TISSUE CHANGES FOLLOWING DEPRIVATION OF FAT-SOLUBLE A VITAMIN.

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Plates 37 to 40.

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INTRODUCTION.

No adequate account of the histopathology of experimental fat-soluble (vitamin) A deficiency has hitherto been published, and no conception of the pathogenesis of the striking gross effects observed in this condition has been possible.

Few pathological studies have been made, and the majority of these have resulted in wholly negative results and, therefore, erroneous conclusions as to the sequence of events and importance of infections.

Analysis of the following papers in most instances reveals as cause for failure either improperly composed diet or insufficient duration of the experiments. A number of workers have assumed that the eye and its glands alone deserved study—organs which we have found to exhibit much less striking lesions than the respiratory and genitourinary tracts and certain glands. In light of the pathology described in this paper it is no longer tenable to characterize the condition of fat-soluble A deficiency by names referring to the eye pathology; *i.e.*, xerophthalmia—keratomalacia. Atrophy of many glands, arrest of growth, emaciation, and replacement of many different epithelia by stratified keratinizing epithelium actually characterize fat-soluble A avitaminosis. The specific pathology is the widespread keratinization.

Emmett and Allen[1] in a comparison of changes due to vitamin A and B deficiency respectively in the rat report "no special outstanding pathological findings," in the absence of fat-soluble A, in contrast to atrophies and hypertrophies found in B deficiency. The duration of their experiments was not given.

Stephenson and Clark[2] failed to find a distinctive pathology in keratomalacia in rats.

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[1] Emmett, A. D., and Allen, F. P., *J. Biol. Chem.*, 1920, xli, p. liii.

[2] Stephenson, M., and Clark, A. B., *Biochem. J.*, 1920, xiv, 502.

Tsuji,[3] although his thesis was the effect of complete vitamin deficiency upon the thyroid, noted only atrophy of organs which he regarded as secondary to atrophy of the thyroid.

Davis and Outhouse[4] studied only the kidneys, spleen, heart, lungs, pancreas, liver, and testes. As they report that the testes were normal in most of their cases, it is certain that either their diet was not deficient in fat-soluble (vitamin) A or that the duration of the experiments was too short.

Cramer, Drew, and Mottram[5] in a study of the effects of vitamin deficiency in rats upon the function of lymphocytes and lymphoid tissue found no pathology in fat-soluble A deficiency. The duration of their experiments is not given.

Wason[6] found no lesions in any organ other than the eyes. She regarded the changes in the cornea as secondary to bacterial invasion.

Meyerstein[7] failed to find any characteristic pathology in either vitamin A or vitamin B deficiency in rats.

Outstanding papers dealing with the pathology are those of Mori[8] and Lambert and Yudkin.[9,10]

Mori's paper was published from the department of Chemical Hygiene in the School of Hygiene and Public Health of Johns Hopkins University so that, presumably, his animals were upon diets approved by McCollum. The composition of the diet employed is not stated, nor are protocols given. Information in regard to diet, age, and weights of animals and duration of the experiments and constancy of results would have greatly enhanced the value of this very short but informing paper. Mori, though he studied the internal organs, regarded the changes in the lacrimal gland as the "most important in the pathological picture of this disease." The change he described was a shrinkage in the cells with other evidences of loss of power of secretion. To the resultant dryness he attributed the xerosis (keratinization) of the cornea and conjunctiva. He also mentions xerotic (keratinizing) changes in the mucous membrane of the larynx and trachea, the principal ducts of the submaxillary, sublingual, and parotid glands and in the ducts of the Meibomian glands. The various salivary glands all showed shrinkage (atrophy) of the cells. The Harderian gland showed changes which can probably be summed up as atrophic. The liver, pancreas, "bowel," kidneys, and thyroid were reported as showing no remarkable change. He made an important conclusion to the effect that "it is necessary to consider the clinical picture of xerophthalmia as a series of secondary symptoms which are indicative of hypofunction of a certain part of the

[3] Tsuji, K., *Acta scholæ med. univ. imp. Kioto*, 1920-21, iii, 713.

[4] Davis, M., and Outhouse, J., *Am. J. Dis. Child.*, 1921, xxi, 307.

[5] Cramer, W., Drew, A. H., and Mottram, J. C., *Lancet*, 1921, ii, 1202.

[6] Wason, I. M., *J. Am. Med. Assn.*, 1921, lxxvi, 908.

[7] Meyerstein, A., *Arch. path. Anat. u. Physiol.*, 1922, ccxxxix, 350.

[8] Mori, S., *Bull. Johns Hopkins Hosp.*, 1922, xxxiii, 357.

[9] Yudkin, A. M., and Lambert, R. A., *J. Exp. Med.*, 1923, xxxviii, 17.

[10] Lambert, R. A., and Yudkin, A. M., *J. Exp. Med.*, 1923, xxxviii, 25.

secretory apparatus of the organism." It is difficult to account for Mori's failure to pay more attention to and to pursue further the keratinization of epithelium.

Yudkin and Lambert and Lambert and Yudkin in their two papers reported observations made upon white rats which were fed under the direction of Osborne and Mendel. They gave all essential data and a number of protocols of typical experiments. They studied only the eye and the paraocular glands. They could not confirm Mori's conclusion that a drying of the eye and a cornification of the epithelium are the first events in the eye changes. Their studies led them to conclude that the earliest lesions consist of focal inflammatory lesions in the conjunctivæ of the lids and nictitating membrane. The cornification of the cornea they regarded as a secondary phenomenon. "Pathologically, the ocular manifestations of a deficiency of vitamin A are referable to a low grade inflammatory process, originating in the palpebral conjunctiva and spreading to the cornea." Infection and lowered resistance are important factors according to these authors.

In the paper on the paraocular glands, they fail to confirm Mori's finding in the Meibomian glands. They found the most marked changes in the Harderian gland, degenerative changes consisting of swelling, vacuolation, and, occasionally, complete epithelial degeneration. In the lacrimal gland (intraorbital) they found conditions similar to those described by Mori in normal rats as well as in their experimental rats. In the Meibomian gland they could find no appearances not duplicable in normal rats. In this paper they again call attention to inflammatory reactions and occasionally suppuration as a part of the disease picture.

Because of the very meagre total of pathological findings in experimental fat-soluble A deficiency, and because of some conflicting reports, we have decided to state as fully as possible the conditions under which our experiments were made.

The Rats.

The white (albino) rats were obtained from the animal colony of The Wistar Institute of Anatomy and Biology. Two strains, known as the Tyler Strain and the Experimental Colony Strain, were furnished us.

The Diet.

The diet employed was almost identical with that used by Yudkin and Lambert,[9] and designed by Osborne and Mendel.

We used a larger amount of yeast and gave McCollum's[11] salt mixture 185 for inorganic salts.[12]

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[11] McCollum, E. V., Simmonds, N., and Becker, J. E., *J. Biol. Chem.*, 1922, liii, 313.

[12] In a preliminary publication (Wolbach, S. B., and Howe, P. R., *Proc. Soc. Exp. Biol. and Med.*, 1925, xxii, 402) we have incorrectly recorded the diet we employed as regards yeast and inorganic salt mixture.

The diet as employed by us was

Casein	18
Starch	51
Salt mixture	4
Lard	24
Brewer's yeast	3
	100

The casein, starch, lard, and salt mixture were prepared by the Harris Laboratories. The brewer's yeast (trade name Majestic) came from the Liberty Yeast Company, Cambridge, Massachusetts. Distilled water, sufficient to make a dough, was added to the ingredients; small cakes were moulded, each containing five gm. of material, and dried in an oven. The final temperature was sufficient to melt the lard.

The formula for the salt mixture is

NaCl	0.173
$MgSO_4$ anhydrous	0.266
$NaH_2PO_4 + H_2O$	0.347
K_2HPO_4	0.954
$CaH_4(PO_4)_2 + H_2O$	0.540
Ferric lactate	0.118
Calcium lactate ($5H_2O$)	1.300

This mixture of inorganic salts was found by McCollum, Simmonds, and Becker to be adequate in the prevention and cure of a form of ophthalmia described by them, and which develops in rats supplied with fat-soluble A.

It is interesting to note that Mori [13] regards this form of ophthalmia as identical with that produced by deprivation of fat-soluble A, but his very brief description of the pathology does not support this conclusion.

The daily ration per rat was 5 gm.

