

## THE RELATION OF VITAMIN C DEFICIENCY TO INTESTINAL TUBERCULOSIS IN THE GUINEA PIG

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PLATES 26 TO 28

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Intestinal tuberculosis is one of the most frequent and serious complications of phthisis. Up to the present time attempts at prevention and treatment have been empirical and unsatisfactory, because the factors involving the production of the intestinal ulcers have not been well understood. The swallowing of sputum laden with bacilli cannot be the sole cause of the condition since many patients swallow such sputum in large quantities for years without developing intestinal lesions, and the problem resolves itself with the question of why the intestines of some patients are susceptible and others resistant to the bacilli passing through them.

Numerous attempts have been made in the past by many workers to produce intestinal tuberculosis in the guinea pig by feeding either tubercle bacilli or tuberculous material. As a rule, the animals develop enlarged cervical or mesenteric lymph glands and die of generalized tuberculosis without developing ulcers in the intestinal tract, but success has been claimed by a few workers. From the reports of the following investigations it is difficult to determine whether or not the diets of their experimental animals were adequate in vitamins.

Villemin (8) in 1868 fed 40 gm. of tuberculous sputum to guinea pigs and killed the animals 3 months later. Lesions were found in the small bowel and cecum. Klebs (4) in 1870 fed guinea pigs tuberculous matter and found tuberculous ulceration in the intestines in two animals. Redman (7) in 1922 reported that in every instance in which he fed virulent tubercle bacilli to guinea pigs, the mesenteric lymph nodes became involved in 34 to 41 days. There were tuberculous nodules in the cecum and colon at 81 and 86 days. They were not open ulcers and no microscopic examinations were reported. Medlar and Sasano (6) in 1924 inocu-

lated eight guinea pigs subcutaneously with human tubercle bacilli (No. H 37) and kept them for a period of 65 to 145 days. One animal had several ulcers in the cecum. Serial sections revealed tubercles in the lymphoid tissue of the small intestines in six of the remaining pigs. All the animals had extensive disease of the liver, and the authors felt that bacilli were discharged through the bile and thus infected the lymphoid tissue of the intestines. Griffith (3) in 1924 fed twelve guinea pigs with emulsion made from tuberculous tissue of animals infected with bovine tubercle bacilli. All of the animals were killed between the 43rd and 44th days, and all showed from one to many small caseous areas in Peyer's patches, the majority of which were ulcerated on the mucous surface. In the large intestines they were situated in the solitary follicles of cecum or colon and many were ulcerated.

One of us (5) has shown that patients with intestinal tuberculosis frequently make rapid and permanent improvement of their intestinal condition when given cod liver oil and tomato juice in addition to the regular hospital diet, while similar patients given the hospital diet alone do badly. Furthermore we have noticed that the addition of these vitamin carriers to the regular hospital diet seems to be a valuable prophylactic measure. These observations encouraged us to undertake the experiments on the rôle of certain vitamin deficiencies in the production of intestinal tuberculosis that are the subject of this paper.

*Preliminary Experiments.*—In February, 1928, we conducted a series of preliminary experiments. Forty-five guinea pigs were given a basic diet of hay, rolled oats, and autoclaved milk *ad libitum*. Of these animals, some were sensitized with non-virulent human tubercle bacilli (No. R 1 Saranac Lake Laboratory) and fed virulent bacilli (No. H 37 Saranac Lake Laboratory) in gelatin capsules; others were fed No. H 37 in capsules without being sensitized; some were confined to the basic diet, while others received in addition small quantities of cod liver oil and tomato juice.

Of twenty-five animals that were fed tubercle bacilli with a diet deficient in vitamins A, C, and D, two developed tuberculous ulcers of the cecum in addition to extensive tuberculosis elsewhere; while none of ten infected guinea pigs that received an adequate diet showed intestinal lesions at necropsy.

We learned that this basic diet was far too severe for our purpose, most of our animals dying of scurvy before the tubercle bacilli had time to produce intestinal lesions. We also found that adult guinea pigs withstood the deficiency of vitamins in their food much better than

young animals. These preliminary experiments were far from conclusive but were sufficiently suggestive to induce us to continue the investigation.

