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THE INFLUENCE OF A VIRAL ENTERIC INFECTION ON CAROTENE METABOLISM

Thesis for the Degree of M. S.
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Robert R. Maronpot

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ABSTRACT

THE INFLUENCE OF A VIRAL ENTERIC INFECTION
ON CAROTENE METABOLISM

by Robert R. Maronpot

Research was conducted using a total of 24 pigs in 3 experiments to determine carotene metabolism during a specific viral enteric infection, transmissible gastroenteritis (TGE).

Clinical signs and gross and microscopic lesions were not noticeably different between infected animals fed carotene and those not fed carotene. Fecal carotene excretion was significantly reduced within 24 hours after oral inoculation with the TGE virus. Fecal carotene excretion was greater in pigs fed water-dispersible beta-carotene beadlets than in pigs fed an oil-soluble beta-carotene.

The virus of TGE produced sprue-like lesions characterized by villous atrophy in the jejunum and ileum of infected pigs. Diarrhea was present within 72 hours after inoculation with the infective agent and continued until the pigs were killed for necropsy.

THE INFLUENCE OF A VIRAL ENTERIC INFECTION ON CAROTENE METABOLISM

By

Robert R. Maronpot

A THESIS

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in partial fulfillment of the requirements
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INTRODUCTION

Most studies on the metabolism of therapeutic agents and nutrients are carried out using normal animals. Consequently, little basic information is available on the metabolism of specific nutrients during the course of an infection.

For many years vitamin A deficiency has been associated with infections of the respiratory and gastrointestinal tracts. Evidence has been published on the interrelationship of vitamin A and disease resistance and severity (Ershoff, 1952; Ludovici and Axelrod, 1951). This information has raised additional questions concerning the therapeutic value and metabolism of carotene in an animal with a specific infection. This research was an attempt to obtain information on the metabolism of carotene during the course of an experimentally produced gastrointestinal infection.

The objectives of this study were (1) to determine the metabolism of beta-carotene during the course of transmissible gastroenteritis (TGE), (2) to determine the effect of TGE upon pigs fed a diet devoid of vitamin A and vitamin A precursors, and (3) to correlate TGE infection and carotene metabolism in the pig.

REVIEW OF LITERATURE

In recent years a renewed interest has been stimulated in the role of vitamin A and its precursors in maintaining animal health. Of special interest are the metabolism and roles of these substances in specific diseases, especially those involving the gastrointestinal tract. Voluminous amounts of information concerning vitamin A and its precursor, carotene, are available in such books as Moore's (1957). This review deals primarily with the role and metabolism of carotene in disease.

Only small amounts of vitamin A pass the porcine chorioallantoic placenta and, consequently, at birth the baby pig has a low vitamin A reserve. Hjarde (1961) and Heaney (1963) have reported that young pigs are dependent upon colostrum and milk for their early source of vitamin A.

Since the pig is an efficient converter of carotene to vitamin A (Braude et al., 1941), this vitamin A precursor may be fed to young pigs to meet their vitamin A requirements. Hentges et al. (1952a) found that a young pig's minimum requirement for a purified source of carotene was 25 mcg. per kilogram of body weight daily. This level allowed for good weight gain and adequate liver storage of vitamin A. However, the National Academy of Sciences - National Research Council (Nutrient Requirements of Swine, 1962) recommends 1.2 mg. of beta-carotene daily for growing pigs under 10 lb. of body weight.

Evidence for the intestinal conversion of carotene to vitamin A was given by Mattson, Mehl and Deuel (1947), who noted that vitamin A first appeared in the small intestine wall following oral carotene ingestion by vitamin A-deficient rats. That same year Wiese, Mehl and Deuel measured the *in vitro* transformation of carotene to vitamin A in rat small intestine incubated in Ringer's solution. Employing thoracic-duct fistulas, Goodwin and Gregory (1948) found increased concentrations of vitamin A in goat and rabbit lymph 2 hours after orally dosing them with carotene. More detailed information on the mechanism of carotene conversion to vitamin A is provided by several British investigators (Glover, Goodwin and Morton, 1948; Alexander and Goodwin, 1950; Thompson *et al.*, 1950). In 1950, Thompson and Kon at the National Institute for Research in Dairying in England determined the intestine to be the site of carotene conversion to vitamin A in the pig. They concluded that carotene was converted to vitamin A ester in the intestinal wall, transported by the lymph to the blood stream and carried to the liver and throughout the body by the blood.

Additional evidence for the intestinel-conversion view was presented by Olson (1959) using C^{14}-labeled beta-carotene and by Popper and Greenberg (1941), who photographed fluorescing vitamin A in the intestinal mucosa 4 hours after vitamin A-deficient rats consumed a carotene meal.

Although many body tissues can convert carotene to vitamin A in small amounts (Bieri and Pollard, 1954; Olson, 1959; McGillivary, 1961), the small-intestine wall, probably the mucosa, is the primary site of this conversion.

Some early workers have studied the interaction of carotene metabolism and infection. Heymann (1936) studied the fecal output of carotene in 24 healthy infants and in 17 infants with various types of childhood infections. The normal absorption rate of 70% was diminished to 35% in those infants with infections. Heymann hypothesized that the decreased absorption associated with infection was due to a toxic factor which directly inhibited fat absorption or affected bile production. After studying more than 500 patients, Clausen (1933) noted that carotene was poorly absorbed in the presence of fever or diarrhea. Spielman et al. (1946) reported that diarrhea and infection in calves shortly after birth reduced absorption and utilization of carotene regardless of the amount fed. When diarrhea was minimized, the calves could utilize carotene more efficiently.

In cattle, horses, pigs and man not ingesting preformed vitamin A carotene is the chief precursor of vitamin A. Since the vitamin A status of the body is associated with resistance to infection and stress (Keener et al., 1942; Boynton and Bradford, 1931; Harmon et al., 1963; Stowesand and Scott, 1964), proper and adequate carotene metabolism may be of paramount importance to the organism's well being.