The suitability of the experimental diet we used is shown by the facts that none of the rats studied showed gross or histological evidence of rachitic or scorbutic changes, that young rats continued to increase in weight for 2 to 3 months, that the pathological effects were constant, and finally by the completely normal growth and fertility of rats fed upon the control diet in which the only difference was the substitution of butter fat for lard. The control diet was tested for its curative properties and its administration was always followed by prompt amelioration and disappearance of the signs and symptoms of the deficient state.

Controls.

Control rats on the same diet, but with butter fat (prepared from fresh butter by melting and washing in hot water) in place of lard, grew normally and have been maintained in normal healthy condition for a year.

[13] Mori, S., *Am. J. Hyg.*, 1923, iii, 99.

The following experiment shows also that rats were fertile and able to produce healthy offspring while upon this control diet.

The rats were from The Wistar Institute, Tyler Strain, and all four were born on Feb. 28, 1924. They were received on Apr. 9, 1924, and kept on a mixed diet of various grains, white bread, and greens until Aug. 22, 1924, when they were placed upon the control diet. On Apr. 14, 1925, that is after 235 days on control diet, Female 93 and Male 94, and Female 95 and Male 96 were placed together.

On May 12, 1925, Female 95 gave birth to six young, five females and one male. The control diet ration was increased to 15 gm. and supplemented by milk, 15 cc. daily. All six offspring were raised to maturity.

On July 4, 1925, Female 93 gave birth to two normal young which she killed, one on July 6, the other on July 10. This female was kept on a 5 gm. ration of the control diet. We neglected to make certain that lactation was possible.

Clinical Course.

The effects of diet deficient in fat-soluble A upon standard bred albino rats as affecting the growth, nutrition, and gross changes occurring in the eye have been described so often and so well that a repetition here is not desirable. Our observations confirm the established order of events, though in no instance did we observe ulceration of the cornea or hypopyon. Loss of power to smell was a late but constant symptom. In male rats, priapism was almost constantly present for 1 to several weeks before death.

Each experimental animal was kept in a separate cage. Excelsior was used for bedding and the cages and vessels for water were kept scrupulously clean. Each animal was fed and watched and after loss of smell occurred, the food was actually placed in its mouth until the ration was consumed.[14]

In common with other investigators we have observed that a much longer period of deficient diet is required to produce symptoms in full grown adult rats than with young, partially grown rats. Similarly, the histological changes occurred more promptly and more extensively in immature than in full grown rats.

The following table of rats which died in direct consequence of the diet illustrates the effect of age and weight.

[14] The success of this investigation is in large measure due to the meticulous care used in the preparation of the diets and feeding of the animals by M. Otto Heinzer.

No. of rat.	Initial weight.	Duration.	Histological changes.
	gm.	*days*	
224	47	63	+++
227	52	61	++
219	56	78	++
216	73	81	
220	75	91	++++
215	81	80	
206	84	77	++
200	86	81	
217	90	78	+
214	91	75	
221	91	109	
222	92	97	++++
211	94	77	++
206	101	89	+++
213	106	109	
204	116	100	+++
203	134	98	+++

The ages of these rats were between 45 and 60 days. Full grown adult rats, aged 150 to 260 days, survived the diet for periods of 150 to 186 days, and on the whole showed less striking histological changes than the younger rats which survived shorter periods. One rat, aged 139 days at the beginning of the experiment and killed after 157 days of deficient diet, showed no keratinization in any organ.

Gross Pathology.

The external appearances of white rats in advanced fat-soluble A avitaminosis have been well described and illustrated. The humped posture, rough coat, emaciation, and encrusted eyelids are familiar signs.

Careful post mortems on thirty-seven rats have revealed few distinctive lesions besides the effects of retardation of growth, the emaciation and the atrophy of certain organs.

We did not record weights and our statements in regard to atrophy or reduction of size of organs are based upon visual comparison only and in the case of small organs by comparing the entire cross-sections as prepared for microscopical study with the normal for corresponding age and weight.

Gross changes which probably are not peculiar to vitamin A deficiency are the

disappearance of fat in adipose tissue throughout the body, diminution of size of liver and spleen, and actual atrophy of the following glands; submaxillary, parotid, lacrimal, Harderian, extraorbital, pancreas, thyroid, pituitary, thymus, and testes The kidneys, parathyroid, and adrenals show no decided decrease in size. The liver becomes small because of absence of stored fat and glycogen, the spleen because of depletion of lymphoid cells. The organs described as atrophic show changes in individual cells when studied microscopically. The Harderian gland becomes paler than normal, the extraorbital deeper colored.

The degree of atrophy varies with different organs. In the case of the submaxillary gland and the Harderian gland, we have occasionally found almost complete disappearance of secreting tissue. Enlarged lymph nodes may be mistaken for submaxillary glands. The atrophy of the testes, parotid, extraorbital gland, and the anterior lobe of the pituitary may be greater than 50 per cent, we have estimated. The thymus gland practically disappears. Marked edema of the submaxillary gland, and, in males, of the testes, takes place during the progress of the atrophic changes.

The distinctive or peculiar gross changes are those due to mechanical factors sequential to the formation and retention of desquamated keratinized epithelial cells. Thus cysts are produced in the submaxillary gland and accessory salivary glands in the base of the tongue and were often found to reach a diameter of 5 mm. Interference with swallowing, because of the large size of the tongue cysts, has been a cause of death in some rats. In the lungs, bronchiectatic cavities were common and occasionally reached a size of 6 mm. in diameter and were so numerous as to be the cause of death. In the urinary tract, great dilatation of pelves and ureters was not infrequent, and complete blockage with desquamated cells was regarded as the cause of death in several rats. The bladder, even more frequently, was found partially filled with desquamated cells.

In male rats, small cysts of similar origin were common in the prostate glands, while the process in the seminal vesicles led to marked contraction and an opaque yellow coloration, almost constant findings in advanced conditions.

The only gross change in the skeleton that we observed was a dorsoventral bowing of the lower cervical and upper thoracic spine, with ventral convexity. Posture, plus athrepsia, we believe accounts for this bowing as the microscopical study after curative treatment as well as of the untreated showed no evidence of rachitic or scorbutic processes in the vertebræ or elsewhere. Individual vertebræ, however, did show alteration in shape in conformity with the spinal curvature.

Histopathology.

Technique.—Zenker's fixative was employed for routine purposes. The routine stain was a modification of Giemsa's,[15] and was used because we believe it has decided advantages in the study of cell changes and is in no respect inferior to the

[15] Wolbach, S. B., *J. Med. Research*, 1919–20, xli, 1.

eosin-methylene blue method. The bone marrow was studied in sternum and ribs, which were fully decalcified in Zenker's fluid. These bones and all soft tissues were embedded in paraffine. The skull, teeth and nares, and large bones were decalcified in 5 per cent nitric acid, embedded in celloidin, and stained with hematoxylin and eosin.

Mitochondria studies were made upon tissues fixed in Regaud's fluid and mordanted further in 3 per cent potassium bichromate. The sections were stained in Altmann's aniline oil, acid-fuchsin, and counterstained with picric acid.

General Account of the Histopathology.

As we have pointed out in a preliminary report,[16] the specific tissue change due to deprivation of fat-soluble vitamin A is replacement of various epithelia by stratified squamous keratinizing epithelium. Atrophy of glands, including ductless glands and the failure to store fat are other striking phenomena demonstrable histologically.

The formation of keratinizing epithelium takes place in the following locations.

1. *Respiratory Tract.*—In the nares including turbinate bones, nasal septum; in the sinuses communicating with the nares; in the larynx, trachea, and bronchi.

2. *Alimentary Tract.*—In the submaxillary glands, the parotid gland, the accessory salivary glands of the tongue and pharynx, and in advanced conditions only in the pancreatic ducts. The stomach, liver, and small and large intestines are not involved.

3. *Genitourinary Tract.*—In the bladder, ureter, and pelvis of the kidney; in the uterus and oviducts, in the epididymis, prostate, seminal vesicles, and coagulating glands. The kidney epithelium and the epithelium of the seminiferous tubules do not keratinize.

4. *Eyes and Glands.*—In the conjunctiva, Meibomian gland ducts, in the cornea, lacrimal gland, Harderian gland, and the extraorbital (lacrimal) gland.

5. *Ductless Glands.*—In the thymus where occasionally we have found greatly enlarged Hassall corpuscles, veritable small cysts filled with desquamated keratinized epithelium.

