cannulated carotid artery by means of a pressure transducer (P-1000 B, Narco Bio-systems) connected to a MK-III Physiograph recorder (Narco Bio-systems). The various doses of each of the five biogenic amines were administered in a volume of 200 μl (in isotonic saline) over a period of 1 min through a cannulated vein. The different doses (three or four) used for each biogenic amine were given to each of the animals used (five to nine per biogenic amine) with an interval of at least 30 min.

Acute oral toxicity studies

The biogenic amines were administered orally as a 25% aqueous dilution at various dose levels to groups of two male and two female rats (Cpb:WU; Wistar random) 8–13 wk old and weighing 184–390 g (males) and 130–232 g (females). The exact amount of the test substances to be dosed in mg/kg was calculated for each animal and administered by gavage. Prior to dosing, the rats were fasted overnight. About 4 hr after dosing they received food again. The rats were observed for 14 days, and clinical signs and deaths were recorded. The animals that died and the survivors killed after 14 days were examined for pathological changes.

Subacute oral toxicity studies

After an acclimatization period of 10, 17 or 24 days, weanling Wistar rats (Bor:WISW) were randomly assigned to groups each of 10 male and 10 female 10 rats. The rats were fed diets containing 0, 200, 2000 or 10,000 ppm tyramine or cadaverine, 0, 200, 2000 or 5000 ppm spermine or putrescine (the high-dose level of spermine was initially 10,000 ppm but was decreased to 5000 ppm from day 4 onwards) or 0, 20, 200 or 500 ppm spermidine (500 ppm was increased to 1000 ppm after 2 wk). In addition, two groups each of 10 male and 10 female rats were fed diets containing 0 or 10,000 ppm spermidine to find an effect level. Diets and tap-water were given *ad lib.* for a period of 5–6 wk.

Observations and analyses

The rats were weighed once weekly, and observed daily for condition and behaviour. Food intake was measured weekly, on a cage basis by weighing the feeders. Water intake was determined daily during the first week of the study in a similar fashion. Systolic blood pressure was measured by an indirect tail-cuff method (Kuijpers *et al.*, 1986). During the acclimatization period, the rats were accustomed to the procedures involved in measuring blood pressure. Systolic blood pressure of all rats was measured once prior to the start of the study and twice per week during the morning (males on days 0, 3, 5, 10, 12, 17, 19, 24 and 26 and females on days 0, 4, 6, 11, 13, 18, 20, 25 and 27). Four successive systolic blood pressure readings were taken of each rat on the days of measuring. The mean value of the last three successful readings from each rat was regarded as the systolic blood pressure of that rat for that day.

Haematology and clinical chemistry. Blood samples were collected from the tip of the tail of all rats early in wk 5 and examined for haemoglobin concentration, packed cell volume and erythrocyte, leucocyte and thrombocyte counts (Sysmex K-1000 Haematology Analyzer, Toa Medical Electronics Co, Ltd, Kobe, Japan), differential leucocyte count (blood smears stained according to Pappenheim) and prothrombin time (Normotest, Nyegaard & Co. A/S, Oslo, Norway). Whole blood taken from all rats after overnight fasting in wk 5 was examined for glucose (Boehringer Glucoquant No. 245-178; Boehringer Mannheim GmbH, Mannheim, Germany). Blood samples taken from the abdominal aorta of all rats at autopsy were centrifuged at 1250 *g* for 15 min and then analysed for alkaline phosphatase (ALP), aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), γ-glutamyl transferase, total protein, albumin, urea, creatinine, total bilirubin, calcium and inorganic phosphate (Cobas-Bio Centrifugal Analyzer, Hofmann-La Roche, Basle, Switzerland), chloride (Chloro Counter, Marius, Utrecht, The Netherlands) and sodium and potassium (Electrolyte-2 Analyzer, Beckman Instruments, Brea, CA, USA).

Urinalysis. In wk 5 all rats were deprived of water for 24 hr and of food for 16 hr. Urine was collected during the last 16 hr of the deprivation period and its volume (calibrated tubes) and density (refractometer; Bellingham and Stanley, London, UK) were measured.

Pathology. The rats were killed in wk 5 or 6 by exsanguination from the abdominal aorta while under light ether anaesthesia, and a thorough autopsy was performed. The following organs of each rat were weighed and the organ/body weight ratios were calculated: adrenals, brain, heart, kidneys, liver, ovaries, pituitary, spleen, testes, thymus, thyroid and uterus. Samples of these organs and of the coagulating glands, epididymides, seminal vesicles, spinal cord, mesenteric lymph nodes, prostate, peripheral nerve, spinal cord and gross lesions were fixed in 10% neutral buffered (pH 7.0) formalin, embedded in paraffin wax, sectioned at 5 μm, and stained with haematoxylin and eosin. Detailed microscopic examinations were carried out on all above-mentioned organs of all rats of the control and high-dose groups. The kidneys, liver, testes, spleen, thymus and heart of all rats of the low- and mid-dose groups in the spermine study were also examined, because effects were noted in the high-dose rats. A thorough autopsy was also performed on rats that were found dead or were killed when moribund during the study. The organs of these animals were not weighed, but tissues were preserved and examined microscopically.

Statistical analysis

Data on body weight were evaluated by one-way analysis of covariance, followed by Dunnett's multiple comparison tests. The laboratory determinations and organ weights were evaluated by one-way analysis of variance, followed by Dunnett's multiple comparison tests. The differential white blood cell counts were analysed by Kruskall–Wallis non-parametric analysis of variance followed by Mann–Whitney *U*-tests. Data on food and water intake were evaluated by analysis of variance, followed by least significant difference tests (experimental unit: the cage). Pre and post treatment data on blood pressure were analysed using paired *t*-tests. The mortality incidence and the histopathological changes were examined by Fisher's exact probability test. All comparison tests were two-tailed, and a probability level of $P < 0.05$ was considered significant.