In the following experiments we used only adult guinea pigs fed on a vitamin-poor basic diet without fruit or fresh vegetables.

This diet was composed of white bread (dried and ground) 20 per cent, chipped beef (ground and dried) 20 per cent, potato chips (ground) 20 per cent, rolled oats 15 per cent, skimmed milk powder 15 per cent, and white cane sugar 10 per cent. The various ingredients were thoroughly mixed and fed to the animals *ad libitum* along with hay and water. This diet was adequate in calories, in protein of good quality, in mineral salts, and in vitamin B<sub>1</sub> and B<sub>2</sub> (G). It was relatively deficient in vitamins A, C, and D. The animals ate the diet with great relish and gained weight for the first 2 or 3 weeks. The appetite then failed and they died in 6 to 8 weeks with evidence of scurvy. If, however, this basic diet was supplemented by 0.3 cc. of cod liver oil and 10 cc. of tomato juice daily, the pigs remained strong and vigorous and lived for months. Young pigs have been reared to maturity on this diet. Some became pregnant but aborted. There were necrotic changes in the placenta, which Evans and Bishop (2) have shown are characteristic of vitamin E deficiency. That there was nothing inherently injurious in this diet is shown by the experiment in which we replaced cod liver oil and tomato juice with cabbage leaves, and the animals gave birth to and reared normal young. By supplementing this basic diet with 2 or 3 cc. of tomato juice daily after the appetite began to fail, the guinea pigs were maintained for months in a prescorbutic state.

*Experiment I. Jan. 4, 1929.*—Fifty-two adult guinea pigs were used in this experiment.

1. Twenty-six normal adult guinea pigs (Groups A, B, C, and D) were used to determine the effect of various diets on healthy animals.

Group A. Ten normal adult pigs received a basic deficient diet only. These animals died between the 6th week and the 4th month. Two had duodenal ulcers at necropsy. Several developed a pyorrhea-like condition about the teeth; pus from the lesions showed the presence of spirochetes, fusiform bacilli, and cocci. One developed a pulmonary lesion in which these organisms were also present. Five of the ten animals lost one or more incisor teeth. Some of the animals presented evidence of scurvy and some did not.

Group B. Five normal adult pigs received a basic diet plus 0.3 cc. of cod liver oil daily. The oil was given by mouth with a tuberculin syringe. These animals died between the 6th week and the 3rd month. Several had a pyorrhea-like condition of the gums and lost incisor teeth. One animal had a few small necrotic areas in the cecum.

Group C. Five normal adult pigs received a basic diet plus 10 cc. of tomato juice daily. The juice was given with a pipette. These animals did very well for 2 months. Then two of them died immediately after giving birth to young. The

mothers showed no evidence of scurvy but the new-born pigs showed marked signs of this disease. The remaining three animals were killed in the 5th month at the end of the experiment and found to be normal.

Group D. Six normal adult pigs received a basic diet plus 0.3 cc. of cod liver oil and 10 cc. of tomato juice daily. These animals did well for 2 months. Two pigs died of spontaneous pulmonary disease between the 2nd and the 3rd month, and the remaining four were killed in the 5th month at the end of the experiment and found to be normal.

2. Twenty-six adult guinea pigs (Groups E, F, G, and H) were used to determine the effect of various diets on tuberculous animals. Each pig was sensitized by a subcutaneous injection of non-virulent human tubercle bacilli (No. R1 Saranac Lake Laboratory) and subsequently fed 0.5 cc. of tuberculous sputum with a tuberculin syringe daily.

Group E. Ten of the above pigs received a basic diet and 0.5 cc. of tuberculous sputum daily. These animals died between the 2nd and 5th months. Three animals which died early were found to have a normal intestinal tract. Five animals presented one or more open tuberculous ulcers in the intestines and two animals showed caseous tubercles in the lymph follicles of the ileum which had not ulcerated. The ulcerative lesions were most common in the ileum, with cecum next, and jejunum and colon least frequently involved.

