to the gasserian ganglion and adjacent CNS, as has been suggested (*3*).

Acute herpetic encephalitis is a well-known clinical entity in man, and diagnosis of this disease has been confirmed by electron microscopy and by isolation of herpes simplex virus in brain tissue from both surgical (*4*) and autopsy material (*5*). The gasserian ganglion of the trigeminal nerve in man may be involved in recurrent herpetic infections (*6*). Spread of herpes virus to the gasserian ganglion in man may be expected to follow primary oral or perioral herpes infection as a result of centripetal infection primarily within Schwann cells of the trigeminal nerve. In this regard, the gasserian ganglion may act as a viral reservoir, with reactivation of the virus resulting in such clinical entities as trigeminal neuralgia, herpetic perioral fever blisters, and even recurrent encephalitis (*7*).

Herpes simplex virus has not yet been recovered from the gasserian ganglion in man, but attempts have been few (*7*); its chronic multiplication has been demonstrated in the lacrimal and salivary glands of man, and these tissues may well be important in recurrent herpetic disease (*8*).

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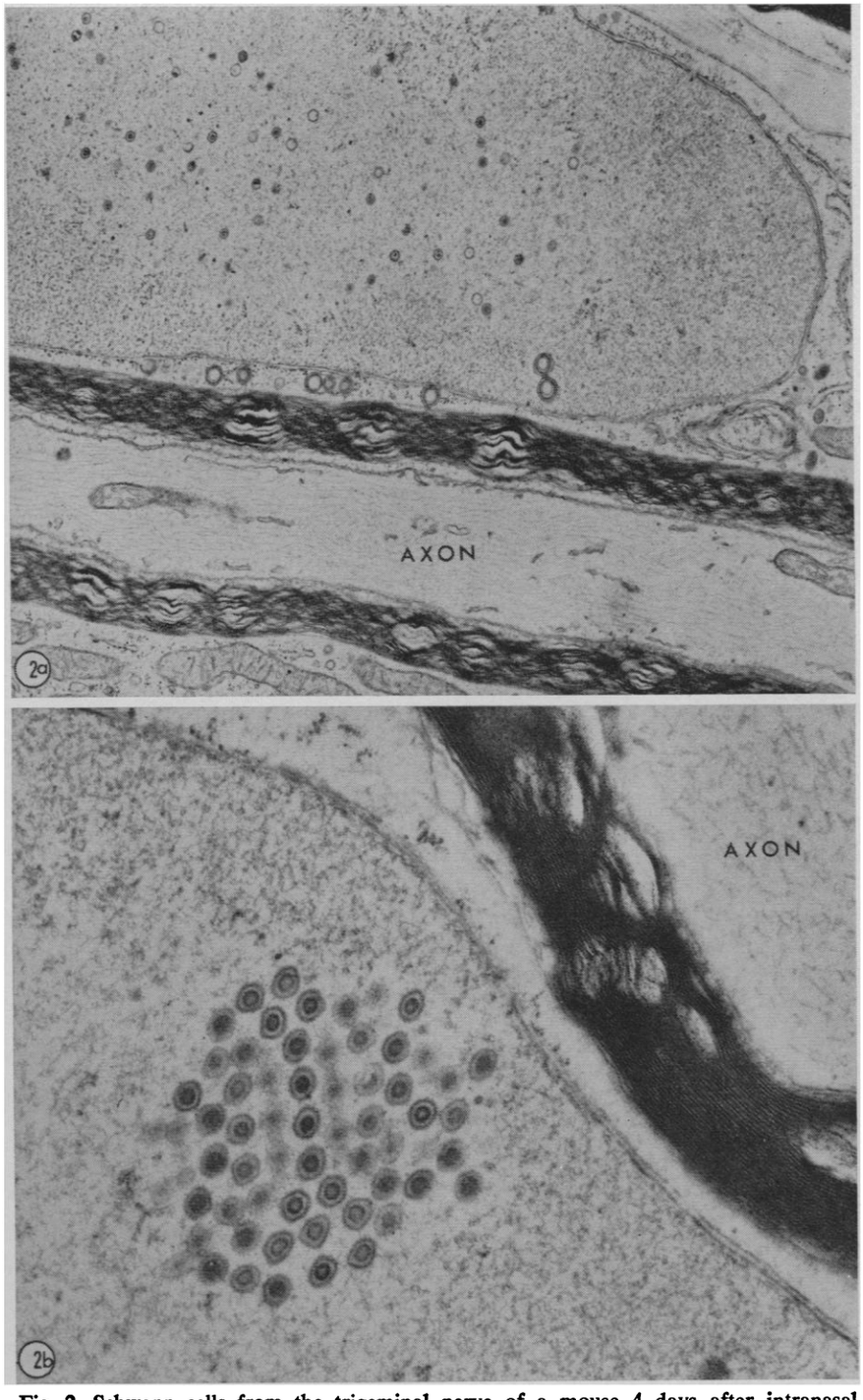

