Renal Toxicity of Tetracycline Degradation Products. (29082)

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Reports on a reversible Fanconi-type syndrome observed after ingestion of improperly stored tetracycline capsules were published by Gross(1), Frimpter *et al*(2), Ehrlich and Stein(3,4), and Rosenthal (5). This subject was also summarized in an editorial of the *Journal of the Am. Med. Assn.*(6). It would be of great interest to determine whether a similar nephrotic syndrome could be produced in laboratory animals. There are 3 degradation products of tetracycline (4-epi-tetracycline,* anhydrotetracycline, and anhydro-4-epi-tetracycline) formed under the influence of heat, moisture, and low pH. No information was available as to whether one or a combination of these tetracycline degradation products may be responsible for this syndrome.

Material and methods: Rats. Materials used, supplied by Dr. N. E. Rigler, Dept. of Chemical Process Improvement, Lederle Laboratories, Pearl River, N. Y., were tetracycline hydrochloride (tetracycline), ammonium quatramycin (4-epi-tetracyline ammonium salt), anhydrodeschloroaureomycin hydrochloride (anhydrotetracycline hydrochloride), and anhydroquatrimycin hydrochloride (anhydro-4-epi-tetracycline hydrochloride). While 4-epi-tetracycline was given as the ammonium salt, tetracycline, anhydrotetracycline, and anhydro-4-epi-tetracycline were given as the hydrochlorides. All doses were calculated in terms of tetracycline base. Table I shows the experimental design of the study using 72 adult male rats (Royal Hart, Wistar strain). Renal function studies were performed following the procedures reported by Balazs *et al*(7), and by Sharratt and Frazer(8). The renal function tests performed are listed in Table II.

All rats were tested prior to dosing to insure normal kidney function. Following an 18-hour fast, the test compounds were administered as a single 1000 mg/kg dose by gavage. The renal function tests were then repeated at intervals up to 72 hours following dosing.

The animals were sacrificed by chloroform inhalation and autopsied 24 hours (Exp. I) or 72 hours (Exp. II) after dosing. They were weighed, and the kidneys were inspected *in situ,* dissected, weighed separately, and split lengthwise for inspection of cut surfaces. The left kidney was fixed in Zenker's solution. One-half of the right kidney was frozen in dry ice while the other half was reserved for determination of renal edema. The only exception to this procedure was made for the first 18 animals sacrificed after 72 hours for which half of the right kidney was fixed in Zenker's solution, and no wet-weight/dry-weight determinations were done. Wet-weight/dry-weight determinations were obtained by weighing the fresh specimens. The tissue samples were dried at 110°C for 96 hours. The samples were weighed again and the volatile matter, essentially water, was calculated as the difference and expressed in per cent.

Results. Definite changes were noted in the first experiment 24 hours after dosing only in animals that received anhydro-4-epi-tetracycline hydrochloride. These changes consisted of an increase in urinary glutamic-oxalacetic transaminase activity (UGOT). There were no pathological changes 6 or 24 hours after a single oral dose of tetracycline hydrochloride, the other degradation products, or a mixture of the 4 compounds. All values for urinary protein, urinary glucose and urinary glutamic-oxalacetic transaminase were within normal limits. These rats were sacrificed after 24 hours; no morphological changes were noticeable in the kidneys.

* In the chemical sense 4-epi-tetracycline is not a degradation product; however, for purposes of this paper it is regarded as such because it has no appreciable antibacterial activity.

TABLE I. Number of Male Rats Receiving a Single Oral Dose of Tetracycline and/or Its Various Degradation Products.

Treatment			No. of rats per group 24 hr Exp I	72 hr Exp II
Controls (H_2O) 10 ml/kg			6	12
Tetracycline hydrochloride 1000 mg/kg			6	6
4-epi-tetracycline ammonium salt 1000 mg/kg			6	6
Anhydrotetracycline hydrochloride 1000 mg/kg			6	6
Anhydro-4-epi-tetracycline hydrochloride 1000 mg/kg			6	6
Combination 500 mg/kg	Tetracycline	65 mg	6	—
	4-epi-tetracycline	10 "		
	Anhydrotetracycline	150 "		
	Anhydro-4-epi-tetracycline	275 "		

TABLE II. Renal Function Tests.

