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DEMONSTRATION OF VIRAL ANTIGEN IN GIANT CELLS FORMED IN MONKEYS EXPERIMENTALLY INFECTED WITH MEASLES VIRUS

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SUMMARY Immunofluorescent studies were carried out on frozen sections of various tissues of 2 cynomolgus monkeys experimentally infected with an attenuate live vaccine strain of measles virus, and of 6 cynomolgus monkeys infected with wild virus materials from patients with measles.

The inguinal lymph nodes removed by operation 3 days after inoculation of attenuate virus showed areas containing antigen. Most of the lymph nodes and the spleen of the monkeys killed after 3 and 5 days and the pulmonary epithelium of the monkey autopsied 7 days after virus inoculation also showed many antigenic areas. However, no multinucleate giant cells were found in the preparations of any of these tissues on staining with hematoxylin-eosin.

Findings were similar in monkeys inoculated with wild virus from patients, but fluorescence was generally greater. Findings in preparations stained with fluorescent antibody were carefully compared with findings in the same preparations stained with hematoxylin-eosin. Many multinucleated giant cells were found in these lymphatic tissues and in the pulmonary epithelium. The giant cells of both tissues contained much viral antigen. Thus giant cell formation could be used as an indicator of virus multiplication in preparations stained with hematoxylin-eosin. However, the absence of giant cells does not necessarily imply that viral multiplication does not occur in tissues infected with either attenuate virus or virulent virus.

INTRODUCTION

A very characteristic pathological change in monkeys infected with measles virus is the formation of multinucleated giant cells in lymphatic tissues and in the bronchial epithelium of the lungs. Experimentally Gordon and Knighton (1941) first found Warthin-

Finkeldey type multinucleated giant cells (Warthin, 1931, Finkeldey 1931) in the tissues of inguinal lymph nodes excised from monkeys inoculated with material containing measles virus. Taniguchi et al. (1954a, b) reported multinucleated gaint cells in the bronchial epithelium of monkeys infected experimentally with measles virus, and noted that the characteristics of these giant cells are identical with those of the cells found at autopsy in the lungs of human cases of measles by Masugi and Minami (1938). Taniguchi et al. (1954a, b) also confirmed the presence of Warthin-Finkeldey giant cells in lymphatic tissues of various organs of monkeys. They considered these pathological findings as evidence of the susceptibility of monkeys to measles virus. Later morphological details of the multinucleated giant cells found in the lymphatic tissues of monkeys with measles were reported by Sergiev et al. (1960) and Nii et al. (1964). Multinucleated giant cells in cell cultures infected with measles virus were reported by Enders and Peebles (1954). Toyoshima et al. (1960) found that both active and UV-inactivated measles virus could produce giant cells in FL cultures.

This work was on the relation of giant cell formation in monkeys inoculated with measles to virus antigen.

MATERIALS AND METHODS

1. *Monkeys*

Eight cynomolgus monkeys weighing 1.5–2.5 kg and confirmed to be free from neutralizing antibody against measles virus, were used.

2. *Inocula*

a) As virulent samples, swabs of nasopharyngeal secretions were collected from 6 patients showing Koplik's spots in the oral mucosa. The swabs were suspended in 0.15 M NaCl, containing 0.05% gelatin, 200 μg of Kanamycin and 2 μg of Amphotericin B.

b) Blood samples were also taken from 2 patients when a rash began to appear.

c) Attenuated measles virus (Toyoshima strain) which had been passages 150 times in chick amnion and several times in primary monkey kidney cells in the Department of Virology of this Institute was used. This infective titer was $10^{5.5}$ $TCID_{50}$/ml in Vero cells.

3. *Virus inoculation*

Five ml samples of (a) were injected subcutaneously into the left thigh of 4 monkeys, 2.5 ml samples of (b) were injected subcutaneously into the left thigh of 2 monkeys and 1 ml samples of (c) were injected subcutaneously into the right thigh of 2 monkeys.

4. *Autopsy and histological examinations*

The inguinal lymph nodes were removed by operation from two monkeys 3 days after virus inoculation. These 2 monkeys were killed on the 5th and 7th days after inoculation and autopsied. The other monkeys were killed anesthetically with ether or choloroform 7, 11 and 12 days after inoculation. At autopsy, the lymph nodes, lung, spleen, liver and kidney were cut in half and one half was immediately stored at -70 C for examination by the fluorescent antibody technique. The other half was fixed with Bouin's fixative or 10% formalin. For the fluorescent antibody technique organs or tissues were treated with n-hexane in a dry-ice acetone bath. Histological preparations were stained with hematoxylin and eosin.