Transmissible gastroenteritis in young pigs is characterized by histopathologic changes remarkably similar to those accompanying the human malabsorption syndromes of tropical and nontropical sprue. In the latter there is a shortening and blunting of the small-intestinal villi, lengthening of the crypts of Lieberkühn and infiltration of the lamina propria with granulocytes (Shiner, 1960; Padykula et al., 1961; Townley, Cass and Anderson, 1964). Electron-microscopic examination reveals that prior to these changes, the microvilli, which

comprise the brush border, are lost in part or completely (Padykula et al., 1961).

The histopathologic lesions of the small intestine in TGE range from a reduction in the height of the mucosal epithelium to necrosis of the mucosa depending upon the animal's resistance and the duration of the infection. In addition, the small-intestinal villi often become swollen, and infiltration of the lamina propria with polymorphonuclear leukocytes is seen on histopathologic sections (Wallace and Whitehair, 1965; Doyle, 1958; Bay, Doyle and Hutchings, 1951).

Previous work and current clinical evaluation indicate that vitamin A has a role in maintaining health in man and animals. The pathogenicity of the TGE virus has been well established. Thus, the use of this infective agent seems appropriate to obtain additional information on the metabolism of carotene in an experimentally produced infection.

MATERIALS AND METHODS

General plan. Experiments were conducted to compare the absorption of beta-carotene in pigs exposed to the TGE virus with that in clinically normal pigs. The pigs were fed a synthetic vitamin A-free diet with controlled amounts of beta-carotene added. Records of body weight, feed consumption, fecal excretion and clinical signs were maintained throughout the study. Blood, liver and fecal samples were collected at selected intervals throughout the experiments for routine hematologic examination as well as for carotene and vitamin A assay. Tissue samples for histopathologic study were routinely taken from the liver, kidneys and from selected areas of the gastrointestinal tract at the time of necropsy.

Animals. Three-week-old Yorkshire pigs raised on the premises were used for all experiments. The usual experiment consisted of an entire litter of pigs using some as control animals and some as test animals in accepted experimental design. The pigs were weaned at 2 weeks of age and given several days to become accustomed to the synthetic diet. No infections or gross abnormalities were present in either the pigs or the sows at the start of the experiments.

Housing and care. All pigs were housed in individual, grated-bottom, galvanized cages and fed from crocks. The fecal collection pans beneath the grating were covered with sheet vinyl plastic perforated with small holes to allow for separation of urine and feces.

Infected and control groups were housed in separte rooms but under otherwise similar environmental conditions. The ambient temperature in each room was 85 F.

Infective agent. The TGE virus, obtained from Dr. E. O. Haelterman of Purdue University, was employed as the infective agent. Its virulence in the 3-week-old pig and the clinical signs produced are well characterized by previous workers (Doyle, 1958; Bay, Doyle and Hutchings, 1951; Wallace and Whitehair, 1965).

Synthetic diet. A complete synthetic diet for young pigs was prepared according to the formulation of Miller et al. (1964), except that vitamin A was not included in this diet.

Blood and tissue analyses.

Blood analyses. Ten-milliliter amounts of blood were collected from the anterior vena cava according to the method of Carle and Dewhirst (1942). All pigs were bled prior to the time of inoculation, 48 hours after inoculation, and terminally. Total and differential white-cell counts were carried out as described by Benjamin (1964). Hemoglobin estimation was by the cyanmethemoglobin method (Benjamin, 1964). Serum vitamin A levels were determined by the method of Neeld and Pearson (1963). This technique was evaluated by comparison with the Carr-Price assay method (Kaser and Stekol, 1943) and was found satisfactory for porcine serum.

Liver analyses. The livers from all pigs were weighed and analyzed for vitamin A by the following modification of the trifluoroacetic

acid method (Neeld and Pearson, 1963): (1) preparation of aqueous liver homogenates in a Waring blendor, (2) alkaline saponification for 10 minutes at 40 C., and (3) substitution of a measured portion of saponified aqueous homogenate for serum. Using the livers from 4 non-experimental pigs, trials were conducted to compare this assay procedure with the techniques described by Diplock, Green and Bunyan (1963). Based on these trials this method proved satisfactory for liver vitamin A assay.

Fecal analysis. Carotene was extracted from an aqueous suspension of feces with petroleum ether (30-60 C.), and the optical density was read in a Coleman Jr. spectrophotometer set at 450 mu against a petroleum-ether blank. The carotene content was then calculated from a previously prepared standard curve.

Using 4 nonexperimental pigs, pilot trials were conducted to determine (1) if carotene is eliminated in the urine and (2) if it is destroyed in the intestine. The results of these pilot studies indicate that pigs with TGE do not eliminate carotene in the urine and that carotene is not destroyed when incubated with the intestinal contents of the infected pigs for 4 hours at 37 C.

Histopathologic technique. The pigs were euthanatized by exsanguination (severing the axillary blood vessels). Tissues of approximately 2 cm. x 0.5 cm. were fixed in 10% formalin and processed for examination by procedures described in the Manual of Histologic and Special Staining Techniques of the Armed Forces Institute of Pathology, Washington, D.C. (1957).

To determine the histologic effect of distention of the intestine with gas, air was injected into an intestinal loop of an anesthetized pig. The pig was killed 4 hours later. Histologic examination of the distended intestine suggests that mere distention of the intestine with gas might cause the villi to shorten and become blunt.

EXPERIMENTS AND RESULTS

Experiment 1

Experimental plan. Ten 3-week-old pigs were randomly divided into 4 groups as follows:

Group	No. of Pigs	Diet
1 - Basal Control (noninfected)	2	Basal vitamin A-free diet, water, 30 ml. of skim milk daily.
2 - Carotene Control (noninfected)	3	Basal vitamin A-free diet, water, 30 ml. of skim milk containing 1.2 mg. beta-carotene* daily.
3 - Basal TGE	2	Basal vitamin A-free diet, water, 30 ml. of skim milk daily.
4 - Carotene TGE	3	Basal vitamin A-free diet, water, 30 ml. of skim milk containing 1.2 mg. beta-carotene* daily.

