rulonephritis and experimental nephritis a fall in serum complement 1 to 2 weeks later was not observed. Instead there was sometimes seen a significant rise in serum complement following homotransplantation.

The authors thank Doctor Frank Adler for valuable help.

Addendum

After this work was submitted for publication we learned that a similar study of serum complement levels in dogs undergoing kidney homotransplantation had been presented by M. Simonsen, Biological Incompatibility in Kidney Transplantation in Dogs, (E. Munksgaard. Copenhagen 1953).

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Received March 24, 1953. P.S.E.B.M., 1953, v83.

Phase Contrast Microscope Study of Oral Epithelium of Normal and Vitamin A-Deficient Rats.* (20358)

LOUIS J. BAUME AND ASGER M. FRANDSEN. (Introduced by Karl F. Meyer.)

From the George Williams Hooper Foundation and the Division of Dental Medicine, College of Dentistry, University of California.

A recent investigation into the structure of the epithelial attachment to the tooth revealed that in fresh, undenatured oral epithelium prominent tonofibrils secure cohesion of the epithelial cells among themselves and with neighboring tissues such as the lamina propria and the surface of the tooth(1). These observations, made in man and monkey with the phase contrast microscope, suggested a corollary study of the behavior of the tonofibrils in oral epithelium of normal and vit. A deficient rats.

Experimental. Twenty male rats of the Long-Evans strain were fed a vit. A-deficient diet† beginning the week before weaning at 14 days of age. Their weight curves showed a plateau between the ages of 50 to 60 days. A maintenance dose of 3 μg of vit. A alcohol in oil was administered *per os* when they showed signs of extreme exhaustion. Four rats which survived the deficient diet for 86 days (100

* Aided by grants from the Research Board of the University of California and the California State Dental Assn.

† The vit. A deficient diet had the following percentage composition: vit.-free casein 24, sucrose 62, salts 4, cottonseed oil, vit. D, mixed tocopherol 10. The following vit. were added per 10 g diet: vit. D 10 USP units, tocopherol (alpha and delta) 3 mg, menadione 50 μg, thiamine 20 μg, pyridoxine 20 μg, riboflavin 50 μg, p-aminobenzoic acid 50 μg, niacin amide 100 μg, inositol 2 mg, choline citrate 10 mg, biotin 6 μg, pteroylglutamic acid 20 μg, pantothenic acid 350 μg, B_{12} 0.2 μg.

days old) were selected for the present study. A control group of 28 male rats were weaned and given a purified diet (30 μg vit. A alcohol to each 10 g of the deficient diet). Twenty of these rats were pair fed and used only for dietary evaluation. The other 8 rats were offered the control diet *ad libitum* and served in this study as normal controls when they were 100 days old. Fresh biopsies and briefly formalin-fixed specimens of the gingival papilla situated between the upper incisors were frozen (dry ice), sectioned and examined in a suspension of saline with the phase contrast microscope. Images of marked contrast can be obtained this way by illuminating the colorless, transparent objects according to the optical principles developed by Zernike(4). Photomicrographs were taken immediately.

Results. In the normal rat the dental papilla is bordered by a broad epithelial sheet (Fig. 1A). This epithelium consists of 10 to 15 cell layers and is occasionally enlarged by rete pegs at the free gingival margin. A wide stratum corneum is a physiologic characteristic.

At high magnification a continuous network of tonofibrils is evident throughout the strata of living epithelial cells (Fig. 2A). A distinct structure conventionally called basement membrane adjoins the germinal layer of epithelium. This membrane is partly translucent and partly interwoven by reticular fibrils continuous with those of the lamina propria and the delicate tonofibrils of the basal cells of oral epithelium. These cells have voluminous nuclei containing prominent nucleoli and globules of chromatin (Fig. 3A). The nuclei gradually disappear toward the surface layers at the expense of an increasingly larger cytoplasm. Tonofibrils, seemingly looser oriented and finer in texture in the rat than in man and monkey, make up the bulk of the cytoplasm of these cells of the stratum spinosum (Fig. 4A). Their intracellular portions are somewhat compressed alongside the nucleus. Extracellular portions forming the intercellular bridges are only slightly larger than the intracellular parts and therefore give the entire network a rather homogenous appearance. As toward the outer surface of the epithelial sheet the cells become more dense and their nuclei no longer are seen, the tonofibrils disappear abruptly. Several layers of transparent, keratinized cells interconnected by few remaining intercellular bridges line the surface. The successive loss of cohesion results in the final shedding of the uppermost layer.