Group F. Six sensitized adult pigs received 0.5 cc. of tuberculous sputum daily, and were fed in addition to the basic diet 0.3 cc. of cod liver oil daily. These animals died between 6 weeks and 4 months. One animal had a normal intestinal tract, and one had non-ulcerated caseous tubercles in the ileum, while the remaining four presented from one to twenty open tuberculous ulcers in the ileum, cecum, jejunum, and colon.

Group G. Five sensitized adult pigs received 0.5 cc. of tuberculous sputum daily, and were fed in addition to the basic diet, 10 cc. of tomato juice daily. These animals all did well and were killed in the 5th month at the end of the experiment. One animal had a caseous tubercle in the ileum which had not ulcerated. The other four animals presented normal intestinal tracts, although there was extensive tuberculosis in the liver, spleen, and lungs.

Group H. Five sensitized adult pigs receiving 0.5 cc. of tuberculous sputum daily were fed in addition to the basic diet 0.3 cc. of cod liver oil and 10 cc. of tomato juice daily. These animals all did well except one which died of unknown causes in the 10th week. The remaining four were killed in the 5th month at the end of the experiment. The intestinal tracts of all five animals were normal although considerable tuberculosis was present in the liver, spleen, and lungs.

#### *Discussion of Experiment I*

Normal adult guinea pigs kept on the vitamin poor diet described died in from 6 to 16 weeks; some showed definite evidence of scurvy, while others presented a variety of lesions ascribable to the deficient

diet. Cod liver oil did not prevent the development of these lesions, but the addition of 10 cc. of tomato juice daily to the basic diet protected the animals completely from all signs of deficiency disease.

Sensitized guinea pigs maintained on the basic diet and fed 0.5 cc. of tuberculous sputum daily developed ulcerative tuberculous lesions in the intestines in five out of ten cases. When cod liver oil was added to the basic diet, four out of six animals developed ulcerative intestinal tuberculosis. When, however, the basic diet was supplemented with 10 cc. of tomato juice daily, the animals remained in good health and only one out of ten showed any trace of intestinal tuberculosis.

To recapitulate—of the sixteen sensitized guinea pigs on diets deficient in vitamin C or in vitamins C, A, and D, which received 0.5 cc. of tuberculous sputum daily, nine presented one or more open tuberculous ulcers of the intestines and three presented caseous non-ulcerative tubercles while only four animals presented normal intestines at necropsy. In contrast, nine out of ten guinea pigs presented normal intestinal tracts when the basic diet was supplemented either with tomato juice or cod liver oil and tomato juice. The remaining animal showed a few non-ulcerative tubercles in the ileum.

*Experiment II. Mar. 15, 1929.*—Forty-six normal adult pigs were used in this experiment (Groups I, J, K, L, and M). With the exception of ten animals in Group M, all the pigs received three doses of approximately 1 mg. of virulent human tubercle bacilli (No. H 37, Saranac Lake Laboratory) by mouth between Mar. 15 and 20. They were given our regular stock diet until Apr. 15 when a number of the pigs presented palpable glands in the neck. The animals were then divided into five groups.

Group I. Sixteen of these animals were given the basic diet described in Experiment I, and 0.5 cc. of tuberculous sputum daily. Eleven of the sixteen pigs died between the 6th week and the 3rd month. The remaining five were killed at the end of the 4th month. Necropsy showed that thirteen pigs had from one to ten tuberculous ulcers in the intestines, two presented caseous non-ulcerative tubercles in the ileum, and only one intestinal tract was normal. In addition, three of the animals had duodenal ulcers and one a pyloric ulcer, all of which were shown to be non-tuberculous on microscopic examination.

Group J. Five adult guinea pigs received 0.3 cc. of cod liver oil and 0.5 cc. of tuberculous sputum in addition to the basic deficient diet. Three of the five pigs died between the 2nd and 3rd months, the remaining two were killed at the end of the 4th month. One animal had numerous caseous non-ulcerative tubercles in the ileum and cecum, and the other four had from one to fifteen tuberculous ulcers in the intestines. One animal had a small duodenal ulcer that was not tuberculous.

