probably as a result of denaturation of the antigenic determinants on the tissue-bound nucleotide.

In conclusion, our results suggest that the fraction of cyclic GMP localized by immunofluorescence to astrocytes in CNS tissue sections does not reflect biochemical changes of the nucleotide resulting from drug stimulation.

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FITC–Protein A–Gold Complex for Light and Electron Microscopic Immunocytochemistry[1]

Protein A (pA) from *Staphylococcus aureus* interacts with the Fc portion of IgG molecules from several mammalian species (7, 8, 13) and has been used for the localization of cellular antigens by light and electron microscopy (1, 4–6, 10). Recently, we reported on the use of a pA-gold complex for the electron microscopic demonstration of intracellular antigenic sites on thin sections of Epon-embedded tissue (12). The present study deals with the preparation and use of pA labeled by both fluorescein isothiocyanate (FITC) molecules and gold particles. This FITC-pA-gold complex can be used in both light and electron microscopic immunocytochemistry.

For light microscopy, normal rat pancreatic tissue from the splenic part of the organ was fixed in Bouin's fluid for 24 hr and embedded in paraffin. For electron microscopy, tissue fragments were fixed in 1% glutaraldehyde–0.1 M phosphate buffer pH 7.4 for 2 hr and embedded in Epon 812. Antiamylase and antitrypsinogen were raised in guinea pigs as already described (2). Anti-insulin, antiglucagon, and antisomatostatin (donated by Drs. P. H. Wright, R. Unger, and S. Ito, respectively) were used for the localization of these pancreatic hormones.

Colloidal gold suspensions (particle size approximately 200 Å) were prepared according to the method of Frens (9) and adjusted to pH 6.9 with 0.2 M K_2CO_3. For the preparation of the FITC-pA-gold complex the following method was used. One milligram of FITC-pA (Pharmacia Fine Chemicals, Uppsala, Sweden) was dissolved in 0.2 ml of distilled water and mixed with 10 ml of the colloidal gold in a plastic tube. After 3 min, 1 ml of 1% polyethylene glycol (mol wt 20,000) was added and the mixture was centrifuged at 60,000 *g* for 1 hr at 4°C. The supernatant was discarded and the red sediment was resuspended in 2 ml of PBS containing 0.2 mg polyethylene glycol per ml. For light microscopy, the FITC-pA-gold complex was used without further dilution. For electron microscopy, it was diluted threefold with PBS. The cytochemical labeling was performed in two steps. Deparaffinized and rehydrated 5 μm sections on glass slides or ultrathin Epon sections on nickel grids were first incubated with the specific antisera for 2 hr at room temperature in a moist chamber. After three washes in PBS, the sections were then incubated with the FITC-pA-gold for 1 hr at room temperature. Following three further washes in PBS, the sections from the paraffin-embedded tissue were stained with 0.01% Evan's blue for 2 min, mounted in glycerol/PBS (1:1) and examined under a Leitz orthoplane microscope equipped with an HBO 200 high-pressure mercury vapor lamp and a Ploemopak reflected light illuminator. The thin sections from the Epon-embedded tissue were stained with 5% aqueous uranyl acetate for 20 min and examined in a Philips EM 300.

To test the specificity of the staining, three types of cytochemical controls were performed: 1) antigen-adsorbed antiserum in the first step, 2) nonlabeled pA in the second step, followed by the FITC-pA-gold complex, and 3) omission of the antiserum step.

By the use of the FITC-pA-gold complex, the specific antibodies bound to the corresponding cellular antigens were detected by both light and electron microscopy. Figure 1 shows paraffin-embedded pancreatic tissue stained for amylase. The acinar cells are brightly fluorescent. Similar results were obtained for trypsinogen (not shown). These findings are indistinguishable from those obtained with the classical indirect immunofluorescence technique using FITC-anti-

