

tumor growth was noted in this study. The failure of antiprolactin to influence metastases may represent a quantitative problem. This view is suggested by noting a lesser incidence of metastases in hypophysectomized rats treated with both prolactin and antiprolactin than in those receiving only the former. It seems unlikely that this effect resulted from a neutralization of prolactin at the injection site by antiprolactin since these agents were administered at different time intervals. It is apparent that an effective tumor-inhibiting dose of antiprolactin in intact rats would be at least greater than twice the dose necessary to induce mammary atrophy.

*Summary.* Neither prolactin nor antiprolactin affected the incidence or size of hepatic metastases in intact rats following the intraportal injection of Walker tumor cells. These failures were unrelated to the mammary changes induced by this hormone or its antibody. On the other hand, antiprolactin moderately inhibited the tumor growth-

promoting effect of prolactin in hypophysectomized rats. This suggests that one factor concerned with the failure noted in intact members may be related to the quantity of antibody required to inhibit tumor growth when endogenous prolactin secretion is maintained.

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### Effect of Tetracycline on Intestinal Absorption of Various Nutrients By the Rat.\* (31490)

S. D. J. YEH AND M. E. SHILS

*Division of Experimental Surgery and Physiology, Sloan-Kettering Institute for Cancer Research,  
Memorial Hospital and Cornell University Medical College, New York City*

Tetracycline administered to rats has been found to decrease incorporation of amino acids into the proteins of many tissues including those of the gastrointestinal tract(1). Other inhibitors of protein synthesis such as puromycin and acetoxycycloheximide(2) have been reported to decrease fat absorption in rats. This study was undertaken in order to determine the effect of tetracycline on the absorption of fat, fatty acid, and the water-soluble nutrients, ferrous ion, D-xylose, and vitamin B<sub>12</sub>.

*Materials and methods.* Adult rats were given tetracycline HCl (TC) i.m. at 250

mg/kg body weight in 2 injections at an interval of 16 hours. Controls received an equal volume of saline adjusted to pH 2 with N/10 HCl. A single dose of each test substance (in the amount and solvent stated for each experiment) was given by stomach tube 2 hours after the second TC injection. The experiment was terminated at the time indicated in the pertinent table by ether anesthesia with blood obtained by cardiac puncture. Various organs were removed and dissolved in aqueous 30% KOH. Unless stated otherwise, the entire G.I. tract was removed and dissolved with its luminal contents. Radioactivity was measured in a well-type scintillation counter and xylose by a colorimetric method(3). When <sup>131</sup>I-con-

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TABLE I. Effect of Tetracycline (TC) on Triolein Absorption.\*

TC (mg/kg)	Radioactivity recovered (% initial dose)				
	Blood/ml	Thyroid (total)	Liver (total)	G.I.	
				Total wall	Lumen contents
0 (6)†	.190 ± .010‡	.268 ± .056	1.97 ± .17	3.91 ± .32	63.2 ± 2.7
250 (7)	.116 ± .009§	.047 ± .009§	.67 ± .07§	3.76 ± .36	79.2 ± 5.4§

\*  $^{131}\text{I}$  triolein in 1 ml corn oil fed by stomach tube 2 hr after last injection of TC and rats sacrificed 6 hr later.

† No. in parentheses is number of animals used.

‡ Mean ± S.E.

§  $P < .05$  as compared to control group.

taining compounds were to be given, thyroid uptake was blocked by previous intragastric administration of 2 ml of aqueous KI (1 or 10 mg/ml). Food was withheld from the time of the first TC injection until time of sacrifice with the stated exception of a 4-day iron absorption study; water was given *ad lib*.

**Results. Lipid absorption:** When  $^{131}\text{I}$  triolein or oleic acid was fed intragastrically, the TC-treated rats were found to have significantly lower radioactivity as compared to

controls in certain organs such as whole blood, liver and thyroid (Table I; Fig. 1). The concomitant finding that the  $^{131}\text{I}$  activity in the intestinal contents was higher in the treated rats than in the controls was further evidence for decreased absorption of the labeled lipids as a result of TC. The differences between groups persisted for many hours (Fig. 1). The effect of the antibiotic on fat absorption was so marked that one could differentiate fat-fed rats receiving TC from the controls simply by inspecting the plasma for turbidity. Six hours after administering 2 ml of corn oil intragastrically, the optical density of plasma measured at  $620\text{ m}\mu$  was  $0.31 \pm 0.09$  for TC-treated as compared to  $1.09 \pm 0.18$  for controls.