Groups 3 and 4 were inoculated at 3 weeks of age by administering 1 ml. of the TGE virus suspension in milk. One pig from each group was killed 72 hours after inoculation. The remaining pigs were killed 120 hours after inoculation.

Results.

Clinical signs. Ration consumption was essentially the same for all 4 groups. Groups 1, 2, and 4 had gains in body weight, while Group

*Assayed Dry Beta-Carotene Beadlets (Type 2.4S) were supplied by Hoffmann-LaRoche, Inc., Nutley, N.J.

3 (Basal, infected) lost weight. Vomiting and diarrhea started in the infected pigs 12 to 24 hours after inoculation. Vomiting persisted for 48 hours. Diarrhea was still evident in the infected pigs 120 hours after inoculation. A moderate leukopenia developed in the infected pigs 48 hours after inocuation, which was essentially the same as reported by Wallace and Whitehair (1965). Groups 1 and 2 had no abnormal clinical signs throughout the experiment.

Gross pathology. The following gross pathologic changes were seen in the infected pigs: distention of the stomach and small intestine with gas, mild to moderate congestion of the mesenteric blood vessels, and yellow, watery fluid in the small and large intestines. In addition, 2 infected pigs had moderately engorged epigastric blood vessels. No gross lesions were evident in the noninfected pigs.

Histopathology. Only the infected pigs had histopathologic lesions. The lesions were confined to the small intestine and included the following: partial to total villous atrophy with loss of the epithelial brush border, villous edema, congestion of the blood vessels in the villi and in the tunica muscularis, infiltration of the lamina propria by neutrophils, eosinophils and macrophages. Infected pigs, killed at 120 hours after inoculation, had a more pronounced cellular infiltration of the lamina propria than those killed at 72 hours. Many of the atrophic villi had cuboidal rather than columnar epithelium, the more cuboidal epithelial cells being at the villous apex. The villous atrophy was confined to the jejunum and ileum and was most marked in those intestinal segments greatly distended with gas (Figures 3, 4, 7, 8, 11, 12).

Analyses. Comparison of fecal carotene excretion in Groups 3 and 4 is summarized in Figure 1. Hepatic vitamin A stores are presented in Figure 2. Serum vitamin A levels ranged from 40 to 58 mcg. per 100 ml. of serum, with no significant differences between any 2 groups.

Experiment 2

Experimental plan. Eight 3-week-old Yorkshire pigs were assigned to 2 groups as follows:

Group	No. of Pigs	Diet
1 - Carotene, Control	4	Basal carotene ration*, water, 30 ml. of skim milk daily.
2 - Carotene, TGE	4	Basal carotene ration*, water, 30 ml. of skim milk daily.

One milliliter of the virus suspension was mixed with the milk and given to each pig in Group 2 as the inoculum.. One pig from each group was killed 72 hours after inoculation; the remaining pigs were killed 120 hours after inoculation.

Results.

Clinical signs. Although feed consumption was uniform within each group of pigs, there was a distinct difference between the groups. Both groups started consuming 150 Grams of ration per pig per day. Group 1 pigs were consuming 250 Grams each at 120 hours after inoculation, while each pig in Group 2 was consuming only 75 Grams. The following table gives the mean body weight changes in kilograms for each group:

*4.29 mcg. of beta-carotene per Gram of feed. The beta-carotene, supplied by Distillation Products Industries, Rochester, N.Y., was a 24% solution in vegetable oil.

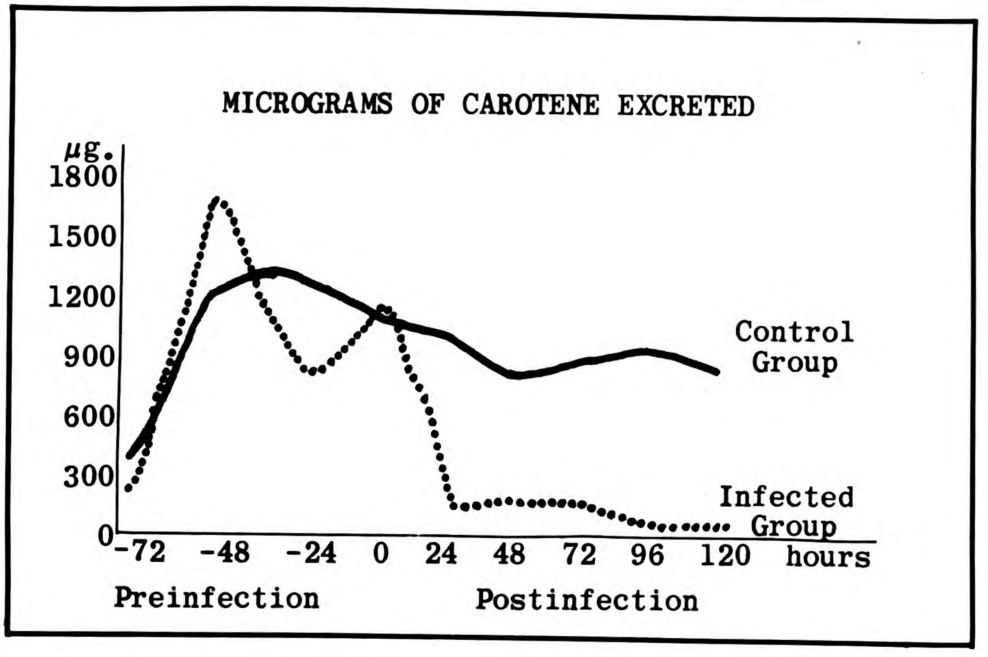

Figure 1.