In oral epithelium of vit. A deficient rats some characteristic deviations from this normal pattern are observed. In general the entire epithelial sheet is thinner (Fig. 1B). A distinct border between epithelium and connective tissue is not discernible as the basement membrane is absent. At various places connective tissue appears to invade the epithelium reducing it to one single layer of living cells, the germinative cells; these contain exceedingly large nuclei (Fig. 2B). The life cycle of the epithelial cells is definitely shortened, evidenced by early karyolysis. However, in all viable epithelial cells tonofibrils are present and display the same morphologic features as in normal epithelium. Their connection with the reticulum of the connective tissue is obviously altered; without forming a network some tonofibrils fuse directly with delicate reticular fibrils of the connective tissue of the lamina propria (Fig. 3B).

Tonofibrillar continuity is lost already in the stratum spinosum (Fig. 4B). Before the nuclei have disappeared completely the tonofibrils decay into a granular mass. This granulated zone in the stratum spinosum is not encountered in normal epithelium and seems to be typical for vit. A deficiency. The succeeding keratinization is also disturbed, resulting in irregular and only slightly cohesive surface layers. It is noteworthy that this keratinous zone is approximately as thick as that of normal oral epithelium.

Discussion and conclusion. Undenatured oral epithelium of the normal rat possesses tonofibrillar structures similar to those in man and monkey(1). These tonofibrils, however, are finer in texture and more irregular in course, but are equally inscrutable to conventional histologic methods. Furthermore, the nuclei of the germinal zone of normal oral rat epithelium are much larger than in man; the keratinous surface zone is wider. Deprivation of vit. A engenders the following pathologic changes in oral epithelium: a) aplasia of the

PLATES I and II. Biopsies of gingival papilla between upper incisors of normal rats, 100 days of age (A) and vit. A deficient rats, 100 days of age (B) frozen sectioned in median sagittal plane at thickness 6-8 μ, suspended in saline, photographed without staining at various magnifications with phase contrast microscope.