Group K. Five adult guinea pigs received 10 cc. of tomato juice daily and 0.5 cc. of tuberculous sputum in addition to the basic diet. These animals were killed at the end of the experiment. Although they had extensive generalized disease, the intestinal tracts of all of them were normal.

Group L. Ten adult guinea pigs received 0.3 cc. of cod liver oil, 10 cc. of tomato juice, and 0.5 cc. of tuberculous sputum daily as well as the basic diet. One of the ten animals died in the 6th week; this animal had refused to swallow any but a small amount of the tomato juice and necropsy revealed numerous open tuberculous ulcers in the intestines. The remaining nine animals were killed at the end of the 4th month. One animal had an open tuberculous ulcer and one had a few caseous non-ulcerative tubercles in the ileum. The remaining seven guinea pigs presented normal intestinal tracts.

Group M. Ten adult guinea pigs which had not been given No. H 37 by mouth received a limited amount of cabbage leaves and 0.5 cc. of tuberculous sputum in addition to the basic diet. These animals did well and were killed at the end of the experiment. At necropsy, none of them presented open tuberculous lesions. However, five had from one to ten caseous non-ulcerative tubercles in the lymph follicles of the ileum. The remaining five guinea pigs showed normal intestinal tracts.

#### *Discussion of Experiment II*

The non-sensitized animals in Experiment II showed much more extensive generalized disease than the sensitized animals in Experiment I, in which the animals had been previously sensitized by a subcutaneous injection of R1. Intestinal lesions developed more frequently in the non-sensitized than in the sensitized animals when fed tuberculous sputum and maintained on a deficient diet. Of the twenty-one animals which were given a diet deficient in vitamin C or in A, C, and D, seventeen developed open tuberculous ulcers, three caseous non-ulcerative lesions, and the intestinal tract of only one animal was normal at necropsy. It was also more difficult to protect these non-sensitized animals against the lesions in the intestines even when adequate diet was given. Twenty-five guinea pigs received either tomato juice, cod liver oil and tomato juice, or cabbage leaves in addition to the basic diet. Seventeen of these animals had normal intestinal tracts at necropsy. Six presented one or more caseous non-ulcerative tubercles in the ileum, while two presented from one to ten open tuberculous ulcers. Two of the animals with ulcers were known to have consistently refused to take the full amount of tomato juice. Summarizing the results of Experiments I and II,

we obtain the following figures: of thirty-seven animals on diets deficient in vitamin C or in vitamins A, C, and D, twenty-six developed from one to fifteen open tuberculous lesions of the intestines, six developed caseous tubercles which were not ulcerative, and five presented normal intestinal tracts. In contrast, of thirty-five animals which received either tomato juice, cod liver oil and tomato juice, or cabbage leaves, twenty-six presented normal intestinal tracts at necropsy, seven showed from one to ten caseous tubercles which were not ulcerative, and only two had open ulcers.

#### *Pathology*

The gross and microscopic appearance of the tuberculous lesions in the intestines of the guinea pig are similar to the tuberculous lesions described by Gardner (1) in man, and by Medlar and Sasano (6) in both man and the guinea pig. The primary lesion is a submucous tubercle in the lymphoid tissue of the intestinal tract. In the early stages, the mucosa over the lesion is intact (Fig. 2). Later the submucous tubercle becomes caseous, ruptures through the mucosa, discharges its contents, and becomes an ulcer (Figs. 1 and 3). The earliest stage of the process which we have studied is shown in Fig. 4. In gross, this solitary follicle seemed somewhat swollen, but otherwise normal. On section, numerous tubercle bacilli were found in large mononuclear cells, some of which showed evidence of degeneration. No giant or epithelioid cells were present in this lesion (Fig. 5). In Fig. 6 an acute ulcerating lesion is shown in which tubercle bacilli were found in large mononuclear cells and in giant cells. Very little necrosis was present. In a more advanced stage of the process, as shown in Fig. 7, the cells near the surface were necrotic. Beneath this was typical tuberculous tissue with giant cells, epithelioid cells, lymphocytes, leucocytes, and fibroblasts. A more chronic tuberculous ulcer is shown in Fig. 3. The necrotic zone has been expelled, fibroblasts were present, and there was a marked infiltration of the lesion with lymphocytes.