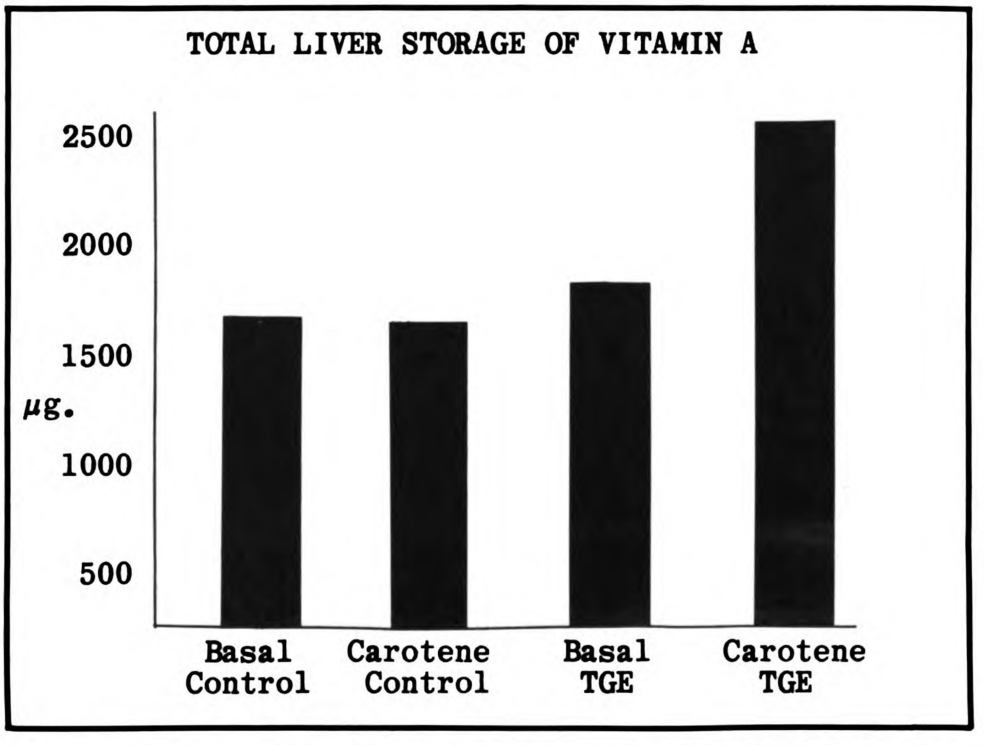

Figure 2.

Figure 3. Jejunum from a carotene, noninfected pig. (A) normal villi, (B) glands of Lieberkühn. Hematoxylin and eosin. x 75.

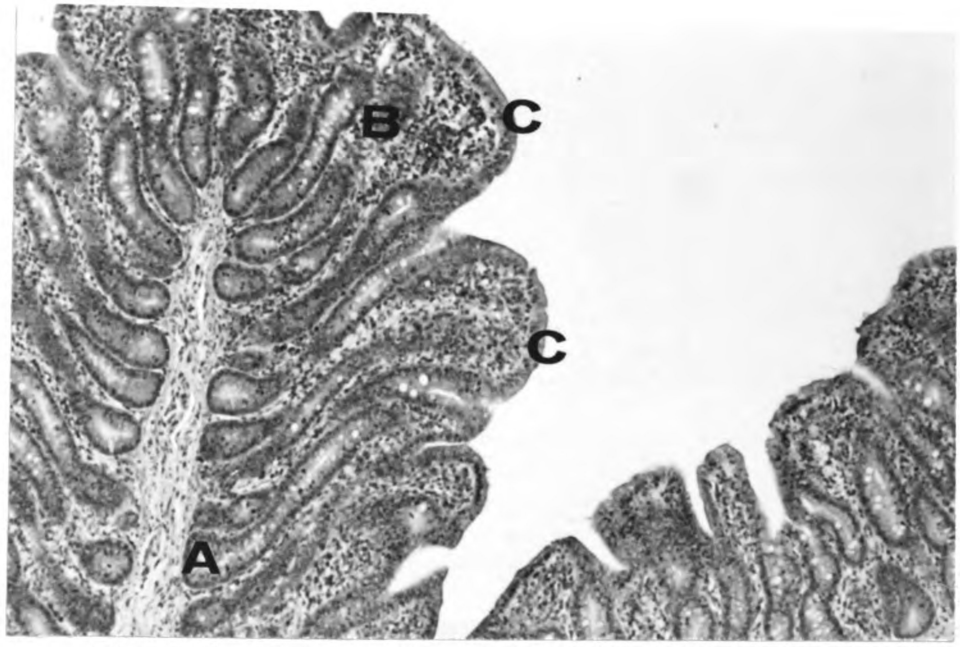

Figure 4. Jejunum from a pig with TGE showing partial villous atrophy. (A) increased length of the glands of Lieberkühn, (B) cellular infiltration of the lamina propria, (C) blunted, atrophic villi. Hematoxylin and eosin. x 75.

	Initial Weight	Terminal Weight
Group 1	2.9 kg.	3.4 kg.
Group 2	2.4 kg.	2.0 kg.

The clinical signs of vomiting and diarrhea were evident 24 to 48 hours after inoculation in the infected pigs. In this experiment only 1 pig in Group 2 vomited. All infected pigs developed a persistent, watery diarrhea which lasted for the duration of the experiment. Forty-eight hours after inoculation there was moderate leukopenia in the infected pigs. Control pigs had no abnormal clinical signs throughout the experiment.

Gross pathology. The only gross pathologic changes seen at the time of necropsy were a mild congestion of the mesenteric blood vessels in 3 of the infected pigs and gas-filled small intestines in all the pigs in the infected group.

Histopathology. Three of the 4 infected pigs had significant histopathologic lesions in the small intestines. The lesions included a shortening and blunting of the intestinal villi in the jejunum and ileum, leukocytic infiltration of the intestinal lamina propria, and a morphologic alteration of the intestinal epithelium from simple columnar to simple cuboidal, with loss of the epithelial brush border. In 1 of the infected pigs the villi were almost nonexistent in some portions of the ileum. All pigs in Group 1 and 1 of the pigs in Group 2 had no significant histopathologic alteration in the gastrointestinal tract.

Analyses. A comparison of the fecal carotene excretion for the control and infected groups is presented in Figures 5 and 6. The mean vitamin A content of the livers was 2742 mcg. for Group 1 and 2358 mcg. for Group 2. There was no apparent difference between the serum vitamin A values of the pigs in Groups 1 and 2. The serum vitamin A values ranged from 33 to 46 mcg. per 100 ml. of serum.

