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Severe Metabolic Acidosis in the Rat Induced by Toxic Doses Of Tetracycline.* (32311)

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Adverse reactions to the tetracycline group of antibiotics have been described in increasing numbers in recent years(1). In particular, serious and frequently fatal illnesses have been reported after intravenous administration of excessive amounts of tetracycline(2-5). In most cases a typical syndrome developed, characterized by jaundice, metabolic acidosis, and azotemia. These reports have again emphasized that tetracycline may have metabolic effects which are potentially deleterious to patients. In this regard, it is not generally appreciated that tetracycline antibiotics can cause inhibition of protein synthesis in mammalian cells(6,7).

In earlier toxicology studies(8,9), the mechanism of death resulting from large doses of tetracycline was not clearly defined. Therefore, in the present investigation, studies were carried out in an attempt to delineate further the toxic effects of tetracycline and determine the mechanism of death. It was found in the rat that intraperitoneal administration of tetracycline in massive doses resulted in a severe and overwhelming metabolic acidosis and marked hyperkalemia.

Materials and methods. Female Wistar rats (Harlan Farms, Cumberland, Ind.) weighing 140-160 g were fasted 16 hours prior to study. A single injection of tetracycline hydrochloride was given, intraperitoneally, in doses of 20, 200, 300, or 400 mg/kg; rats that lived for 14 days were considered to be survivors. In other studies animals were sacrificed at 4, 8, and 16 hours after intraperitoneal injection of tetracycline and serum electrolytes determined using the Technicon Autoanalyzer. Arterial pH, carbon dioxide tension, and bicarbonate concentrations were determined by the Astrup method using a revised nomogram(10). The tissue and plasma levels of tetracycline were determined by a bio-assay method employing *Bacillus cereus* as the test organism(11). Sections of small intestine, kidney, and liver obtained from tetracycline-treated rats at necropsy were examined by light microscopy. Statistical analysis was performed using the Student's t test(12).

Results. Survival rates of rats after intraperitoneal injection of tetracycline in various dosages are shown in Table I. All of 10 rats given 20 mg/kg and 7 of 10 rats (70%) that received 200 mg/kg survived 14 days. The survival rate decreased to 44% and 38% in rats injected with doses of 300 and 400 mg/kg, respectively. Most deaths occurred within 16-24 hours after injection of tetracycline. In preliminary studies in this laboratory, survival rates were similar in nonfasted rats and in male rats.

Data on changes in serum electrolytes and blood gases after administration of 400 mg/kg of tetracycline are shown in Table II. Hematocrits and serum sodium concentrations were not affected during an 8-hour period. Blood urea nitrogen (BUN) increased from 15 $\pm$ 3 (1 S.D.) to 41 $\pm$ 7 mg/100 ml at 8 hours ($p<0.001$). Serum potassium increased from 4.8 $\pm$ 0.4 to 7.6 $\pm$ 2.4

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TABLE I. Survival Rates After Treatment with Intraperitoneal Tetracycline.

Tetracycline dosage, mg/kg	No. rats	Survivors 24 hr	Survivors 72 hr	Survivors 14 days
20	10	10 (100%)	10 (100%)	10 (100%)
200	10	7 (70%)	7 (70%)	7 (70%)
300	24	12 (50%)	11 (44%)	11 (44%)
400	24	11 (44%)	9 (38%)	9 (38%)

mEq/l ($p<0.01$) with 5 of the 11 potassium values being greater than 9.0 mEq/l. Serum carbon dioxide combining power decreased from 25 ± 2 to 13.8 ± 3 mEq/l ($p<0.02$). The presence of a severe metabolic acidosis only 4 hours after the injection of tetracycline was further corroborated by decreases in arterial pH, carbon dioxide tension, and bicarbonate levels. After 8 hours arterial pH had decreased further to 7.16 ± 0.15 units, and the bicarbonate level had remained stationary at 12.9 ± 6 mEq/l. Three of 5 arterial pH values were below 7.10, reflecting the severity of the metabolic acidosis. To exclude the possibility that the metabolic acidosis was due to hydrochloric acid and ascorbic acid contained in the tetracycline preparation, studies were carried out in which 10 rats received an intraperitoneal injection of 1.0 ml of a solution containing .1 N HCl and 0.15 g of ascorbic acid, the final pH being 1.94. These amounts of acid were equivalent to the amount found in the tetracycline preparation used. The mean arterial pH value 8 hours after injection of the acid solution was 7.34 ± .03 units which is not significantly different from the control value of 7.37 ± .02 units ($p>0.1$).