In all of the glands, alimentary tract, genital tract and paraocular, the appearance of keratinizing epithelium is preceded by atrophy

[16] Wolbach, S. B., and Howe, P. R., *Proc. Soc. Exp. Biol. and Med.*, 1925, xxii, 402.

of the original epithelium and occurs first in the ducts and later in the gland acini. Infection and suppuration are very common, but not invariable and have nothing to do in initiating the epithelial change. The transformation of epithelium in gland acini is seen easiest in the Harderian gland in very late stages of the avitaminosis.

The sequences of the process have been easiest to follow in the respiratory mucosa upon the turbinate bones, in the trachea and bronchi and in the epithelium of the ducts of the submaxillary gland. In all of these locations the process begins in numerous foci and each focus spreads. The rate of growth is rapid as attested by numerous mitoses of the basal cells. The adjacent viable columnar epithelium becomes undermined by the new stratified epithelium and completely separated from its original mesenchymal support before it has lost the appearances and staining reactions of viability. We emphasize the multiple origin of keratinizing foci. (Figs. 11—17.) The foci do not always begin in ducts or structures nearest the outlet where duct or gland epithelium comes in contact with stratified epithelium. Thus keratinization was often found in bronchi in the absence of the change in the trachea, while careful study of the nares has shown no evidence that the epidermis invades. In the uterus, the earliest changes toward keratinization were seen in the uterine glands and not upon the surface epithelium.

Details affecting different organs will be given in the descriptions of the individual organs. A few general considerations are, however, appropriately considered first.

Infection as the immediate cause of the eye changes is generally held at present, on the basis that resistance or natural immunity is impaired by the avitaminosis. We have made no direct attempts to study resistance in infection in this condition. Our own experiences in the care of the rats are in complete opposition to the importance of infection, either as an initiating factor in the pathology or as a cause of death. Our microscopical studies, while giving frequent evidence of bacterial invasion of the nares, the salivary gland, conjunctiva, bladder, and renal pelvis show quite the contrary in other locations.

Advanced keratinization of conjunctiva, cornea, and Harderian gland usually occurred without microscopical evidence of infection. The same is true of the bronchi and bladder and renal pelvis. In

spite of extreme degrees of bronchiectasis, pneumonia was very rare in our rats. We had not a single example of ulceration of the cornea or of hypopyon, though occasional instances of pyogenic reactions in conjunctiva and cornea. In spite of evidence of bacterial invasion, in some instances of the renal pelvis, following keratinization, there was no instance of bacterial invasion of the substance of the kidney.

The disappearance of stored fat in the face of a liberal amount of lard in the diet plainly indicates focussing chemical and physiological studies upon the fat metabolism. Clinical signs and microscopical evidence of the deficiency do not occur until the bulk of stored fat has disappeared. Yet, we have found extensive keratinization in rats with undiminished fat in bone marrow and the presence of occasional plump fat cells in small numbers in many locations, subcutaneous, perirenal, mediastinal, and omental. We have not devoted special study to the so called glandular adipose tissue.[17]

Though familiar with the appearances and locations of this lobular fat tissue we are not convinced of its having special chemical functions and we believe that adequate study of its presence in relation to age and nutrition has not been made. However, some "glandular" fat may still be present in microscopic amounts in well established pathological conditions of fat-soluble A avitaminosis. The presence of minute amounts of stored fat does not, however, exclude the possibility that the missing factor responsible for the cell changes is one derived from fat.

Mucus is another substance which may be followed microscopically, and inasmuch as the commonest seats of the keratinizing changes are mucus-secreting epitheliums, we have tried to establish some association between the two. This has not been possible, the formation of mucus continues in epithelium in which many foci of keratinization are established, while in the intestinal tract secretion of mucus in typical goblet cells appears to be unaffected up to the death of the animal from avitaminosis.

Mitochondria Studies.

All of the organs susceptible to the changes described as well as liver and kidneys of one control rat and two experimental rats were compared for mitochondria content.

[17] Cramer, W., *Brit. J. Exp. Path.*, 1920, i, 184.

One of the experimental rats, No. 100, was an adult 175 days old at the beginning of the experiment. This rat was killed after 181 days on the deficient diet and showed only early lesions of the deficiency, very slight atrophy of glands and small foci of keratinization in the submaxillary gland, the renal pelvis, and epithelium of the turbinates. Plump fat cells were present in abundance in retroperitoneal tissues, poles of testes, and subcutaneous tissue.

The other rat, No. 205, weighed 104 gm., initial age between 45 and 60 days, was killed after 89 days and showed extensive keratinization in turbinates, posterior lingual, submaxillary glands, and moderate keratinization in Meibomian gland ducts, the Harderian glands, bronchi, bladder, and prostate. This rat showed almost complete loss of stored fat, though a few plump fat cells were found microscopically in the polar fat of testes and subcutaneous tissues and many in the bone marrow.

In the liver, pancreas, kidneys, and adrenals all three rats showed similar mitochondria pictures. In some instances the mitochondria were more numerous in the experimental rats as in the pancreas and kidney of Rat 205, and liver of Rat 100 than in the control, due probably to vagaries of technique.

In thyroid, larynx, trachea, bronchi, Harderian glands, extraorbital, lacrimal, submaxillary glands, and intestinal mucosa there was little if any difference between the three rats.

In the submaxillary gland of Rat 205, where the cell atrophy was most marked, the mitochondria were grouped in close apposition to the nuclei and seemed to be reduced in numbers. However, in the keratinizing epithelium in ducts mitochondria were abundant in the basal layer of cells. In a primary bronchus of Rat 100 where only small islands of keratinizing epithelium were established, and in the larynx of Rat 205, mitochondria were found in normal numbers in the ciliated epithelium in contact with the keratinized patches, and also in the basal cells of the latter.

These observations, while few, seem conclusive and indicate that the mitochondrial apparatus is not primarily affected in fat-soluble A deficiency.

Histological Sequences.

In order to avoid constant repetition of objective findings, we shall present much of our microscopical studies in narrative form, basing our account on the study of tissues from rats in succession of progress of the fat-soluble avitaminosis.

The order in which keratinization involves different organs gives no clue to the possible chemistry involved.

In twenty-five rats showing keratinization, studied histologically, the epithelium of the turbinate bones and the submaxillary gland were

always involved. As a rule, keratinization of trachea and bronchi preceded that of the urinary tract, but in four instances the reverse was true. The pancreas was the last organ to show keratinization. The Harderian glands, and the intra- and extraorbital lacrimal glands were affected much later and in lesser degree than salivary glands, respiratory tract, urinary tract, and uterus in the female. In the submaxillary glands, the ducts of purely mucus-secreting alveoli, mixed types, and pure serous types are simultaneously affected by the sequence leading to keratinization.

The atrophy preceding keratinization may be so slight as to require comparison with normal controls for detection. The diminution in size affects the cytoplasm. The nuclei remain unaltered in size. The original cells of an epithelium do not change their characteristics. The cells destined to produce the foci of stratified keratinizing epithelium may be recognized as clumps of two or more deeply basic staining cells beneath the original epithelium. (Fig. 11.) These cells appear at first to form a syncytial mass. Their nuclei are richer in chromatin than those of the original epithelium and contain conspicuous chromatin "knots." Mitotic figures are numerous and growth may take the form of a thin layer of cells underlying the original epithelium or of a circumscribed clump.

In the trachea, these newly formed cells have been found in a layer of single cells completely encircling the lumen underneath the original and still apparently normal epithelium. With growth which is rapid, as attested to by the number of mitotic figures, the cells form an orderly stratified layer applied in normal fashion to the tunica propria; the superficial cells exhibit the sequences of normal keratinization as regards presence of keratohyaline globules and staining reactions. Throughout the duration of life, multiplication of the basal layer of cells continues at a rapid rate, and large numbers of keratinized cells are thrown off. The original epithelium is apparently able to survive for a considerable period upon the surface of the keratinizing epithelium and the microscopic pictures indicate that it is often cast off in sheets. Where the initial growth is in the form of a clump of cells, and this is the more common finding, Figs. 11, 12, 14, and 15, the focus grows by peripheral extension beneath the adjacent and often apparently normal epithelium. Fig. 16. Thus, in the nares and trachea,

mucus-secreting ciliated epithelium with normal staining reactions and normal appearing nuclei may lie upon a keratinizing layer of cells.

The most striking examples of rapidly continued proliferation of the keratinizing epithelium have been found in the bladder, ureters, and renal pelvis and in these locations we have found responses on the part of the supporting tissues suggestive of the behavior of the stroma in epithelial new growths. The orderly apposition of epithelium and tunica propria has been lost, epithelial downgrowths are present and the greatly thickened layer of epithelium has become vascularized by the incorporation of blood vessels of capillary and precapillary size.