Fig. 2. Schwann cells from the trigeminal nerve of a mouse 4 days after intranasal inoculation with herpes virus. (a) Nucleus of a cell contains scattered complete and empty particles; note the enveloped herpes particles, many of which appear empty (× 18,400). (b) Nucleus of another cell contains an aggregate of herpes nucleocapsids; note the myelin sheath of the cell, enveloping the axon (× 53,600). Virions were not observed within axonal cylinders.

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29 May 1968

Human Prothrombin Activation: Immunochemical Study

Abstract. *Antiserums to human prothrombin contain antibodies directed against at least two different antigenic sites. During blood coagulation, prothrombin is cleaved into two major antigenically distinct fragments—thrombin and a "pro" fragment. The latter is present in normal serum, whereas thrombin loses its immunologic reactivity, presumably because of its combination with the natural thrombin inhibitors in blood.*

The mechanism of the conversion of the zymogen prothrombin to the active enzyme thrombin is poorly understood. Important observations have been made of prothrombin conversion in 25 percent sodium citrate solutions (*1*), with tissue extracts (*2*), and with activated factor X preparations (*3*). These studies indicate that major cleavages occur in the prothrombin molecule during thrombin formation. I now report studies of

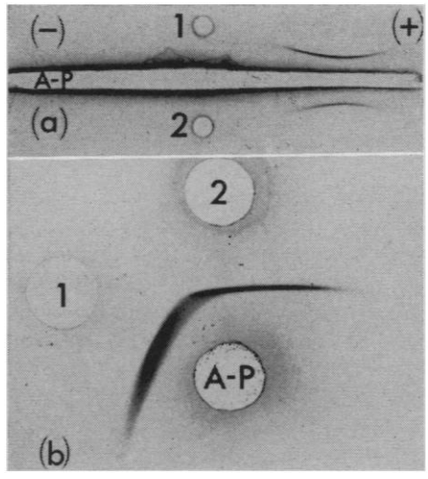

Fig. 1. Immunoelectrophoresis (a) and immunodiffusion (b) of human plasma and purified prothrombin. Well 1, plasma; well 2, prothrombin; A-P, antiserum to prothrombin.

prothrombin conversion under more physiological conditions, using immunologic techniques, and the identification of a cleavage product of prothrombin in normal human serum.

Human prothrombin was prepared and chromatographed on freshly poured columns of the carboxylic resin Biorex 70 (*4*). The product is homogeneous by physicochemical and immunologic criteria and is devoid of all other clotting-factor activities save for a trace of factor X, representing less than 0.002 percent of the preparation. Rabbits were immunized by the subcutaneous injection of 1 mg of prothrombin in complete Freund's adjuvant once a week for 3 weeks. Control serum was collected from all rabbits prior to immunization. Five days after the last injection, and periodically thereafter, serum was harvested. Precipitin titers generally remained high for several months after the third injection. Before use, all serums were absorbed with one-tenth their volume of aluminum hydroxide suspension (*5*) and incubated at 60°C for 30 minutes. This procedure did not alter precipitin potency of the antiserums, but did eliminate nonspecific effects in clotting assays. Antiserums contained 0.5 to 2.0 mg of antibody to prothrombin per milliliter, as measured by quantitative precipitin tests (*6*). On immunoelectrophoresis (*7*), antiserums developed a single arc against the provoking antigen and against normal citrated human plasma (Fig. 1a). Prior aluminum hydroxide absorption of the plasma, a procedure that removes prothrombin and the other vitamin-K dependent factors (*8*), removes its capacity to react with antiserum. On immunodiffusion (*9*), the precipitin lines of plasma and purified prothrombin give a reaction of immunologic identity (Fig. 1b). Species specificity of the antibody is indicated by the total lack of reaction of antiserum to prothrombin with rabbit or bovine plasma. Incubation of rabbit antiserums with normal human plasma at 37°C for 1 hour results in the total neutralization of prothrombin activity without change in the activity of any other coagulation factors, particularly the other vitamin-K dependent coagulation factors VII, IX, and X, the residual activity being 103, 105, and 97 percent, respectively. Prothrombin activity was measured by the two-stage technique of Ware and Seegers (*10*), while the other factors were measured by one-stage assays based on the correction of the clotting defect of congenitally deficient plasmas.