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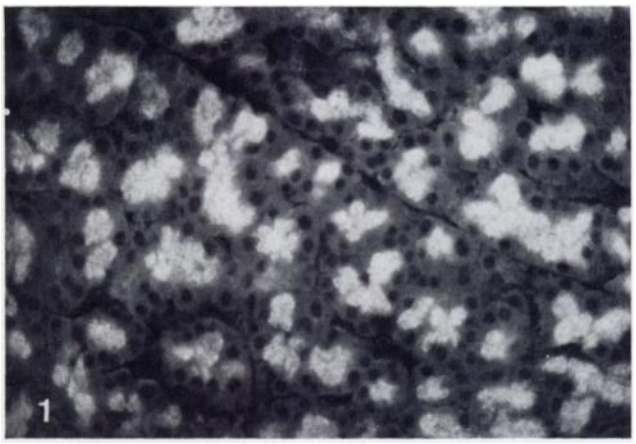

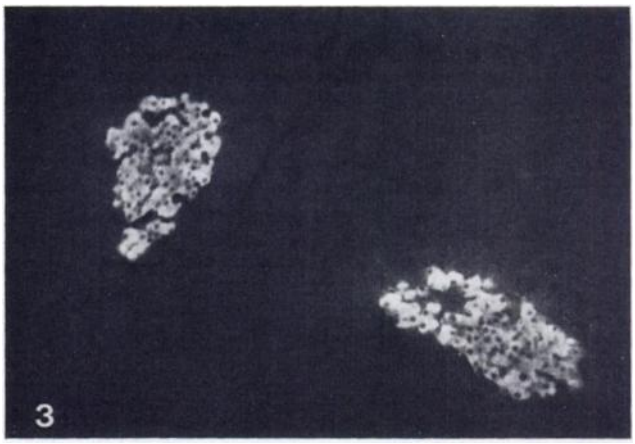

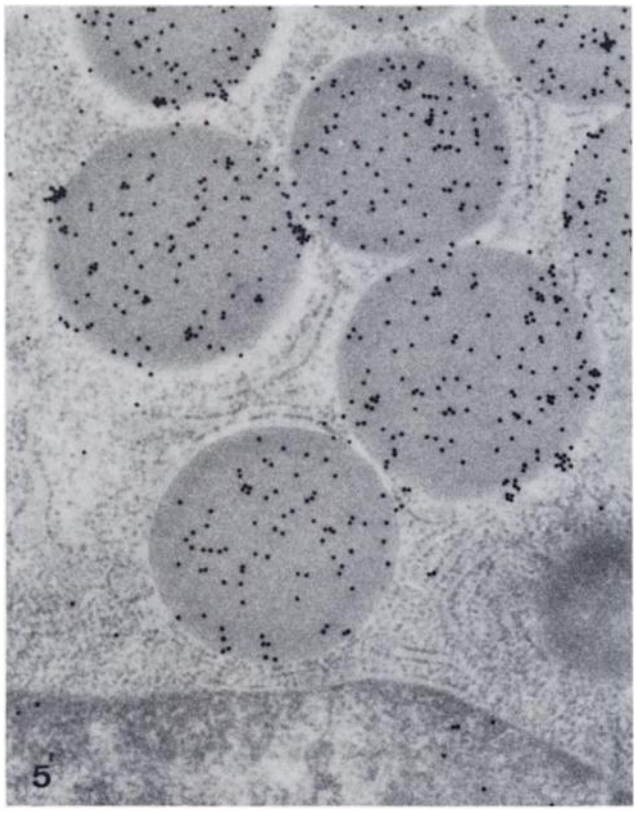

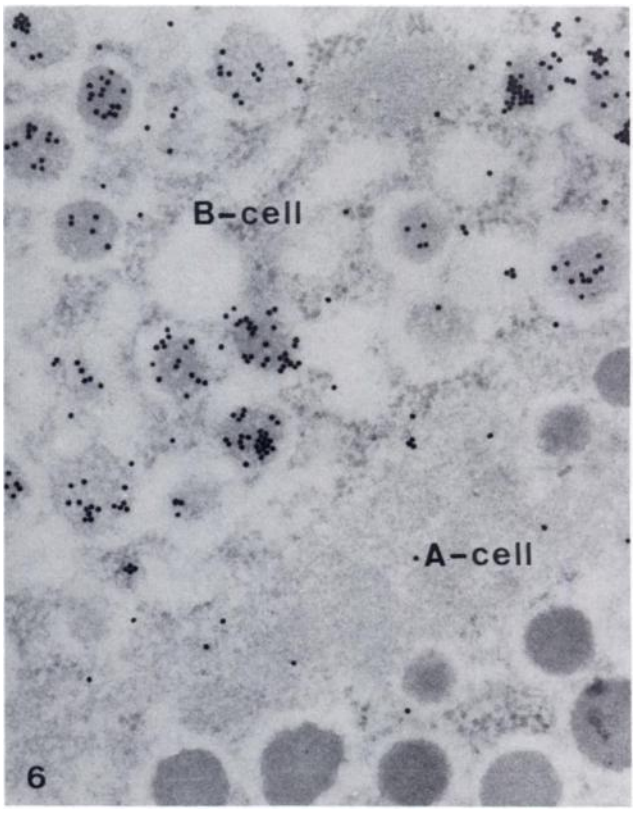

Figures 1 and 2. Bouin-fixed and paraffin-embedded tissue from the splenic part of normal rat pancreas. Staining for amylase results in a bright fluorescence in the cytoplasm of the acinar cells. × 350 (Figure 1). Control experiment for amylase with nonlabeled pA in the second step followed by FITC-pA-gold. No fluorescence is visible. × 350 (Figure 2).

Figures 3 and 4. Bouin-fixed and paraffin-embedded tissue from the splenic part of normal rat pancreas. Consecutive serial sections showing two islets of Langerhans. The fluorescent insulin-containing cells are centrally located (Figure 3), while the glucagon-containing cells (Figure 4) are less numerous and situated at the periphery of the islets. × 150.

Figures 5 and 6. Glutaraldehyde-fixed and Epon-embedded rat pancreatic tissue. Staining for amylase results in a labeling of the zymogen granules of an acinar cell by numerous gold particles. × 33,500 (Figure 5). Staining for insulin. The secretory granules of a B-cell are labeled by gold particles, whereas the secretory granules of an A cell are negative. × 39,500 (Figure 6).

IgG (2). Figures 3 and 4 present the results obtained for insulin and glucagon, respectively. The characteristic topographical distribution of the two endocrine cell types is visible (11). The staining of thin sections for exocrine enzymes (Figure 5 shows amylase) or endocrine hormones (Figure 6 shows insulin) with the FITC-pA-gold technique gave results similar to those obtained with the pA-gold technique (3, 12). When the specificity of the staining procedure was tested no tissue fluorescence was observed in any of the controls (Figure 2). By electron microscopy, very few gold particles were seen over the cells under all control conditions.