Experiment 3

Experimental plan. Experiment 3 was a replicate of Experiment 2 using 3 pigs in each group. All animals were killed 96 hours after inoculation.

Results.

Clinical signs. Feed consumption was essentially the same for both groups of pigs. Two of the infected pigs had a loss of weight, 1 infected pig had no weight change, and the control pigs all gained weight. In the infected group, diarrhea was evident in 1 pig in 48 hours after inoculation with the TGE virus, and 1 of the infected pigs had leukocytosis with a left shift. The 3 pigs in the control group had no abnormal clinical signs throughout the experiment.

Gross pathology. The infected pigs were all characterized by gas-filled small intestines which contained a yellowish fluid. A diphtheroid membrane covered the tongue and lined the esophagus of 2 of the infected pigs. Hypermotility of the small intestine was still present at necropsy in 2 of the infected pigs.

Histopathology. Partial to complete villous atrophy was evident in 2 of the 3 infected pigs. The villi were blunted and the brush

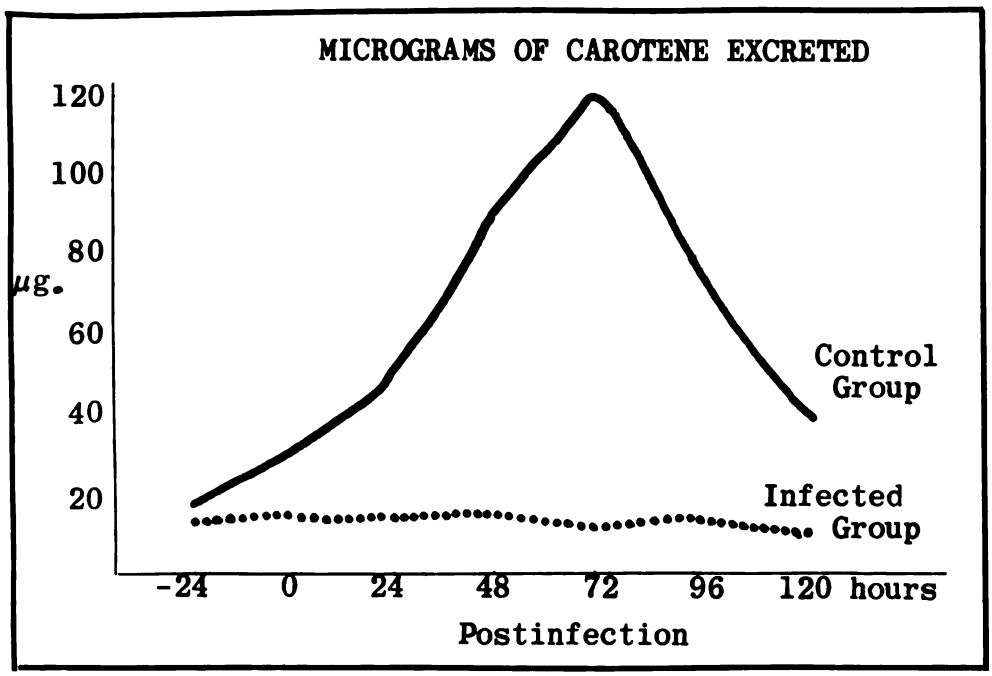

Figure 5.

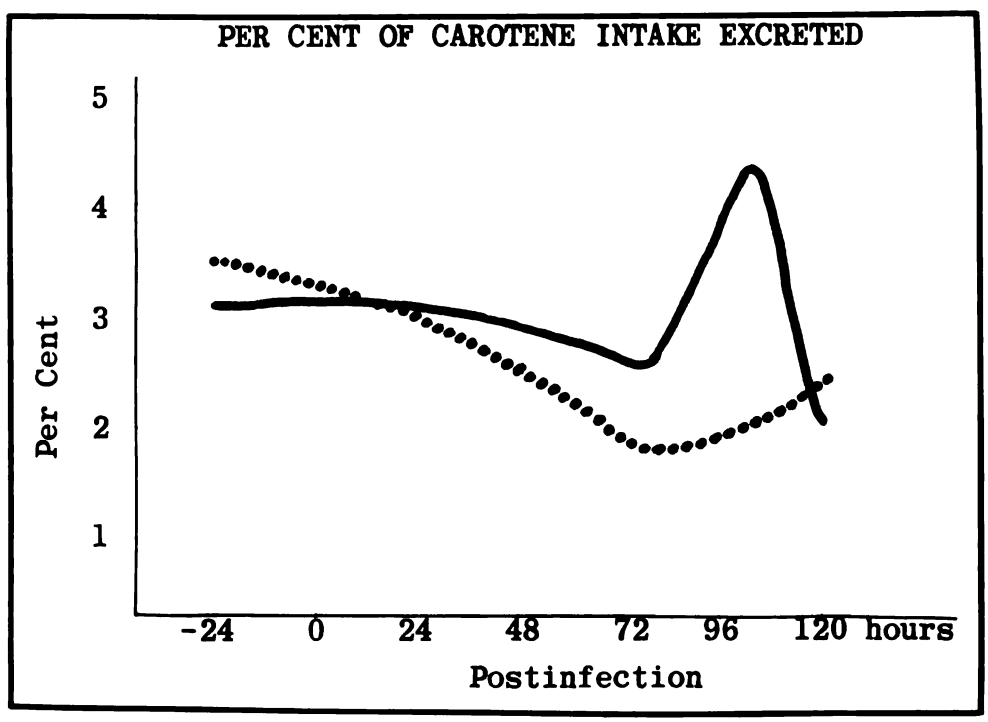

Figure 6.