Serum tetracycline levels at various time intervals are shown in Table III. Levels decreased from a mean value of 171 ± 69 (± 1 S.D.) μg/ml at 2 hours to 81 ± 27 μg/ml at 8 hours. Tissue concentrations of tetracycline in animals dying after intraperitoneal injection of the antibiotic are shown in Table IV. Rats given 300 mg/kg and dying within 24 hours were found to have the highest concentrations of tetracycline in the kidney and liver. In rats injected with 400 mg/kg and dying within 24 hours, there were greatly increased amounts of tetracycline in all tissues with the highest levels

TABLE II. Alterations in Serum Electrolytes and Blood Gases After Tetracycline Treatment.*

	Normal values		Time after tetracycline: 4 hr		t test	P value	8 hr		t test	P value
Hematocrit, %	46	± 3† (6)	43	± 3 (6)‡	1.73	NS	43	± 2 (5)	1.87	NS
BUN, mg/100 ml	15	± 3 (6)	38	± 11 (12)	4.95	<.001	41	± 7 (10)	8.28	<.001
Serum sodium, mEq/l	145	± 3 (6)	142	± 5 (7)	1.28	NS	143	± 8 (4)	.56	NS
" potassium, mEq/l	4.8	± .4 (6)	6.6	± 1.1 (7)	3.82	<.005	7.6	± 2.4 (11)	2.814	<.02
" CO_2 c.p., mEq/l	25	± 2 (6)	14	± 4 (7)	5.88	<.001	13.8	± 3 (6)	7.619	<.001
Arterial pH, units	7.37	± .02 (5)	7.22	± .13 (9)	2.83	<.02	7.16	± .15 (5)	2.774	<.02
" pCO_2, mm Hg	37	± 6 (5)	21	± 9 (9)	3.80	<.005	28	± 10 (5)	1.77	NS
" bicarbonate, mEq/l	22	± 1 (5)	12.6	± 4 (9)	4.90	<.001	12.9	± 6 (5)	3.34	<.02

* 400 mg/kg injected intraperitoneally.
† Mean ± 1 S.D.
‡ Figures in parentheses represent number of determinations performed.
NS = Not significant.

TABLE III. Serum Tetracycline Levels After Intraperitoneal Injection of Tetracycline.*

Rat No.	Time after tetracycline 2 hr	4 hr	8 hr
	Serum tetracycline, μg/ml		
1	313	128	89
2	177	127	100
3	147	115	92
4	108	117	21
5	127	119	85
6	153	125	96
Mean ± 1 S.D.	171 ± 69	122 ± 5	81 ± 27

* 400 mg/kg.

again being found in the kidney and liver.

All of the organs examined at necropsy were grossly normal. Histologic examination of sections of small intestine revealed focal blunting of the villi and a slight increase in the number of mononuclear cells within the lamina propria. The hepatic Kupffer cells in the liver of one rat contained numerous prominent dark granules, but otherwise there were no changes in the liver as determined by light microscopy. There were no significant alterations visible in the kidneys.