When antiserum to prothrombin is reacted against normal human serum on immunoelectrophoresis a new arc appears, with a more anodal mobility than prothrombin (Fig. 2). This arc is never seen in normal plasma. The serum component is nondialyzable, and can be removed from serum by aluminum hydroxide absorption. The serum component shows a reaction of partial identity with plasma and purified prothrombin on immunodiffusion, as indicated by spur formation (Fig. 3, wells 4 compared to 5 and 1 compared to 2). Purified human thrombin (*11*) also gives a reaction of partial identity with plasma and purified prothrombin (Fig. 3, wells 6 compared to 5 and 3 compared to 2). Thrombin and the serum component, however, appear to have no antigenic sites in common, since their precipitin lines cross without any evidence of interaction (Fig. 3, wells 3 compared to 4 and 6 compared to 1).

Although the antiserum to prothrombin which was used in these studies evidently contained antibodies to thrombin, no thrombin precipitin line could be detected in serum with the usual immunologic techniques. When purified thrombin is incubated with serum or plasma at 37°C for 1 hour, the antigenic reactivity of thrombin is lost almost entirely. During the incubation period, the clotting activity of thrombin also disappears. Studies of the activation of ^{125}I-labeled prothrombin (*12*) suggest strongly that both changes are due to complex formation with α_2-macroglobulin, a well-known natural antithrombin (*13*).

As expected from the foregoing, anti-

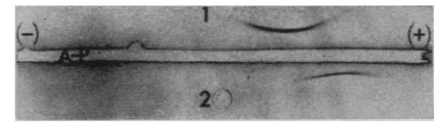

Fig. 2. Immunoelectrophoresis of plasma (well 1) and serum (well 2) against antiserum to prothrombin (A-P).

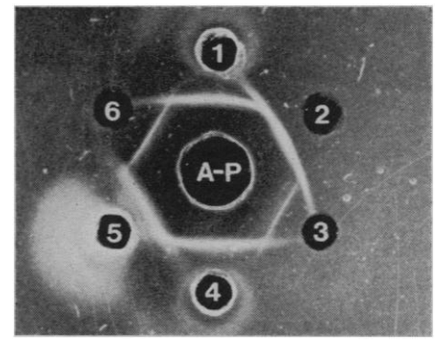

Fig. 3. Immunodiffusion of prothrombin and its cleavage products, against antiserum to prothrombin (A-P). Wells 1 and 4, serum; wells 3 and 6, thrombin; well 2, prothrombin; well 5, plasma.

serum to prothrombin, after absorption with human serum, loses its ability to react with human serum, while retaining reactivity toward prothrombin and thrombin. Josso *et al.* have reported an immunologic analysis of human prothrombin and thrombin (*14*). They found a reaction of partial identity between enzyme and zymogen. Because they routinely absorbed rabbit antiserums with human serum before use, they were unable to demonstrate the nonthrombin cleavage product of prothrombin activation, which I call the "pro" piece, normally present in human serum.

The recognition of this "pro" piece has already proven useful for testing alterations in the prothrombin molecule during purification, and should be valuable for the study of prothrombin activation under a variety of conditions.

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Corneal Calcification

Abstract. *Superficial calcification was produced in the normal rabbit cornea by mild irradiation with a carbon dioxide laser. The calcification was entirely extracellular and closely resembled that observed in human band keratopathy, which was characterized as hydroxyapatite by x-ray diffraction. The electron-microscopic appearance of calcific spherules and conglomerates in early corneal calcification is presented. The calcific spherules arise at the basal plasma membrane surface of the epithelial cells in close relation to their basement membrane.*

In studies on the effects of continuous carbon dioxide laser irradiation (wavelength 10.6 μ) (*1*), a number of rabbit eyes developed clinical and histopathologic changes identical with band keratopathy as observed in the human cornea. In the human cornea, this calcification appears to be wholly extracellular and is generally most prominent in the region, known as Bowman's membrane, lying beneath the epithelium. In the rabbit cornea, which does not have a structure completely analogous to Bowman's layer in the human cornea, early calcification also appears by light microscopy to lie beneath the epithelium and is wholly extracellular.