Test	Method	Normal range male rats
Urinary glutamic-oxalacetic transaminase (UGOT)	Sigma Kit* 505-OP	up to 50 Sigma-Frankel units/ml
Urine protein	Albustix®	to 100 mg %
" glucose	Tes-Tape®	0
Microscopic examination		no cellular material

* Sigma Kit 505-OP, Sigma Chemical Co., St. Louis, Mo. Albustix®, Trademark of Ames Co., Inc., Elkhart, Ind. Tes-Tape®, Trademark of Eli Lilly Co., Indianapolis, Ind.

In the second experiment the rats were observed for 72 hours and urinalyses were done 24, 48, and 72 hours after dosing. Results are presented in Table III. Minor changes were noted in 2/6 rats dosed with 4-epi-tetracycline ammonium salt 24 hours after dosing and in 1/6 animals dosed with anhydrotetracycline hydrochloride 72 hours after dosing.

The UGOT values for the animals dosed with tetracycline hydrochloride, 4-epi-tetracycline ammonium salt, and anhydrotetracycline hydrochloride were consistently within the normal range (0-50 Sigma-Frankel units) for this strain of rats in our laboratory. Although statistically significant differences were found in this particular experiment, these findings were not considered indicative of renal damage. This conclusion was substantiated by the post-mortem studies using ordinary light microscopy.

However, marked functional changes were noticeable in most animals at 24, 48, and 72 hours following administration of anhydro-4-epi-tetracycline hydrochloride. These changes consisted of proteinuria, glucosuria, cellular debris in the urine sediment, and markedly elevated UGOT activity. At 24 hours after dosing, 2 animals of this group had high levels of UGOT activity (195 and 1310 Sigma-Frankel units). At 48 hours after dosing the UGOT activity of these 2 rats was reduced to the normal range. At this time 3 additional animals revealed high UGOT activity. At 72 hours after dosing the UGOT activity of these 3 rats had returned to the normal range. The high UGOT activity for a relatively brief period can be explained by the fact that necrotic cellular material containing glutamic-oxalacetic transaminase in the mitochondria [Boyd(9), Borst (10), and Segal *et al*(11)] was washed out with the urine. The damage found in the kidneys of rats in these studies consisted of necroses of the tubular epithelium as a result of which cellular contents (including GOT) were liberated from necrobiotic cells and carried out in the urine. Once the tubular epithelial enzyme had been excreted in the urine, the UGOT values returned to the normal range.

Morphological findings in the kidneys of rats 72 hours after a single dose of 1000

TABLE III. Results of Urinalysis in Rats 24, 48, and 72 Hours After a Single Oral Dose of 1000 mg/kg of Tetracycline and/or Its Degradation Products.