5. *Antiserum*

Vero cells were infected with attenuated measles virus of the Toyoshima strain and infected cells were desrupted by sonication at 10 kc for 5 min and centrifuged at low speed (3,000 rev/min) for 15 min. The supernatant was centrifuged at $77{,}000 \times g$ for 2 hr. The precipitate was resuspended in PBS and used as viral antigen. This infective titer was $10^{6.3}$ $TCID_{50}$/ml in Vero cells. Volumes of 5–10 ml of viral antigen preparation were injected intravenously into healthy cynomolgus monkeys four times at weekly intervals. One week after the final immunization, the animals were bled. At this time the CF antibody titer was 1 : 256.

6. *Preparation of fluorescein conjugated antibody*

The standard ammonium sulfate method was used for separation of the γ-globulin fraction from these immune sera. Conjugation with fluorescent dye was performed by the method of Marshall et al. (1958). Thus, 0.01 mg of fluorescein isothiocya-

nate (Baltimore Biological Laboratories) was mixed with 1 mg of γ-globulin fraction adjusted to pH 9.5 with 0.5 M carbonate buffer. The mixture was allowed to conjugate with stirring at room temperature for 3 hr. Unconjugated dye was removed by passage through a Sephadex G-25 column. The conjugated fraction was collected and applied to a column of DEAE-cellulose equilibrated with 0.005 M phosphate buffer, pH 7.0, in 0.1 M NaCl. This staining titer was 1 : 8.

7. *Fluorescent antibody technique and hematoxylin-eosin staining*

Tissues (lymph nodes, lung, liver, kidney and spleen)were treated with n-bexane at −70 C for 15 min and frozen sections of 4–6 μ thickness were cut by cryostat. Sectioning was done at −18 to −20 C. Sections were immediately fixed by immersion in acetone at −20 C for 15 min and dried in air. Sections were overlaid with labeled antibody, placed in a moist chamber and incubated at 37 C for 1 hr. Unreacted labeled antibody was washed off gently with three changes of PBS. Specimens mounted with PBS were examined with a Nikon fluorescent microscope. Microphotographs were made of various fields of the sections stained with fluorescent antibody and these areas were marked. Then the samples were rinsed with PBS, refixed with ethanol and restained with H-E. The microphotographs of fluorescent antibody were carefully compared with those of the same fields in the sections after H-E staining.

RESULTS

1. *Relationship between giant cells and viral antigen*

Various lymph nodes, and the lungs and spleens were removed from 3 monkeys on the 7th day, from one monkey on the 11th day and from 2 monkeys on the 12th day after inoculation of wild virus material. Sections of these tissues were examined both by the fluorescent antibody technique and after restaining with H-E. Specific fluorescence was found in tissues from all the lymph nodes, spleens and lungs examined. Multinucleated giant cells were found in these lymphatic tissues and in the pulmonary epithelium. Results are shown in Table 1. Results on the various tissues are described below.

1) Lymph nodes and spleens

Various flourescent areas were observed in these lymphatic tissues, and on restaining with H-E, some fluorescent spots were found to correspond to multinulcleated giant cells. Generally intensely fluorescent areas were found in germinal centers. Many fluorescent spots were also found around germinal centers. These spots seemed to be single infected cells. Comparative photographs are shown in Figs. 1, 2, 3 and 4 for lymph nodes and in Figs. 5, 6, 7 and 8 for spleens.

2) Lungs

Specific fluorescent areas were found in the epithelium lining some bronchi and bronchioli of the lungs. After restaining with H-E, some fluorescent areas were found to correspond to multinucleated giant cells, as shown in Figs. 9–14. Fluorescent areas were also seen in the area corresponding to the peribronchial lymphatic tissues.

3) Livers and kidneys

No fluorescence was observed in these tissues.

2. *Detection of specific fluorescence in tissues of monkeys infected with attenuated virus*

The inguinal lymph nodes were removed by operation from 2 monkeys 3 days after inoculation of attenuated virus. These monkeys were killed on the 5th and 7th day, respectively, after inoculation. Lymph nodes and other tissues were removed and subjected to FAT. Specific fluorescence was seen in the inguinal lymph nodes on the side of inoculation on the 3rd day after inoculation and in all lymph nodes examined, and the epithelium in the bronchi of the lungs and in the white pulp of the spleen on the 5th and 7th days after inoculation. But unlike the case after inoculation of wild virus, no multinucleated giant cells were detected in these samples. Results are shown in Table 1.