Our object in these experiments was to determine if possible the part played by vitamin C deficiency in the pathogenesis of intestinal tuberculosis in the guinea pig. In order to do this we compared the intestinal lesions due to scurvy with those of tuberculosis. We found

that in the early scorbutic lesions submucosal hemorrhage was the outspoken feature; the mucosa overlying the area of hemorrhage becomes necrotic, sloughs, and forms an ulcer (Figs. 9-11). The initial tuberculous lesion also occurs in the submucosa (Figs. 2, 12) and apparently goes on to ulceration (Fig. 3) when there is not sufficient vitamin C in the diet. Apparently, an adequate supply of vitamin C in the diet protects the guinea pig against ulcerative intestinal tuberculosis, even in the presence of submucous tubercle.

The guinea pig and man appear to be similar in their vitamin C requirements. Patients with intestinal tuberculosis improve when tomato juice is added to their diet, while our tuberculous guinea pigs develop intestinal lesions unless protected by an adequate supply of vitamin C in their food.

Our experiments however, do not explain the clinical value of cod liver oil in intestinal tuberculosis. Man is quite susceptible to vitamin A and D deficiencies, while the guinea pig is not. It is thus possible that a deficiency of vitamins A and D (the vitamins of cod liver oil) plays a part in the development of intestinal tuberculosis in man and not in the guinea pig. Before this part of the problem can be settled it will be necessary to repeat our experiments utilizing an animal such as the monkey which is susceptible to the same vitamin deficiencies as man and also to tuberculosis.

#### CONCLUSION

1. Seventy-two adult guinea pigs were fed tuberculous sputum daily for periods ranging from 6 weeks to 4 months.
2. Thirty-seven of these were maintained on a diet partially deficient in vitamin C; twenty-six developed ulcerative intestinal tuberculosis.
3. In the remaining thirty-five animals whose diet was supplemented by an adequate amount of vitamin C only two developed tuberculous ulcers in the intestines.
4. From these studies we conclude that the ingestion of tubercle bacilli by the guinea pig is not the sole factor in the production of intestinal tuberculosis.
5. In our opinion, an adequate supply of vitamin C usually protects the guinea pig against ulcerative intestinal tuberculosis.



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## EXPLANATION OF PLATES

## PLATE 26

FIG. 1. Photograph of tuberculous ulcers from the small intestine of a guinea pig.  $\times 2$ .

FIG. 2. Low power photomicrograph of a submucosal tubercle in the intestine of a guinea pig. The mucosa is still intact.  $\times 35$ .

FIG. 3. Low power photomicrograph of a chronic tuberculous ulcer in the intestines of a guinea pig.  $\times 35$ .

FIG. 4. Low power photomicrograph of an early tuberculous involvement of a solitary follicle in a guinea pig. See Fig. 5.  $\times 20$ .

## PLATE 27

FIG. 5. Camera lucida drawing of a section from early tuberculous ulceration of a solitary follicle in the intestines of a guinea pig. The tubercle bacilli are present only in monocytes. Some of the monocytes are degenerating. A low power photomicrograph of the section from which this drawing was made is shown in Fig. 4. Approximately  $\times 800$ .

FIG. 6. Camera lucida drawing of a section from an early tuberculous ulcer in the intestines of a guinea pig. Tubercle bacilli are seen in monocytes and in giant cells. Note the dilatation of the capillaries and the proliferation of fibroblasts. Approximately  $\times 800$ .