RESULTS

Blood pressure after iv administration

The results of the iv injection of the five biogenic amines at various dose levels on systolic and diastolic blood pressure are presented in Table 1. Administration of tyramine (0.01–0.3 mg/kg body weight) resulted in acute dose-related increases in systolic and diastolic blood pressure already at a rather low level of 0.01 mg/kg body weight, whereas iv administration of the other four biogenic amines resulted in acute dose-related decreases in systolic and diastolic blood pressure. With spermine a significant decrease was seen in systolic and diastolic blood pressure at 1 mg/kg and above, with spermidine at 3 mg/kg and above, with putrescine at 10 mg/kg and above and with cadaverine at 30 mg/kg and above.

Acute oral toxicity studies

Most of the animals showed sluggishness and piloerection on single oral treatment with each of the five biogenic amines examined. Exophthalmus was seen in the 1000 and 2000 mg/kg groups with

Table 1. Systolic and diastolic blood pressure of rats treated iv with various dose levels of five biogenic amines

Dose level (mg/kg body weight)	No. of rats	Blood pressure (mmHg) in the various groups†			
		Systolic		Diastolic	
		(pre)	(post)	(pre)	(post)
		Tyramine			
0.01	7	106 ± 9	115 ± 7**	43 ± 4	55 ± 4**
0.03	9	96 ± 7	112 ± 8**	44 ± 5	60 ± 8**
0.1	6	93 ± 10	122 ± 12**	46 ± 2	77 ± 9**
0.3	7	84 ± 10	110 ± 12**	37 ± 3	76 ± 8**
		Spermidine			
1	7	133 ± 5	131 ± 5	85 ± 5	85 ± 5
3	7	133 ± 5	122 ± 6*	83 ± 5	72 ± 4**
10	7	125 ± 7	105 ± 8**	73 ± 7	49 ± 6***
30	7	120 ± 7	84 ± 8***	69 ± 7	35 ± 6***
		Spermine			
0	8	124 ± 5	128 ± 4*	64 ± 5	67 ± 5**
1	7	120 ± 5	113 ± 6	59 ± 5	52 ± 5*
3	7	111 ± 4	87 ± 12*	44 ± 1	36 ± 1***
10	7	105 ± 4	53 ± 15**	45 ± 3	21 ± 4**
30	5	110 ± 4	28 ± 11**	44 ± 3	11 ± 3***
		Putrescine			
0	8	119 ± 5	123 ± 5*	75 ± 6	77 ± 5
10	8	124 ± 6	118 ± 4	75 ± 5	65 ± 3*
30	8	107 ± 7	100 ± 7**	54 ± 4	41 ± 3***
100	7	101 ± 4	72 ± 11**	49 ± 4	26 ± 4***
		Cadaverine			
0	6	114 ± 9	120 ± 9**	73 ± 6	77 ± 7*
3	6	105 ± 11	111 ± 11**	59 ± 8	61 ± 9
10	6	94 ± 9	95 ± 9	51 ± 6	48 ± 7
30	6	100 ± 11	84 ± 8*	51 ± 7	38 ± 6*
100	5	101 ± 9	73 ± 7**	52 ± 7	32 ± 7*

†Blood pressure was recorded continuously and the maximum reaction was generally measured within 30 sec after iv injection (pre = blood pressure before treatment; post = blood pressure after iv injection). Asterisks indicate significant differences between pre and post values (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; paired t-tests).

tyramine, spermidine and spermine. The animals treated with putrescine that died showed tremors. The mortality data and 'approximate LD_{50}' values of the five biogenic amines are presented in Table 2. Tyramine and cadaverine showed the lowest acute toxicity of more than 2000 mg/kg body weight. The 'approximate LD_{50}' of putrescine was 2000 mg/kg, while spermidine and spermine showed the highest acute toxicity, namely 600 mg/kg body weight for each of the compounds.

Subacute oral toxicity studies

The main results of the subacute studies are presented in Tables 3–9. In view of the substantial amount of data collected, only those results of the haematological, clinical chemistry, urine and histopathological examinations are shown that might point to potentially treatment-related effects.

Tyramine. Systolic blood pressure was relatively low at 10,000 ppm tyramine in females, although the difference from the control was not statistically significant (Table 3). In males, mean body weights were slightly lower at 10,000 ppm, whereas food and water intake did not show consistent changes among the various groups (Table 3). Clinical chemistry (Table 5) revealed decreased plasma alkaline phosphatase (ALP) activity in both sexes with 10,000 ppm and also in males with 2000 ppm. The relative weight of the liver was increased at 10,000 ppm in males only (Table 6). At microscopic examination the incidence of aggregates of reticulo-endothelial (RES) cells and of necrotic hepatocytes in the liver and that of basophilic tubules in the kidneys were slightly higher in males of the 10,000 ppm group than in those of the controls, although the differences were not statistically significant (Table 7).

Spermidine. Mean body weights and food intake were significantly decreased with 10,000 ppm in

Table 2. Mortality figures and 'approximate LD_{50}' values of male and female rats administered single oral doses of five biogenic amines

Dose levels (mg/kg)	No. of rats that died in the various groups†									
	Tyramine		Spermidine		Spermine		Putrescine		Cadaverine	
	M	F	M	F	M	F	M	F	M	F
125	0	0	0	0	0	0				
250	0	0	0	0	0	0				
500	0	0	0	1	1	0				
1000	0	0	2	2	2	2				
2000	0	0	2	2	2	2	0	2	0	0
5000							2	2	1	1
'Approximate LD_{50}' value (mg/kg)	> 2000		600‡		600‡		2000		5000	

†No. of deaths in groups, which initially consisted of two male (m) and two female (f) rats per group.
‡Values were calculated according to the method of Weil (1952).

