THE INFLUENCE OF A VIRAL ENTERIC INFECTION ON ABSORPTION OF CHLORTETRACYCLINE

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ABSTRACT

THE INFLUENCE OF A VIRAL ENTERIC INFECTION ON ABSORPTION OF CHLORTETRACYCLINE

by Larry J. Wallace

Research was conducted using 18 pigs assigned to the following groups: (1) control, (2) control plus chlortetracycline, (3) transmissible gastroenteritis (TGE) virus-infected, and (4) TGE virus-infected plus chlortetracycline.

Signs of TGE were present within 12 hours after infection. The disease was characterized by clinical signs, leukopenia, and gross and microscopic lesions.

The virus of TGE had an inhibitory effect on absorption of orally administered chlortetracycline (CTC) on the first two days of treatment. On the third day of treatment, CTC blood levels of infected pigs approximated those of control pigs.

Microscopic lesions in infected, CTC-treated pigs included regeneration of intestinal epithelium on the third and fourth days. Infrequently, small areas resembling proliferation were observed in the intestinal epithelium during and after the third day in infected pigs which did not receive CTC.

Absorption of CTC appears to be involved in an active transport system.

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By

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Sincere thanks are given to the technical staff and animal caretakers in the Department of Pathology who helped in many ways during this research project.

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The author is most sincerely thankful and indebted to his wife, Eileen, for her continual patience, understanding,

and encouragement throughout the research, and for her assistance in the preparation of this thesis.

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INTRODUCTION

Most drugs and other therapeutic agents are evaluated in experiments using the normal animal. It is rather obvious that in practice these products are not used in the normal animal but are used by the clinician in the treatment of a specific disease process. Therefore, it is of importance to have information on the metabolism of drugs during the course of a specific infection. This more closely approximates the information of interest to the clinician.

Probably of most importance is the role of the gastrointestinal tract in the metabolism, espécially the absorption, of the orally administered drugs. Information on the influence of a specific lesion of the gastrointestinal tract on the absorption of a drug would not only be of value for the product tested but this information could be extrapolated and applied in evaluating other drugs.

This study was designed to determine the influence of a specific viral infection (transmissible gastroenteritis) and the resulting lesions on the absorption of chlortetracycline (CTC). This information would have wide application in clinical medicine as well as basic biomedical interest.

REVIEW OF LITERATURE

Interest in the absorption and distribution of chlortetracycline (CTC) throughout the body is indicated by the large amount of material which has been published on this subject. In preparing this literature review, only those references which seemed pertinent to serum and/or blood levels of CTC have been cited. Although much has been published on the general subject, very little has been published on CTC absorption in swine. Information regarding the influence of a specific enteric infection on absorption of CTC is limited.

In one report on swine (Maddock et al., 1953) the highest concentration of CTC in the serum was 0.83 mcg./ml. which resulted from an oral dose of 4.5 mg./lb. Lepper et al. (1949) reported that, when the drug was given orally, the highest concentrations were obtained in the majority of individual patients at the third and a few at the sixth hour. Drill (1958) mentions that following oral administration of the tetracyclines, peak concentrations after a single dose are reached in about 2 to 4 hours and decrease slowly during the succeeding 12 to 24 hours. Jones (1957) reported that the tetracyclines are absorbed readily from the stomach and first part of the small intestine to give a peak plasma level

within 2 to 4 hours in carnivorous animals. Putman et al. (1953) compared serum concentrations between tetracycline and chlortetracycline after 4 oral doses of 0.5 Gm. of each drug every 6 hours. In their study there appeared to be a gradual increase in the serum concentration of both drugs when multiple doses were given. Whitlock and co-workers (1950) gave children single oral doses of 11 mg./kg. and 22 mg./kg. body weight. They found the peak level was attained slowly somewhere between 1 and 4 hours after drug ingestion. This peak level was low, but was maintained at a relatively constant value until 6 and sometimes 8 hours after ingestion. These same investigators stated that the absence of progressing or marked cumulation of Aureomycin in the serum after prolonged oral administration, together with the failure of increased oral dosage to produce significantly higher serum levels, suggests some limiting factor in the ability of the gastrointestinal mucosa to absorb the antibiotic. In a study by Gray et al. (1953), dogs were given CTC orally at a dosage of 5, 10 and 25 mg./kg. body weight. At the end of 4 hours the mean blood serum concentrations in micrograms per ml. were 0.1 (0.1 - 0.2), 0.8 (0.1-2.0), and 2.2 (0.8 - 5.0) respectively. They state that variability emphasizes the unreliability of such determinations as indices of CTC absorption from the gastrointestinal tract of the dog. Brainerd et al. (1951) reported that, following a 250 mg. oral dose in humans, concentrations of CTC in serum reached a peak at 2 hours followed by a decrease at 4 and 6 hours. In this

same study it was found that at 6 hours there was relatively little difference in serum concentration between oral doses of 250 mg. and 1 Gm. Therefore, they concluded that there is a maximal rate of absorption from the gastrointestinal tract which can not be exceeded by increasing the dose four-fold. Ibsen and Urist (1962) state that metal ions alter the absorption of CTC. They also state that tetracycline and related compounds form complexes with calcium and other metal ions and are deposited in all newly calcifying tissue and can be used to label the growing skeleton.

Sirota and Saltzman (1950) found that 70% of Aureomycin is bound to plasma components and, of that, approximately 85% is albumin-bound. As the free (therapeutically active) component of the drug is dissipated by excretion and metabolism, more of it is liberated from the protein-aureomycin complex. They state that it is these characteristics which explain the prolonged therapeutic blood levels and relatively high urine concentrations obtained following a given dose of the drug. Malek and Kolc (1960) studied tissues by fluorescence using ultraviolet light and found there can be no doubt that inflammatory hyperemia makes possible increased penetration of CTC into the tissue and increased resorption of CTC by the lymphatic system. They believed that the increase of CTC in the lymphatic vessels is probably made possible by the binding of CTC on the proteins of the inflammatory exudate, which causes CTC to be more lymphotrophic. An additional factor is probably stasis of the lymphatic system in the areas of the inflammation.

In vitro studies have indicated that CTC is most stable near pH 2 (Dornbush and Pelcak, 1948). The serum concentrations observed in chickens following oral administration of penicillin, Aureomycin, and terramycin were much lower than those that have been reported in man and other animals (Smith, 1954).

Harrell and Heilman (1949) found that, regardless of the duration of treatment in patients who were given 750 mg. every 6 hours, the maximum concentration of CTC in serum demonstrated by their technique was about 8 mcg./ml. They report this would indicate that the rate of excretion is rather constant and that repeated administration of 750 mg. every 6-8 hours does not result in any "piling up" of CTC in the serum.

Eisner *et al.* (1953) using guinea pigs found that aureomycin concentration in the serum was not directly proportional to the dose. They also found that when the same doses were administered each day for nine consecutive days, higher serum levels were found than with the single doses.

EXPERIMENTAL PROCEDURE

Objectives.

1. To determine the blood levels of CTC treated pigs after inducing a specific infection of the gastrointestinal tract.
2. To compare the blood levels of CTC in infected pigs with noninfected control pigs.
3. To compare these results (under 1 and 2) with information found in the literature.
4. To observe the clinical and pathological effects of a viral enteric infection in the pig.

Housing and Care. Baby pigs were the experimental animals used in this study. Two litters of third-generation specific pathogen-free (SPF) Yorkshire pigs born August 1, 1963, were purchased from a local swine farm on August 9, 1963, and brought to the Department of Pathology's Disease-Free Laboratory at Michigan State University. These pigs were given a liquid iron compound orally by the owner at three days of age to prevent anemia. No infection or gross abnormality was present in the pigs or dams of either litter at the time of purchase. The pigs were allowed to become accustomed to a controlled environment and a milk ration for 3 days.

The two litters of 8-day-old pigs were assigned to 4 groups (Table I) by random numbers according to Goulden (1956). Group I (Controls) consisted of 4 uninfected pigs given no CTC. Group II consisted of 4 uninfected pigs given CTC. Group III consisted of 5 infected pigs given no CTC. Group IV consisted of 5 infected pigs given CTC.

The infected (Groups III and IV) and uninfected (Groups I and II) pigs were housed in two separate isolation rooms under similar environmental conditions. Temperatures in each room were maintained at 82 F. The pigs were placed in individual galvanized metabolism cages to allow recording of feed and water consumption* and clinical observation of each pig. Rectal temperatures were taken daily at 8 a.m. Feed and water were offered to the pigs in crocks. A separate caretaker was assigned to the pigs in each isolation room as a precaution against spreading infection.