**Absorption of water-soluble nutrients:**  
**Iron:** The distribution of  $^{59}\text{Fe}$  after being given as ferrous sulfate either intragastrically or i.p. was measured in TC-treated and control groups. When the intestinal route was involved, the radioactivity in blood, liver and kidneys was markedly reduced in the TC group both at 1 and 4 days after feeding. These findings correlated well with higher residual activity in the G.I. tract and its contents (Table II). When  $^{59}\text{Fe}$  was given parenterally, the TC group had somewhat more labeled iron in blood and kidneys; there were no group differences in liver and G.I. tract activity (Table 3).

**Xylose:** 150 mg of this carbohydrate was administered intragastrically and urine was collected in the following 6-hour period. TC-treated rats were found to excrete significantly more xylose than did the controls ( $73 \pm 6.8$  mg as compared to  $49 \pm 6.3$  mg).

**Vitamin B<sub>12</sub>:** No significant differences

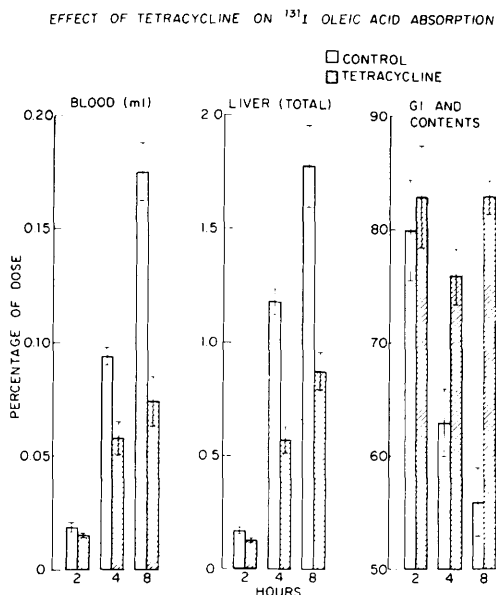


FIG. 1. Effect of tetracycline (TC) on  $^{131}\text{I}$  oleic acid absorption and distribution. Radioactivity (as % of intragastrically administered dose) was measured in blood, liver and GI tract in treated (250 mg TC/kg given i.m. twice at an interval of 16 hr) and control rats. 2  $\mu\text{C}$  of oleic acid in 2 ml of corn oil were given 2 hr later and rats sacrificed after fat feeding at times indicated. Vertical line = mean ± S.E.

TABLE II. Effect of Tetracycline on Iron Absorption and Distribution.\*

TC (mg/kg)	Duration (days)	Radioactivity recovered (% initial dose)			
		Blood/ml	Liver (total)	Kidneys	Absorbed¶
0 (6)†	1	.22 ± .03‡	1.24 ± .14	.09 ± .008	20.4 ± 4.4
250 (6)	1	.08 ± .01	.46 ± .05§	.05 ± .005	9.8 ± 1.3§
0 (5)	4	2.47 ± .30	5.12 ± .91	.45 ± .066	61.6 ± 4.1
250 (6)	4	.46 ± .07	1.64 ± .21	.17 ± .015§	32.1 ± 4.4

\* 20  $\mu\text{g}$   $\text{Fe}^{++}$  as sulfate with 0.5  $\mu\text{c}$   $^{59}\text{Fe}$  given intragastrically 2 hr after last TC dose and rats sacrificed 1 or 4 days later.

†,‡,§ Footnotes as in Table I.

|| P < .01 as compared to control group.

¶ Fed—(G.I. contents + feces).

TABLE III. Effect of Tetracycline on Tissue Distribution of Parenteral Iron.\*

TC (mg/kg)	Blood/ml	Radioactivity recovered (% initial dose)		
		Liver (total)	Kidneys (total)	G.I. (total)
0 (6)†	.99 ± .12‡	19.3 ± .3	1.21 ± .05	21.9 ± .6
250 (7)	1.61 ± .22§	19.9 ± .8	1.55 ± .09	22.1 ± .8

\* 10  $\mu\text{g}$   $\text{Fe}^{++}$  as sulfate with 0.25  $\mu\text{c}$   $^{59}\text{Fe}$  i.p. 2 hr after last TC dose. Animals sacrificed 24 hr later.

†,‡,§,|| Footnotes as in Tables I and II.

TABLE IV. Effect of Tetracycline on  $^{57}\text{Co}$  Vitamin  $\text{B}_{12}$  Absorption.\*

TC (mg/kg)	Blood/ml	Radioactivity recovered (% initial dose)		
		Liver (total)	Kidneys (total)	G.I. (total)
0 (6)†	.04 ± .005‡	2.40 ± .19	4.51 ± .33	80.7 ± 1.2
250 (6)	.04 ± .004	3.28 ± .38	3.85 ± .37	79.3 ± 2.6

\*  $^{57}\text{Co}$  vitamin  $\text{B}_{12}$  20  $\text{m}\mu\text{g}$  and 40  $\text{m}\mu\text{c}$  given intragastrically 2 hr after last TC dose and rats sacrificed 8 hr later.