We are endeavoring to learn the consequences of "pyramiding" this effect in rats by alternating the deficient diet with the control diet. These observations on the sequences, particularly the focal character of the initial changes, suggests an unequal rationing of cells with the resources remaining in advancing avitaminosis. It is also evident that factors essential to epithelial proliferation are abundantly present in advanced fat-soluble A avitaminosis. Function, possibly to be expressed as maintenance of differentiation is interfered with.

The Integument Muscle and the Supporting Tissues.—Observations have been made only on the skin of the eyelid and base of the ear where the skin was included incidentally in the preservation of the eye and parotid and extraorbital glands. In these locations no striking changes were found. In the most advanced cases of the avitaminosis, slight but demonstrable atrophy of the hair follicles and sebaceous glands was found. The epidermis in a few instances was thinner than in controls and the keratinized layer less pronounced.

The connective tissue throughout the body is unchanged with the exception of three locations where edema was found in the interstitial connective tissue of testes, submaxillary gland, and lungs. This edema in testes and submaxillary gland reaches a height before the atrophy becomes extreme. Exact time relationship has not been worked out, but the edema apparently occurs during the period of early rapid atrophy which, we presume, follows the exhaustion of stored vitamin, and may disappear before death.

In the case of the mucous type of submaxillary gland, the edema liquid stains bluish, without doubt due to admixture of mucus derived from disintegrated cells. Mori has observed this edema with admixture of mucus, and offered substantially the above explanation.

The inference that this edema is the consequence of inability of

epithelium to utilize the materials brought to it by the blood is a natural one but implies functions on the part of the vascular endothelium, as yet not recognized. In the lungs, the connective tissue of the pleura, septa, and perivascular and peribronchial supports is the seat of moderate degrees of edema, never recognized in gross, and as yet not to be correlated with other findings in the body.

Skeletal muscle was studied in preparations of the skull, tongue, thorax, scapula, spine, and long bones. No change was found attributable to the avitaminosis. Waxy degeneration was found in a few cases, and only in association with infection. Bone and cartilage were studied routinely in costochondral junctions of ribs, in the sternum, the skull, and in a few extreme cases of the avitaminosis in vertebral and long bones. No specific change was found. Evidence of complete cessation of growth was the rule in advanced cases, but there was no evidence of osteomalacia, rachitis, or scorbutus.

Hess, McCann, and Pappenheimer[18] failed to produce gross skeletal changes in rats on a diet deficient in vitamin A. They have described the microscopic appearances due to lack of bone growth and we cannot add to their account.

The Teeth.—Routine sections of the skull through the nares at three levels were made from twenty-two rats, thus affording opportunity to study the incisor teeth of the upper jaw. Gross changes in the teeth were not noted in life except that the rate of growth was much slower than in rats upon other diets, water-soluble B deficiency and high protein diets, for instance. Actual wearing down of the incisors in a few rats indicates that complete cessation of growth may occur. Microscopical changes, usually of minor degree were found regularly in the odontoblast layer and frequently in the pulp. Our series affords a complete sequence from the earliest demonstrable shrinkage of the odontoblasts to complete disappearance. Preceding complete disappearance, irregular downgrowths of odontoblasts occur and these produce irregular deposits of dentine. Odontoblasts, while undergoing atrophy continue to produce dentine and presumably, due to the separation of the individual cells, become incorporated in the dentine matrix. Another less frequent finding was the presence of islands of osteoid tissue, incorporating and surrounded by osteoblasts. A careful study of early stages of this lesion has convinced us that the connective tissue cells of the pulp take up the function and morphology of osteoblasts because the first step in the process is the deposit of a hyaline homogeneous matrix between normal appearing

[18] Hess, A. F., McCann, G. F., and Pappenheimer, A. M., *J. Biol. Chem.*, 1921, xlvii, 395.

connective tissue cells. While superficially these changes resemble those in latent scorbutus in guinea pigs,[19] the fundamental phenomenon is probably different, as we have shown[20] that in scorbutus inability to produce intercellular matrices is the first phenomenon and not cell atrophy. Again, as in the consideration of edema of the testes, we may make the query, do these deposits of osteoid tissue in the pulp represent the utilization of transported material designed for odontoblasts. Enamel formation ceases. The enamel-forming cells in advanced stages are either shrunken and atrophic or replaced by a narrow layer of stratified, non-keratinizing epithelium.

The Respiratory Tract.—The replacement of the respiratory mucosa of the nares, (Figs. 1 and 2) accessory sinuses, larynx, trachea (Figs. 3 and 4) and bronchi (Fig. 17) by stratified squamous keratinizing epithelium has been mentioned and the histological sequences described. In the nares the olfactory glands (Bowman's glands) also become replaced by keratinizing epithelium. A purulent exudate in the nares is common, but not constant. In numerous instances there was extensive replacement of the respiratory mucosa by keratinizing epithelium without the presence of an exudate. Again, an extensive puriform infiltration and exudation was present in rats killed before keratinization had begun. From our material we are able to conclude that infection of the nares is common in this avitaminosis, but that the specific epithelial changes occur wholly independently of infection.

In the larynx, trachea, bronchi, and their glands, evidences of infection may or may not be present, with or without replacement of normal mucosa by keratinizing epithelium. The lungs show the pathology of secondary changes, chiefly due to occlusion of bronchi with desquamated keratinized cells, *i.e.* bronchiectases which may or may not show evidences of infection. The occasional minor degrees of bronchitis and bronchopneumonia which we found are without distinctive pathology.

The olfactory epithelium and the sensory epithelium of Jacobson's organ were always intact except for instances of inflammatory changes and invasion by growth of adjacent keratinizing epithelium.

The Alimentary Tract.—In the esophagus, stomach, and intestines no changes were found except in rare instances of a slight degree of atrophy, perceptible only by comparison with controls of the glands (Brunner's) of the duodenum.

The pancreas in advanced cases showed moderate atrophy of the secreting cells. The islands of Langerhans were consistently normal in the entire series. In the main branches of the pancreatic duct in a few rats there were foci of early formation of keratinizing cells.

The liver showed no lesions peculiar to the avitaminosis.

The Submaxillary Gland. Fig. 5.—The earliest changes to be noted, but only by careful comparison with controls, in the series, is slight atrophy of the gland acini, both mucous and serous types, and atrophy of the duct epithelium. Islands

[19] Höjer, J. A., *Acta pædiat.*, 1924, iii, suppl.

[20] Wolbach, S. B., and Howe, P. R., *Proc. Soc. Exp. Biol. and Med.*, 1925, xxii, 400.

of keratinizing cells appear in the complete absence of evidences of infection or any cellular infiltration whatever; and before apparent atrophy of the gland itself has occurred. Edema of the submaxillary gland occurs apparently in the most active period of atrophy and like that of the testes is transient—disappearing before maximum atrophy. The edema is strictly localized and is confined by the capsule of the gland. The liquid in mucus-secreting lobules is basic staining and study has shown that this property is due to admixture of mucus from gland cells which disintegrate. We are inclined to attribute the cell disintegration to the effects of the edema, sequences in the tunica propria can be followed from hyaline swelling and fragmentation to disappearance. Cells separate from their acini and become free in the liquid. The nuclei of the epithelial cells degenerate, the cells become rounded and finely vacuolated. Other cells present are lymphoid cells, small numbers of polymorphonuclear leucocytes, spindle cells, presumably from the supporting tissue of the gland, and rarely eosinophilic leucocytes and red blood corpuscles. Capillaries and larger blood vessles may traverse edematous spaces, but hemorrhages do not occur and the disposition of the blood vessels as edema and atrophy progress has not been determined.

As the avitaminosis progresses, the keratinization of ducts and atrophy of gland tissue proceed, in most instances complicated in later stages by infection. Without infection we find an infiltration of the gland stroma with lymphoid and plasma cells. The gland cells become vacuolated and occasionally invaded by polymorphonuclear leucocytes. The connective tissue of the stroma increases. In mucus-secreting alveoli there is a noticeable increase of small, deeply staining, non-vacuolated epithelial cells which persist after desquamation and disappearance of the original cells. The same process occurs later in the serous alveoli, and in the most severe grades of the deficiency these replacing cells become squamous and keratinizing. Eventually the gland as a whole becomes markedly fibrosed and filled with cysts lined with keratinizing epithelium. Infection complicates the picture as it causes complete destruction of the epithelium lining cysts and repair by granulation tissue accompanied by foreign body reactions to the retained keratinized cells. As the cysts, derived from ducts, dermoid in character, reach dimensions of 5 to 6 mm. pressure atrophy effects ensue in adjacent gland tissue.