Table 3. Body weight, food consumption, food efficiency, water consumption, systolic blood pressure and mortality of rats fed various levels of five biogenic amines for 5–6 wk

Dietary level (ppm)	Body weight (g) on day 0	Body weight (g) on day 28	Food consumption (g/rat/day) mean wk 1–4	Food efficiency (g/gain/g food) mean wk 1–4	Water consumption (g/rat/day) in wk 1	Systolic blood pressure	Mortality† (no. of rats)
Tyramine							
			Males				
0	134 ± 3.8	300 ± 5.6	20.6	0.29	25.6	111	0
200	134 ± 3.4	290 ± 4.8	19.4	0.29	23.6	118	1
2000	134 ± 3.2	290 ± 4.6	20.9	0.27	25.8	112	0
10,000	134 ± 4.2	285 ± 7.6*	19.7	0.28	27.0	111	0
			Females				
0	112 ± 2.7	180 ± 4.6	14.0	0.17	22.1	112	0
200	112 ± 3.0	190 ± 4.3	14.8	0.19	21.1	110	0
1000	112 ± 2.7	182 ± 5.2	14.5	0.17	23.4	111	0
10,000	112 ± 2.3	181 ± 4.8	13.9	0.18	21.2	104	0
Spermidine							
			Males				
0	178 ± 2.5	317 ± 4.1	20.7	0.24	25.8	119	0
20	178 ± 2.0	314 ± 7.1	20.5	0.24	26.5	113	0
100	178 ± 3.2	307 ± 5.0	20.0	0.23	25.1	116	0
500/1000	179 ± 3.3	314 ± 6.2	20.2	0.24	26.5	124	0
0	134 ± 3.8	300 ± 5.6	20.6	0.29	25.6	111	0
10,000	134 ± 3.5	271 ± 7.1**	18.5*	0.27	24.2	111	0
			Females				
0	131 ± 2.0	186 ± 4.2	13.6	0.15	22.2	111	0
20	131 ± 2.7	185 ± 4.0	14.0	0.14	22.1	110	0
100	131 ± 2.2	184 ± 3.3	13.8	0.14	21.9	108	0
500/1000	131 ± 2.5	185 ± 5.0	13.7	0.14	19.7	110	0
0	112 ± 2.7	180 ± 4.6	14.0	0.17	22.1	112	0
10,000	112 ± 2.8	184 ± 4.1	13.9	0.19	22.7	109	0
Spermine							
			Males				
0	84 ± 1.4	226 ± 5.1	15.1	0.34	14.0	109	0
200	84 ± 2.0	231 ± 7.5	14.7	0.36	14.5	107	0
2000	84 ± 2.0	221 ± 6.1	14.5	0.34	13.9	106	0
10,000/ 5000	84 ± 2.8	110 ± 7.0**	7.9*	0.11	9.4**	91*	6*
			Females				
0	78 ± 0.8	149 ± 4.2	11.0	0.23	12.8	99	0
200	78 ± 1.4	146 ± 4.2	10.9	0.23	13.0	107	0
2000	78 ± 1.4	150 ± 2.2	10.9	0.24	12.9	106	0
10,000/ 5000	78 ± 1.7	110	8.8	0.13	10.3	92	9***
Putrescine							
			Males				
0	100 ± 1.4	245 ± 4.52	16.0	0.33	17.9	114	0
200	99 ± 1.5	254 ± 4.6	16.3	0.35	17.2	119	0
2000	100 ± 1.6	257 ± 6.0	16.8	0.34	18.1	111	0
5000	99 ± 1.7	236 ± 6.2	14.8*	0.33	18.1	112	0
			Females				
0	91 ± 1.3	179 ± 3.3	12.0	0.26	16.1	108	0
200	91 ± 1.2	173 ± 2.9	11.8	0.25	16.1	110	0
2000	91 ± 1.1	172 ± 4.5	11.6	0.25	15.4	108	0
10,000	91 ± 1.1	161 ± 3.0**	11.4	0.22	15.9	112	0
Cadaverine							
			Males				
0	106 ± 2.8	260 ± 5.1	16.9	0.33	21.9	111	0
200	107 ± 1.6	256 ± 7.5	16.4	0.33	22.9	114	0
2000	106 ± 1.8	259 ± 6.8	16.6	0.33	22.0	111	0
10,000	107 ± 1.4	231 ± 3.2**	15.2	0.30	28.2	106	0
			Females				
0	93 ± 1.1	169 ± 3.3	12.1	0.23	16.4	102	0
200	93 ± 0.9	164 ± 2.9	11.9	0.22	18.0	104	0
2000	93 ± 1.6	167 ± 3.8	11.8	0.22	17.1	108	0
10,000	93 ± 1.2	150 ± 3.8**	10.9**	0.19	16.6	98	0

†Number of deaths at the termination of the study.
Body weight values are means ± SEM for groups of 10 rats. Food consumption, food efficiency and water consumption are the means for two cages of initially five animals each.
Asterisks indicate significant differences from corresponding control groups [$*P < 0.05$; $**P < 0.01$; $***P < 0.001$; covariance-Dunnett's test (body weight); ANOVA-LSD test (food consumption and water consumption); ANOVA-Dunnett's test (systolic blood pressure); Fisher's exact test (mortality)].

males only, while food efficiency and water intake of males were slightly lower in this group, although the differences from the controls were not statistically significant (Table 3). The 10,000 ppm group showed significant increases in plasma activities of ALP, aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) in females, and decreases in plasma levels of calcium and potassium in males and in plasma protein levels in females (Table 5). In the 10,000 ppm group, the relative weights of the

brain, testes and kidneys were increased in males and that of the liver was decreased in both sexes (Table 6).

Spermine. During the first 4 days, when the top-dose rats consumed diets with 10,000 ppm spermine, they ate little food and became emaciated. Some rats showed aggressive behaviour and convulsions. Several died, even after the level had been decreased to 5000 ppm on day 4 (Table 3). Thereafter some stabilization in condition occurred. Towards the end of the study all survivors in the top-dose group showed abdominal distension and slight paralysis of the hind legs. Growth rate, food intake and water intake were considerably decreased in the top-dose group, but not in the lower dose groups (Table 3). Systolic blood pressure was decreased in males of the top-dose group and also in the female that survived in this group (Table 3).

In males of the top-dose group, haemoglobin concentration, packed cell volume, mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) were decreased and mean corpuscular haemoglobin concentration (MCHC), prothrombin time and thrombocyte count were increased (Table 4). Packed cell volume, MCV and MCHC showed the opposite changes in the surviving female in this group.