FIG. 7. Camera lucida drawing from a section of an extensive tuberculous ulcer in the intestines of a guinea pig. The superficial portion of the lesion is entirely necrotic. Beneath this layer one sees polymorphonuclear leucocytes in various stages of degeneration. Fibroblasts, lymphocytes, monocytes, and giant cells are seen in the depth of the lesion. Approximately  $\times 800$ .

FIG. 8. Camera lucida drawing of a giant cell in a submucosal tubercle in the intestines of a guinea pig. Low power photomicrograph of the lesion from which this drawing was made is shown in Fig. 2. Approximately  $\times 800$ .

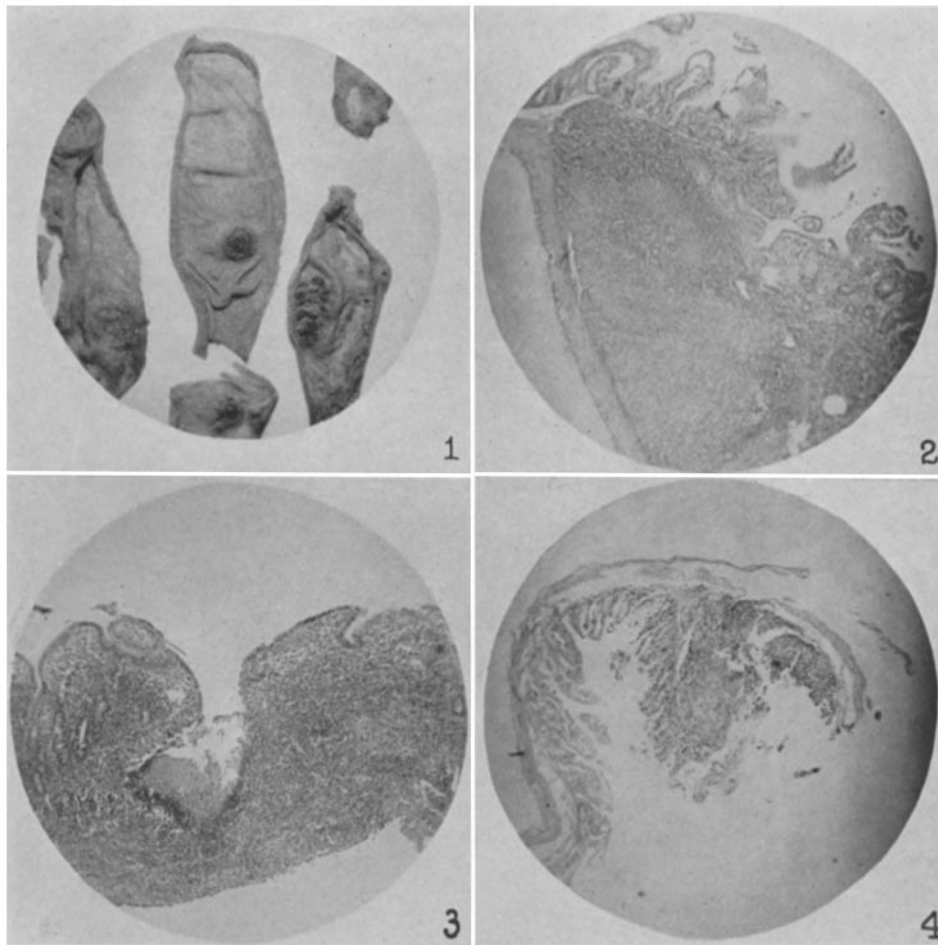
## PLATE 28

FIG. 9. Low power photomicrograph of acute scorbutic ulcer in the ileum of a guinea pig.  $\times 15$ .