The accessory salivary glands of the base of the tongue and the pharynx show the same sequences as those of the submaxillary gland and in these locations the gamut of events may take place independently of infection. (Fig. 6.)

The parotid glands rarely showed evidence of infection. Keratinization of ducts and atrophy of the gland occurred much later in the progress of the deficiency than in the submaxillary gland, and but few instances of cyst formation, due to accumulation of keratinized cells occurred in the series. The sequences, though occurring late, are the same as in the submaxillary gland.

The Urinary Tract.—In the epithelium of the bladder, Fig. 10, ureters, and pelvis of the kidney (Fig. 8) the original epithelium becomes replaced by keratinizing epithelium which develops as in other locations from underlying nests of cells. In

the kidneys, the apices of the pyramids become covered by a thick layer of keratinizing epithelium, while the epithelium of the pelves, ureters, and bladder show the most remarkable pictures encountered in this study. In these latter locations there is evidence of very rapid growth of the epithelium; in some instances keratinization ceased. Mitotic figures are to be found in every field of a 3 mm. immersion objective and frequently there are two to four mitoses per field. In the bladder invaginations and dermoid cyst-like formation occur. In ureter, pelves, and bladder epithelial downgrowth resulting in the incorporation of blood vessels is frequent. The behaviors indicate growth power suggestive of neoplastic potentiality.

The Male Genital Tract.—Edema of the testes during atrophy of the seminiferous tubules reaches extreme degrees. The liquid lies outside the basement membrane, the connective tissue between the tubules does not seem to be permeated by liquid, so that the appearance in sections is that of tubules floating in a liquid medium. The liquid is evidently rich in protein material as it furnishes a heavy precipitate of coarse hyaline globules. The atrophy of the tubules when complete leaves apparently only cells derived from the sustentacular cells. The cells frequently have two to four nuclei. Early stages in the atrophy show spermatids with scattered chromatin particles and a structureless layer of material, probably derived from the cytoplasm of these cells.

In the epididymis a more diffuse interstitial edema occurs. The cells of the ductus epididymidis become slightly atrophic but retain their normal characteristics. The cells of the efferent ductules of the testis apparently increase in number in early stages of the avitaminosis inasmuch as they form a deep epithelium layer, often with large vacuoles between cells and occasionally with secondary gland-like formation of cells with cuticular borders bounding a lumen. Subsequently shrinkage of cells takes place and rarely islands of squamous keratinizing cells make their appearance in the basal layer of cells. Fig. 15. In one instance the ducts of the epididymis and testis were filled with pus.

The prostate gland, Fig. 9, seminal vesicles, and coagulating gland exhibit with regularity the sequence of atrophy, keratinization, and fibrosis, and the prostate frequently becomes the seat of a puriform exudation.

The Female Genital Tract.—The mucosa of the uterus, and later that of the oviducts, becomes replaced by stratified, keratinizing epithelium. The process originates in numerous foci as in other organs and begins in the uterine glands as well as in the surface mucosa.

No characteristic pathology has been found in the ovaries. We have not made sufficiently careful comparisons with normal ovaries to be certain that some degree of retardation of follicle formation does not take place, but even in advanced stages of the avitaminosis, ova and follicles in all stages of development up to maturity are found. Mitotic figures are present in the follicular cells, and apparently ovulation occurs as corpora lutea are found in various stages of formation and regression.

The Circulatory System.—We have found no change in blood vessels or in the ability to form new capillaries in repair by granulation.

Focal lesions in the myocardium were found with considerable regularity in sixteen rats out of nineteen, which showed keratinizing epithelial changes. Control rats and three adult experimental rats kept on the deficient diet for periods of 50 to 120 days, but which did not develop the characteristic epithelial changes of the deficiency, did not show lesions of the myocardium. The lesions are focal, usually not numerous, and represent the sequences of degeneration of a few contiguous muscle fibres—acidophilic staining, loss of striations, vacuolization, and fragmentation, and the consequent reparative processes, terminating in cicatrization. Correlations of these myocardial lesions with epithelial changes show that the more advanced the latter the more numerous the former.

Blood and Lymphoid Tissues.—No study of the morphology of the blood cells was made in life. Bone marrow was studied in sections from ribs and sternum from every autopsy. We could find no characteristic differences between the marrow of normal and control diet rats and the marrow from rats with advanced lesions of vitamin A deficiency. In advanced deficiency, fat completely disappears and the fat cells are represented by large connective tissue-like cells. Slight edema with fibrin threads were seen in a few cases. There is no decrease in numbers of megacaryocytes. Reduction in numbers of cells concerned in hematopoiesis was apparent in a few marrows, but a greater depletion of blood-forming cells was found in the marrows of two rats kept upon a water-soluble B deficient diet (casein 18 gm., butter fat 20 gm., starch 58 gm., McCollum's salt mixture 185, 4 gm.) for 3 and 4 months respectively.

The spleen as the deficiency progresses, becomes more and more depleted of lymphoid and erythrocyte-forming cells. Megacaryocytes completely disappear. Phagocytic cells laden with hemosiderin pigment become more numerous so that the end-result is a spleen with no trace of hematopoiesis or lymphopoiesis, containing as many pigmented cells as lymphoid cells. The secondary follicles have disappeared. The reticulum is not increased. The spleens of normal rats vary in number of hemosiderin-containing cells, and frequently contain many. The spleen of a water-soluble B deficient rat showed a comparable degree of atrophy and pigmentation to many of the fat-soluble A rat spleens. The atrophic changes and pigmentation of the latter are accordingly not characteristic of the A deficiency.

Lymph Nodes.—The lymph nodes in proximity to the submaxillary gland and the bronchial lymph nodes usually were larger than in control rats. The enlargement is due in part to the accumulation in lymph sinuses of cells concerned in the disposal of products from atrophying and occasionally infected tissues, and in part to lymphoid hyperplasia. In mesenteric lymph nodes we found no departure from the normal.

The Ductless Glands: Thymus.—Extreme reduction in size, owing to almost complete loss of small thymic cells is constant in established vitamin A deficiency.

The thymic corpuscles (Hassall's corpuscles) from two rats are greatly enlarged and duplicate the human type. They consist of cyst-like structures lined with flattened epithelium and filled with masses of concentrically arranged hyaline scales, which we believe to be desquamated keratinized cells. In one out of four thymuses from control rats we found very much smaller cavities with hyaline material in Hassall's corpuscles. Our observations are too few to be conclusive, but indicate that the thymic corpuscles are epithelial and respond to the vitamin A deficiency as do other epithelial structures.

The Adrenal Gland.—We found no noteworthy response, gross or histological, to the deficiency.

The thyroid, parathyroid, and anterior portion of the pituitary gland all respond by atrophy. In the thyroid, this is evidenced by reduction in size of the cells and diminution of colloid content of the acini. In the parathyroid, moderate, and in the pituitary, striking reduction in size of the gland cells occurs, but we could find no other change and the architecture of both glands remains normal.

The Nervous System.—As the rats exhibited no symptoms pointing to nervous system lesions, the peripheral nerves were not studied. No lesions were found in brain, cerebellum and sympathetic ganglia. The ganglion cells of the myenteric plexus were normal whenever found.

The Eye and Paraocular Glands.—The eyes, including the globe, the lids, nictitating membrane, the intraorbital lacrimal gland, the Harderian glands, and the extraorbital lacrimal glands from eighteen rats were studied.

We have found no qualitative differences in the behavior of the eye and its glands as compared with other organs. Mori and Lambert and Yudkin have described the important featues of the pathology, but disagree in their interpretation. Lambert and Yudkin give a much greater importance to infection than does Mori who ascribes the keratinization of the cornea to dryness secondary to loss of secretion. Lambert and Yudkin state that the corneal lesions are secondary to the lesions of the conjunctiva of the lids and nictitating membrane. These authors have missed entirely the specific nature of the keratinizing change which they saw only on the cornea, and treat the pathology of the eye as results of infection secondary to loss of immunity caused or favored by atrophy of the paraocular glands.

Our own observations disprove that infection plays an important rôle in producing the clinical picture of the ophthalmia or that it is responsible for initiating the keratinizing change. In only one rat, initial weight 134 gm., which died on the 98th day of the experiment, did we find the histology of an infectious process.