Values in ( ) = ranges

Treatment	No. of rats	24 hr after dose			48 hr after dose			72 hr after dose		
		UGOT Sigma-Frankel units/ml	Protein (mg %)	Glucose (%)	UGOT Sigma-Frankel units/ml	Protein (mg %)	Glucose (%)	UGOT Sigma-Frankel units/ml	Protein (mg %)	Glucose (%)
Control (H_2O)	12	17.2 (7–27)	Trace–30 in 12/12	0 in 12/12	19.5* (16–25)	Trace–30 in 12/12	0 in 12/12	22.8 (16–30)	30 in 12/12	0 in 12/12
Tetracycline hydrochloride	6	30.5† (20–42)	Trace–30 in 6/6	0 in 6/6	27.3‡ (23–30)	30 in 6/6	0 in 6/6	31.0 (18–43)	Trace–30 in 5/6 30–100 in 1/6	0 in 6/6
4-epi-tetracycline ammonium salt	6	36.2§ (9–48)	<30 in 1/6 30–100‖ in 5/6	0 in 4/6 0.1 in 2/6	20.3‡ (19–22)	Trace–30 in 6/6	0 in 6/6	25.3 (22–28)	30 in 6/6	0 in 6/6
Anhydrotetracycline hydrochloride	6	34.5† (19–43)	30 in 6/6	0 in 6/6	21.3‡ (19–26)	Trace–30‖ in 6/6	0 in 6/6	23.7 (20–28)	Trace–30 in 5/6 30–100 in 1/6	0 in 6/6
Anhydro-4-epi-tetracycline hydrochloride	6	271.5§ (14–1310)	30–100‖ in 3/6 300 in 1/6 1000 in 2/6	0 in 4/6 2.0 in 2/6	165.8† (27–395)	300–1000‖ in 6/6	0.25 in 1/6 2.0 in 5/6	25.5 (15–35)	30 in‖ 2/6 100–300 in 4/6	0 in 1/6 0.25 in 1/6 2.0 in 4/6

* 6 animals only.
† Significantly different from controls (rank test $P \leq 0.01$).
‡ 3 animals only.
§ Significantly different from controls (rank test $P \leq 0.05$).
‖ Cellular material in urine sediment.

TABLE IV. Summary of Morphologic Findings in Kidneys of Rats 72 Hours After a Single Dose of 1000 mg/kg of Tetracycline and Its Degradation Products.*

Treatment	Control	Tetracycline hydrochloride	4-epi-tetracycline ammonium salt	Anhydro-tetracycline hydrochloride	Anhydro-4-epi-tetracycline hydrochloride
No. of animals	12	6	6	6	6
Body wt (g)	252 (192–348)	252 (170–342)	253 (177–318)	259 (177–346)	241 (156–321)
Absolute kidney wt (mg)	2304 (1829–3123)	2276 (1670–2911)	2484 (1786–3047)	2411 (1833–3611)	2683 (2329–3032)
Relative kidney wt (mg/100 g)	929 (825–1057)	922 (847–1075)	989 (934–1029)	944 (792–1054)	1174† (931–1637)
Water content (%)	77.9 (77.1–79.0)	77.1 (76.3–77.2)	78.0 (77.4–79.0)	78.0 (77.2–78.0)	81.4† (80.0–82.3)
Gross findings	Not remarkable 12/12	Not remarkable 6/6	Not remarkable 6/6	Bilateral hydronephrosis 1/6 Not remarkable 5/6	Brownish red color with nutmeg appearance, renal cortex slightly swollen, line of demarcation between cortex and medulla not distinct 6/6
Degree of tubular necroses	Not remarkable 12/12	Not remarkable 6/6	Not remarkable 6/6	Not remarkable 6/6	Marked 2/6 Moderate 2/6 Slight 1/6 None 1/6

* Values in ( ) = ranges. † Significantly different from controls (rank test $P \leq 0.05$).

mg/kg of tetracycline hydrochloride or its degradation products are summarized in Table IV. Anhydro-4-epi-tetracycline hydrochloride was the only compound producing toxicity under these conditions. It caused renal edema and various degrees of cortical, tubular necroses which were grossly recognizable. The changes consisted of severe sloughing of remnants of the necrotic epithelium (Fig. 1, 2).

Material and methods: Dogs. The studies in dogs were limited to administration of tetracycline hydrochloride, anhydrotetracycline hydrochloride, and anhydro-4-epi-tetracycline hydrochloride to one dog each because of the limited amount of the 2 degradation products that could be prepared from the same batch of tetracycline hydrochloride used in this study. Saline was used to prepare solutions for intravenous injections which were adjusted with 4 N NaOH to a pH of 8.9-9.4. The compounds were administered as a single daily dose for 2 days. Rate of injection was 2.5 mg/kg/min keeping the volume of injection constant at 16 ml/dog for the first dose of 10 mg/kg. The second dose, administered on the following day, was 20 mg/kg using 32 ml/dog as the volume of injection. The animals were observed for physical appearance during the intravenous administration and at several intervals thereafter. Urine was obtained by catheterization 6 hours following dosing for the first 2 days and analyzed for the presence of protein (Combistix®), glucose (Benedicts Reagent), and occult blood (Hematest®).† On subsequent days voided urine was collected in a container which was placed in dry ice. These samples were analyzed only if no vomitus was present as a contaminant.