Ration. Pigs were fed pasteurized, homogenized, whole milk fortified with 400 USP units of Vitamin D obtained from the Michigan State University Dairy. The first two days the pigs were offered 100 ml. of milk per feeding. This was increased to 130 ml. per feeding on the third day and kept at this level throughout the experiment. Feeding was done at 8 a.m., 12 noon, and 4 p.m. Any milk left over from the previous feeding was measured and recorded. The pigs had

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*Feed and water consumption values are approximate due to spillage and evaporation.

access to water at all times. At 8 a.m. and 4 p.m., the amount of water remaining from the previous period was measured and recorded and a fresh 300 ml. placed in each clean crock.

Infective Agent. Transmissible gastroenteritis (TGE) virus* was used as the infective agent. Virulence of the original sample was determined by methods employed by Keahey (1963). Virus material was maintained at -20 C, delivered to the Disease-Free Laboratory on experimental day 0 and allowed to thaw at room temperature. Immediately after thawing, 2 ml. of virus material** were added to 10 ml. of whole milk and given to each pig in the assigned groups. All pigs consumed the virus-bearing milk within five minutes except pig 14. The milk was administered to this pig orally with a dose syringe.

Antibiotic. The antibiotic selected was crystalline chlortetracycline (CTC).+ Dosage was 1 mg./50 Gm. live weight, before each morning feeding.++ Weights of the pigs were taken each morning at 8 a.m., prior to feeding. The

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*Original sample obtained through the courtesy of Dr. E. O. Haelterman, School of Veterinary Science and Medicine, Purdue University, Lafayette, Indiana.

**Virus material was given to assigned groups of pigs at 8 a.m. on August 12, 1963.

+Chlortetracycline (Aureomycin), supplied by the Agricultural Division, American Cyanamid Company, Princeton, New Jersey.

++Chlortetracycline administration to the assigned groups of pigs began at 8 a.m. on August 13, 1963.

assigned dose of CTC was weighed out on a Mettler Balance and placed in size 00 gelatin capsules. Oral administration of gelatin capsules containing CTC was by hand or by balling gun.

Tissue Analysis. Blood samples were collected from the anterior vena cava, as described by Carle and Dewhirst (1942), using heparinized 10 ml. luer-lok syringes with 1½ inch, 20 gauge needles. The samples were taken each day at 8 a.m., 10 a.m., and 2 p.m., with the 8 a.m. bleeding being prior to the administration of CTC. Ten ml. of blood were collected at each bleeding except at 8 a.m. when 11 ml. were taken of which 1 ml. was utilized for hematologic study. The 10 ml. of blood for CTC assay were placed in heparinized, screw-cap tubes and quick-frozen in dry ice within 4 minutes. Hematologic studies included hemoglobin (Hb), packed cell volume (PCV), total and differential leukocyte counts. At the termination of the experiment the blood samples for CTC assay were packed in dry ice and sent to Dr. L. A. Shor*, for microbiological assays. These were conducted according to the modified FDA cylinder-plate methods of Abbey and Hewel (1962).

Pathologic Procedures. At 2 p.m. on experimental day 1 and each day thereafter except experimental day 4, 1 pig was selected from each group for necropsy. These pigs

*Assays were done through the courtesy of the Agricultural Division, American Cyanamid Company, Princeton, New Jersey.

were euthanatized with Lethol.* On experimental day 4, necropsies were performed on all the remaining pigs. These pigs were euthanatized by exsanguination (severing the axillary blood vessels). Necropsies were performed immediately after euthanasia. Pigs chosen for necropsy on the first, second and third days from the infected Groups III and IV were those showing the most severe clinical signs. Pigs were chosen from the uninfected Groups I and II at random. Immediately prior to death, the pigs were weighed and terminal blood samples were taken for hematologic study and CTC assay. Tissue sections of approximately 2 cm. in length were taken from the fundic region of the stomach, duodenum, jejunum, ileum, and spiral colon. The sections were collected at the same site for all pigs. The tissues were placed in Zenker's fixative for 24 hours, washed for 24 hours, and stored in 80% ethyl alcohol until processed for embedding in paraffin blocks. Sections were cut at 5 microns and stained with Harris' hematoxylin and eosin. In addition periodic acid-Schiff (PAS) stain was used, when necessary, to demonstrate mucus and fibrinoid material. All histopathological procedures used are described in the Manual of Histologic and Special Staining Technics of the Armed Forces Institute of Pathology, Washington, D.C. (1957).

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* Lethol, Pitman-Moore Company Allied Laboratories, Division of The Dow Chemical Company, Indianapolis, Indiana.

RESULTS

The details of the results are given in tables in the appendix. The average body temperature, feed and water consumption are given in Table II. The weight changes are recorded in Table III, chlortetracycline blood values in Tables IV and V, amount of chlortetracycline given to each pig in Table VI and hemogram studies in Tables VII and VIII.

Clinical Signs

Definite clinical signs of TGE were present in all infected pigs (Groups III and IV) after a 12-hour incubation period. Body temperatures remained in the normal range for all groups of pigs (Table II). This parallels the findings of Reber and Whitehair (1955), who found that TGE infection did not influence the average body temperature. Prominent signs were vomiting in a few infected pigs, and diarrhea in all of them. Vomiting persisted in some of the infected pigs for 48 hours. Diarrhea persisted throughout the experiment in all infected pigs. Feces of Group III pigs (infected, non-treated) were watery with blood-tinged mucous strands and varied from light greenish-yellow to light brown in color. Feces from the Group IV pigs (infected, treated) were similar in consistency and color to those of the pigs in

Group III until the third day. At that time they had more consistency with a brown color and remained that way throughout the experiment. The rear quarters and tails of all infected pigs were pasted with a fetid, yellowish-brown fecal material. No vomiting or diarrhea occurred in the non-infected pigs. Anemia became apparent in all pigs (Tables VII and VIII) as the experiment progressed. Dehydration became apparent in the infected pigs as evidenced by a gaunt appearance and dry skin (Fig. 1). Dehydration was more severe in Group III than in Group IV. This was noticed especially during and after the third day when the latter group showed signs of recovery in the way of alertness, activity, increasing milk consumption and decreasing water consumption (Table II). The water consumption of Group IV remained essentially within the amounts consumed by the uninfected groups as compared to Group III which had an evident polydipsia especially on days 1, 3, and 4 (Table II). Partial anorexia was evident in both infected groups during the first 2 days after infection. All groups experienced a slight weight gain during the experiment. To keep the weight gains from being misleading they were calculated as the difference between the terminal and day -1 weights. This kept the expected initial weight loss due to environmental change from influencing the net results (Table III).

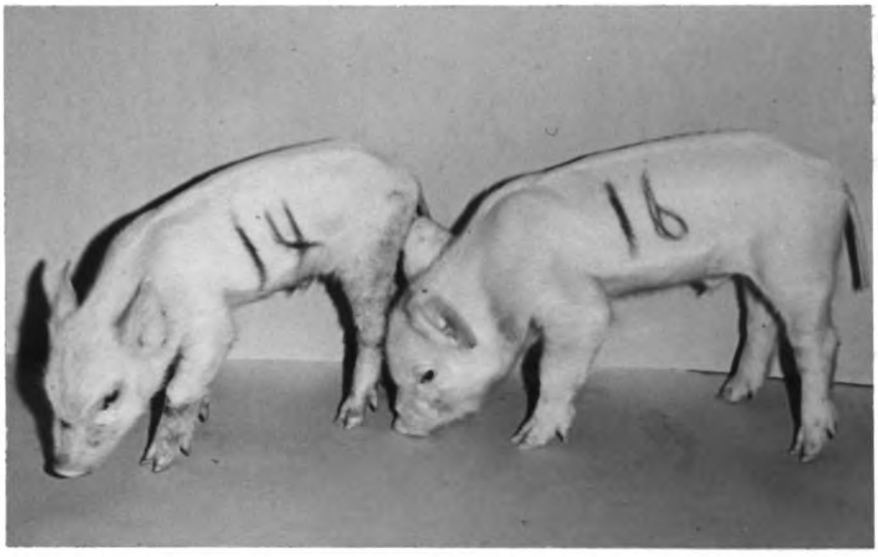

Fig. 1. Pigs 14 (TGE infected) and 16 (Control) on day 2. Pig 14 shows signs of dehydration and has fecal material adhering to the extremities and rear quarters.