Clinical chemistry (Table 5) revealed statistically significant increases in plasma ASAT and ALAT activities and in inorganic phosphate and sodium levels at 5000 ppm in both sexes, and in ALP activity in females. Plasma calcium level was decreased in males of the 5000 ppm group and in the surviving female of this group, while plasma potassium concentration was decreased in males of the mid- and high-dose group groups. Fasting glucose and urea levels were increased in the top-dose males, whereas albumin and chloride levels were decreased in the surviving female of this group. In the high-dose group, the concentration test showed a considerable increase in urinary volume accompanied by a considerable decrease in density (Table 8).

The relative weights of the testes, brain, thyroid, adrenals, spleen and heart were increased and that of the liver decreased in males of the 5000 ppm group (Table 6).

Gross examination did not reveal any abnormalities that could be ascribed to the feeding of spermine. On histopathological examination, treatment-related changes were seen in the testes, kidneys, liver, heart, spleen and thymus of the 5000 ppm group (Table 7). The testicular changes consisted of considerable reduction of spermatogenesis. Mature spermatocytes were virtually absent. Spermatogenesis seemed to be arrested in one of the end stages, since the main cell type present in the tubules contained acrosome-granules. Depletion of spermatozoa was also observed in the epididymides, where hardly any mature spermatids were detected. The kidneys of the survivors showed slight enlargement of the nuclei of the proximal tubular epithelial cells. The rats that died showed slight to severe necrosis of the proximal convoluted tubules, and a small to moderate number of basophilic tubules mainly located in the outer

Table 4. Effects on haematological parameters in wk 5 of rats fed various levels of biogenic amines for 5–6 wk

Parameters	Sex	Values for rats receiving biogenic amines at dietary levels (ppm) of: 0	200	2000	10,000†
Spermine					
HB (mmol/litre)	M	8.5 ± 0.1	8.4 ± 0.1	8.8 ± 0.1	7.8 ± 0.2**
	F	9.0 ± 0.1	9.1 ± 0.1	8.9 ± 0.1	8.9
PCV (litre/litre)	M	0.438 ± 0.007	0.434 ± 0.007	0.452 ± 0.005	0.386 ± 0.006**
	F	0.434 ± 0.005	0.445 ± 0.004	0.435 ± 0.004	0.480
Thromb ($10E^9$/litre)	M	1010 ± 35	989 ± 22	989 ± 51	1236 ± 80*
	F	874 ± 28	946 ± 19	888 ± 32	788
PTT (sec)	M	35.4 ± 0.5	34.2 ± 0.7	35.1 ± 0.4	42.5 ± 1.1**
	F	34.6 ± 0.6	34.2 ± 0.6	33.5 ± 0.4	-
MCV (fl)	M	69.9 ± 1.1	69.7 ± 0.9	71.6 ± 0.7	61.4 ± 1.4**
	F	65.8 ± 0.8	66.4 ± 0.7	64.7 ± 0.7	72.7
MCH (fmol)	M	1.36 ± 0.02	1.35 ± 0.02	1.40 ± 0.01	1.24 ± 0.02**
	F	1.36 ± 0.01	1.35 ± 0.01	1.33 ± 0.01	1.35
MCHC (mmol/litre)	M	19.5 ± 0.2	19.4 ± 0.1	19.5 ± 0.2	20.3 ± 0.3*
	F	20.7 ± 0.2	20.3 ± 0.2	20.5 ± 0.2	18.5
Cadaverine					
HB (mmol/litre)	M	9.1 ± 0.1	9.1 ± 0.1	9.1 ± 0.1	9.3 ± 0.1
	F	9.0 ± 0.1	9.0 ± 0.0	9.0 ± 0.1	9.2 ± 0.0*
PCV (litre/litre)	M	0.449 ± 0.003	0.450 ± 0.006	0.456 ± 0.004	0.470 ± 0.004**
	F	0.458 ± 0.003	0.452 ± 0.004	0.449 ± 0.003	0.466 ± 0.005
Thromb ($10E^9$/litre)	M	896 ± 30	856 ± 20	861 ± 34	898 ± 38
	F	856 ± 27	878 ± 32	880 ± 20	964 ± 15*
MCHC (mmol/litre)	M	20.4 ± 0.2	20.2 ± 0.1	19.9 ± 0.1	19.7 ± 0.2*
	F	19.7 ± 0.1	19.9 ± 0.1	20.0 ± 0.1	19.7 ± 0.2

HB = haemoglobin concentration PCV = packed cell volume Thromb = thrombocytes PTT = prothrombin time MCV = mean corpuscular volume MCH = mean corpuscular haemoglobin MCHC = mean corpuscular haemoglobin concentration

†The dietary level of 10,000 ppm spermine was decreased to 5000 ppm from day 4 onwards.

‡ = not determined.

Values are means ± SEM for groups of nine or 10 rats, except for the 5000 ppm spermine group, which shows the means of four males and one female. Asterisks indicate significant differences from the corresponding control values (*$P < 0.05$; **$P < 0.01$; Dunnett's test). No other haematological analysis revealed any significant change whatsoever.

Table 5. Clinical chemistry values in plasma of rats fed various levels of five biogenic amines for 5–6 wk