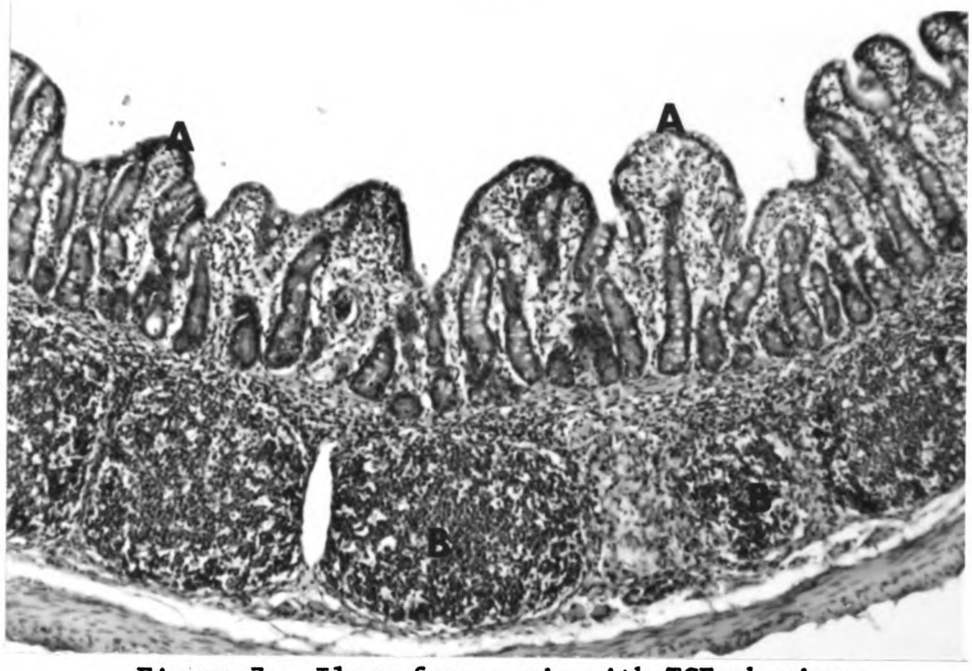

Figure 7. Ileum from a pig with TGE showing partial villous atrophy. (A) atrophic villi, (B) Peyer's patches. Hematoxylin and eosin. x 75.

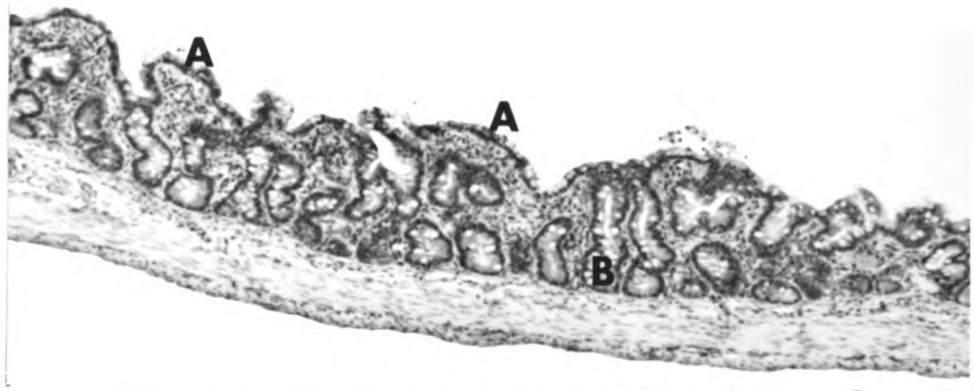

Figure 8. Ileum from a pig with TGE showing total villous atrophy. (A) atrophic villi, (B) glands of Lieberkühn. Hematoxylin and eosin. x 75.

border was absent from many of the villi. The diphtheroid membrane found covering the tongue and lining the esophagus of 2 infected pigs was composed largely of sloughed epithelium, neutrophils and fungi.

Analyses. Figures 9 and 10 illustrate the fecal carotene excretion for this experiment. The mean hepatic vitamin A content was essentially the same for both groups of pigs. The control pigs had 3014 mcg. of vitamin A as a group mean, while the infected pigs had 2929 mcg. Serum vitamin A values ranged from 36 to 46 mcg. per 100 ml. of serum with no apparent differences between the control and infected groups.

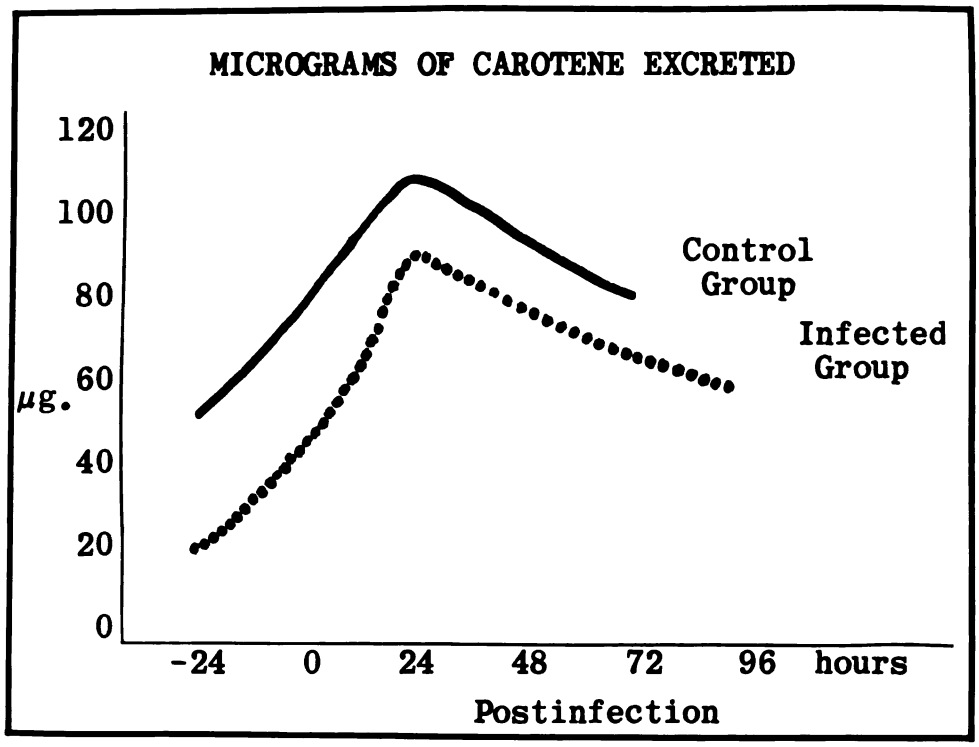

Figure 9.