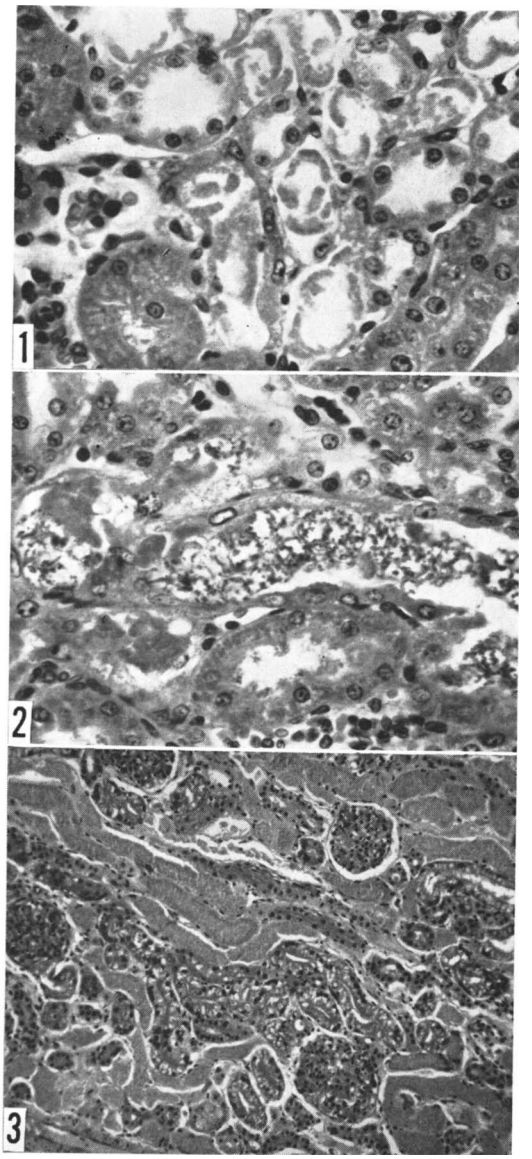
Only one animal which was moribund 4 days after the last injection was sacrificed and autopsied. The 3 body cavities were inspected *in situ* and their contents dissected *en bloc*. Representative samples of all major organs were fixed in suitable fixatives and paraffin sections were stained with hematoxylin and eosin.

Results. The 2 animals given either tetracycline hydrochloride or anhydrotetracycline hydrochloride were apparently in good health,

† Combistix® and Hematest® are registered tradenames of Ames Co., Inc., Elkhart, Ind.

were not sacrificed, and are still alive. The dog given anhydro-4-epi-tetracycline hydrochloride had frequent episodes of emesis during 80 hours after the second dose (20 mg/kg). The urine of this animal was very dark and gave a strongly positive result for occult blood up to 6 hours following each intravenous dose. The analyses for glucose and protein were negative in all of the samples except those that contained large amounts of hemoglobin. This animal was found moribund 96 hours after the second dose. The blood urea nitrogen (B.U.N.) at this time was greater than 70 mg%. The normal range for dogs of our colony is 10-20 mg%.