Parameters	Sex	Values for rats receiving biogenic amines at dietary levels (ppm) of: 0	200	2000	10,000†
Tyramine					
ALP (U/litre)	M	268 ± 8	249 ± 7	226 ± 6**	20 ± 9**
	F	209 ± 7	213 ± 13	178 ± 11	162 ± 5**
Spermidine					
ALP (U/litre)	M	268 ± 8	—	—	2.73 ± 15
	F	209 ± 7	—	—‡	239 ± 12*
ALAT (U/litre)	M	42 ± 2	—	—	45 ± 1
	F	36 ± 2	—	—	43 ± 2*
ASAT (U/litre)	M	58 ± 2	—	—	59 ± 2
	F	59 ± 2	—	—	67 ± 3
TP (g/litre)	M	59 ± 1	—	—	59 ± 1
	F	62 ± 1	—	—	58 ± 1*
Ca (mmol/litre)	M	2.59 ± 0.03	—	—	2.49 ± 0.02**
	F	2.43 ± 0.02	—	—	2.37 ± 0.02
K (mmol/litre)	M	3.55 ± 0.08	—	—	3.18 ± 0.06**
	F	3.17 ± 0.08	—	—	3.08 ± 0.05
Spermine					
ALP (U/litre)	M	308 ± 13	320 ± 6	299 ± 11	308 ± 19
	F	280 ± 11	263 ± 13	240 ± 10	397*
ALAT (U/litre)	M	28 ± 2	29 ± 2	28 ± 1	76 ± 12**
	F	24 ± 1	27 ± 2	28 ± 2	49**
ASAT (U/litre)	M	57 ± 2	58 ± 2	56 ± 1	158 ± 22**
	F	56 ± 2	56 ± 3	57 ± 2	85**
Glucose (mmol/litre)	M	3.3 ± 0.1	3.4 ± 0.1	3.2 ± 0.1	4.1 ± 0.4**
	F	4.0 ± 0.1	3.8 ± 0.1	3.8 ± 0.1	4.7
Urea (mmol/litre)	M	6.5 ± 0.4	5.8 ± 0.3	6.4 ± 0.3	8.6 ± 0.7**
	F	9.0 ± 0.6	8.8 ± 0.2	9.4 ± 0.5	9.6
Albumin (g/litre)	M	31.5 ± 0.3	31.2 ± 0.3	31.1 ± 0.2	30.7 ± 1.4
	F	34.1 ± 0.4	34.6 ± 0.7	33.6 ± 0.3	29.9*
Inorg. P (mmol/litre)	M	2.23 ± 0.06	2.14 ± 0.05	2.05 ± 0.06	3.91 ± 0.26**
	F	2.13 ± 0.06	1.98 ± 0.05	1.88 ± 0.08*	4.36**
Ca (mmol/litre)	M	2.50 ± 0.02	2.56 ± 0.02	2.58 ± 0.02	1.03 ± 0.04**
	F	2.53 ± 0.03	2.59 ± 0.03	2.53 ± 0.02	1.45**
K (mmol/litre)	M	3.59 ± 0.07	3.54 ± 0.05	3.32 ± 0.03*	2.97 ± 0.20**
	F	3.46 ± 0.12	3.27 ± 0.05	3.41 ± 0.10	4.15
Na (mmol/litre)	M	144 ± 0.4	143 ± 0.5	143 ± 0.4	146 ± 0.6*
	F	140 ± 0.6	139 ± 0.6	140 ± 0.7	146**
Cl (mmol/litre	M	105 ± 0.8	105 ± 0.9	106 ± 0.5	104 ± 2.0
	F	106 ± 0.5	107 ± 0.8	105 ± 0.7	99**
Putrescine					
ALAT (U/litre)	M	28 ± 1	31 ± 2	32 ± 2	33 ± 2
	F	25 ± 1	28 ± 2	25 ± 1	31 ± 2*
Cadaverine					
ALAT (U/litre)	M	27 ± 2	27 ± 2	32 ± 3	39 ± 2**
	F	24 ± 1	23 ± 1	23 ± 1	28 ± 2
ASAT (U/litre)	M	51 ± 2	57 ± 2	52 ± 2	59 ± 2*
	F	57 ± 3	52 ± 1	53 ± 1	60 ± 2
K (mmol/litre)	M	3.62 ± 0.05	3.52 ± 0.04	3.64 ± 0.05	3.29 ± 0.08**
	F	3.16 ± 0.06	3.16 ± 0.08	3.16 ± 0.12	3.30 ± 0.15

ALP = alkaline phosphatase ALAT = alanine aminotransferase ASAT = aspartate aminotransferase Inorg. P = inorganic phosphate TP = total (plasma) protein

†The dietary level of 10,000 ppm spermine was decreased to 5000 ppm from day 4 onwards. The highest dose level of putrescine was 5000 ppm instead of 10,000 ppm.

‡ = not determined in the second study with spermidine.

Values are means ± SEM for groups of nine or 10 rats, except for the 5000 ppm spermine group, which shows the means and SEM of four males and one female. Asterisks indicate significant differences from the corresponding control values (*$P < 0.05$; **$P < 0.01$; ANOVA-Dunnett's test). No other clinical chemistry analysis revealed any significant change whatsoever.

cortical region. The liver had a slight to moderate decrease in glycogen content. In the heart, slight to moderate focal myocardial degeneration accompanied by mononuclear cell infiltration occurred in the high-dose rats and in one mid-dose male. The rats that died showed severe depletion of the white pulp in the spleen and, in the thymus, acute involution characterized by loss of normal architecture of cortex and medulla, and severe necrosis of lymphocytes.

Putrescine. Mean body weights, food intake and food efficiency were slightly decreased with 5000 ppm in both sexes, although the differences from the controls were statistically significant for body weights in females and for food intake in males (Table 3). Plasma alanine amino-transferase activity was slightly increased in females of the 5000 ppm group (Table 5). In the 5000 ppm group, the relative weight of the brain was significantly increased in females (Table 6).

Cadaverine. Mean body weights, food intake and food efficiency were slightly decreased with 10,000 ppm, although the differences were not always statistically significant (Table 3). Haematology (Table 4) revealed slight increases in packed cell volume and MCHC in males and in haemoglobin concentration and thrombocyte count in females at

10,000 ppm. Clinical chemistry (Table 5) revealed slightly increased plasma ASAT and ALAT activities in males at 10,000 ppm, while the plasma potassium level was slightly decreased in this group. The relative weights of the testes and brain were significantly increased in the 10,000 ppm group. Relative liver weight was decreased in the top-dose males (Table 6).

DISCUSSION

A summary of the major findings that were obtained in the acute and subacute studies with the five biogenic amines examined is presented in Table 9.

The acute studies showed that tyramine and cadaverine had a low acute oral toxicity, namely more than 2000 mg/kg body weight. Putrescine had an 'approximate LD_{50}' of 2000 mg/kg and spermidine and spermine had the lowest value of 600 mg/kg for each of the compounds.