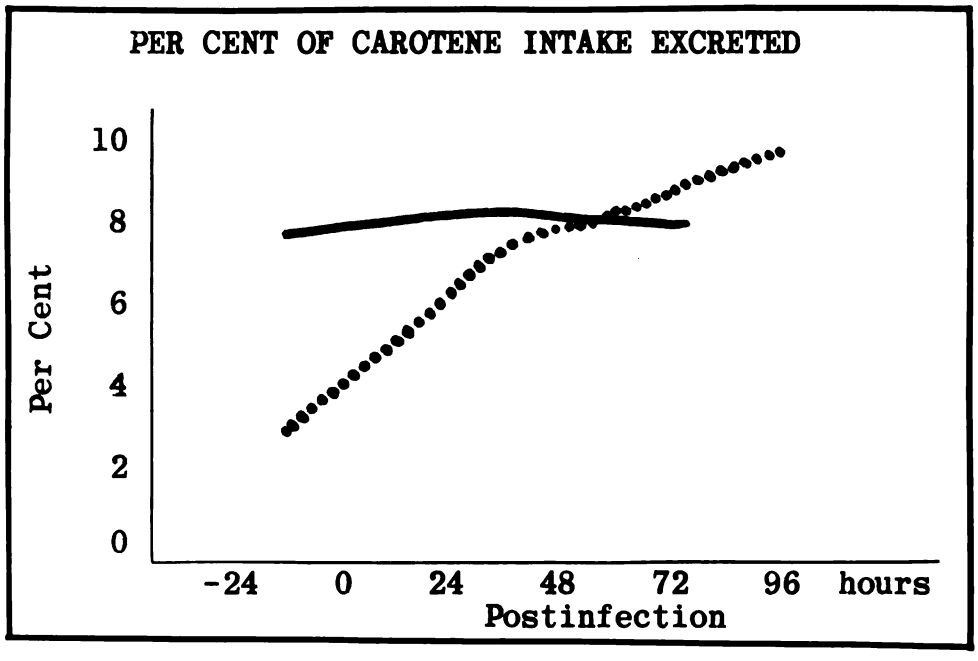

Figure 10.

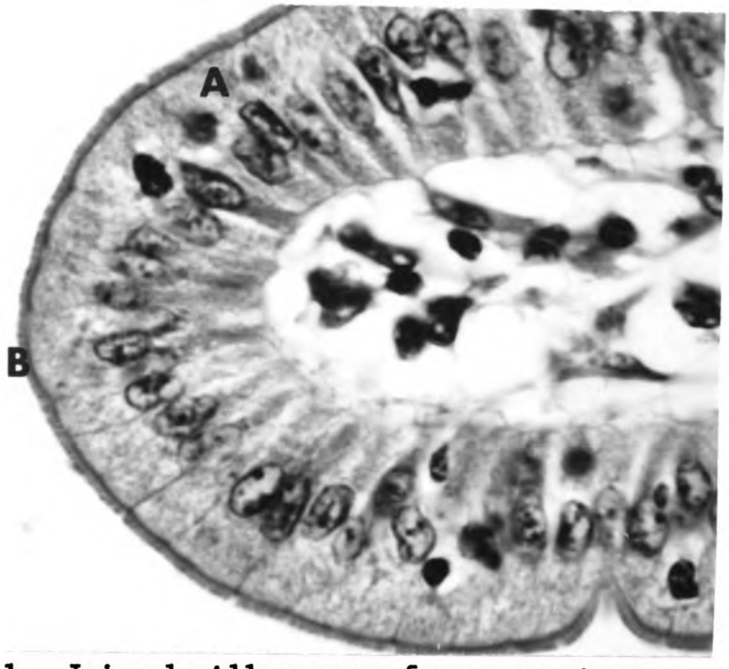

Figure 11. Jejunal villous apex from a carotene, noninfected pig. (A) normal columnar epithelium, (B) brush border. Hematoxylin and eosin. x 768.

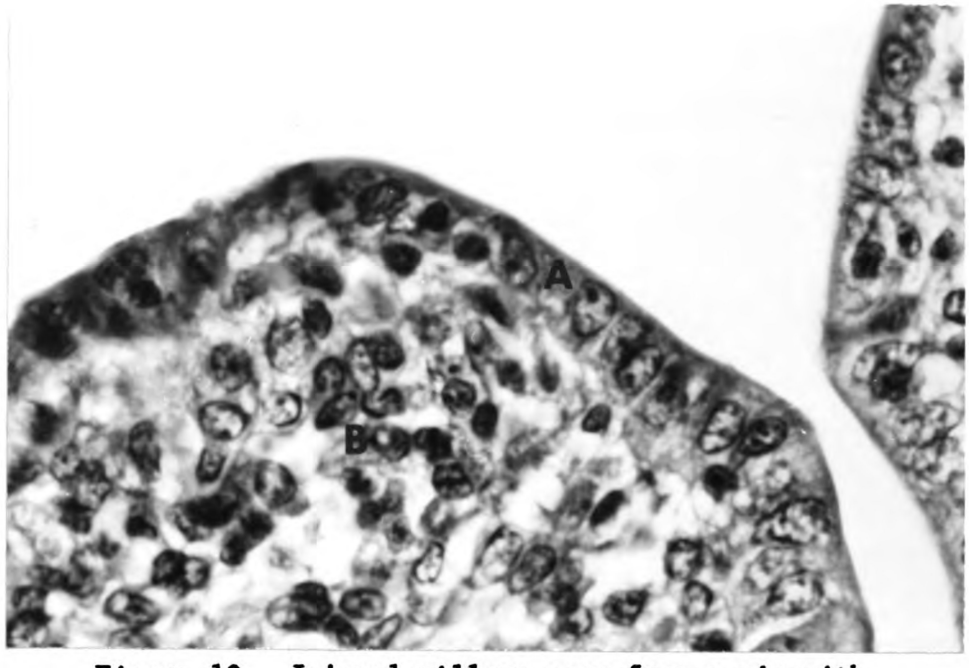

Figure 12. Jejunal villous apex from a pig with TGE. (A) cuboidal epithelium without brush border, (B) cellular infiltration of the lamina propria. Hematoxylin and eosin. x 768.