TABLE 1. *Appearance of measles viral antigen and giant cells in the tissues of cynomolgus monkey infected with attenuated and wild measles viruses*

Virus materials	A[a]		A		WS[b]	WB[c]	WS	WS	WS	WB
Number of monkeys	1		2		5X-4	5X-10	5X-1	5X-5	5X-8	5X-11
Days after inoculation	3	5	3	7	7	7	7	11	12	12
Submandibular lymph node (right)		+[d]		++	+	+	+++(G)[f]	++	+	+++
Submandibular lymph node (left)		+		++	+	−[e]	+++(G)	+++	+	+++
Bronchopulmonary lymph node		++		++	++	+	NT[g]	NT	++	+++
Axillary lymph node (right)		+		+	+	−	+++	+++(G)	+	+++(G)
Axillary lymph node (left)		+		+	+	±	+++(G)	+++(G)	+	+++(G)
Inguinal lymph node (right)	+	+	+	+	+	−	+++(G)	+++	+	+++
Inguinal lymph node (left)		+		+	++(G)	+	+++	+++(G)	+	+++
Mesenteric lymph node (root)		±		+	±	−	+++(G)	+++	+	+++(G)
Mesenteric lymph node (peripheral)		+		+	±	−	+++(G)	+++	+	+++(G)
Lung		+		++	+	+	+++(G)	+++(G)	+	+++(G)
Liver		−		−	−	−	−	−	−	−
Spleen		+		++	++(G)	+	+++(G)	+++	+	+++(G)
Kidney		−		−	−	−	−	−	−	−

a: Attenuated measles virus
b: Swabs from patients with measles
c: Blood from patients with measles
d: Degree of viral antigen
e: No viral antigen
f: Giant cells detected
g: Not tested

DISCUSSION

Immunofluorescent studies on monkeys infected experimentally with measles virus have been reported by Nii and Kamahora (1964) and Yamanouchi et al. (1969). The former authors demonstrated the existence of measles viral antigen in affected lymph nodes. The latter authors reported the widespread existence of measles viral antigen in various lymphatic tissues and in the bronchail epithelium of monkeys infected either with virulent strains or with attenuated vaccine strains of measles virus. On careful comparison of microphotographs of fluorescent areas of affected tissues and microphotographs of the same areas of the preparations after restaining with hematoxylin-

eosin, we found that the areas of multinucleated giant cells in either lymphatic tissues or bronchial epithelium showed specific fluorescence. Therefore, the Warthin-Finkeldey giant cells in the lymphatic tissues and giant cells in the bronchial epithelium are closely related with multiplication of measles virus. However, the absence of the giant cells does not necessarily indicate the absence of viral antigens. The significance of giant cell formation in monkeys infected with measles virus is unknown. It may be related with the virulence or titer of the virus propagated in the tissues.

Sections from various tissues of a monkey (No. 5X-1) 7 days after inoculation of wild virus material. Odd numbers are photographs by the fluorescent antibody technique, and even number are photographs of sections restained with H-E.

FIGURE 1. *Section from a left axillary lymph node. Specific viral antigen is seen.* ×300

FIGURE 2. *The same microscopic field as Fig. 1, restained with H-E. Some fluorescent areas were found to correspond to multinucleated giant cells (Warthin-Finkeldey type).* ×300

FIGURE 3. *Section from a right submandibular lymph node. Specific viral antigen is seen.* ×300