A single iv administration of tyramine to rats (0.01–0.3 mg/kg body weight) induced a dose-related increase in systolic and diastolic blood pressure. Tyramine is known to be a vasoactive amine in humans, inducing increased blood pressure. It has been reported that excessive intake of tyramine releases noradrenaline from the sympathetic nervous system, which increases blood pressure by constricting the vascular system and stimulating the heart muscle (Askar and Treptow, 1986; Smith, 1980–81). The increase in blood pressure, known as the 'cheese reaction' in humans, may cause severe headache and may induce brain haemorrhage and heart failure (Smith, 1980–81). In the subacute study, the oral ingestion of even 10,000 ppm tyramine by the diet did not induce an increase in systolic blood pressure. This may be ascribed to the fact that under normal circumstances amines absorbed from food are rapidly detoxified by monoamine oxidases (MAOs) present in the intestine and liver. When MAO inhibitors were used, it was shown that accumulation of tyramine occurred, resulting in hypertension (Smith, 1980–81).

A single iv administration of spermidine (3 mg/kg), spermine (1 mg/kg), putrescine (10 mg/kg) and cadaverine (30 mg/kg) induced a reduction in systolic and diastolic blood pressure. A transient fall in blood pressure after iv injection of spermine (0.15 M/kg; 52 mg/kg) was reported by Tabor and Rosenthal (1956). In the oral feeding studies no decreases in systolic blood pressure were observed, except for a slight decrease in systolic blood pressure in the

Table 6. Relative organ weights of rats fed various levels of five biogenic amines for 5–6 wk

Parameters	Sex	Values (g/kg) for rats receiving biogenic amines at dietary levels (ppm) of: 0	200	2000	10,000†
Tyramine					
Liver	M	44.6 ± 0.7	45.6 ± 0.8	45.0 ± 1.2	50.2 ± 0.76**
	F	40.8 ± 0.5	40.9 ± 0.7	41.7 ± 0.8	42.8 ± 0.6
Spermidine					
Liver	M	44.6 ± 0.7	-‡	-	41.0 ± 0.9
	F	40.8 ± 0.5	-	-	38.7 ± 0.8*
Kidneys	M	6.44 ± 0.11	-	-	6.92 ± 0.13*
	F	7.19 ± 0.14	-	-	7.46 ± 0.23
Brain	M	5.88 ± 0.15	-	-	6.42 ± 0.16*
	F	9.12 ± 0.31	-	-	9.12 ± 0.21
Testes	M	10.26 ± 0.22	-	-	11.40 ± 0.44*
Spermine					
Liver	M	46.0 ± 0.6	46.3 ± 0.5	45.0 ± 0.6	40.8 ± 0.9**
	F	41.6 ± 0.7	40.4 ± 0.5	42.1 ± 0.5	45.2
Heart	M	3.73 ± 0.07	3.68 ± 0.07	3.73 ± 0.12	4.60 ± 0.10**
	F	4.11 ± 0.06	4.19 ± 0.05	4.03 ± 0.08	4.59
Spleen	M	2.12 ± 0.04	2.03 ± 0.05	2.18 ± 0.06	2.85 ± 0.37**
	F	2.28 ± 0.10	2.26 ± 0.06	2.23 ± 0.09	2.63
Thyroid	M	0.069 ± 0.006	0.063 ± 0.004	0.071 ± 0.005	0.111 ± 0.005**
	F	0.088 ± 0.005	0.098 ± 0.007	0.095 ± 0.007	-
Adrenals	M	0.165 ± 0.006	0.173 ± 0.006	0.165 ± 0.005	0.270 ± 0.028**
	F	0.279 ± 0.014	0.313 ± 0.010	0.304 ± 0.010	0.386*
Brain	M	7.43 ± 0.18	7.21 ± 0.24	7.65 ± 0.14	15.11 ± 0.97**
	F	10.47 ± 0.29	10.69 ± 0.29	10.32 ± 0.11	13.42
Testes	M	11.39 ± 0.34	11.52 ± 0.37	12.16 ± 0.24	13.98 ± 2.24
Putrescine					
Brain	M	7.21 ± 0.13	6.97 ± 0.10	6.90 ± 0.14	7.18 ± 0.14
	F	9.33 ± 0.15	9.72 ± 0.16	9.58 ± 0.20	10.30 ± 0.19**
Cadaverine					
Liver	M	39.7 ± 1.0	40.2 ± 0.6	40.9 ± 0.6	36.1 ± 0.7**
	F	38.1 ± 0.5	37.9 ± 0.6	38.2 ± 0.9	36.7 ± 0.7
Brain	M	6.46 ± 0.17	6.36 ± 0.20	6.60 ± 0.18	7.15 ± 0.11*
	F	9.13 ± 0.16	9.35 ± 0.17	9.23 ± 0.27	10.02 ± 0.21*
Testes	M	10.92 ± 0.24	10.97 ± 0.42	11.03 ± 0.32	12.17 ± 0.29*

†The dietary level of 10,000 ppm spermine was decreased to 5000 ppm from day 4 onwards. The highest dose level of putrescine was 5000 ppm instead of 10,000 ppm.

‡ = not determined.

Values are means ± SEM for groups of nine or 10 rats, except for the 5000 ppm spermine group, which shows the means and SEM of four males and one female. Asterisks indicate significant differences from the corresponding control values (*$P < 0.05$; **$P < 0.01$; ANOVA-Dunnett's test). No other organ weight value revealed any significant change whatsoever.

Table 7. Type and incidence of histopathological changes found in the kidneys and liver of rats give tyramine at dietary levels of 0-10,000 ppm and in the kidneys, liver, spleen, thymus, heart, testes and epididymides of rats given spermine at dietary levels of 0-5000 ppm for 5–6 wk