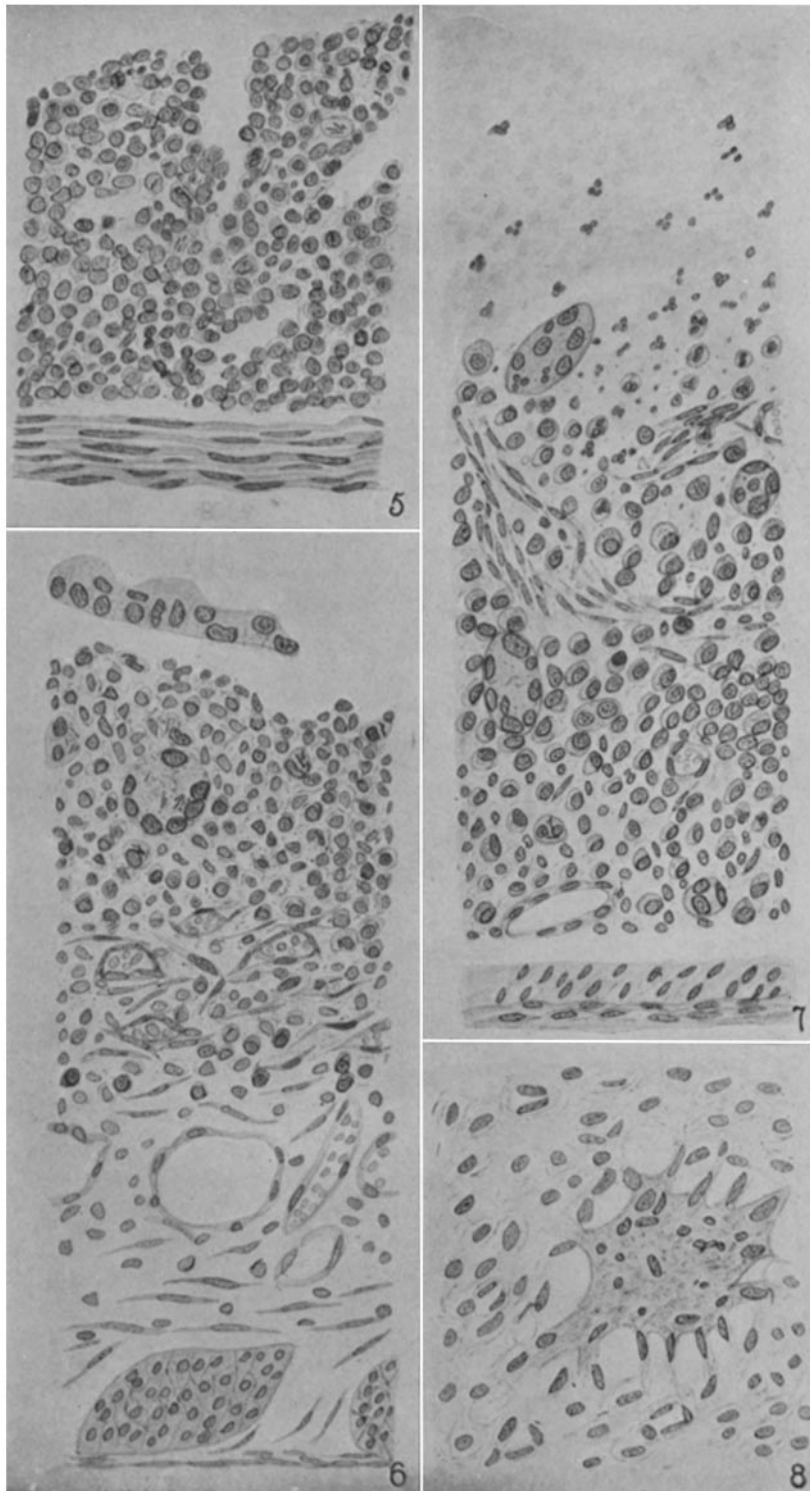
FIG. 10. Low power photomicrograph of acute scorbutic ulcer in the ileum showing hemorrhage in mucosa and submucosa.  $\times 90$ .

FIG. 11. High power photomicrograph of section shown in Fig. 10. Note marked degeneration of mucosa and absence of cellular reaction.  $\times 390$ .