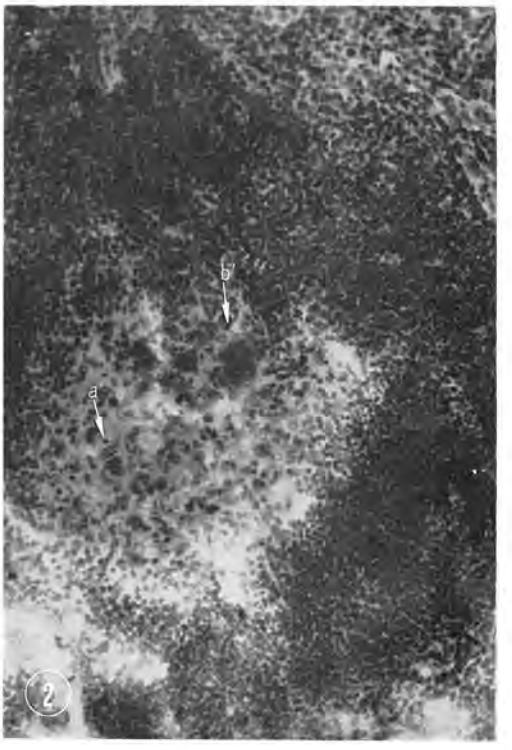
FIGURE 4. *The same microscopic field as Fig. 3, restained with H-E. Areas of intense fluorescence were found to correspond to multinucleated giant cells (Warthin-Finkeldey type).* ×300

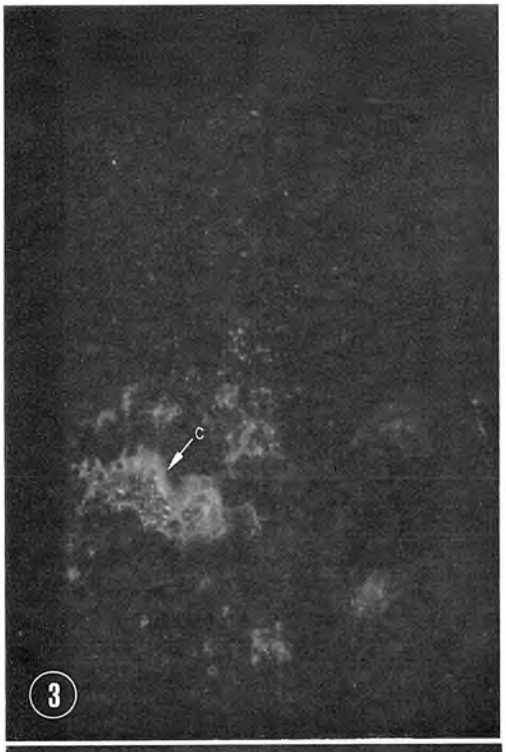
FIGURES 5 and 7. *Sections from the spleen. Specific viral antigen is seen.* ×300, 400

FIGURES 6 and 8. *The same microscopic fields as Figs. 5 and 7 respectively, restained with H-E. Some fluorescent areas were found to correspond to multinucleated giant cells (Warthin-Finkeldey type).* ×300, 400

FIGURES 9, 11 and 13. *Sections from a lung. Specific viral antigen is seen on the lining epithelium of bronchi and inside the bronchi.* ×400, 1,000, 1,000

FIGURES 10, 12 and 14. *The same microscopic fields as Figs. 9 and 11, 13 respectively, restained with H-E. Some fluorescent areas were found to correspond to multinucleated giant cells.* ×400, 1,000, 1,000