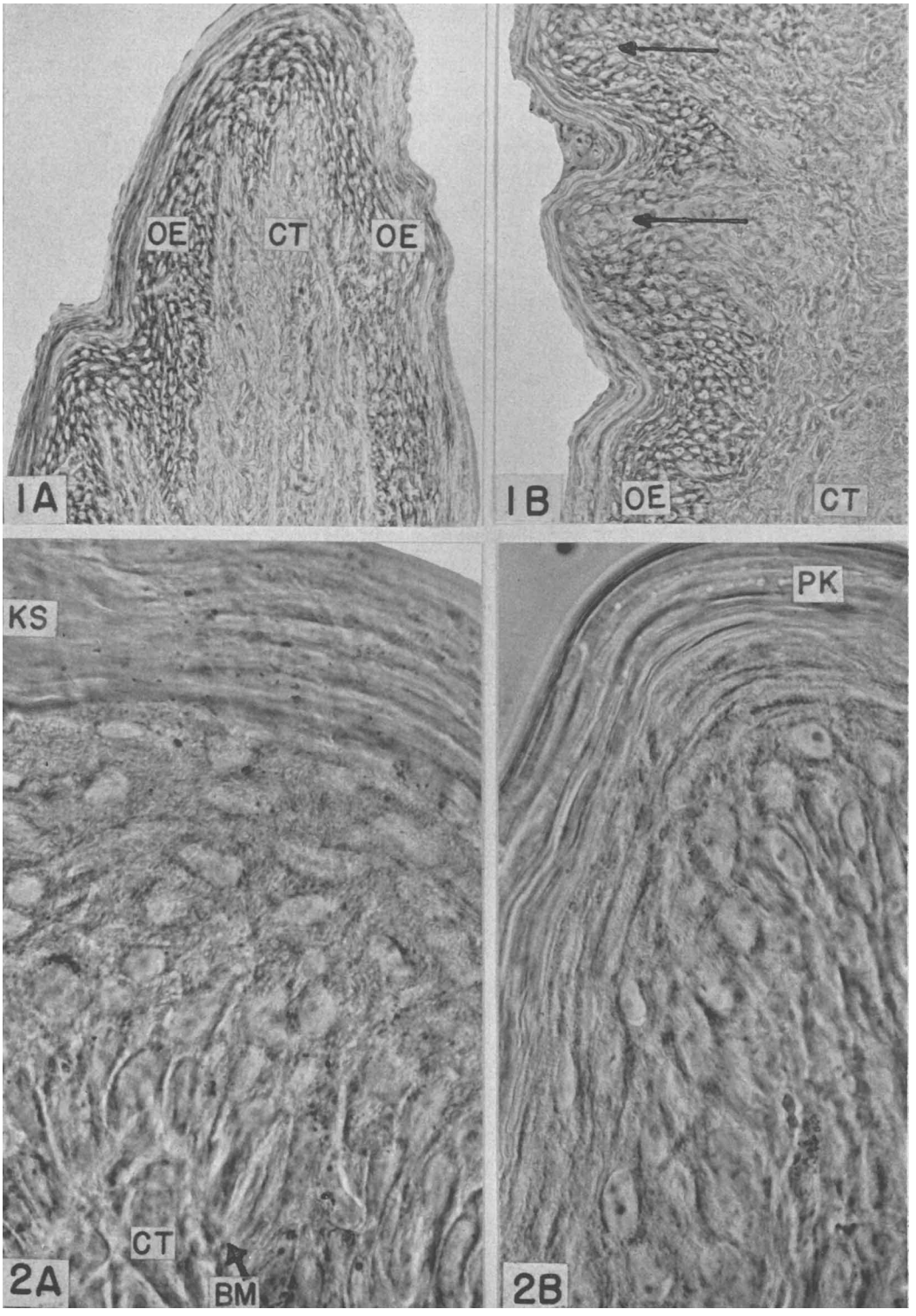

FIG. 1. Overall aspect of gingival papilla, orig. mfg. × 120. A. Normal rat shows broad epithelial sheet (OE) with rete pegs at the vestibular side. CT = connective tissue. B. Vit. A deficient rat shows connective tissue focally penetrating into epithelium (arrows).

FIG. 2. Epithelial sheet is shown, orig. mfg. × 1600. A. In the normal rat oral epithelium shows wide zone of keratinized cells at surface (KS), prominent tonofibrils in stratum spinosum and basement membrane (BM) at epithelio-connective tissue junction. B. In the vit. A deficient rat only few layers of living epithelial cells are present, the surface is parakeratotic (PK) and the epithelio-connective tissue junction is not discernible.

