DETECTION OF VIRUS ANTIGENS IN AN INFECTED CELL USING I^{131} - LABELED ANTIBODIES

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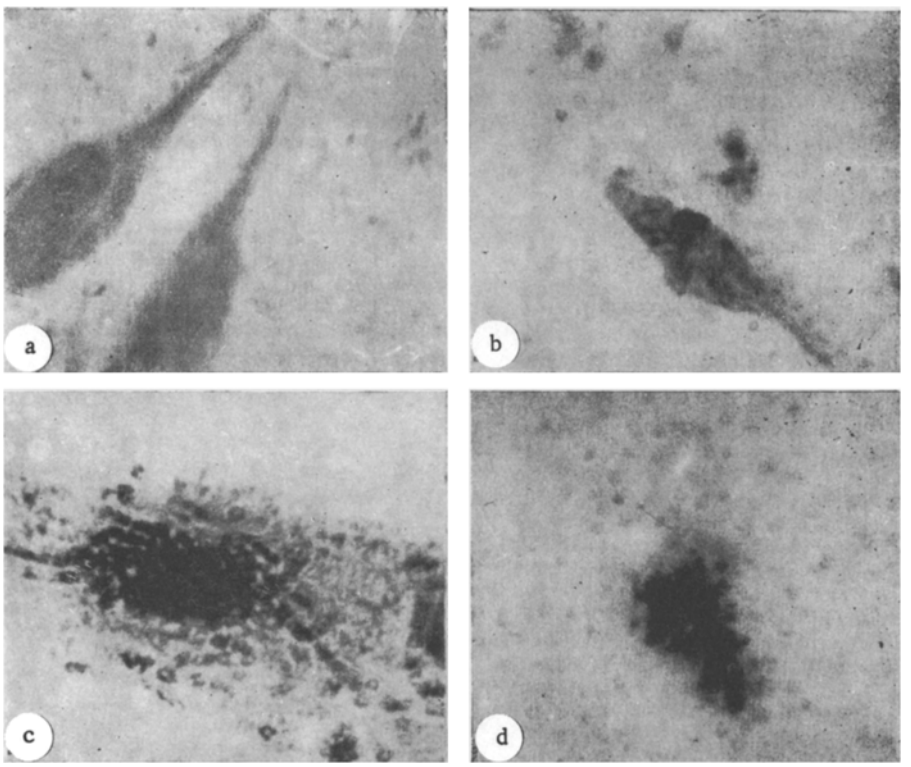
Radioactive isotopes (P^{32}, S^{35}, I^{131}) have frequently been used for labeling protein [3, 5, 7, 9, 10]. The least disturbing method is that using I^{131} [5, 6, 9]. During iodization of protein, I^{131} is incorporated into the 3:5 position of the tyrosine residue, and also into histidine. Sera labeled with radioactive substances have been used in immunological experiments [2, 5]. I^{131}-labeled protein has been used in autoradiographic experiments [8].

We have attempted to determine the intracellular localization of a specific antigen by means of labeled anti-influenzal sera, using the method of autoradiography.

EXPERIMENTAL METHOD

Viruses of human influenza A – PR_8 (ID_{50} 10^7) and of duck influenza A (Kosice) 550 (ID_{50} 10^7) were selected for the experiments. A skin-muscle culture of human embryonic cells and a culture of chick fibroblasts were used. Cells in a concentration of $2 \cdot 10^5$/ml of medium were seeded on to glass slides and used on the 3rd-4th day of incubation at 37°. A rat serum immune to PR8 virus with an antihemaglutinin titer of 1:320 was selected.



Cell culture treated with labeled serum. a) control (uninfected culture); b) infected with virus PR8 and incubated for 1 h; c) the same after incubation for 3 h; d) the same after incubation for 24 h.

The γ-globulin was labeled by the method of Francis [5] and Ziebster [10]. Sodium iodide was used as carrier. The activity of the radioactive iodine (I^{131}) was 0.3 μCi/ml. Autoradiography was carried out by the usual method, with fine-grain MP emulsion.

EXPERIMENTAL RESULTS

After saturation of its globulins with iodine, the serum was carefully freed from residual radioactivity by dialysis in physiological saline, the latter being frequently changed. Serum with an activity of 2.47 μCi/ml was used in the experiment. To determine the activity of the globulins, they were precipitated by a saturated solution of ammonium sulfate. The activity of the serum globulins was 0.76 μCi/ml of serum. To reveal the presence of intracellular virus protein, a monolayer of cells was infected with the above-mentioned viruses in a dilution of 10^{-3}. After incubation for 1, 3, and 24 h at 37°, cells were washed free from medium, and labeled immune serum was applied to them for 30 min. The cells were subsequently washed several times with Hank's solution, fixed in Carnoy's mixture, and embedded in a thin layer of emulsion. As controls, cells not infected with virus and cells infected with virus heterologous in relation to the serum antibodies were used. After exposure for 14 days the slide was developed. The results are shown in the figure.

Uninfected cells treated with serum are shown (see figure, a). No autographs may be seen.

In the figure (b, c, d) can be seen cells infected with PR8 virus (incubated for 1, 3, and 24 h). Autographs are readily seen as black granules. After 1 h only a few could be counted, but after 3 h they were numerous and occupied nearly all the cytoplasm. After incubation for 24 h the cell could not be seen, for it appeared to consist of granules of autographs. These findings agree with the results of the detection of intracellular influenzal antigen by Coons's method [1, 4], using fluorescent antibodies.

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SUMMARY

Labeling of antibodies with I^{131} with subsequent autoradiography was used to detect the viral antigen in the cell.

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