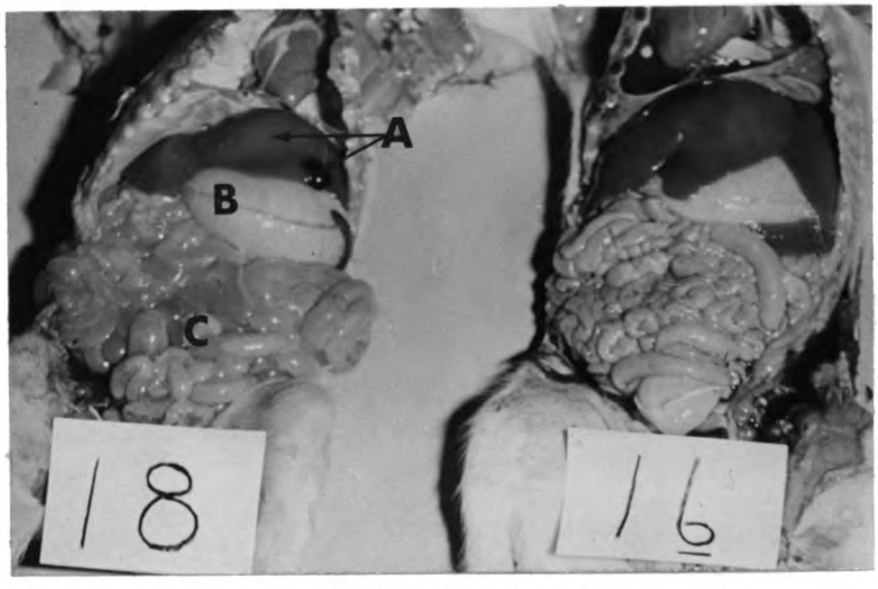

Fig. 2. Pigs 18 (infection plus antibiotic) and 16 (Control) on day 2. In pig 18, note the greatly distended gall bladder with dark bile and a liver slightly lighter in color than pig 16 (A). Stomach distended with gas and slightly congested blood vessels over the greater curvature (B). The intestinal tract is distended with gas (C). Pig 16 has normal appearing viscera.

Gross Pathology

All pigs had some skin abrasions from cage trauma, and pale mucous membranes as evidence of anemia. Carcasses of uninfected pigs were in a normal condition of flesh. Carcasses of infected pigs were in gaunt to emaciated condition and were dehydrated, especially in Group III. Carcasses of Group IV pigs appeared less dehydrated during and after the third day. At the time of euthanasia the stomachs of all uninfected pigs contained a normal milk curd undergoing digestion except pigs 7 and 13 whose stomachs contained a more particulate curd. The colons of all uninfected pigs contained yellow, pelleted feces. Pigs in the infected groups all showed typical lesions of TGE (Fig. 2) in varying degrees of severity that resembled the lesions reported by Doyle (1958), Smith (1956) and Runnells *et al*. (1960). Not all of the following lesions were necessarily observed in each pig.

Gastrointestinal tract. The stomach was distended with gas, and in some pigs there was moderate congestion over the greater curvature of the stomach especially noticeable in the serosa. The stomach contained solid to cheesy, bile-stained, undigested, food material, in some instances adhering to the gastric mucosa. A mild to moderate catarrhal gastritis was present. Pig 8 (Group IV) had an ulcer, 1 cm. in diameter, near the squamoglandular junction in the esophageal region of the stomach. The pyloric sphincters

were relaxed to strongly contracted. The entire intestinal tracts were flatulent and showed mild to moderate serosal and mucosal congestion. Atony was especially prominent in the jejunum and ileum. Intestinal contents had a foamy, watery to mucoid consistency, and were clear to yellowish or greenish-brown in color.

In some pigs blood-tinged mucus was mingled with the ingesta from the jejunum posteriorly. On the third and fourth days no blood was present in the intestinal contents of Group IV pigs and the intestinal contents were soft in consistency and brownish in color.

Other prominent gross lesions. The mesentery and mesenteric lymph nodes were mild to moderately congested and edematous. In several of the infected pigs the gall bladder was slightly to markedly distended with a dark colored bile. Moderately hemorrhagic areas were present in the subcutaneous tissues and musculature of all (infected and uninfected) pigs at the bleeding sites. The livers and kidneys of most pigs were pale in appearance.

Histopathology

Lesions in the infected pigs paralleled those reported by Bay, Doyle, and Hutchings (1951). However, due to the nature of this experiment, the histopathology of the gastrointestinal tract has been recorded, as there were obvious differences between the two infected groups (III and IV) of pigs. These differences were noted especially at the

latter phase of the trial. Not all the lesions were necessarily observed in each pig.

Stomach. Hypersecretion of mucus, coagulation necrosis, erosion, desquamation and in some areas, reduction in height of the gastric epithelial cells were observed (Fig. 3). A constant finding was generalized congestion with endothelial nuclei in some of the larger blood vessels of the tunica muscularis appearing enlarged. There was patchy distribution of fibrin in the lamina propria, predominantly around small blood vessels immediately below the epithelial basement membrane. The lamina propria was edematous and contained an increase in neutrophils, lymphocytes, plasma cells and occasionally a few macrophages. Lymphocytic infiltration into the tunica mucularis was observed in some pigs. Division figures did not appear to be present in excessively high numbers in the mucosal and glandular epithelium.

Duodenum. The most outstanding lesion was the congestion of all blood vessels, especially at the tip of the villi (Fig. 4). Pathologic alterations were not seen in the mucosal epithelial cells in any pig. The lamina propria was edematous and contained an increase in neutrophils, lymphocytes, plasma cells and occasionally macrophages. The same inflammatory cells mentioned above were present in fewer numbers in the submucosa.

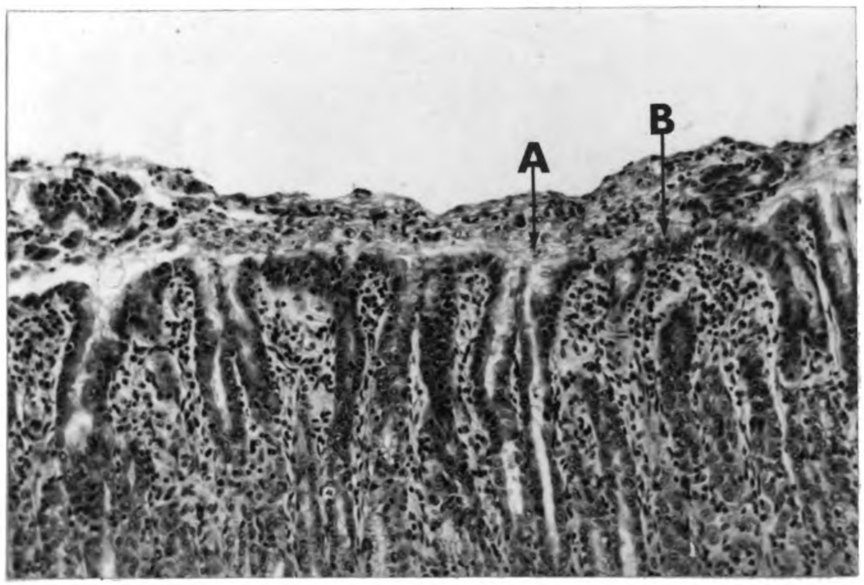

Fig. 3. Stomach from a Group III (TGE virus) pig on day 3. Hypermucous secretion (A), desquamation and erosion (B) in the gastric mucosal epithelium, with leukocyte infiltration in the lamina propria. H.& E. stain; x 101.

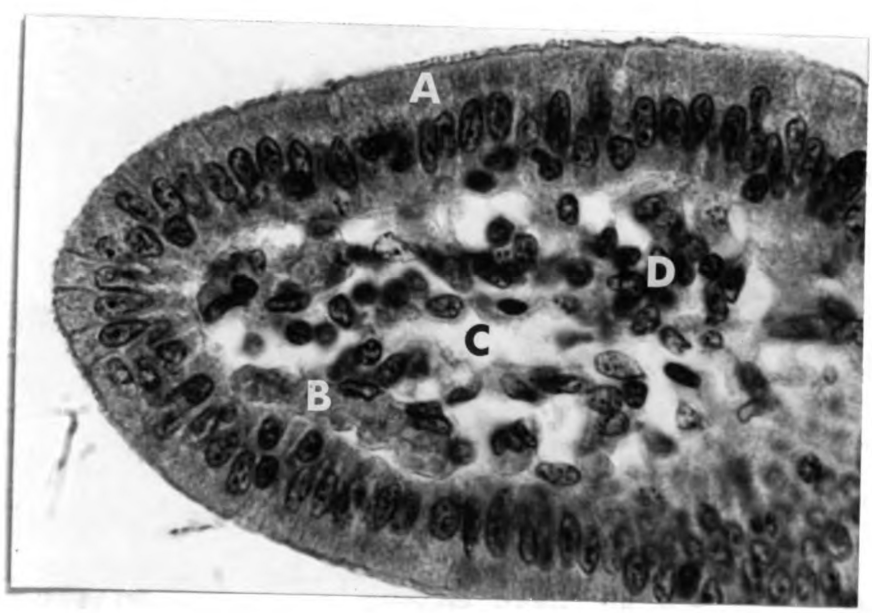

Fig. 4. Duodenum from a Group III (TGE virus) pig on day 3. Normal mucosa with brush border (A). Slight congestion of blood vessels (B), edema (C), and leukocyte infiltration (D) in lamina propria. H. & E. stain; x 507.

