

IMMUNOELECTRON MICROSCOPY OF ANTIBODIES TO A TONOFILAMENT PROTEIN.

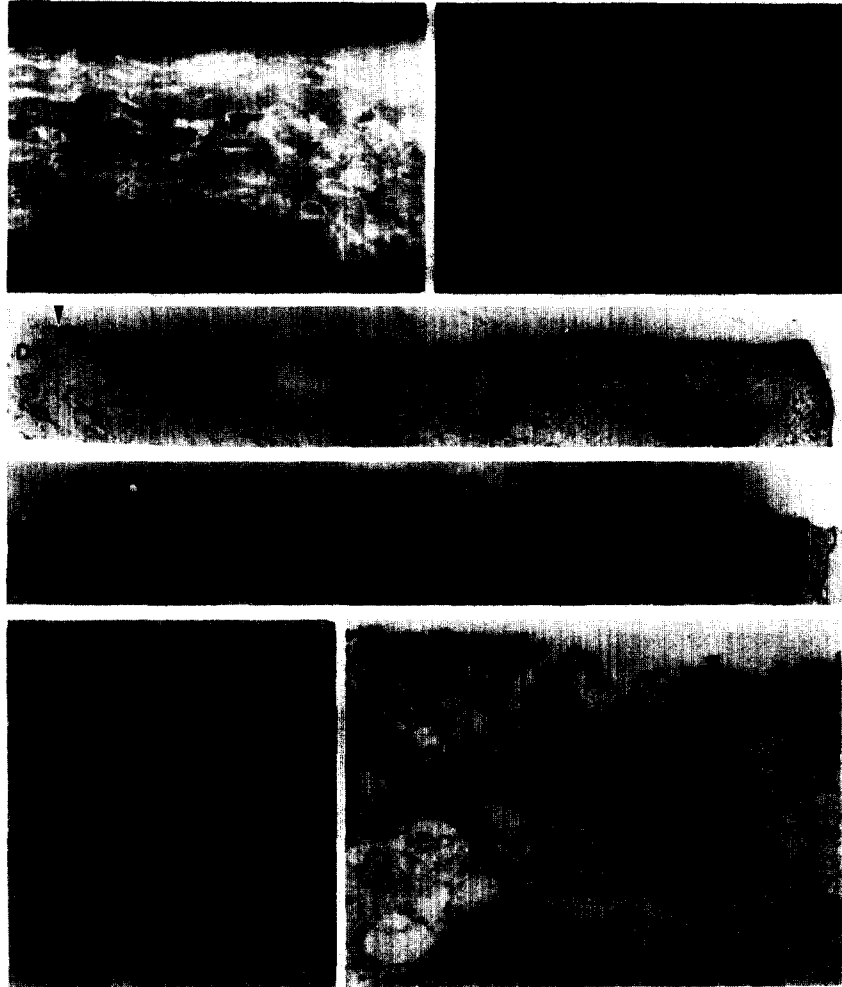
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A protein of 58,000 daltons was isolated from newborn rat epidermis which was high in specific activity after short-term exposure to tritiated leucine (Brysk, et al., 1977). A specific antibody to this protein was prepared in rabbits. The aim of this investigation was to determine the sites of localization of the specific antibodies in the epidermis. Immunolocalization studies by the indirect method were done at the light microscopic level with FITC labeled goat anti-rabbit (GAR) IgG and at the ultrastructural level with the peroxidase antiperoxidase (PAP) method (Gray, et al., 1977). Cryostat sections incubated with immune serum and preimmune serum are shown in Figs. 1 and 2, respectively. Fluorescent labeling over the entire epidermis was observed with immune serum whereas no label, above the normal autofluorescence, was observed with preimmune serum. These results were confirmed ultrastructurally using the PAP unlabeled antibody method (Gray, et al., 1977). The epidermis was labeled when tissues were incubated with immune serum (Fig. 3) but not labeled when incubated with preimmune serum (Fig. 4). At higher magnifications the peroxidase reaction products can be seen associated with individual tonofilaments (Figs. 5 and 6). A gradual decrease of peroxidase labeling is seen in the stratum corneum away from the cut edge of the tissue (Fig. 5). In the basal cell layer (Fig. 6), tonofilaments attached to desmosomes near the cut edge of the tissue are labeled whereas those on the opposite side of the desmosomes in an adjacent cell are not labeled. The dermis was not labeled in any of the above experiments. The results of this study demonstrate that a protein isolated from newborn rat epidermis is a component common to the tonofilaments in all epidermal cell layers.

Brysk, M. M., Gray, R. H. and Bernstein, I. A. 1977. *J. Biol. Chem.* 252(6):2127.

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Figures 1 and 2. Immunofluorescent stained cryostat sections of newborn rat epidermis with immune serum (Fig. 1) and preimmune serum (Fig. 2), X600. Figures 3-6. Electron micrographs of immune peroxidase treated skin strips. Figure 3. Immune serum, X2500. Figure 4. Preimmune serum, X2500. Figure 5. Stratum corneum showing peroxidase labeled tonofilaments, X7500. Figure 6. Lower epidermis with labeled tonofilaments, X7500. Arrowheads indicate dermis (D)-epidermis (E) junction, unlabeled tonofilaments (TF) PAP labeled tonofilaments (TF*), desmosomes (d), and basal cell nucleus (N).