DISCUSSION

These experiments indicate that there is reduced fecal carotene excretion in pigs infected with TGE virus. Pilot studies (see page 8) indicate the following: (1) TGE infected pigs do not eliminate carotene in the urine and (2) carotene is not destroyed when incubated with the intestinal contents of TGE infected pigs. In view of these findings, it is probable that pigs with TGE absorb more carotene than noninfected pigs. The increased liver stores of the infected pigs with carotene supplementation (Group 4) in Experiment 1 tend to confirm this possibility. The serum vitamin A levels give no indication of an increased carotene absorption in pigs with TGE. This is to be expected, however, since it is well known that serum vitamin A levels remain in a normal range as long as there is an abundant hepatic vitamin A reserve (Hentges et al., 1952b; Caster and Mickelsen, 1955).

The infected pigs fed the basal ration (Group 3) in Experiment 1 had adequate hepatic vitamin A stores as compared to the controls (Groups 1 and 2). Hence, they were not dependent upon dietary carotene to meet their vitamin A needs. In view of this, it is not surprising that the inclusion of carotene in the diet did not affect the susceptibility of pigs to TGE, nor did it affect the severity of the infection.

The histopathologic lesions in the jejunum and ileum of the animals with TGE are remarkably similar to those described in the human malabsorption syndromes of tropical and nontropical sprue

(Shiner and Doniach, 1960; Padykula et al., 1961). For convenience, the intestinal lesions were classified into the categories of partial villous atrophy and total villous atrophy according to Shiner and Doniach (1960). In these experiments the pigs with TGE often had both categories of villous atrophy and in some areas there were normal villi. Whether the TGE virus itself is responsible for the villous atrophy remains to be determined.

The villous atrophy seen in these experiments was most pronounced in the gas-distended segments of the small intestine. A pilot study (see page 9) suggested that mere distention of the intestine with gas might cause the villi to become shortened and blunted. However, this does not explain the loss of the brush border (microvilli), the cuboidal epithelium, cellular infiltration of the lamina propria and congestion of the mesenteric blood vessels in infected pigs. Multiple factors, including TGE virus, distention of the intestine with gas, vascular congestion, and other unknown factors, are possibly responsible for atrophy of the villi.

Certain biochemical alterations, especially decreased enzyme activity in the intestinal epithelium, accompany sprue in humans (Padykula et al., 1961). Diminished activity of enzymes suggests disturbances in energy production and release which would affect certain aspects of active transport. Decreased activity of a chemical substance which normally inhibits excessive carotene absorption might explain the decreased carotene excretion seen in these experiments. However, no such chemical substance has been isolated. Without further investigation, this is mere speculation.

Clinical signs. While TGE in pigs under 1 week of age is usually fatal, older pigs generally survive (Doyle, 1958). In the 3-week-old pigs used in these experiments, the clinical signs of infection with TGE varied in severity. Diarrhea was the major clinical sign in all infected animals. In addition, vomiting, reduced feed consumption, weight loss and leukopenia were noted. The leukocytosis with a left shift in 1 infected pig (Experiment 3) was probably due to a secondary bacterial complication.

Gross pathology. The most marked and consistent finding in the infected pigs was a distention of the small intestines with gas and fluid. As was pointed out previously, this may have a more or less direct relationship to the histopathologic changes seen in TGE. The intestinal hypermotility noted in 2 infected pigs (Experiment 3) would contribute to the diarrhea. Congestion of the mesenteric and epigastric blood vessels is in agreement with the findings of other workers (Doyle, 1958; Bay, Doyle and Hutchings, 1951).

Histopathology. Villous atrophy was first described in detail by Shiner and Doniach (1960), who classified 3 types: partial, subtotal, and total. In describing the villous atrophy in these experiments the partial and total categories were used.

Partial villous atrophy in pigs with TGE was characterized by a shortening and blunting of the villi to as much as one third of the normal height. These villi became thickened and the lamina propria was infiltrated with numerous leukocytes. The epithelial lining was diminished in height and the brush border was absent. In addition, the glands of Lieberkühn were relatively lengthened and the blood

vessels in all layers were congested. Total villous atrophy was similar to the above but with the villi shortened to less than one third of the normal height and covered with a simple cuboidal epithelial lining.

Chemical pathology. These experiments indicate that hepatic vitamin A stores were not noticeably affected by TGE virus infection. If the pigs had been more deficient in vitamin A at the start of the experiment, more group differences might have been anticipated. In Experiment 1 the hepatic vitamin A reserves were lower than in the 2 succeeding experiments. The most pronounced difference in terminal hepatic vitamin A stores was recorded in this experiment.

In Experiment 1, fecal carotene excretion was absolutely higher than in the 2 succeeding experiments. A possible explanation for this might be that in Experiment 1 the carotene was administered in the daily skimmed milk while in the next 2 experiments, carotene was mixed with the ration. In addition, dry, water-dispersible, carotene beadlets were used in Experiment 1 and oil-soluble carotene was used in Experiments 2 and 3.

SUMMARY

Research was conducted using a total of 24 pigs in 3 experiments to determine carotene metabolism during a specific viral enteric infection, transmissible gastroenteritis (TGE).

Clinical signs and gross and microscopic lesions were not noticeably different between infected animals fed carotene and those not fed carotene. Fecal carotene excretion was significantly reduced within 24 hours after oral inoculation with the TGE virus. Fecal carotene excretion was greater in pigs fed water-dispersible beta-carotene beadlets than in pigs fed an oil-soluble beta-carotene.

The virus of TGE produced sprue-like lesions characterized by villous atrophy in the small intestines of all infected animals. Diarrhea was present within 72 hours after inoculation with the infective agent and continued until the pigs were killed for necropsy.

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