Jejunum and Ileum. The most severe lesions were observed at this level of the intestinal tract and the greatest difference in the two groups of infected pigs were evident in this tissue. The lumen contained desquamated epithelial cells, mucus, erythrocytes and fibrin strands. There was a marked reduction in height of the mucosal epithelium with coagulation necrosis, desquamation, and erosion. Goblet cells, although present in the crypts, were almost entirely absent in the surface epithelium. The lamina propria was edematous, had small hemorrhagic areas, and contained increased neutrophils, lymphocytes, macrophages and plasma cells along with several areas of necrosis. The same cells mentioned above, although in fewer numbers, appeared in the submucosa and tunica muscularis. Necrotic debris was observed in the lumens of several glands. An outstanding and constant finding was in the terminal portion of the villi, immediately under the basement membrane, where large accumulations of fibrin were observed around the small blood vessels (Fig. 5 and 6). Some of the blood vessels appeared thrombosed. Numerous pyknotic nuclei were found in the mucosal and glandular epithelium. Small foci of neutrophils along with degenerating leukocytes and nuclear debris were present in the reaction centers of the lymph nodules in Peyer's patches (Fig. 7). A marked difference between the infected groups (III and IV) became evident on the third day. In pig 8 of Group IV, areas suggesting proliferation of mucosal epithelial cells were observed along with an increase

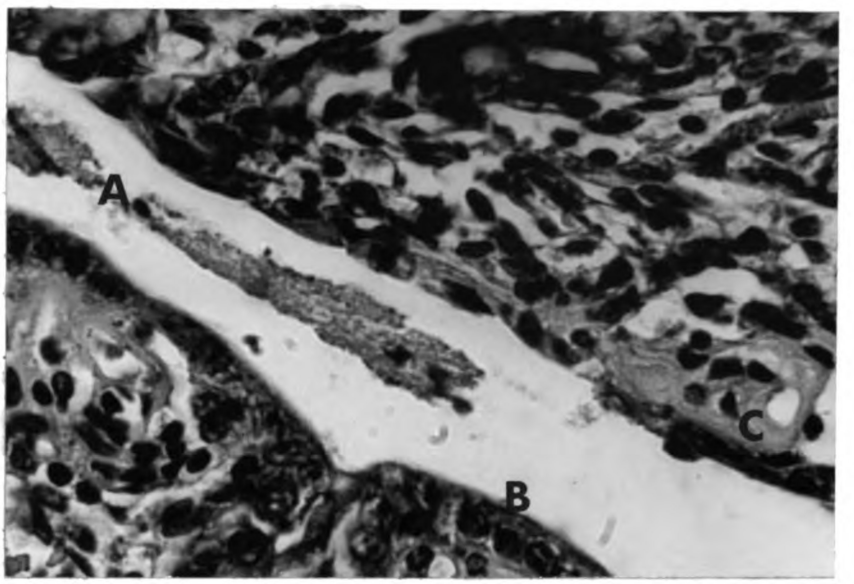

Fig. 5. Jejunum from a Group III (TGE virus) pig on day 4. Lumen contains cellular debris, leukocytes and fibrin (A). Marked reduction in height of epithelium (B), with fibrin and leukocytes around a small blood vessel (C). H. & E. stain; x 507.

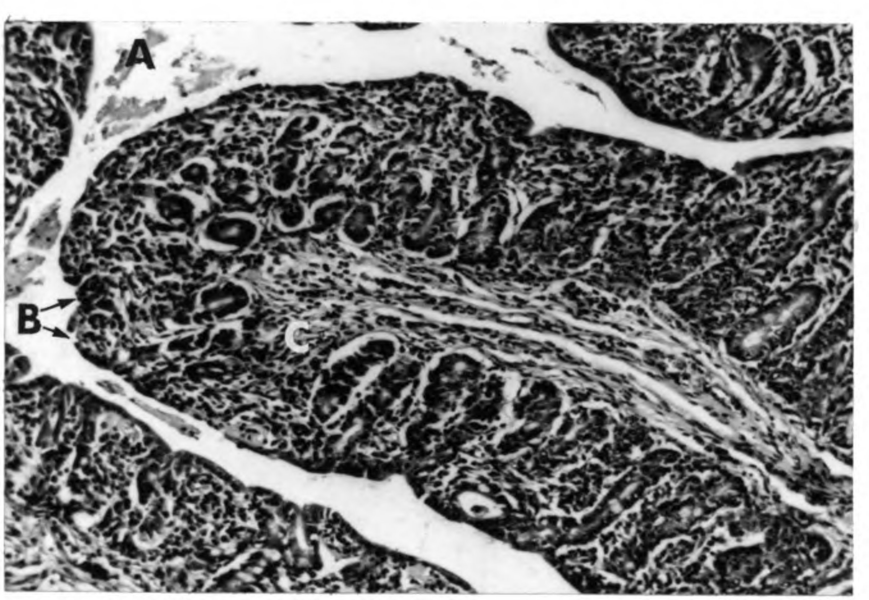

Fig. 6. Jejunum from a Group III (TGE virus) pig on day 4. Erythrocytes, fibrin and leukocytes in the lumen (A), erosion and necrosis of epithelium (B). Leukocyte infiltration in lamina propria (C). H. & E. stain; x 101.

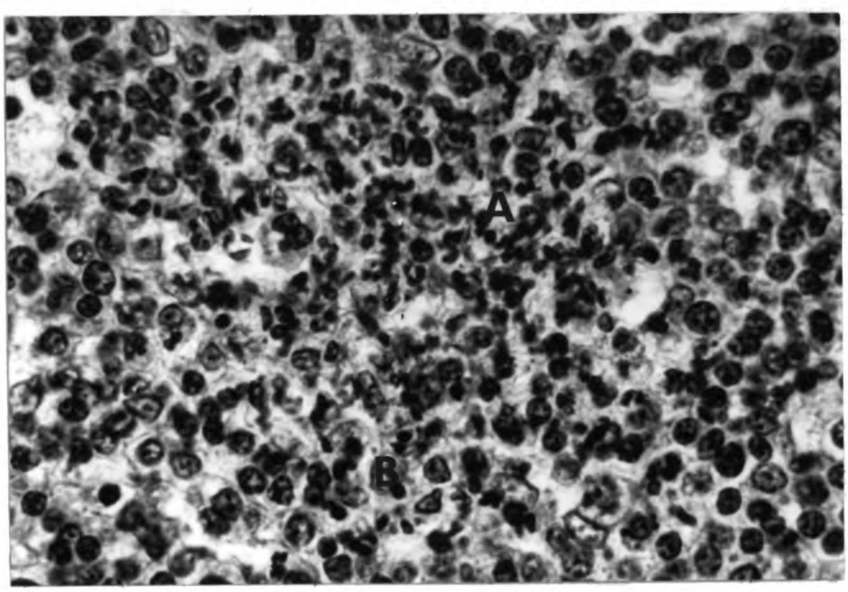

Fig. 7. Peyer's patch from an infected pig on day 4 showing a focus of neutrophils and nuclear debris (A), and degenerating leukocytes (B). H. & E. stain; x 507.

in the number of goblet cells and an increase to almost normal height of the intestinal epithelium. These changes were also found on the fourth day in pigs 10 and 15 (Group IV, infection and antibiotic) with slight improvement over the third day (Fig. 8 and 9). Small areas resembling proliferation were infrequently found in the surface mucosal epithelium of Group III pigs.

Colon. During the first two days there was very little alteration of the mucosal epithelium. On the last two days of the experiment, changes similar to those in the jejunum and ileum were observed but with less severity. The lumen contained desquamated epithelial cells, necrotic debris, leukocytes, erythrocytes, fibrin strands and mucus. Epithelial mucosa was reduced in height, eroded and contained areas of coagulation necrosis. Throughout the entire period of infection there was moderate edema in the lamina propria and infiltration with increased numbers of neutrophils, lymphocytes, macrophages and occasionally plasma cells. All blood vessels were moderately to severely congested. The pathologic changes in the colon were, in most cases, patchy in distribution.

Inclusion bodies were not seen in any part of the gastrointestinal tract.