As Lambert and Yudkin state, lacrimation persists after the clinical signs of the ophthalmia are established. This we confirm and histologically have found that advanced keratinization of cornea and conjunctival epithelium may exist without appreciable atrophy of the Harderian, intra-and extraorbital lacrimal glands.

The substitution of a keratinizing epithelium in conjunctiva and cornea is the first change we have found. The mucous cells of the conjunctiva persist after this

change has occurred and are to be seen overlaid with keratinized cells. Subsequently the mucous glands of the conjunctiva atrophy and disappear. Desquamated keratinized cells are in all probability rapidly removed from the conjunctival sac in the first stages of the deficiency. Eventually they accumulate and may adhere to the lid margins where the keratinized cells cohere to a greater extent than elsewhere. In late stages the basal cells of the conjunctival epithelium show slight, irregular downgrowth and there is an increase of capillaries in the underlying connective tissue. At this stage accumulations of mononuclear cells, lymphocytes, and endothelial leucocytes are common. Polymorphonuclear leucocytes invade degenerating mucous cells after these have become completely embedded by the cells of the keratinizing epithelium, but actual proof of infection occurred but once in the series. The epithelium over the nictitating membrane shares in the general process and we have not found it to be a locus of early infection.

The Meibomian glands we find in accordance with Mori do respond, but not strikingly. In a few cases only were the ducts occluded by keratinizing cells so that we attribute but a minor rôle to these glands in producing the picture of the ophthalmia.

The substantia propria of the cornea after keratinization is established, becomes edematous and vascularized by ingrowth of capillaries from the sclera. (Fig. 7.) This response we believe to be due to the greater physiological demands from the more active and changed epithelium. In very late stages of the deficiency wandering cells of all types, including polymorphonuclear leucocytes appear in the cornea. The external basal membrane (Bowman's) not easily demonstrable in the normal guinea pig eye, becomes wholly unrecognizable. The internal basal membrane (Descemet's) remains unchanged. Within the eye we have seen no pathology. The epithelium of the ciliary process undergoes no change.

The Harderian gland responds earlier than the lacrimal glands, later than the submaxillary glands, and undergoes atrophy and keratinization, first in the ducts, later in the acini. The sequences are identical with those described for the submaxillary gland and will not be repeated here. Early evidence of atrophy as pointed out by Lambert and Yudkin is the disappearance of the yellow deposit in the gland lumina as seen after Zenker fixation. Complete disintegration of gland acini and eventual conversion of the gland into a series of cavities lined by stratified epithelium and filled with desquamated keratinized cells is the end-result as observed by us.

The intraorbital and the extraorbital lacrimal glands respond late, first showing atrophy and finally keratinization of the ducts.

Correlation of the eye pathology with that in other organs shows that the eye changes come later than the changes in the respiratory tract and in the submaxillary glands. The epithelium of the eye and its glands respond as do other epithelial structures and in respect to some other organs show less susceptibility to the vitamin A deficiency. The vascularity of the conjunctiva and the innervation of

the cornea and conjunctiva probably contribute largely to the picture during life. The incrustation of the lids is due to adherence of desquamated cells. Infection with destructive lesions of the cornea must be regarded as accidental and need not occur in animals in which the deficiency has been carried to an end-result.

Vitamin A Deficiency in the Human.—There has been but one pathological description of a fatal human case.[21]

This post mortem, done in the pathological service of one of us at the Children's Hospital, is adequately recorded. We have recently restudied the tissues and can add nothing of importance. Keratinization of the epithelium of the renal pelvis, and large size of Hassall's corpuscles in the thymus were overlooked. The keratinization of the pancreatic ducts is more striking than any seen by us in the pancreas of rats. In respect to the keratinizing change in lungs, uterus, submaxillary gland ducts, and thymus, the picture was identical with that in our experimental rats. The order of changes in this human case was somewhat different in that the salivary glands showed only slight changes. The peculiar swollen epithelial cells with nuclear inclusion bodies in the ducts of the parotid and submaxillary glands are unlike any change seen in rats. The lacrimal gland showed only slight changes and no keratinization. The eyes were not removed for study.

The importance of this human case is that it did show the epithelial changes which we have produced in rats and, therefore, contributes irrefutable evidence for an important part of our thesis to the effect that vitamin A deficiency affects epithelial tissues in a specific manner.

SUMMARY AND CONCLUSIONS.

The specific tissue changes which follow the deprivation of fat-soluble vitamin A in albino white rats and in the human concerns epithelial tissues. This effect is the substitution of stratified keratinizing epithelium for normal epithelium in various parts of the respiratory tract, alimentary tract, eyes, and paraocular glands and the genitourinary tract. We have described the morphological sequences which clearly show that the replacement of epithelium arises from focal proliferation of cells arising from the original epithelium

[21] Wilson, J. R., and DuBois, R. O., *Am. J. Dis. Child.*, 1923, xxvi, 431.

and not by differentiation or change of preexisting cells. Young rats respond to the deficiency more promptly than adults.

Growth activity of epithelium is not diminished. On the contrary, there is convincing evidence that it is greatly augmented. In a few of our animals the behavior of the replacing epithelium in respect to numbers of mitotic figures and response on the part of connective tissue and blood vessels suggests the acquisition of neoplastic properties. While the epitheliums which are the seats of these changes are largely of covering types, glandular epithelium is involved, specifically in the paraocular glands and salivary glands. It is highly probable also that the epithelium of gland ducts, respiratory mucosa, and genitourinary tract have secretory functions so that we conclude that the deficiency results in loss of specific (chemical) functions of the epitheliums concerned, while the power of growth becomes augmented. Explanation for the substitution of a chemically inactive (nonsecretory) epithelium, common in type for all locations, remains a matter of speculation.

We can only speculate also in regard to the absence of change in the epithelium of such organ as the liver, parenchyma of the kidney, stomach, and intestines. The significance of the order or sequence in which different organs exhibit this change has not been determined. In general the respiratory mucosa in nares, trachea, and bronchi respond first, then the salivary glands, eye, genitourinary tract, then paraocular glands and pancreas, although as has been noted there are exceptions to this order.

Our studies show that the mitochondrial apparatus is not primarily affected. Study of individual cells indicates that the first morphological evidence of avitaminosis will be found in the nucleus. We have not devoted sufficient study to be certain, but an increase of chromatin and in some instances in size of nucleoli are early morphological manifestations.

Other important effects of fat-soluble A deficiency are atrophy of glandular organs, emaciation, localized edema of testes, submaxillary gland, and connective tissue structures of the lungs and focal myocardial lesions. From our own limited experience with rats fed on a water-soluble B deficient diet and from work by Cramer, Drew, and Mottram,[5] the loss of fat in water-soluble B deficiency is as great, if

not greater than in vitamin A deficiency, so that for the present we assume that this is not a specific manifestation of any one avitaminosis. The same applies to glandular atrophy. Both of these effects probably concern the nutrition as a whole and may be ascribed to inanition.[22]

The occurrence of transient edema in testes and salivary gland coinciding with a period of maximum atrophic change, suggests the hypothesis that this edema is the result of failure of epithelium to utilize transported material, which leads further to the hypothesis that the capillaries of these organs are differentiated in regard to permeability to the respective materials utilized by the cells. It would seem that in the case of the testis we have a unique instance of complete atrophy producible at will without impairment of circulation and supporting tissues. This phenomenon may possibly be followed with advantage in the study of the mechanism of edema.

Vascularization of the cornea, as we have shown it to be independent of infection, must be a physiological response to the increased demands of the rapidly growing epithelium which has replaced the corneal epithelium.

We have assumed throughout this work that the diet on which we kept our animals was deficient in respect to a single substance or group of substances having similar physiological properties, designated by the term fat-soluble vitamin A. Whether or not more than one so called vitamin or accessory substance was missing in the diet we employed does not affect the theoretical importance of the morphological results.

Work by Evans and Bishop[23] would indicate that other factors affecting fertility in addition to the so called antixerophthalmic or vitamin A factor may have been missing.

Our own experience leads us to believe the specific effects we have described upon epithelial tissues were in all probability due to withdrawal of a single factor. We have shown how these effects, that is the replacement of uterine epithelium by keratinizing epithelium can account for sterility in the female. Whether or not the atrophy of the

[22] Jackson, C. M., Inanition and nutrition, Philadelphia, 1925.