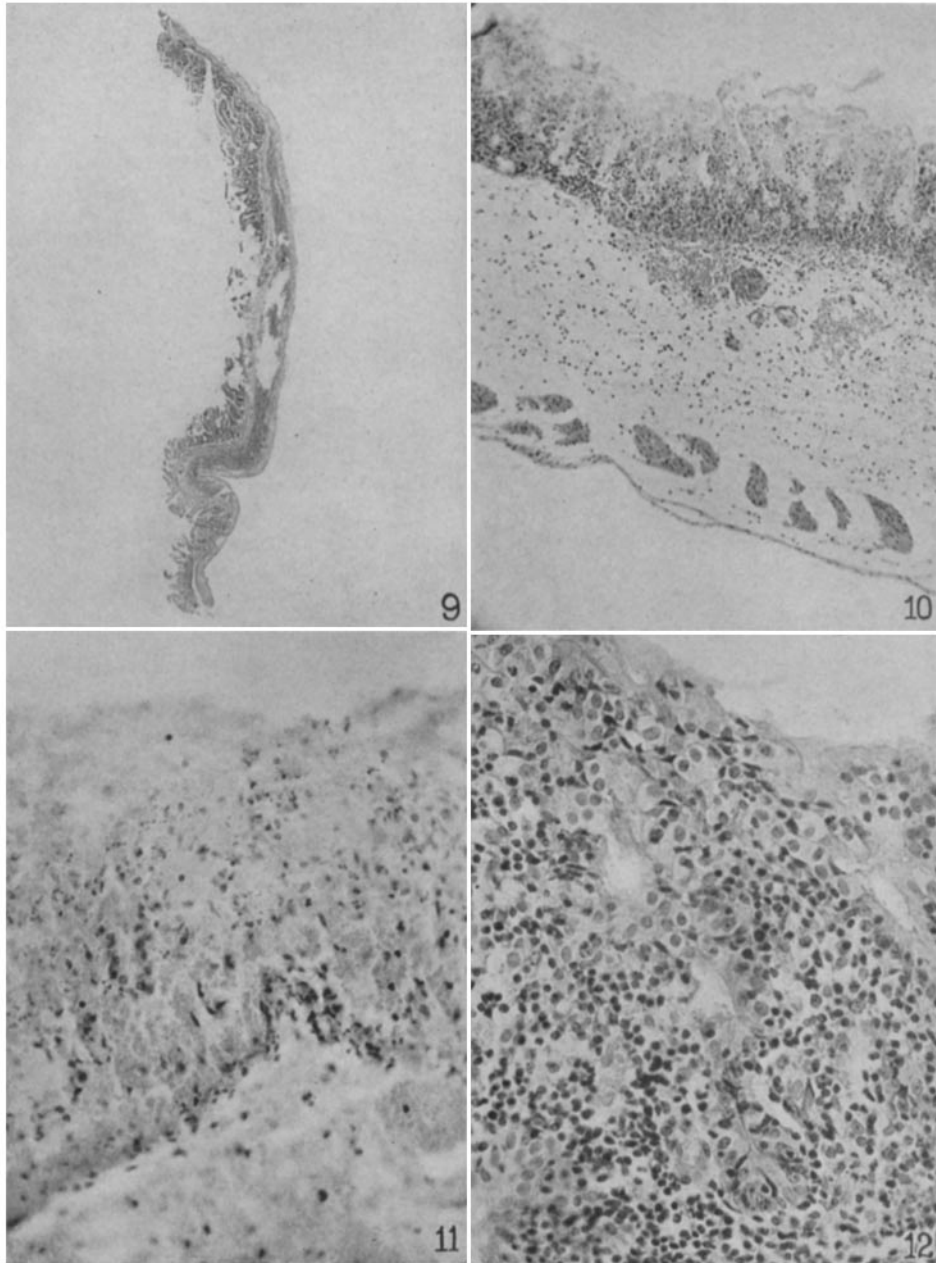
FIG. 12. High power photomicrograph of the mucosa overlying the caseous tuberculous lesion seen in Fig. 2. Note the accumulation of well preserved lymphocytes and monocytes in contrast to the ischemic necrosis found in the scorbutic lesion shown in Fig. 11.  $\times 430$ .



(McConkey and Smith: Vitamin C lack and intestinal tuberculosis)



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