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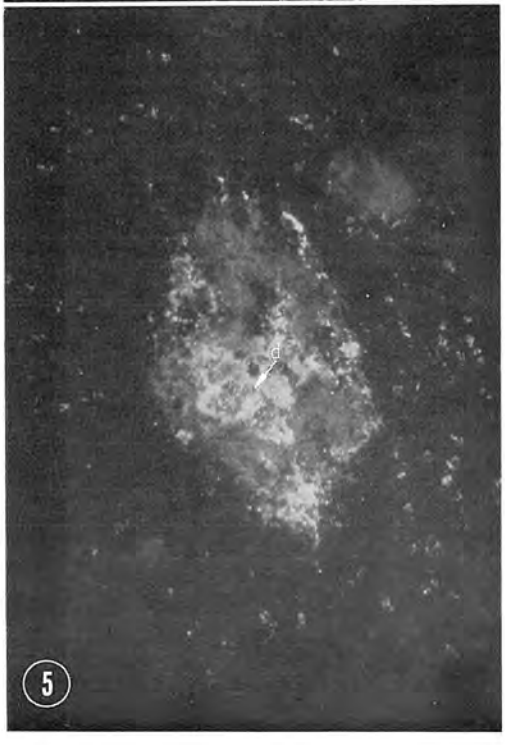
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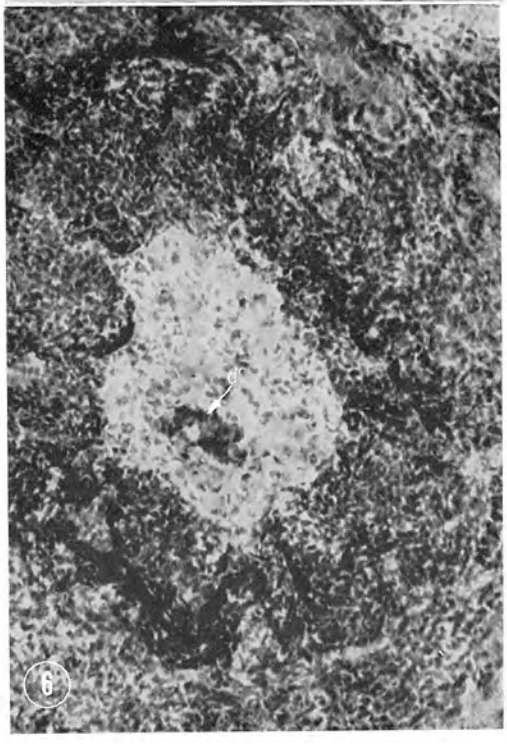
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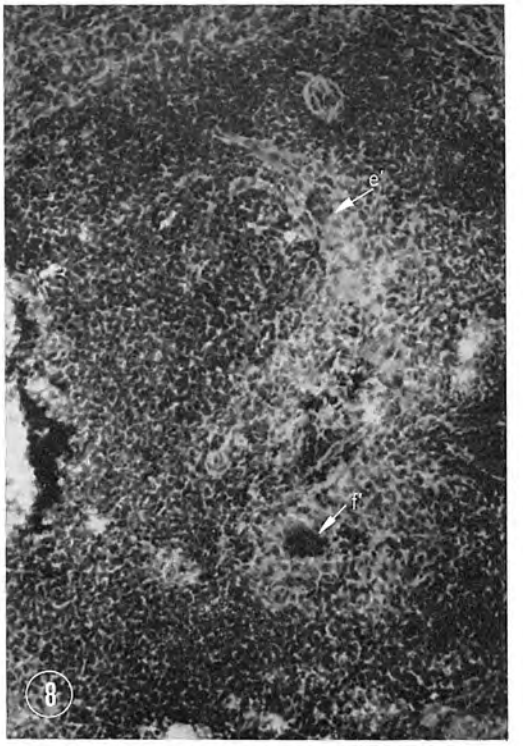
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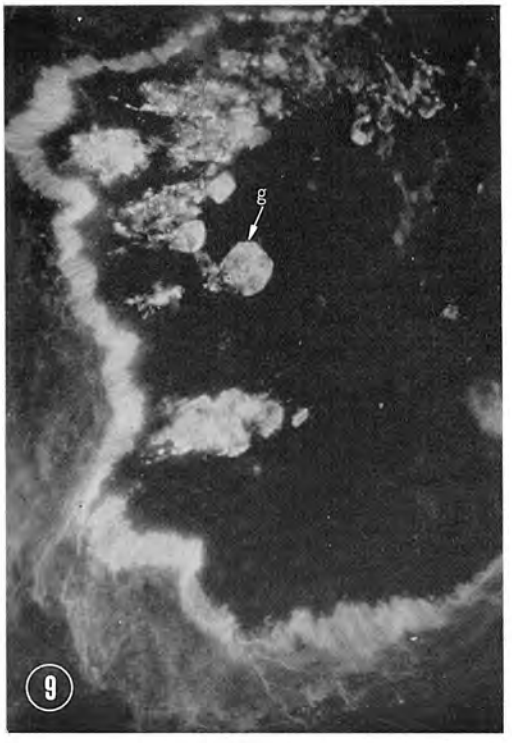
