carry a subsequent normal litter within 131 days is evidence of lack of intoxication. This is supported by an average weight gain of 41.4% of mother animals from onset of first aborted pregnancy to first day of the last carried through litter.

Summary. (1) One or 2 doses of 7 mg/kg of TC or 2.5 mg/kg of MC destroyed the litters of rats when given after 10th day of gestation period. (2) Placentas survived the fetuses. (3) Maternal response to dosage consisted in depression of myeloid tissues of bone marrow, peripheral WBC and prothrombin time. (4) Four consecutive litter destructions *in utero* with TC were well tolerated by a group of rats, did not impair their subsequent fertility or reproduction, nor cause abnormalities in subsequent offspring. (5) The same doses of TC or MC given prior to fertilization, or implantation were less effective in litter destruction.

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Cytochemistry of Male Reproductive Tract in Scurvy and Inanition.* (24083)

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Investigations have related ascorbic acid to the male reproductive tract. Lindsey and Medes(1) found degeneration of the germinal epithelium in acute scurvy. The inanition which accompanied scorbutus produced the same alterations(1). Goettsch(2) noted similar changes in the testes of scorbutic guinea pigs. Mukherjee and Banerjee(3) observed degeneration and fibrosis of germinal epithelium and Leydig cells in chronic scurvy. They related these alterations specifically to ascorbic acid deficiency rather than to the accompanying inanition. Vit. C studies on animals other than the guinea pig indicate that ascorbic acid is necessary to maintain male reproductive function(4). In the present investigation, cytochemical studies were carried out on the effects of avitaminosis C on the reproductive tract of the male guinea pig, and an attempt was made to differentiate these changes from those which result from inanition in the presence of an adequate dietary intake of ascorbic acid.

Materials and methods. A total of 80 male guinea pigs were used in this investigation. Each experimental group consisted of 4 animals which were fed as follows: (a) Vit. C deleted diet, *ad lib.*, (b) Vit. C deleted diet, *ad lib.*, plus 10 mg ascorbic acid daily, (c) Vit. C deleted diet plus 10 mg ascorbic acid daily with food consumption adjusted in order that the animal's weight losses would follow those of the scorbutic guinea pig, and (d) commercially prepared Vit. C fortified diet *ad lib.* The animal on the restricted diet (c) was used to differentiate between changes brought about by caloric restriction and those induced by ascorbic acid deficiency. The Vit. C deleted diet was prepared according to Woodruff *et al.*(5) with the following additions: 10 g $CaCo_3$, 2 g K acetate, 40 g Ca pantothenate, 12 mg folic acid and 200 mg ferric citrate per kg of diet. Groups remained on their respective diets until the scorbutic animal appeared

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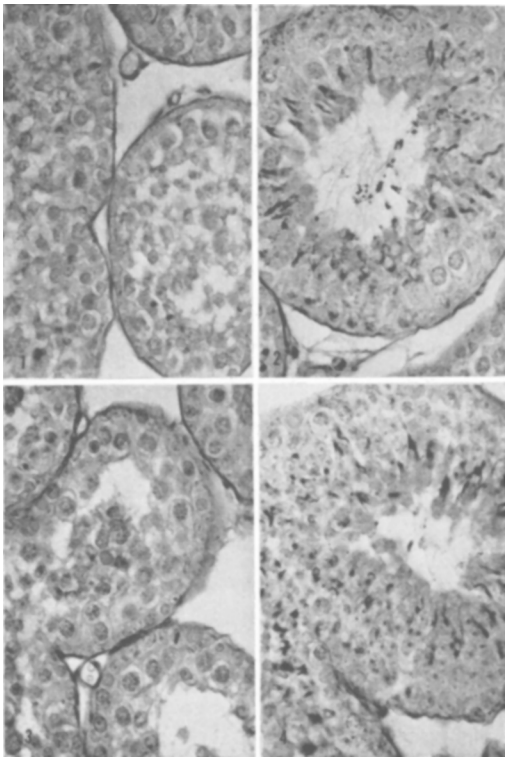