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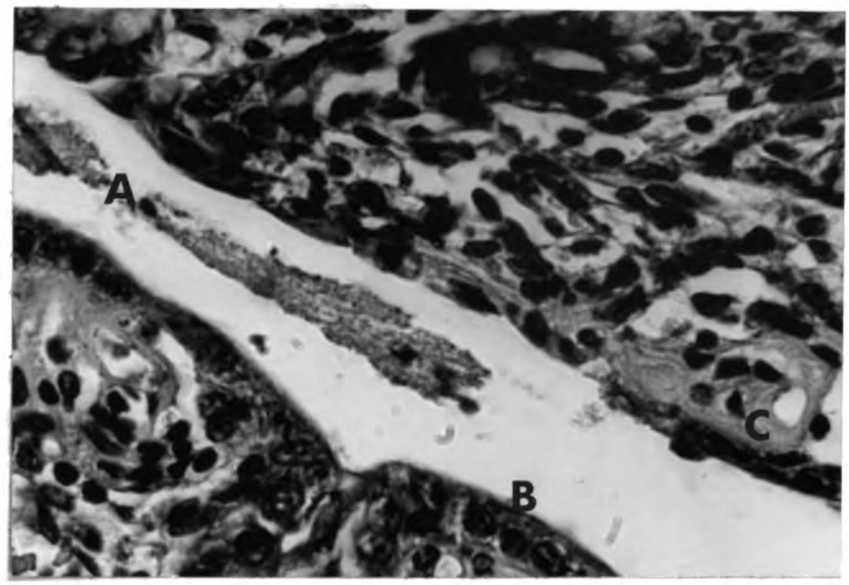

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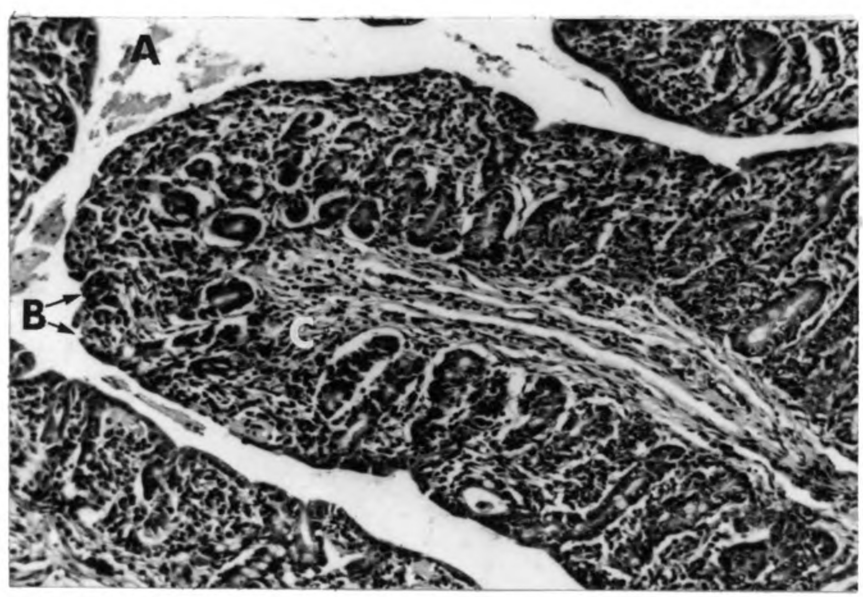

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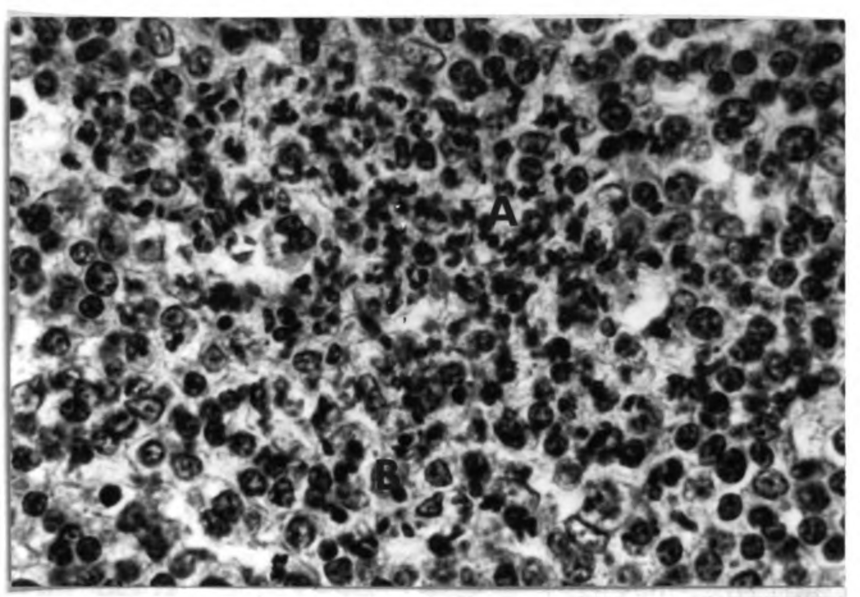

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Colon. During the first two days there was very little alteration of the mucosal epithelium. On the last two days of the experiment, changes similar to those in the jejunum and ileum were observed but with less severity. The lumen contained desquamated epithelial cells, necrotic debris, leukocytes, erythrocytes, fibrin strands and mucus. Epithelial mucosa was reduced in height, eroded and contained areas of coagulation necrosis. Throughout the entire period of infection there was moderate edema in the lamina propria and infiltration with increased numbers of neutrophils, lymphocytes, macrophages and occasionally plasma cells. All blood vessels were moderately to severely congested. The pathologic changes in the colon were, in most cases, patchy in distribution.

Inclusion bodies were not seen in any part of the gastrointestinal tract.

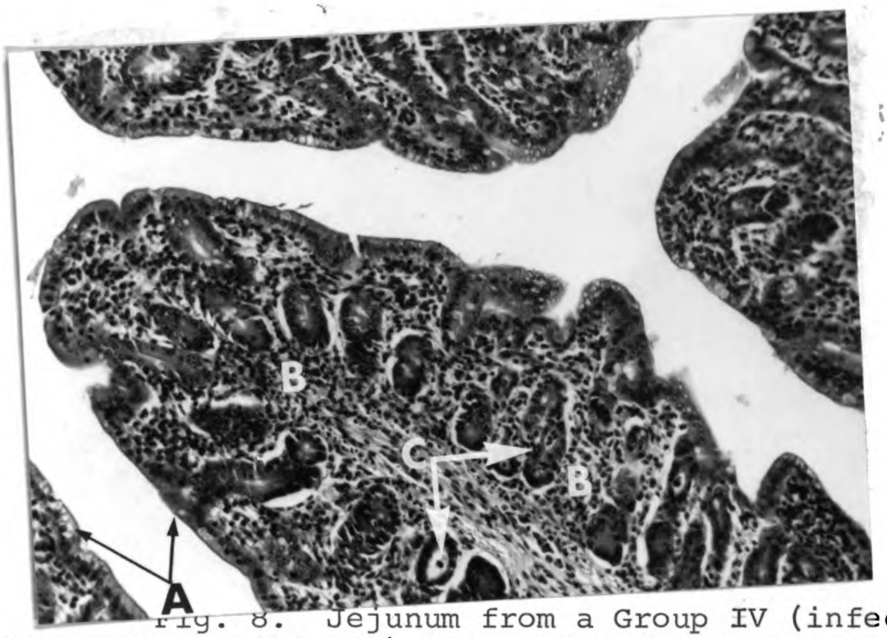

Fig. 8. Jejunum from a Group IV (infection plus antibiotic) pig on day 4. Increase in height of mucosal epithelium with increasing numbers of goblet cells (A). Leukocyte infiltration into the lamina propria (B). Necrotic debris in the lumen of some glands (C). H. & E. stain; x 101.

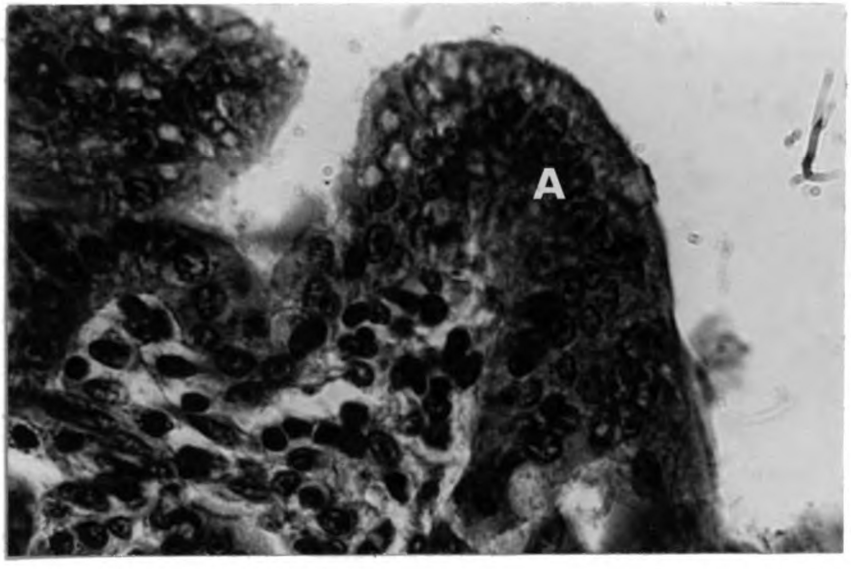

Fig. 9. Jejunum from a Group IV (infected plus antibiotic) pig on day 4 with an area of proliferation of mucosal epithelial cells (A). H. & E. stain; x 507.