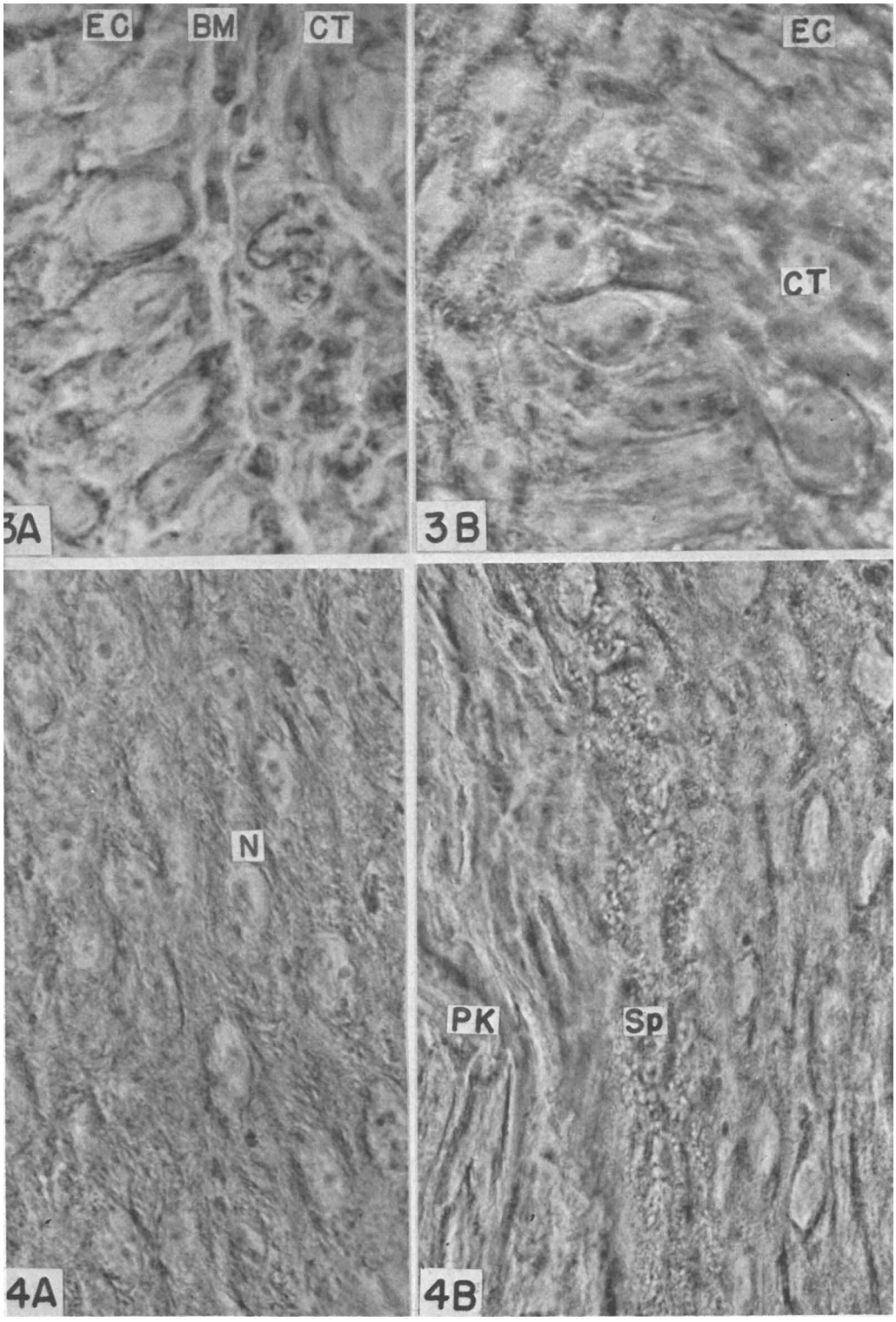

FIG. 3. Epithelio-connective tissue junction is illustrated, orig. mfg. × 2200. A. The normal rat shows translucent basement membrane interwoven with reticulum in continuity with tonofibrils of epithelial basal cells (EC). B. In the vit. A deficient rats few fine tonofibrils of basal cells fuse with delicate reticulum of connective tissue (CT) in absence of basement membrane.

FIG. 4. Stratum spinosum is shown, orig. mfg. × 1800. A. In the normal rat tonofibrils extend irregularly from one cell into another, compressed alongside the nucleus (N); the extracellular portions identical with the intercellular bridges are slightly enlarged forming desmosomes. B. In the vit. A deficient rat tonofibrils decay precociously effecting spongiosis (Sp) and parakeratinous surface layers (PK).

basement membrane at the epithelio-connective tissue junction, b) precocious decay of the tonofibrils in the stratum spinosum, and c) parakeratosis of the surface layers. a) Absence of a distinct border structure between epithelium and supporting connective tissue could indicate that one point of attack of vit. A deficiency possibly lies in the connective tissue. The basement membrane belongs to the ground substance of connective tissue and encloses a network of reticular fibers(2). The connection of this reticulum with the tonofibrils of the epithelium is altered in the mucosa of vit. A deficient rats. At various places connective tissue penetrates the epithelial sheet, limiting the latter to one layer of living cells. This points to a block in the trophic supply of the epithelium by the connective tissue in vit. A deficiency. The role of vit. A in the metabolism of connective tissue obviously warrants histochemical studies. b) Precocious, granular involution of the tonofibrils is related to the well-described metabolic dysfunction of epithelium in vit. A deficiency(3). Loss of tonofibrillar continuity within cells with a nucleus constitutes a specific disturbance that is not a stage of the normal cytomorphic changes of oral rat epithelium. Apparently it is identical with the histopathologic condition termed spongiosis. These observations on undenatured oral epithelium throw some new light on the nature of the prominent, but little known, protoplasmatic cell structures called tonofibrils. They corroborate the views previously expressed(1) that the presence of tonofibrils is bound to the vitality of the cell. Since vit. A, probably through intercession of connective tissue, controls differentiation and longevity of the epithelium, it directly or indirectly secures maintenance of tonofibrillar continuity. c) Keratinizing metaplasia, usually described as the main effect of vit. A deficiency, is a physiologic condition in oral rat epithelium. However, this keratinizing process is also disturbed in vit. A deficiency. Due to precocious decay of tonofibrils the surface layers are only slightly cohesive and very irregular in width.

Summary. Biostructures of oral epithelium such as tonofibrils and basement membrane were ascertained in normal rats with the help of phase contrast microscopy. Vit. A deficiency effected in oral rat epithelium 1) aplasia of the basement membrane, 2) precocious disintegration of the tonofibrils in the stratum spinosum, and 3) parakeratosis of the surface layer.

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Received April 21, 1953. P.S.E.B.M., 1953, v83.

Cooling of Embryonated Eggs to Produce an LD_{50} for *Coccidioides immitis*. (20359)

JOSEPH R. GOODMAN, JOAN FOUNTAINE, AND JOHN VINCENT.
(Introduced by Charles M. Carpenter.)
From the Department of Investigative Medicine, Veterans Administration Hospital, Long Beach, Calif.

In studying a chronic disease process, an investigator is faced with the problem of choosing an objective criterion that is suitable for the evaluation of the progress of the disease or the effect of a therapeutic procedure. Procedures that utilize the evaluation of the LD_{50} are frequently used. However, agents that produce chronic diseases usually do not kill a large percentage of infected individuals in a relatively short period of time. Therefore, especially susceptible host species must be used or some other modifying procedure employed. Frequently physical agents such as X-ray(1) or chemical substances such as