FIG. 1. Scorbutic seminiferous tubules indicating aspermiogenesis. Allochrome staining. ×380.
FIG. 2. Allochrome stained section of dietary control testis. Note presence of developing spermatids. ×380.
FIG. 3. Seminiferous tubules of inanition control animal. Observe absence of spermatids. Allochrome stain. ×380.
FIG. 4. Seminiferous tubule of normal guinea pig. Stained as above. ×380.

moribund showing a typical scorbutic facies, neuritis of the hind limbs, bloody diarrhea, anorexia, malaise and severe weight loss. The time for the development of this syndrome was 25-36 days. Testes were fixed in Orths' fluid and seminal vesicles and epididymides in Lillie's acetic acid alcohol formalin. These tissues were stained by the periodic acid-Schiff technic (PAS) to demonstrate the acrosomic derivatives and differentiate the various stages of spermiogenesis(6). Two types of controls were employed(7). These included: (a) treatment in salivary amylase for one hour at 37°C in order to remove glycogen and (b) staining without previous passage through periodic acid. Reactions were considered positive only if they stained after treatment with periodic acid. The allochrome technic was used to study nuclear morphology and connective tissue elements. In order to demonstrate ascorbic acid, testes, epididymides and seminal vesicles were placed in an acetic acid-silver nitrate alcoholic solution for 30 minutes followed by acid fixation in sodium bisulfite and sodium thiosulfate for 2 hours(8).

Results. Testicular and epididymal morphology. Following application of the PAS reaction to the testes of normal guinea pigs, Schiff-positive staining was localized in the cytoplasm of the interstitial cells, walls of blood vessels, lamellated connective tissue and the basement membrane of seminiferous tubules. Glycogen was present in some of the blood vessel walls. The cytoplasm of the spermatogonia and spermatocytes gave a slight reaction, and numerous brightly staining granules were observed in the latter cells. Throughout spermiogenesis a weak cytoplasmic staining was evident. It was observed that the idiosome was present as a slightly reactive region in the Golgi zone and its granulation was fine and diffuse. With the technics employed it was possible to follow the stages of spermiogenesis in the guinea pig (Fig. 4). Results obtained are in agreement with earlier studies(6). Twelve of the 20 scorbutic animals showed spermiogenic arrest indicated by deficiency of spermatids and spermatozoa in the testes (Fig. 1). Similar changes were noted in 6 inanition guinea pigs (Fig. 3), and 4 of the 8 inanition animals, in which spermatozoa were seen, had large areas of germinal cell aplasia. A single dietary control animal had no spermatozoa and 3 had limited spermiogenic blockage. With these exceptions, the testicular histology of the dietary controls was normal (Fig. 2). Three normal guinea pigs demonstrated aspermiogenesis; however, these particular animals were immature in as much as other pathologic changes consistent with scorbutic or starved animals were not evident. Other indications of spermiogenic arrest in the scorbutic and inanition animals were noted. Nine of each of these guinea pigs had no spermatozoa in the lumen of the ductus epididymis. This was noted in 4 normal animals. Three of these guinea pigs had demonstrated evidence of im-

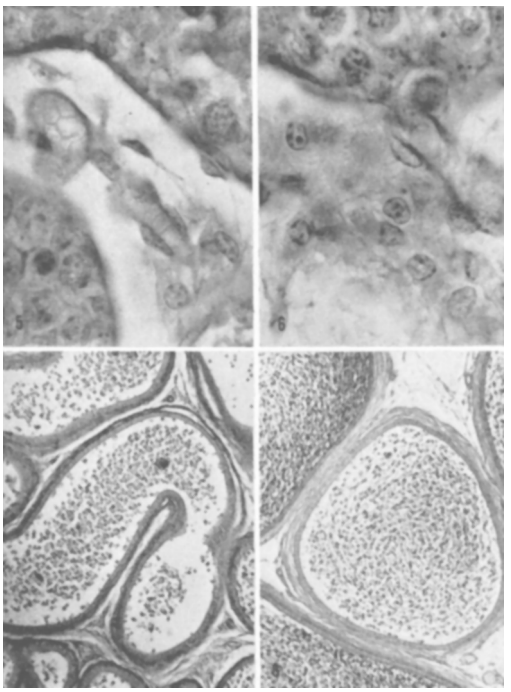

FIG. 5. Leydig cells of scorbutic guinea pig demonstrating pyknotic nuclei and spindling. ×950.