[23] Evans, H. M., and Bishop, K. S., *J. Am. Med. Assn.*, 1923, lxxxi, 889.

testis is due to the same factor remains to be proved, but presumptive evidence is strong that this is the case.

The study of the reverse changes that follow in the rapid amelioration when the rats are restored to an adequate diet has been begun and will be reported later.

We have shown that the substitution of keratinizing epithelium in all locations is not secondary to infections, and presumably is a primary effect of the withdrawal of factors essential for the chemical activities or maintenance of differentiation of the epitheliums concerned. It is, of course, possible that the phenomenon is produced in a roundabout way in that it may be secondary to the effects of the avitaminosis upon the metabolism of tissue-sustaining substances. This possibility is supported by the cessation of growth of the skeleton and teeth, although we know that other avitaminoses produce retardation of growth.

EXPLANATION OF PLATES.

PLATE 37.

FIGS. 1 and 2. Turbinate bone at 43 and 93 diameters respectively. To show keratinizing epithelium and accumulation of desquamated cells. Rat 103. Initial age 150 days, died after 180 days on experimental diet.

FIGS. 3 and 4. Trachea at 43 and 257 diameters respectively. Showing complete replacement of respiratory mucosa by stratified keratinizing epithelium. Rat 206, initial weight 101 gm. Died after 89 days on experimental diet.

PLATE 38.

FIG. 5. Salivary gland. 50 diameters. Atrophy; a dilated duct with keratinizing epithelium. Infected. Rat 105. Initial age 54 days, died after 160 days on experimental diet.

FIG. 6. Base of tongue. Keratinizing cysts of accessory salivary gland origin. 50 diameters. Rat 103.

FIG. 7. Cornea. 180 diameters. Vascularization of substantia propria. Keratinization of epithelium. Rat 105. Initial weight 104 gm. Killed on 89th day on experimental diet.

FIG. 8. Kidney. 50 diameters. Keratinizing epithelium on pyramid. Rat 105.

PLATE 39.

FIG. 9. Prostate gland with atrophy, fibrosis, keratinization. 95 diameters. Rat 103.

FIG. 10. Bladder. Rat 103. 95 diameters.

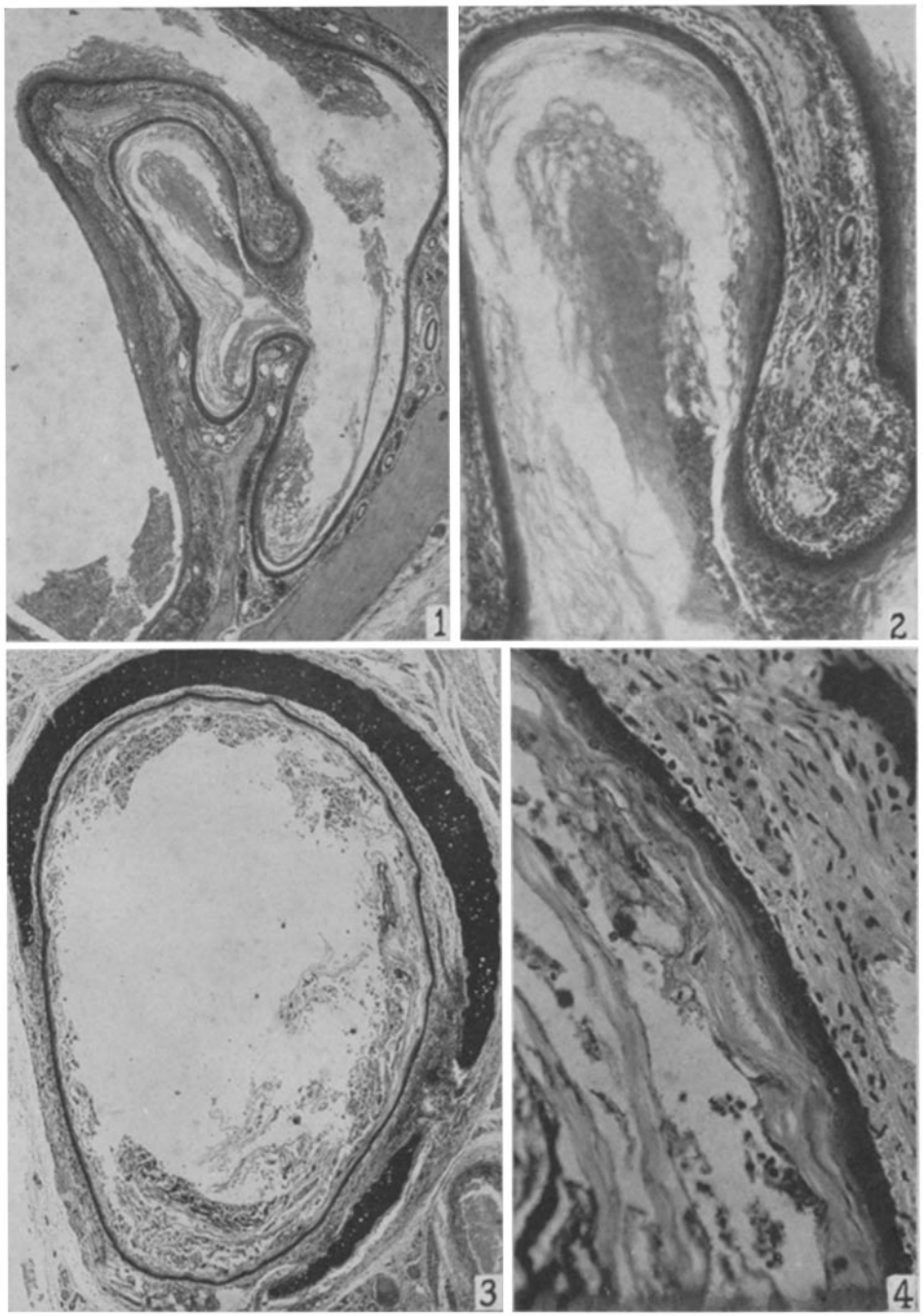
FIGS. 11 and 12. Respiratory mucosa, turbinate bones. 656 diameters. Early foci of keratinization. Rat 100. Initial age 175 days. Killed on 181st day of deficient diet.

FIG. 13. Posterior nares, to show small foci of established keratinization. 173 diameters. Rat 105.

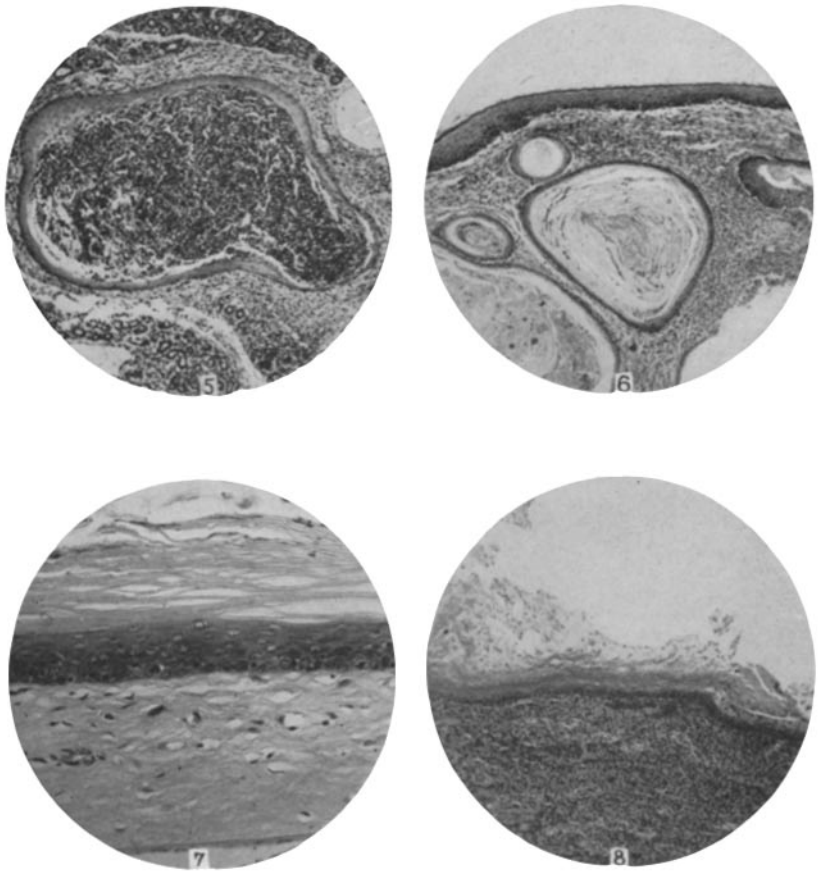
PLATE 40.