Tissue Analysis

Chlortetracycline (CTC) Blood Values. Results from this aspect of the experiment gave some interesting differences between Groups II (CTC, no TGE virus) and IV (CTC, plus TGE virus) (Fig. 10 and Tables IV and V).

1. Group II: Peak CTC blood values were reached between the second and sixth hours after oral administration of the antibiotic. A build-up of CTC was evidenced by higher peak levels each day. A marked drop in CTC blood level from the 2-hour bleeding on one day to the 0-hour bleeding of the following day is clearly evident. Measurable levels of CTC were still present in the blood 24 hours after oral administration. It is of interest to note that the daily amount of CTC in the blood did not correspond to an increasing daily dose of CTC to individual pigs within the group, as is illustrated in pigs 17 and 13 on days 3 and 4 respectively (Table VI).
2. Group IV: This group of pigs presented an entirely different result. High peak CTC blood levels were not reached initially. For the first 26 hours after oral CTC administration the blood levels continued to rise at a low level as compared to Group II. The first decrease in CTC blood level occurred between the 2- and 6-hour bleedings on the second day and remained the same until the 0-hour bleeding on the third day. On day 3, the CTC blood level increased

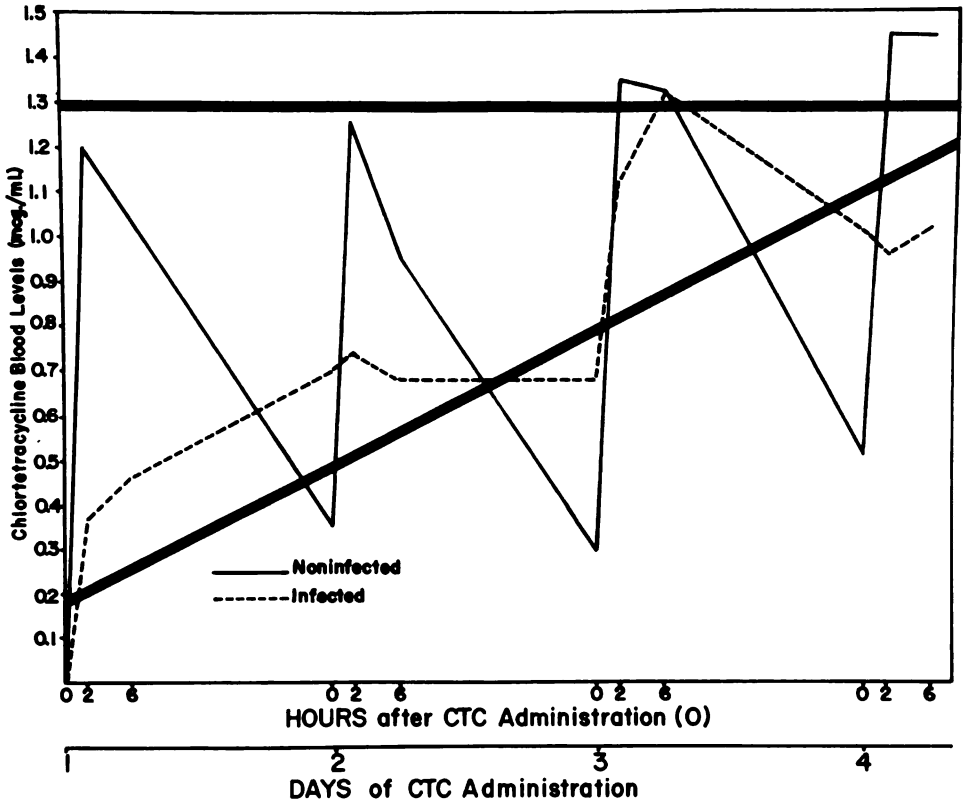

Fig. 10. Average chlortetracycline (CTC) blood levels of infected and non-infected pigs at 8 a.m., 10 a.m., and 2 p.m., recorded on the graph as 0, 2, and 6 hours, respectively. Horizontal (wide, dark) line above illustrates the constant high-peak CTC blood levels and the increasing peak blood levels on succeeding days in the noninfected pigs. Diagonal (wide, dark) line illustrates the progressive increase of CTC blood levels in the infected pigs.

progressively until it almost equalled that of the Group II pigs at the 6-hour bleeding. By use of the Rank Sum Test (Walker and Lev, 1953), there was no difference in CTC blood levels between Groups II and IV on day 3. From the 6-hour bleeding of day 3 to the 0-hour of day 4, the CTC blood level in this group decreased more than it had between any previous bleeding periods. It is difficult to understand why the CTC blood level decreased between the 0- and 2-hour bleedings on day 4; however, it did begin to increase between the 2- and 6-hour bleedings. Pig 18 was omitted from the determinations (Fig. 10) because of having only one measurable CTC blood level prior to euthanasia (Table V).

Hematology

To facilitate evaluation of the results, the hemograms of noninfected pigs (Groups I and II) were averaged together as were the hemograms of the infected pigs (Groups III and IV) (Tables VII and VIII). A pronounced anemia occurred during the experiment as indicated by the low Hb and PCV values. Hemoglobin and PCV values were higher in the infected pigs from about the second through the fourth days, which can be attributed to the pigs' dehydrated condition. Hemoglobin values have been previously reported which were higher in pigs infected with TGE than in noninfected pigs (Reber and Whitehair, 1955). Nucleated erythrocytes (NRBC) remained essentially within the normal limits for pigs of this age (Schalm, 1961). However, by the end of this

experiment the increase in NRBC was probably due to the anemia. A leukopenia was evident in the infected pigs (Groups III and IV) 24 hours after they were infected with the TGE virus. This was supported by the Rank Sum Test (Walker and Lev, 1953). Total and differential leukocyte counts varied widely as can be expected for pigs of this age. Immature leukocytes (lymphoblasts, prolymphocytes, myeloblasts and metamyelocytes) were found in varying numbers in all pigs throughout the experiment. Macrocytes were seen occasionally. Polychromasia, hypochromia and numerous platelets were a constant finding. As illustrated in Table VIII, the leukopenia in Groups III and IV actually persisted during the first 3 days. A leukopenia was present in the noninfected pigs on the second day. Of extreme interest is the progressive neutropenia present on the second day and persisting throughout the experiment in the noninfected pigs (Table VII).

DISCUSSION

General. The results of this experiment demonstrate the inhibitory effects of a viral enteric infection on the absorption of orally administered chlortetracycline. The CTC blood values of infected pigs, although low initially, did not decrease precipitously over a 24-hour period, as did the CTC blood levels in the noninfected pigs. In the non-infected pigs it is obvious that CTC was rapidly metabolized and eliminated. The almost continuous rise in CTC blood levels of infected pigs can be explained in part by the fact that about 70% of CTC is bound to protein components of an inflammatory exudate, making a CTC-protein complex, (Sirota and Saltzman, 1950). Inflammatory hyperemia makes possible increased penetration of CTC into the tissue and increased resorption of CTC by the lymphatic system (Malek and Kolc, 1960). Some of the changes in the gastrointestinal tract produced by the TGE virus (generalized hyperemia and edema throughout the gastrointestinal tract) therefore would enhance CTC penetration into tissue and binding to protein components of the inflammatory exudate.

The build-up of CTC in the blood of the Group II (CTC, no TGE virus) pigs is similar to research done by Brainerd et al. (1949). They found a build-up of CTC in the

serum of humans on a continuous oral dosage of 1 Gm. of CTC every 4 to 6 hours. These same researchers stated that this effect was noted as early as after the second dose of the drug, but usually was more marked after several days.