FIG. 6. Leydig cells of normal guinea pig. ×950.

FIG. 7. Cauda epididymidis of scorbutic animal. Note sloughed immature spermatids in lumen of ductus. ×96.

FIG. 8. Cauda epididymidis of normal control guinea pig. Spermatozoa in lumen of ductus. ×96.

maturity on histological examination of the testes. Three dietary controls had no spermatozoa in the ductus epididymis. Sloughed immature and degenerating spermatids were seen in the epididymides of 15 scorbutic guinea pigs (Fig. 7) and 8 inanition controls. This occurred in 2 normal animals and one dietary control; in all 3 of these cases, spermatid sloughing was moderate. Numerous spermatozoa were present in the ductus epididymis of normal guinea pigs (Fig. 8). Pathologic changes in the Leydig cells were noted in 16 of the 20 scorbutic animals (Fig. 5). These cellular alterations consisted of pyknotic nuclei, diminished size and varying degrees of elongation. In some cases, these cells were similar to fibroblasts. These changes were more pronounced in guinea pigs with complete spermiogenic arrest. Half of the inanition animals and 2 dietary controls demonstrated Leydig cell changes. No Leydig cell alterations were seen in the normal guinea pigs (Fig. 6). There were no pathological changes in Sertoli cells, basement membrane or intertubular connective tissue of the testes.

Glycogen studies. Variable quantities of glycogen were localized in the testes, seminal vesicles, and epididymides. There were testicular glycogen deficiencies in the scorbutic and inanition animals. One-half of these guinea pigs had little or none of the substance; the remainder had moderate amounts. Six dietary and 5 normal controls had testicular glycogen deficiencies. The scorbutic animals had more glycogen accumulation in the epithelial cytoplasm of the caput and cauda epididymidis than other controls. Glycogen was increased in the cytoplasm of the seminal vesicle epithelium of scorbutic and inanition animals. Furthermore, there occurred a corresponding decrease in seminal vesicle muscle glycogen in these 2 groups of guinea pigs. Less glycogen was localized in the epithelium and muscularis of seminal vesicles of normal and dietary control animals. There was no glycogen in the Schiff-positive lamina propria of all seminal vesicles studied.

Ascorbic acid studies. Eight scorbutic guinea pigs had no Vit. C demonstrable in the testes and 9 animals demonstrated small to moderate amounts; 8 of these had granules of ascorbic acid in the Leydig cell cytoplasm. One inanition control had no testicular Vit. C and 7 had small amounts. Moderate to large quantities were found in a similar number of inanition animals, one of which lacked ascorbic acid in the interstitial cells. All dietary controls had moderate to large amounts of Vit. C in Leydig cells. Three normal controls had decreased testicular ascorbic acid; Leydig cell Vit. C was present in all members of this group. One-half of the scorbutic animals had no ascorbic acid in the epithelium of the caput epididymidis. Vit. C was localized in the epididymis in moderate to large quantities in most dietary and inanition controls. These 2 groups had more ascorbic acid than the normal animals. Similarly, smaller amounts of Vit. C were noted in the cauda epididymidis of scorbutic and normal animals.

The distribution of Vit. C was random throughout the seminal vesicles of scorbutic and control animals. No differences were noted in the ascorbic acid content of these glands.

Discussion. Spermiogenesis was blocked or inhibited in the majority of scorbutic guinea pigs (Fig. 1) and in many inanition control animals (Fig. 3). The degree of aspermiogenesis was related to the maximum percentage body weight loss in the scorbutic animals. This relationship was not as well defined in inanition controls. Ascorbic acid may offer some protection from the effects of inanition. It is suggested that avitaminosis C results in an inability to utilize other essential dietary substances resulting in more severe cachexia than that noted in the inanition controls.