The most striking post-mortem changes in this dog were found in the kidneys. They were approximately of the same size (weight of both kidneys, 37 g). The capsules stripped with ease revealing a yellowish-tan surface with a fine, whitish reticulated pattern on a grayish background. The renal cortex was swollen and the cut surface bulged. The cortex was light yellowish-tan and showed prominent, linear, red striae apparently corresponding to cortical, radial blood vessels. Immediately beneath the cortex at the cortical medullary junction the renal substance was slightly sunken and showed marked, reddish, linear, radial discoloration; it was otherwise not remarkable. The renal medulla was rather pale; otherwise not remarkable. The microscopic examination of the kidneys revealed a rather uniform picture. All the convoluted tubules in the renal cortex showed severe damage. Many of these tubules were devoid of lining epithelium. This had apparently become necrotic, sloughed, and been transformed into amorphous granular material which completely filled the lumina of these tubules (Fig. 3). In other places the nuclei were still present in the tubular epithelium but were pyknotic and distorted. In some cases nuclear fragmentation was apparent; in other cases the nuclei appeared to be intact but the cytoplasma of the cells showed vacuolation and granulation. In almost every tubule, whether or not an



remnants of necrotic epithelium following one oral dose of 1000 mg/kg of anhydro-4-epi-tetracycline. H + E, magnification × 180.

FIG. 2. (Rat K 81, Kidney). Early stage of epithelium necrosis of proximal convoluted tubules with few disintegrating nuclei. A more advanced type of necrosis at the left center of the tubules where the lumen is filled with protein material and remnants of nuclear material. Dosage as in Fig. 1. H + E, magnification × 180.

FIG. 3. (Dog L 2737, Kidney). Most of the tubuli completely filled with necrotic, granular material; regressive vacuolization of tubular cytoplasm in other tubuli. Bowman's spaces almost completely obliterated by swollen glomerula. Anhydro-4-epi-tetracycline was given intravenously at a dose of 10 mg/kg on Day 1 and 20 mg/kg on Day 2. H + E, magnification × 105.

FIG. 1. (Rat K 81, Kidney). Some proximal convoluted tubules containing sloughed, hyalinized

epithelium was present, the lumen was filled with granular particulate matter or completely occluded by marked swelling of the epithelium. There appeared to be some relative sparing of the distal convoluted loop but otherwise the damage was rather uniform. There was considerable swelling of the glomerular tufts so that Bowman's space was almost completely obliterated. The glomeruli were otherwise not remarkable. In the renal medulla the collecting tubules were seen, in many cases, to contain casts of granular and hyaline material. The epithelium of the collecting tubules did not appear abnormal, however. The renal pelvis showed some foci of submucosal edema; it was otherwise not remarkable.

Discussion. The cases of the Fanconi-type syndrome reported in man by Gross(1), Frimpter *et al*(2), Ehrlich and Stein(3,4), and Rosenthal(5) were not fatal and the functional derangements disappeared. No morphological changes have been demonstrated. A common feature of the reported cases was the ingestion of improperly stored tetracycline capsules prior to appearance of symptoms. This leads to a presumptive causal relationship but is far from being conclusive proof that the capsules were an etiologic factor in the disease.

The preliminary animal studies reported above indicate that the morphologic concomitant of this syndrome is a necrotic process of the convoluted tubuli in the renal cortex. The ability of the renal tubule to regenerate and to recover function, if the damage is not so extensive as to be rapidly fatal, is well known. Of the degradation products studied only anhydro-4-epi-tetracycline hydrochloride caused tubular damage in rats and in one dog. It is notable that this compound at a lower dosage (275 mg/kg) in combination with the others had no harmful effects in the rat. It appears probable that the degree of functional impairment and morphologic damage is a function of the dose and the duration of administration of anhydro-4-epi-tetracycline.

Summary. The toxic effects of 3 degradation products of tetracycline: 4-epi-tetracycline, anhydrotetracycline, and anhydro-4-epi-tetracycline were studied in rats and dogs. Anhydro-4-epi-tetracycline was the only degradation product which caused abnormal urinary findings similar to the Fanconi-type syndrome observed in man after ingestion of improperly stored tetracycline preparations. Severe, nephrotic changes were found in the kidneys of rats after very large oral doses (1000 mg/kg/day) and in dogs after intravenous doses of 10 and 20 mg/kg respectively on successive days.

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