The rabbits were exposed to CO_2 laser irradiation of approximately 0.35 watt/cm^2 for 10 minutes over most of the corneal surface. The corneas developed superficial opacification. In two eyes the typical clinical appearance of band keratopathy was seen when examined 12 days later by both gross inspection and biomicroscopy. In another animal exposed at 0.48 watt/cm^2 for 7 minutes, calcification within a more dense corneal opacity was noted only upon histologic and electron-micro scopic examination from samples taken 14 days after irradiation. Histopathologic studies revealed the calcareous deposits [stains with alizarin red (*2*) (Fig. 1)] just beneath the epithelium. By electron microscopy, myriad spherules (Fig. 2a) appearing as concentric rings of exceedingly fine crystals (Fig. 2b) were observed within the basement membrane region of the epithelium, in the superficial stroma, and near the superficial keratocytes. Also visible were conglomerates of spherules that were presumably formed by fusion of the spherules. The superficial keratocytes engulfed a number of these spherules, which were then seen lying in membrane-bound cytoplasmic vesicles. No spherules could be found within the cytoplasm of the overlying epithelial cells, although a number could be seen to lie almost against the basal plasma membrane of the cells (Fig. 2c). In more severe cases, the space between the two outer rings of the spherule became more dense. It is possible that the basement membrane of the epithelium plays a major role as the initial nidus for the calcification. This might explain the double outer layering of the spherules or conglomerates, much like that seen in calcification of basement membrane of the peritubular capillary of the kidney in experimental calcinosis (*3*). These electron-microscopic observations were similar to those made in cases of human band keratopathy (*4, 5*). X-ray diffraction studies of samples of human cornea exhibiting band keratopathy revealed a crystal pattern characteristic of hydroxyapatite (*5*).

The cornea is normally a transparent and avascular structure, and band keratopathy can often be clearly observed in the central region, where there is no evidence of vascularization. Because the calcified spherules are found over almost the entire extent of the cornea just beneath the epithelium, and in a single plane beneath the epithelium, it was clear that the deposition of calcium in this region was related to some activity

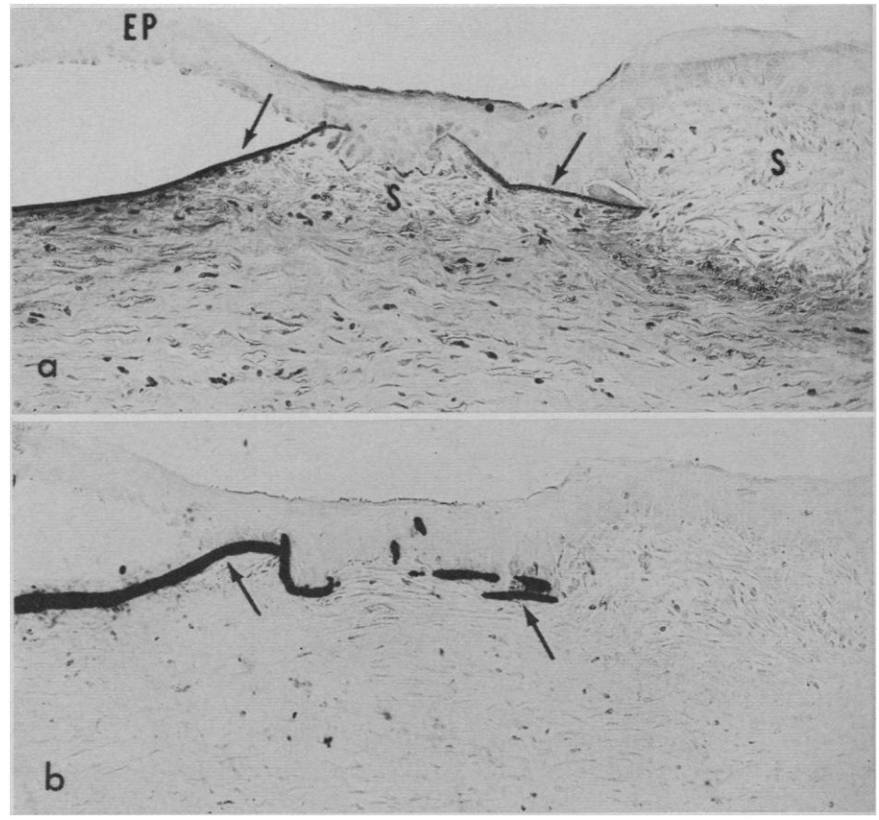

Fig. 1. (a) Thin section (1.5 μ) of Epon-embedded cornea stained with paraphenylenediamine (*7*) to show an interrupted, dark-staining layer in the anterior stroma (free arrows). Subepithelial stromal scarring is present in two locations (*S*), and the epithelium (*EP*) is lifted free of this dense layer on the left (× 115, AFIP negative 68-5142). (b) A section taken from the same block used for Fig. 1a and stained with alizarin red (*2*) to show the heavy concentration of calcium (free arrows) (× 115, AFIP negative 68-5143).