The greatest amount of ascorbic acid is utilized by the guinea pig during its early growth(9). Previous studies(1-3) used animals not exceeding 300 g in weight. In the present investigation, marked changes occurred in the reproductive tracts of scorbutic and starved guinea pigs weighing less than 350 g, less severe changes being noted in more mature animals. The androgen level may offer some protection from inanition and/or scurvy. Guinea pigs maintained on a regimen intermittently free of ascorbic acid had increased resistance to scurvy(10). These animals may have reached sexual maturity before the end of the experiment.

Vit. C is required for proper formative cell function and has been related to the cellular synthetic processes in that it is in greatest concentration in the Golgi apparatus(11). This is substantiated in the present study by spermiogenic arrest. The proacrosomic granule arises from the Golgi apparatus and subsequently forms the acrosomic granule which is the precursor of the acrosome(6,12). Alteration in the chemical composition of the Golgi apparatus may result in spermiogenic arrest as noted in this study. During inanition Vit. C may be unavailable to its target foci in the Leydig cells or germinal epithelium.

In this investigation only scorbutic animals demonstrated absence of Leydig cell Vit. C. Ascorbic acid may be related in androgen production by these cells. However, nearly all scorbutic and inanition animals had early degenerative Leydig cell change, and Vit. C was abundant in inanition controls. Spermatid sloughing may be associated with inability of scorbutic guinea pigs to produce hyaluronic acid(13). Thus the integrity of the germinal epithelium is not maintained. Appearance of normal Sertoli cells in acute scurvy agrees with most work on tissue changes in experimental scurvy. However, degeneration of these cells has been reported (14).

Epithelial glycogen was present in seminal vesicles of scorbutic and starved animals which had sustained severe spermiogenic arrest. Most normal and dietary controls had abundant testicular glycogen. Scorbutic and inanition guinea pigs had less. Glycogen depletion might result in starvation, but an explanation for seminal vesicle deposition of those same animals is difficult. This may be related to the fructose metabolism of these glands which in turn is an indicator of androgenic activity(15). Thus there is evidence suggesting that scurvy and/or inanition plays a role in reproductive physiology as reflected by these carbohydrate metabolism disturbances in testes and seminal vesicles.

Summary. Histochemical studies were conducted on effects of avitaminosis C on the reproductive tract of the male guinea pig, and an attempt was made to differentiate these changes from those which result from inanition in the presence of an adequate dietary intake of ascorbic acid. Results indicate that scurvy may cause an inability to utilize other essential dietary substances resulting in more severe cachexia than that noted in inanition controls. These studies suggest that changes in the cytochemistry of the Golgi apparatus are involved in spermatogenic arrest. Furthermore, as noted in this investigation, the relation of scurvy and/or inanition to reproductive physiology is indicated by altered carbohydrate metabolism in testes and seminal vesicles. These changes were more marked in scorbutic animals than in inanition controls.

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Ciliocytophthoria: Relationship to Viral Respiratory Infections of Humans.*† (24084)

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Papanicolaou(1) described abnormal ciliated epithelial cells found in sputum specimens from some patients with acute and chronic pulmonary disease. The phenomenon was designated ciliocytophthoria, abbreviated as "CCP." The possible significance of CCP as a diagnostic aid in infections of the respiratory tract, and its correlation with the presence or later development of lung cancer were suggested.

The present communication reports observations of sputum cytology, with special emphasis on CCP, in patients with various types of respiratory disease. The results show that CCP is commonly found during pulmonary infections of viral etiology.

Methods. Single specimens produced by deep coughing were collected in 70% ethyl alcohol and smeared and stained by Papanicolaou technic(2). From many patients daily samples were obtained during illness and convalescence. The presence or absence of CCP was ascertained using as criteria the degenerative changes of ciliated epithelial cells described and illustrated in the original report (1). Only those specimens produced by a deep cough (as evidenced in the smear by presence of histiocytes containing carbon particles) were included in the analysis. Smears composed predominantly of squamous epithelial cells and devoid of dust cells were discarded as unsatisfactory. The normal cytogram of deep cough sputum specimens has been described and illustrated by Carabelli (3). Our data were obtained from specimens processed within one or 2 days after sample was received in the laboratory. It may be of practical use to mention, however, that CCP can be demonstrated in positive sputum specimens stored in 70% alcohol at 4°C up to 6 months. Although other cell types are not

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