In the past much work has been done to show what effects different levels of dietary calcium have on the absorption of CTC. It is also well known that calcium and the tetracycline antibiotics can form a complex by the mechanism known as chelation. Therefore one might assume that the calcium in the milk diets of these pigs may have some alterative effect on CTC absorption. In man it has been found that concomitant administration of milk does not influence the absorption of CTC as does aluminum hydroxide gel (Bartholomew and Nichols, 1950). Therefore, since there is a great similarity in the digestive processes in the porcine and human species, it is not unreasonable to believe that calcium would not influence CTC absorption in the pig. It has been suggested that calcium absorption is involved in an active transport system (Palmer and Thompson, 1961). Depending on the absorptive mechanisms by which calcium absorption is involved in this active transport, perhaps the CTC calcium complex could also be actively absorbed from the gastrointestinal tract. The above statement is merely an assumption as it would depend on the chemical binding of calcium in the active transport mechanism and in the CTC-calcium binding. Certainly CTC must in part be involved in some active transport system. Previously cited work has

clearly demonstrated that only a certain amount of CTC is absorbed at a given time. If this absorption was entirely passive, then with all the exposed blood vessels from epithelial erosion and necrosis, hyperemia and inflammatory exudate which were present in this viral infection, it would seem that the initial CTC blood levels in the infected pigs should have been higher than they were. On the third day, at which time the mucosal epithelium was first observed in the recovery phase, the CTC blood levels were found to increase markedly in the infected (Group IV) pigs, almost equalling the CTC blood level in the noninfected (Group II) pigs. In man it has been found that the brush border on the free surface of the absorbing cells in the small intestine increases the absorbing area of the intestinal cell about 14 times (Schiff, 1961). This should also, at least in part, hold true for the pig. Thus some part of the gastrointestinal epithelium must be involved with active absorption of CTC and apparently this viral infection interfered with an active transport mechanism.

The decreasing CTC blood level observed in the Group IV pigs from the 6-hour bleeding of the third day to the 0-hour bleeding of the fourth day could mean that less CTC was bound to the protein of the inflammatory exudate. Therefore, CTC was being metabolized and eliminated from the body faster than it had been previous to this time. From this it could be interpreted that less inflammatory exudate was present due to the apparent recovery of the pigs. However, in the

histopathologic sections the edema was extensive in the lamina propria throughout the experiment.

Clinical. The clinical signs of TGE were present within 12 hours after administration of milk containing the virus. This is perhaps on the early side of the usual incubation period for this disease, but is within the range commonly seen. The change in character of the feces in the Group IV pigs was due to the CTC and perhaps can be explained in part by work done by Reber (1955). He tested the effect of CTC on isolated segments of normal porcine jejunum and found it to have a varied effect upon the motility of smooth muscle but, in general, decreased amplitude of contractions, followed by inhibition of motility, was observed. With enteritis, intestinal peristalsis is increased. It is possible that, as the epithelium was recovering, the effects of CTC absorption in some way also slowed peristalsis, thereby not only allowing for more fluids to be absorbed, but also allowing for the CTC to be in contact with the mucosal epithelium longer, and thus further enhancing the recovery and changing the character of the feces. Dehydration is usually seen in pigs with this disease from the loss of body fluids and was undoubtedly enhanced in this experiment by frequent bleeding of the pigs. Because of essentially normal water and food consumption by Group IV pigs, dehydration from loss of body fluids at the end of the experiment was not as severe as in Group III pigs.

From the results of this experiment, the clinical improvement in the Group IV pigs was evident. As the intestinal epithelium began to recover, this allowed better absorption and assimilation of nutrients, thus strengthening the pigs in their attempt to overcome the disease. Also the presence of CTC undoubtedly inhibited the stress of secondary bacterial infection. The weight gain in the Group III pigs was totally unexpected and perhaps can best be explained on the basis that these pigs were kept in a controlled environment. Had it not been for this type of environment, it is doubtful that any weight gain would have occurred. The controlled environment is undoubtedly the reason for no deaths occurring in the infected pigs, especially those in Group III. Perhaps the controlled environment was why no deaths occurred in the Group IV pigs. However, with this group receiving CTC to control a secondary infection, their chances for survival would be much greater. Even though the infected pigs were not stressed from various environmental conditions, they were markedly stressed from daily repeated bleedings.

The severe anemia present in all pigs by the end of this experiment was primarily a result of the multiple daily bleedings. Blood loss from the enteritis in the infected pigs also influenced the anemia. A leukopenia was observed especially during the first 24 hours after infection and should aid in a differential diagnosis. However, under field conditions and after the initial leukopenia, a secondary bacterial infection may occur and make the hemogram more

difficult to interpret. Hemograms vary markedly as the age changes in baby pigs and must therefore be interpreted carefully.

In the past CTC and several other chemotherapeutic agents have been used in attempts to treat this disease and have proved to be of little or no value. Based on the results obtained from this experiment, it appears that CTC has certain beneficial effects in treating perhaps the secondary effects of this viral enteric infection.

In vitro activity of CTC has shown that the majority of both Gram-negative and Gram-positive bacteria are inhibited by less than 1 microgram per ml. (1:1,000,000) (Kanegis et al., 1950). The average CTC blood level in the infected pigs was 0.47 mcg./ml. at the 6-hour bleeding on the first day of treatment. A higher blood level would be preferred such as the 1.2 mcg./ml. in the noninfected pigs at the 2-hour bleeding on the first day of treatment. The need for a higher CTC blood level is obvious, since the infected pigs are experiencing a leukopenia and other stress factors due to the TGE virus, the severity of which would be much greater under field conditions. Chlortetracycline probably doesn't have any direct effect on the TGE virus, but it might inhibit a secondary bacterial infection which in most instances plays an important role in the deaths of infected pigs in a weakened condition. Chlortetracycline blood levels from the treatment regimen followed in this experiment were not high initially, but at least the intestinal epithelium may have

been helped in its attempt to recover. Better results may have been observed if the dose of CTC used in this experiment were increased or given more frequently, especially in field outbreaks. It is not unreasonable to believe that death losses in baby pigs from this disease could be reduced if oral administration of this antibiotic could be started as soon as the signs are manifested. Along with this, sound management practices should be enforced to relieve other stress factors.

Gross and Microscopic Lesions. Lesions observed at necropsy generally paralleled those seen by other researchers who have studied the effects of TGE in pigs. The gall bladders were found to be distended with dark greenish-brown bile in both groups of infected pigs. The livers in some of the infected pigs, along with being pale, also had a slight yellowish tinge. The big difference in this experiment was the change in the character of the intestinal contents during and after the third day. Perhaps this change occurred in part because the intestinal epithelium was recovering from the effects of the TGE virus under the influence of CTC as was shown in Fig. 8 and 9.

Histopathologic changes were similar to those reported by Bay et al. (1951). Only specific lesions observed in the infected pigs will be discussed in detail. The most severe lesions were in the jejunum and ileum. Coincidentally, these areas of the intestinal tract also revealed the

outstanding differences between the untreated (Group III) and treated (Group IV) pigs. In some areas of the gastrointestinal tract the TGE virus caused severe reduction in height of the gastrointestinal epithelium and in other areas caused total erosion. This reduced the surface area for absorption and probably altered the enzymes which function in transport of substances across the mucosal epithelium.

A common histopathologic observation was the appearance of fibrin, predominantly around the small blood vessels, within the lamina propria. It was seen most frequently around the smaller blood vessels immediately beneath the basement membrane. The reaction closely resembles that of a myoarteritis as described by Hopps (1961), followed by a fibrinoid necrosis and later thrombosis of the affected blood vessel. This same reaction is probably incorporated in the necrobiotic changes observed by Bay et al. (1951). The periodic acid-Schiff (PAS) stain gave evidence of a mucopolysaccharide derived from the ground substance in the fibrinoid material. Some of the superficial epithelial necrosis and erosion was due to thrombosis of blood vessels within the lamina propria.

On the third day of the experiment, in the Group IV pigs areas suggestive of proliferation, increase in height of the surface mucosal epithelium and an increase in numbers of goblet cells were significant indications that the epithelium was in a recovery phase. These same findings were present on the fourth day but were more advanced. In work

done by Abrams et al. (1963), it was stated as a recognized principle that one of the accompaniments of mucosal injury is an abundance of desquamated cells associated with an exudate. They also mentioned that the sharp increase in cellular proliferation in areas of inflammation would be viewed as a secondary adjustment essential to epithelial integrity.

The surface mucosal epithelial recovery, observed in the pigs of Group IV, possibly were from the beneficial effects of CTC. Apparently the inhibition of secondary bacterial invaders provided an environment in which the epithelium could recover from the effects of the TGE virus.

SUMMARY AND CONCLUSIONS

Research was conducted using 18 pigs assigned to the following groups: (1) control, (2) control plus chlortetracycline, (3) transmissible gastroenteritis (TGE) virus-infected, and (4) TGE virus-infected plus chlortetracycline.