	Dietary level (ppm)							
	Males				Females			
Lesions	0	200	2000	10,000†	0	200	2000	10,000†
Tyramine								
Kidneys	(10)			(10)	(10)			(10)
Basophilic tubules	3			7	5			6
Mineralization	0			0	2			5
Liver	(10)			(10)	(10)			(10)
RES-cell aggregates and necrotic hepatocytes	2			7*	7			5
MC infiltrates	0			0	1			0
Spermine								
Kidneys	(10)	(10)	(10)	(9)‡	(10)	(10)	(10)	(8)
Tubular necrosis	0	0	0	5*	0	0	0	7***‡
Basophilic tubules	0	0	0	4*‡	0	0	0	3‡
Karyomegaly	0	0	0	3	0	0	0	1
Small proximal tubules	0	0	0	2	0	0	0	1
Increased mitosis	0	0	0	1	0	0	0	0
Proteinaceous droplets	1	2	3	0	0	0	0	0
Tubular nephrosis	7	7	4	1*	10	9	9	0***
Mineralization	0	0	1	0	10	8	10	1***
MC infiltrates	0	0	0	0	1	1	0	0
Liver	(10)	(10)	(10)	(9)	(10)	(10)	(10)	(8)
Decreased glycogen content	0	0	0	9***	0	0	0	7***
RES-cell aggregates and necrotic hepatocytes	7	6	2*	4	2	4	2	2
Periportal PMC-infiltrates	1	0	0	0	0	0	0	0
MC infiltrates	0	1	0	0	0	0	1	0
Focal hepatocellular necrosis	0	0	0	0	0	0	0	2
Spleen	(10)	(10)	(10)	(9)	(10)	(10)	(10)	(8)
White-pulp depletion	0	0	0	3‡	0	0	0	7***‡
Extra medullary haematopoiesis	2	0	1	0	1	1	0	0
Thymus	(10)	(10)	(10)	(7)	(10)	(10)	(10)	(5)
Acute involution	0	0	0	4*‡	0	0	0	4**‡
Starry sky appearance	0	0	0	0	2	0	1	0
Total epithelial proliferation	0	1	0	0	0	0	0	0
Heart	(10)	(10)	(10)	(9)	(10)	(10)	(10)	(7)
Focal myocardial degeneration	0	0	1	3	0	0	0	1
Thrombus	0	0	0	1	0	0	0	0
Testes	(10)			(9)				
Reduced spermatogenesis	0			8***				
Epididymides	(10)			(9)				
Reduced number of spermatocytes	0			8***				

RES = reticulo-endothelial cells MC = mononuclear cells PMC = polymorphonuclear cells

†The dietary level of 10,000 ppm spermine was decreased to 5000 ppm from day 4 onwards.

‡These changes were seen only in the rats that died.

Values are incidence of lesions at each dose level, with the number of organs examined in each group in parentheses. Asterisks indicate significant differences from the control values (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; Fisher's exact test).

No other organ examined from rats treated with tyramine and spermine nor any organ examined from rats treated with spermidine, putrescine and cadaverine revealed treatment-related histopathological changes. The changes observed were about equally distributed among the control and test groups or occurred in a single animal only. A few organs (the coagulating glands, seminal vesicles, spinal cord, peripheral nerve and uterus) showed no pathological change whatsoever.

top-dose spermine group; this was most probably due to malfunction of the kidneys, as evidenced by a marked decrease in concentrating ability and histopathological renal changes. The increased relative weights of the adrenals and the myocardial degeneration in the top-dose spermine group may have been related to the decrease in blood pressure. Myocardial degeneration conceivably results in decreased cardiac output, and a reduced blood pressure may lead to chronically increased adrenomedullary production of pressor substances, resulting in an increased adrenal weight.

Table 8. Volume and density of the urine concentration test conducted in wk 5 in rats fed various levels of five biogenic amines for 5–6 wk

		Values for rats receiving dietary levels (ppm) of:			
Parameters	Sex	0	200	2000	10,000'
Spermine					
Volume (ml)	M	1.9 ± 0.1	2.1 ± 0.2	1.8 ± 0.2	3.2 ± 0.5** (4)
	F	1.4 ± 0.1	1.1 ± 0.1	1.0 ± 0.1	6.2** (1)
Density (kg/litre)	M	1.070 ± 0.002	1.064 ± 0.003	1.070 ± 0.003	1.031 ± 0.007** (4)
	F	1.065 ± 0.003	1.066 ± 0.005	1.072 ± 0.003	1.016** (1)

Values are means ± SEM for groups of 10 rats (or for the number in parentheses). Asterisks indicate significant differences from the corresponding control values (*$P < 0.05$; **$P < 0.01$; ANOVA-Dunnett's test). The volume and density measurements in the studies with tyramine, spermidine, putrescine and cadaverine showed no significant differences from the corresponding controls.

Table 9. Summary of major findings obtained in acute and subacute studies with the five biogenic amines examined

Observations	Findings† obtained with:				
	Tyramine	Spermidine	Spermine	Putrescine	Cadaverine
'Approx. LD_{50}' (mg/kg)	> 2000	600	600	2000	5000
Blood pressure after iv	+	– –	– – –	– –	– –
NOAEL (ppm)	2000	1000	200	2000	2000
Mortality	0	0	+ +	0	0
Paralysis	0	0	+	0	0
Body weight	–	–	– – –	–	– –
Anaemia	0	0	+	0	0
Haemoconcentration	0	0	0	0	+
Electrolytes and/or Inorg. P	+	+	+	0	+
Impaired renal function	0	0	+ +	0	0
Histopathology					
Kidneys	0	0	+ +	0	0
Liver	0	0	+	0	0
Heart	0	0	+	0	0

'Approx. LD_{50}' = 'approximate LD_{50}' iv = intravenous administration Inorg. P = inorganic phosphate

†The findings were graded as follows: 0 = not affected; + = slightly increased or slightly affected; + + = moderately increased or moderately affected; – = slightly decreased; – – = moderately decreased; – – – = markedly decreased.

The very different dynamics of systemic exposure following iv administration *v.* dietary oral administration is probably a major reason for seeing only a slight effect with spermine and for not seeing any effect with the other biogenic amines.

Decreased body weights associated with diminished food intake and sometimes also with decreased food efficiency were observed in the top-dose group of all five biogenic amines examined. The adverse effects on body weight were most pronounced with spermine, followed by putrescine, cadaverine, spermidine and tyramine. At the next lower levels (e.g. 1000 ppm for spermidine and 2000 ppm for the other four amines), these effects did not occur. Putrescine fed to newly hatched Japanese quail at a dietary level of 2000 ppm for 9 days also had no apparent effect on body weight (Blonz and Olcott, 1978); furthermore, a combination of cadaverine (1710 ppm), putrescine (910 ppm), tyramine (910 ppm) and histamine (540 ppm) fed to rats for 32 days had no effects on growth or nitrogen utilization (Haaland and Njaa, 1989). These previous results support our own findings that levels of up to 1000 or 2000 ppm of the five biogenic amines examined do not influence growth rate. In a study with 7-day-old chickens, however, it appeared that supplementation of tyramine or cadaverine at fairly low levels (33 and 82 ppm, respectively) to basal diets with a high concentration of biogenic amines from fish meal resulted in a decrease in growth, food intake and food efficiency, suggesting a high sensitivity of young chickens to biogenic amines (Bakker, 1994).