The results presented demonstrate that pA labeled by both FITC molecules and gold particles can be used as a second step reagent for the visualization of cellular antigens in the light and electron microscope.

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Are Fast Glycolytic Fibers Present in Slow Loris *(Nycticebus coucang)* Hindlimb Muscles?

The hindlimb muscles of the majority of mammalian species contain three muscle fiber types. These three can be classified as fast-twitch glycolytic (FG), fast-twitch oxidative-glycolytic (FOG), and slow-twitch oxidative (SO) according to the criteria of Peter et al. (7). A few variations from this general condition have been reported, e.g., the ankle extensors of the striped skunk contain no FG fibers (4), and the soleus muscles from several species have also been shown to contain only FOG and SO fibers (1). While studying muscle fiber type populations in prosimian primates, we observed that all muscle fibers of slow loris hindlimb muscles possess high-oxidative activity. This is in contrast to an earlier study by Ariano et al. (1) that reported the presence of a small percentage of FG fibers in slow loris hindlimb muscles. To determine whether FG fibers are present in these muscles, other enzyme tests that differentiate these fiber types were conducted on hindlimb muscles from three slow lorises.

Muscle fiber types were differentiated by demonstrating Ca^{+2}-activated ATPase activity in sections previously exposed to acidic buffer solutions of varying pH. By varying the pH of the preincuba-