Discussion. That the LD_{50} dose for rats after an intraperitoneal injection of tetracycline was about 300 mg/kg and that tetracycline was concentrated primarily in the kidney, liver, and intestinal muscosa are consistent with previous observations(9,10,13, 14). Since histologic changes were minimal, however, the mechanism of death from massive dosages of tetracycline would appear to be metabolic. In this study it was demonstrated that administration of 400 mg/kg of tetracycline to the rat results in a severe and overwhelming metabolic acidosis with decreased arterial pH, carbon dioxide tension, and bicarbonate levels. The acidosis was disproportionately severe for the degree of azotemia present. Since serum potassium levels increased significantly, it is possible that some animals succumbed to cardiac arrhythmias induced by hyperkalemia. The rapid onset of severe acidosis and hyperkalemia within 4-8 hours after toxic doses of tetracycline was impressive. These data are similar to those obtained with the antibiotic, cycloheximide, in animals; rabbits and dogs developed a severe metabolic acidosis and hyperkalemia within 6-9 hours after injection of toxic doses of cycloheximide or acetoxycycloheximide with arterial pH levels as low as 6.81 units, and serum potassium concentrations as high as 10.2 mEq/l(15). There is evidence to suggest that the alterations in blood gases and electrolytes observed in experimental animals receiving toxic dosages of tetracycline and cycloheximide are due to a similar mechanism. Both antibiotics have been shown to inhibit protein synthesis in mammalian cells(6,7,15). It is pertinent that these inhibitory effects on protein synthesis were demonstrated within 4-6 hours after administration of tetracycline(6,7).

It should be reemphasized that massive doses of tetracycline were administered to normal rats in order to cause significant inhibition of protein synthesis(6,7). In the present study the serum tetracycline levels at 2, 4, and 8 hours after administration of 400 mg/kg of tetracycline were 15-30 times therapeutic levels. The pertinence of these observations to humans, therefore, remains uncertain. Patients developing a fatal syndrome after tetracycline received this drug

TABLE IV. Tissue Levels of Tetracycline in Animals Dying Within 24 Hours After Intraperitoneal Injection of Tetracycline.

Rat No.	Tetracycline dosage, mg/kg	Tetracycline, μg/g tissue Jejunum	Heart	Kidney	Liver
1	300	174	263	686	635
2	"	510	223	500	994
3	"	770	246	568	595
4	"	313	290	1045	983
5	"	371	286	718	588
6	"	286	202	515	494
7	"	432	286	806	510
8	"	552	217	879	1086
9	"	750	213	752	707
10	"	484	282	733	780
11	"	569	282	717	386
12	"	749	278	805	820
Mean		497	256	727	690
± 1 S.D.		206	33	147	216
14	400	230	248	360	624
15	"	667	567	1420	796
16	"	562	287	1154	874
17	"	911	353	1056	816
18	"	879	291	1056	863
Mean		610	349	1009	795
± 1 S.D.		250	113	350	90

in normal to excessive amounts in dosages ranging from approximately 20-70 mg/kg (2-5), but no data on tetracycline blood levels were described. However, most of these patients developed a severe metabolic acidosis which was disproportionately severe for the degree of azotemia present. Similarly, patients with impaired renal function given tetracycline developed acidosis, hyperphosphatemia, and azotemia(16). Blood tetracycline levels in these patients were as high as 30 μg/ml. The disproportionate acidosis observed in these studies(2,5,16) does raise the question of protein synthesis inhibition. It is clear that more studies are needed in patients receiving tetracycline to determine whether significant inhibition of protein synthesis occurs.

Summary. Treatment of rats with tetracycline in a dosage of 400 mg/kg results in a syndrome somewhat similar to reported cases in humans of death following intravenous administration of large doses of tetracycline. The rats rapidly developed a severe metabolic acidosis with arterial pH levels frequently decreasing to values below 7.10 units. The acidosis was disproportionately severe for the degree of azotemia present. In addition, marked hyperkalemia frequently developed within 4-8 hours after administration of tetracycline. It is suggested that death resulted from an overwhelming metabolic acidosis or cardiac arrhythmias due to hyperkalemia.

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Mitotic Activity of Rabbit Blood Vessels.* (32312)

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Although endothelial cells of blood vessels are functionally active and subject to considerable stress, previous reports have failed to demonstrate convincing evidence of their mitotic activity or turnover in normal, adult animals.(1). Indeed, mitotic activity has not been established in any layer of the normal vascular wall. Most reported observations have been confined to morphological examination of vascular cells; but a single study by Spraragen *et al*(2) noted the absence of labeling in the normal rabbit aorta following

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