†,‡ Footnotes as in Table I.

were observed in the distribution of  $^{57}\text{Co}$   $\text{B}_{12}$  between the two groups (Table IV).

Sections of jejunum obtained from both groups of rats 6 hours after intragastric administration of 1 ml of corn oil were examined microscopically. Hematoxylin-eosin stained sections revealed no atrophy of the villi or alteration in epithelial cells in either group. There were no significant differences between the two groups in the amounts of fat present in the epithelial cells of Sudan-stained frozen sections. The TC group appeared to have somewhat more sudanophilic material in the lamina propria than did the controls.

*Discussion.* These results indicate that tetracycline in the dosages utilized causes impairment of fat and iron absorption without hindering the intestinal transport of xylose and vit  $\text{B}_{12}$ . The marked differences in the patterns of absorption of these nutrients in-

dicate that the antibiotic did not produce generalized damage of small bowel mucosa; otherwise, all nutrients would have been similarly affected. This view is supported by the histologic evidence of normal villi and epithelial cells in the TC-treated rats. The impaired fat and iron absorption is attributable to either intraluminal alteration or to interference in specific mechanisms of transport. Of the possible intraluminal factors which must be considered, that of pancreatic insufficiency appears unlikely, inasmuch as oleic acid absorption (which does not require lipase action) was as depressed as was that of triolein. Since TC is known to improve fat absorption in the "blind loop syndrome" in various species(4), it is unlikely that this antibiotic interfered with micelle formation which appears to be of importance in fat absorption(5). Alteration in intestinal flora

may affect absorption. While the drug was given i.m., significant bacterial alterations may have occurred in the relatively short period of these experiments. It has been observed that germ-free animals have impaired absorption and utilization of iron(6) and increased xylose absorption(7).

Tetracycline is capable of chelating with various multivalent ions including ferrous and ferric(8), and such chelates may either be poorly absorbed or may be more rapidly excreted following absorption than the free forms. The findings with parenterally administered  $^{59}\text{Fe}$  (Table III) rule out increased excretory loss as the result of TC administration, but the effect on iron absorption is not known.

In considering interference with specific transfer mechanisms across the gut wall, it has been suggested that certain inhibitors of protein synthesis inhibit fat absorption by interfering with  $\beta$ -lipoprotein synthesis in the mucosal cells(2). TC may exert its effect by a similar mechanism since the dosages given are capable of inhibiting amino acid incorporation into proteins of the G.I. tract(1). However, we did not observe the accumulation of fat in epithelial cells described to have occurred with the other inhibitors of protein formation(2). Since i.m. TC causes local irritation with fluid accumulation, the possibility must be considered that hypovolemia occurred which could have depressed intestinal lymph and blood flow with con-

sequent malabsorption. However, this hypothesis seems improbable since decreased intestinal perfusion should have impaired absorption of all nutrients; furthermore, severe mechanical trauma in the limbs of rats did not impair fat absorption (unpublished data).

*Summary.* Tetracycline administration to adult rats (250 mg per kg i.m.) was associated with impaired intestinal absorption of fat (as  $^{131}\text{I}$  triolein) fatty acids (as  $^{131}\text{I}$  oleic) and radioactive iron. The jejunal mucosa of the treated animals was microscopically normal and without lipid accumulation. D-xylose absorption appeared to be increased as the result of tetracycline treatment since urinary excretion was significantly greater than that of control. Vitamin B<sub>12</sub> absorption was not affected. Possible mechanisms accounting for this differential effect are discussed.

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### Comparative Effectiveness of Glucose and Sucrose in Enhancement of Hypersalimentionation and Salt Hypertension.\* (31491)

C. E. HALL AND O. HALL

*University of Texas Medical Branch, Galveston*

Rats prefer drinking certain sugar solutions to water(1) and become hypertensive when given saline to drink(2-4). Consequently, the addition of several sugars in 5% concentration to a 1% saline solution increases the quantity of fluid and electrolyte consumed, and increases severity of salt hypertension in these

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animals(5,6). Although sucrose, fructose and one of two samples of maltose each were found to share this property, sucrose has thus far been the most efficacious(7,8). Curiously, the incorporation of honey is without effect upon either saline consumption or induction of hypertension(8). Since the ability of sugars to increase saline consumption is not a function of their inherent "sweetness"(8),