The mortality, weight loss, decreased food and water intake, aggressive behaviour and convulsions observed in animals fed 10,000 ppm spermine in the first week are probably due to the toxicity of spermine at this high level. After this level had been decreased to 5000 ppm, there was some stabilization in the condition of the surviving animals in this group; however, at the end of the study they showed paralysis of the hind legs. The increased prothrombin time, blood glucose level, plasma ALAT and ALP activities and the relatively low plasma albumin level in high-dose males could not be related to liver damage and may have been due to the considerable decrease in food intake in these rats (Levin *et al.*, 1993; Oishi *et al.*, 1979; Schwartz *et al.*, 1973).

Spermine was the only biogenic amine that showed treatment-related decreases in red blood cell variables in males of the high-dose group. These changes in red blood cell picture might be the result of the marked disturbed renal function observed in the high-dose spermine group.

The increases in packed cell volume, haemoglobin concentration and thrombocyte counts and the decrease in MCHC value with 10,000 ppm cadaverine occurred in only one of the sexes and the differences from the controls were slight. However, at present there is no explanation for these findings and therefore they are regarded as treatment related.

In the high-dose groups with tyramine, spermidine, putrescine and cadaverine, changes occurred in one or more of the plasma enzymes related to the liver (ALP, ASAT and ALAT) together with increases or decreases in the relative weight of the liver; with spermidine a decrease in total plasma protein levels in females was also observed. As all these changes were slight and no treatment-related histopathological changes were observed in the liver, they are considered to be a reflection of changes in the metabolic function of the liver rather than indications of a hepatotoxic effect of these four biogenic amines. With spermine, the decrease in liver weight observed in males of the high-dose group may be related to the decreased glycogen content in the liver seen on microscopic examination.

The top-dose spermine treated rats clearly showed impaired renal function, as evidenced by increased urinary volume and decreased density in the concentration test, which was accompanied by histopathological changes of the kidneys, increased plasma levels of urea and creatinine and changes in plasma electrolytes. This clearly indicates that spermine is nephrotoxic to the rat. The renal lesions

observed in the animals that died during the experimental period consisted of tubular necrosis and the presence of basophilic tubules. It is commonly believed that these basophilic tubules are regenerating tubules, and the minor lesions in the kidneys of the animals that survived the experimental period are most probably related to this regeneration process. Previously, spermine has been found to be a potent nephrotoxicant when administered by ip injection to mice, rats and rabbits (Fisher and Rosenthal, 1951; Rosenthal *et al.*, 1952). Severe desquamative necrosis of the epithelial cells of the proximal convoluted tubules has been reported, which resulted in albuminuria, uraemia and death. Except for the high-dose level of spermine, the present feeding study did not reveal any signs of nephrotoxicity in rats at a dietary level of 2000 ppm (0.2%) or below. This is in line with previous oral studies in which a dietary level of 0.1% spermine fed to mice for 70 days failed to produce renal changes (Fisher and Rosenthal, 1951), whereas a level of 0.15% fed to mice for 40 days caused only slight transient proteinuria (Tabor and Rosenthal, 1956). Tyramine, putrescine and cadaverine showed no evidence of renal toxicity in the present studies, not even at the high-dose levels. Intraperitoneal injection of a single dose of putrescine (483 mg/kg) or cadaverine (525 mg/kg) also did not reveal any nephrotoxicity in mice (Tabor and Rosenthal, 1956). Tabor and Rosenthal (1956) reported that ip injection of spermidine caused proteinuria. In our study with spermidine, a slight increase in relative kidney weight occurred in the high-dose males. However, this was accompanied neither by impaired renal function nor by treatment-related histopathological changes in the kidneys. The increased kidney weights with spermidine are therefore considered to be of little, if any, toxicological significance.

The increased relative testis weights with spermidine and cadaverine and the increased relative brain weights with spermidine (males), spermine (males and females), putrescine (females) and cadaverine (males and females) in the high-dose groups are attributed to the lower body weights in these groups and the well known inverse correlation between body weight and relative testis and brain weight (Feron *et al.*, 1973).

The testes of the high-dose spermine males showed a decrease in size and absolute weight, as well as reduced spermatogenesis. Since an adequate calorie intake is required for normal spermatogenesis to progress, and considering that testicular recovery after an initial insult requires more than 4 wk to be completed (Yuan and McEntee, 1987), the testicular changes observed in the present study could be regarded as a reflection of the low food intake during the first week. However, the possibility that the testicular changes observed are a direct effect of spermine cannot be excluded.

The histopathological changes observed in the spleen (white pulp depletion) and thymus (acute involution) of the high-dose spermine rats that succumbed at an early stage of the study may have been caused by stress due to the preterminal conditional decline. A direct effect of spermine on the immune system is rather unlikely because, at dose levels of spermine showing no effect on body weight gain, no change in white blood cell picture or weight and histopathology of spleen and thymus were noticed.

In conclusion, based on the effects observed in the high-dose groups of the repeated-dose studies, it appeared that spermine is the most toxic of the five biogenic amines examined.

The only significant change observed in the mid-dose groups of all five biogenic amines occurred with 2000 ppm spermine in males and consisted of a slightly decreased plasma potassium level and myocardial degeneration, although the latter change occurred in only one of 10 animals examined. As these findings were more pronounced or occurred at a higher incidence in the high-dose group, they are assumed to be related to treatment.

On the basis of the present results, the NOAEL was 2000 ppm (180 mg/kg body weight/day) for tyramine, cadaverine and putrescine, 1000 ppm (83 mg/kg body weight/day) for spermidine and 200 ppm (19 mg/kg body weight/day) for spermine.

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