FIGS. 14 and 15. From Rat 100. 600 diameters. From trachea and efferent ductule of testis respectively. To show foci of initial change towards keratinization.

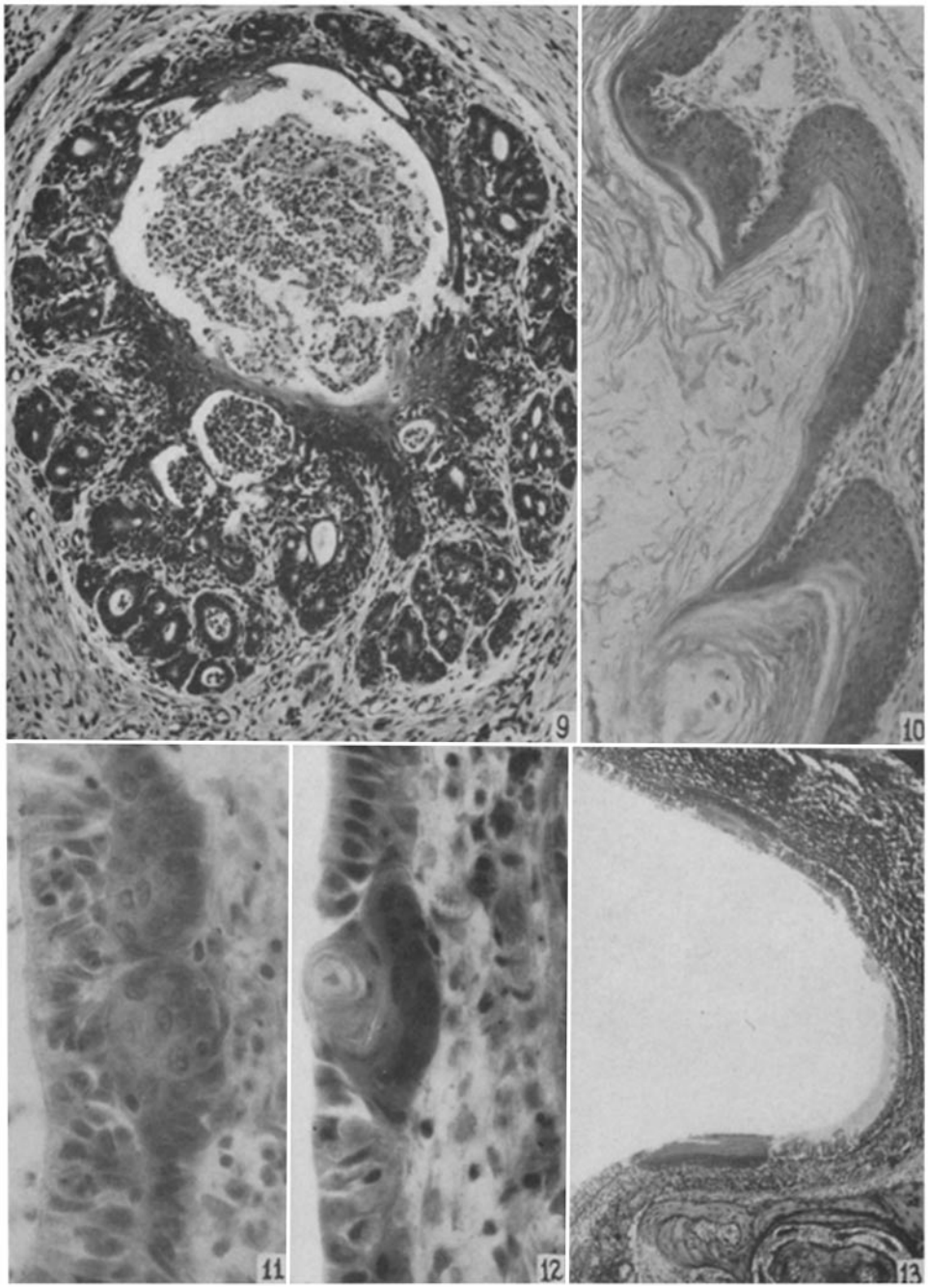
FIGS. 16 and 17. From rat 100. 600 diameters. Fig. 16, from turbinate bone shows small focus of keratinization with extension and undermining of adjacent epithelium. Fig. 17 from a bronchus; a focus of early keratinization.



(Wolbach and Howe: Tissue changes after vitamin A deficiency.)



(Wolbach and Howe: Tissue changes after vitamin A deficiency.)



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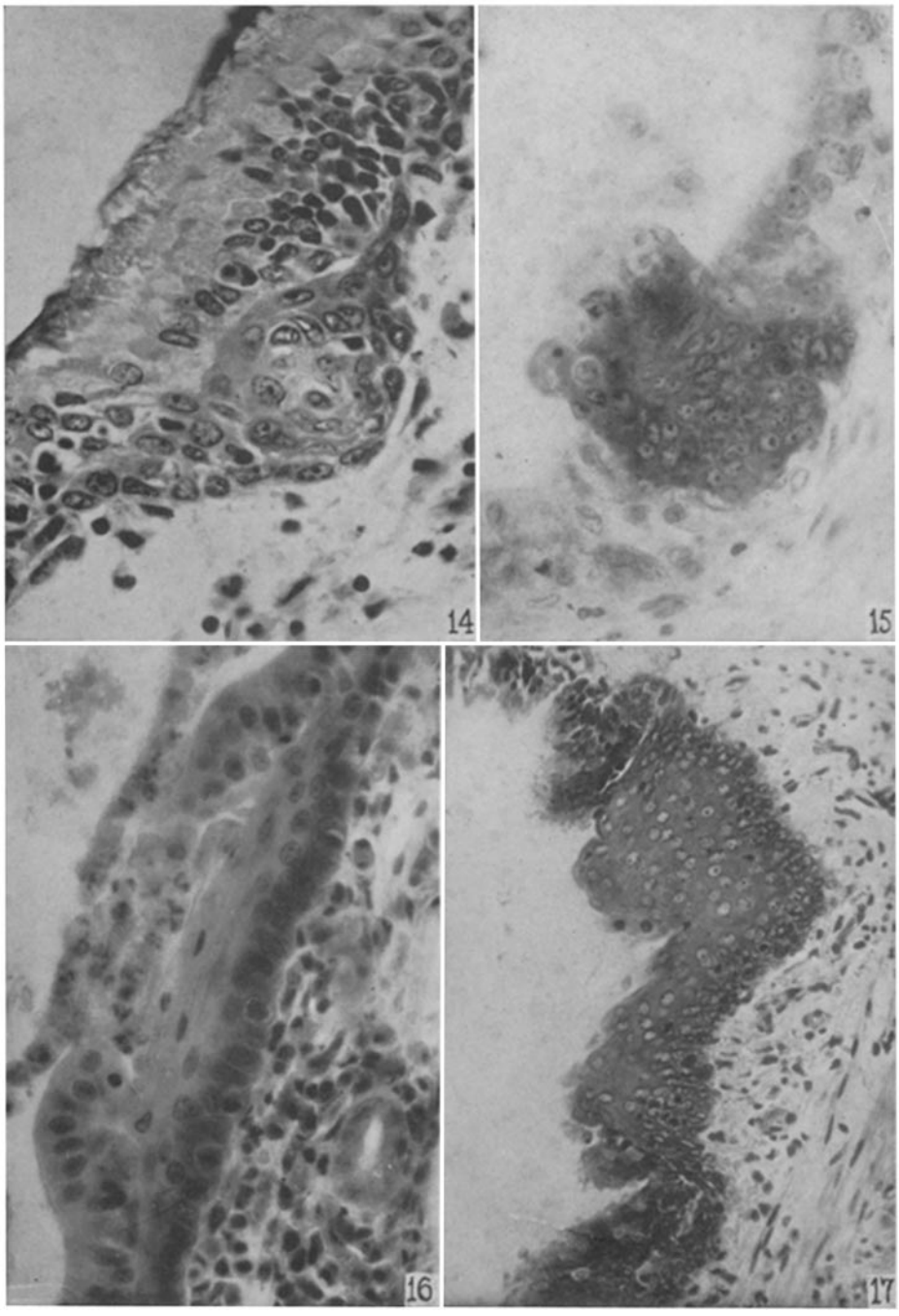

(Wolbach and Howe: Tissue changes after vitamin A deficiency.)