Signs of TGE were present within 12 hours after infection. The disease was characterized by clinical signs, leukopenia, and gross and microscopic lesions.

The virus of TGE had an inhibitory effect on absorption of orally administered chlortetracycline (CTC) on the first two days of treatment. On the third day of treatment, CTC blood levels of infected pigs approximated those of control pigs.

Microscopic lesions in infected, CTC-treated pigs included regeneration of intestinal epithelium on the third and fourth days. Infrequently, small areas resembling proliferation were observed in the intestinal epithelium during and after the third day in infected pigs which did not receive CTC.

Absorption of CTC appears to be involved in an active transport system.

Infected pigs given CTC orally appeared to be in better clinical condition at the end of the fourth day as compared to the untreated, infected pigs.

APPENDIX

TABLE I--Identification of Pigs Assigned to the Four Groups and Their Treatments

Group No.	Pig No.	Treatment
I	3,5,7,16	Control - No CTC, no TGE virus.
II	4,12,13,17	CTC, 1 mg./50 Gm. body weight. (One capsule(s) at 0-hour* on 4 successive days), no TGE virus.
III	1,2,9,11,14	No CTC, TGE virus only.
IV	6,8,10,15,18	CTC, 1 mg./50 Gm. body weight. (One capsule(s) at 0-hour* on 4 successive days), plus TGE virus.

*0-hour is 8 a.m.

TABLE II--Daily Average Body Temperatures (F), and Milk and Water Consumption (ml.)* for Each Group

		DAYS							
	GROUP**	-3	-2	-1	0	1	2	3	4
TEMPERATURES	I	103.4	102.8	101.4	101.4	101.0	100.9	101.1	101.0
	II	102.5	102.6	100.7	100.9	101.8	101.3	100.7	100.8
	III	103.6	103.4	101.3	101.4	102.3	102.4	103.2	102.8
	IV	103.2	103.7	101.5	101.6	102.0	100.8	101.4	101.7
MILK CONSUMPTION	I	133	233	305	306	345	347	325	260
	II	101	241	390	390	358	347	325	260
	III	137	220	321	388	131+	188	157	115
	IV	143	280	370	390	161+	160	227	260
WATER CONSUMPTION	I	---	535	261	245	216	297	158	110
	II	---	444	258	265	148	177	115	80
	III	---	533	329	---	300	258	350	210
	IV	---	505	316	---	216	185	167	75

*Milk and water values are approximate due to spillage and evaporation.

**For complete identification of these groups see Table I.

+Four p.m. feeding not included.

TABLE III--Average Weights of Pigs in Grams

	Days of August, 1963									
	9	10	11	12	13	14	15	16		
	Days of Experiment									
GROUP	-3	-2	-1	0	1	2	3	4	Terminal*	Weight Gained**
I (Control)	2442	2220	2250	2313	2408	2520	2544	2357	2500	250
II (Control: CTC, 1 mg./50 Gm. body weight beginning on day 1)	2221	2172	2150	2205	2335	2450	2558	2485	2565	415
III (TGE virus only on 0 day)	2697	2573	2554	----	2449	2641	2817	----	2639	85
IV (CTC, 1 mg./50 Gm. body weight beginning on day 1 plus TGE virus on 0 day)	2641	2521	2527	----	2505	2393	2557	2735	2803	276

*Weight of pigs immediately prior to euthanasia.

**Based on difference of terminal weight and weight on experimental day -1.

TABLE IV--Chlortetracycline Blood Levels of Noninfected (Group II) Pigs

		Blood Sample Time and Level (mcg./ml.)			
Day	Pig No.	0-Hour*	2-Hour	6-Hour	Terminal**
1	4	neg.	0.65	0.65	0.58
	12	neg.	1.13	0.83	----
	13	neg.	0.75	1.35	----
	17	neg.	2.25	1.35	----
Average		neg.	1.20	1.05	0.58
2	12	0.37	1.44	1.28	0.93
	13	0.35	1.25	1.05	----
	17	0.35	0.98	0.57	----
Average		0.36	1.26	0.97	0.93
3	13	0.35	1.28	1.52	----
	17	0.25	1.42	1.13	1.00
Average		0.30	1.35	1.33	1.00
4	13	0.51	1.45	1.45	1.52
Average		0.51	1.45	1.45	1.52

*Dosage was 1 mg./50 Gm. body weight on 4 successive days after 0-hour sample.

**Not included on the graph (Fig. 10).

TABLE V--Chlortetracycline Blood Levels of Infected (Group IV) Pigs

		Blood Sample Time and Level (mcg./ml.)*			
Day	Pig No.	0-Hour**	2-Hour	6-Hour	Terminal+
1	6	neg.	0.41	0.39	0.48
	8	neg.	0.23	0.34	----
	10	neg.	0.32	0.32	----
	15	neg.	0.51	0.83	----
	18++	neg.	neg.	neg.	----
Average		neg.	neg.-0.37	neg.-0.47	0.48
2	8	0.27	0.52	0.76	----
	10	0.32	0.30	0.35	----
	15	1.50	1.40	0.96	----
	18++	neg.	----	0.44	0.44
Average		neg.-0.70	0.74	0.63	0.44
3	8	0.65	0.82	1.10	0.98
	10	0.58	0.98	1.35	----
	15	0.84	1.55	1.50	----
Average		0.69	1.12	1.32	0.98
4	10	1.22	1.02	0.89	0.78
	15	0.80	0.89	1.15	1.10
Average		1.01	0.96	1.02	0.94

*Negative responses not considered as 0 for computation.

**Dosage was 1 mg./50 Gm. body weight on 4 successive days after 0-hour sample.

+Not included on the graph (Fig. 10).

++Confirmed by re-assay, dropped from experiment, thus average at 6-hour (day 2) is 0.69.

TABLE VI--Milligrams of CTC Given to Each Pig Daily and the Accumulative Total Given to Each Pig During the Experiment

		Mg. CTC weighed and put in capsules DAYS				Accumulative mg. CTC given during the experiment
	Pig No.	1	2	3	4	
GROUP II	4	42.8	----	----	----	42.8
	12	44.7	45.3	----	----	90.0
	13	48.5	49.4	53.1	49.8	200.8
	17	51.0	52.8	49.8	----	153.6
GROUP IV	6	53.0	----	----	----	53.0
	8	44.4	43.0	42.3	----	129.7
	10	53.5	52.4	52.0	51.2	209.1
	15	62.8	60.0	59.5	58.2	240.5
	18	37.1	35.9	----	----	73.0

TABLE VII--Average Hemograms of Noninfected Pigs (Groups I and II)*

Day	PCV (%)	Hb. Gm./100 ml.	Nucleated erythrocytes/ 100 leukocytes	Leukocytes/ cu. mm.**	Absolute Differential Leukocyte Count					
					Band	Neut.	Lymph.	Mono.	Eos.	Bas.
-3	31.3	9.4	10.6	9,461	322	3,690	5,308	24	24	12
-2	----	---	----	-----	---	-----	-----	--	--	--
-1	----	---	----	-----	---	-----	-----	--	--	--
0	30.8	9.2	10.7	7,396	104	2,381	4,852	0	44	0
1	31.6	8.8	4.8	5,978	84	1,650	4,119	48	54	6
2	21.9	6.7	7.2	3,674	22	856	2,733	33	11	11
3	16.3	3.5	15.7	5,517	0	110	4,816	0	17	17
4	11.8	4.3	19.5	4,389	0	329	4,060	0	0	0

*For complete identification of these groups see Table I.

**Corrected for nucleated erythrocytes.

TABLE VIII--Average Hemograms of Infected Pigs (Groups III and IV)*

Day	PCV (%)	Hb. Gm./100 ml.	Nucleated erythrocytes/ 100 leukocytes	Leukocytes/ cu. mm.**	Absolute Differential Leukocyte Count					
					Band	Neut.	Lymph.	Mono.	Eos.	Bas.
-3	30.8	8.7	9.9	6,609	126	2,267	4,144	26	26	7
-2	----	---	----	-----	---	-----	-----	--	--	--
-1	----	---	----	-----	---	-----	-----	--	--	--
0+	34.3	10.0	4.0	6,786	34	2,667	3,888	88	88	20
1	30.6	9.2	5.8	2,471	141	961	1,332	22	10	2
2	31.0	8.9	5.3	4,249	323	731	3,085	25	42	8
3	20.9	6.3	8.1	4,053	57	1,159	2,797	12	24	4
4	17.9	6.0	17.6	6,099	189	1,897	3,989	6	0	0

*For complete identification of these groups see Table I.

**Corrected for nucleated erythrocytes.

+Day on which Groups III and IV were infected with TGE virus.

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