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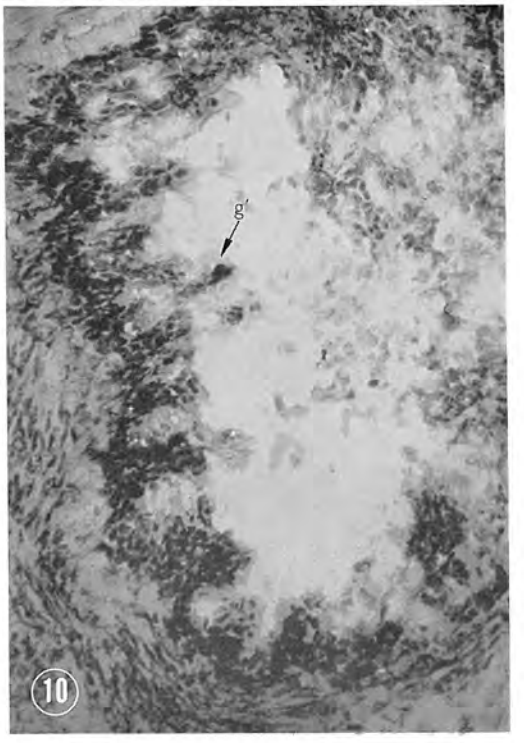
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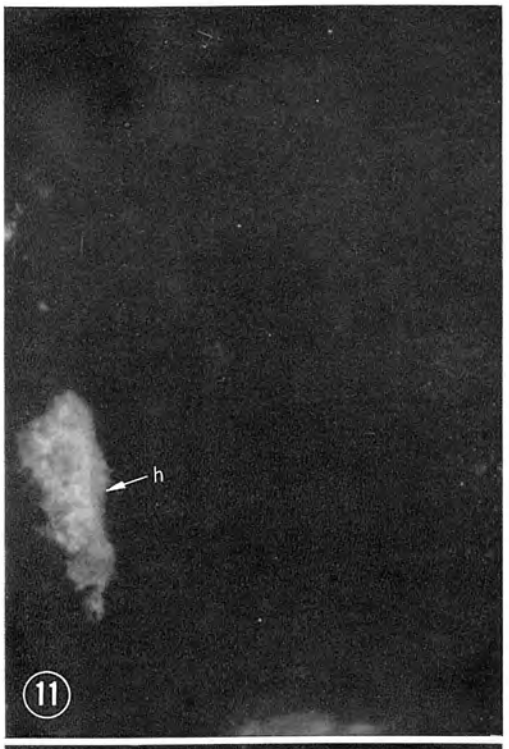
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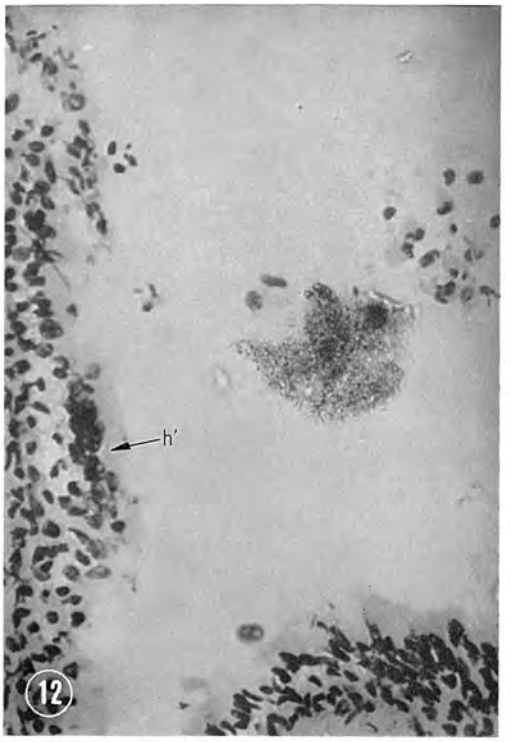
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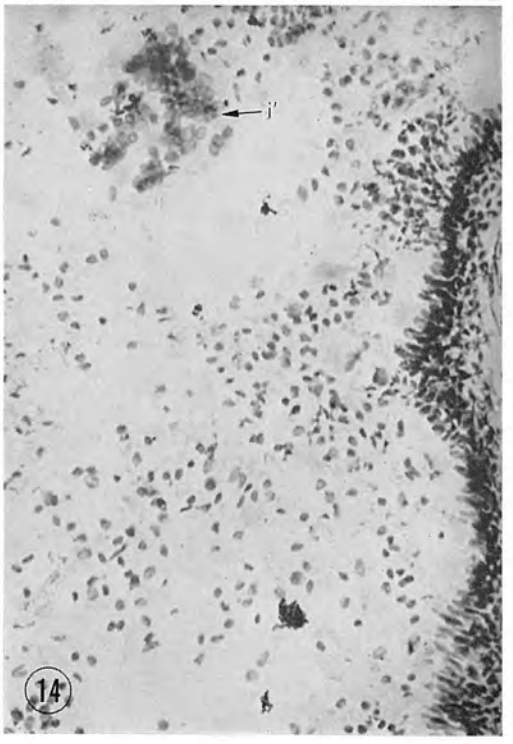
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