3. The lipide-C^{14} recoveries observed with the method described here were compared with those obtained by methods used earlier in this laboratory, namely, that of Chernick *et al.*(3) and that of Felts *et al.*(1). The design and results of these experiments are given in Table III. It is clear that the lipide-C^{14} yields observed with the abbreviated method described here are in good agreement with those obtained by the laborious procedures employed earlier.

Summary. 1. A rapid method for determination of lipide-C^{14} in experiments in which liver slices are incubated with C^{14}-labeled compounds is described. 2. Data on the reliability of the procedure, and a comparison of values obtained by this simplified procedure with those obtained by the previous laborious methods are presented.

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Antigenicity of Canine Distemper Inclusion Bodies as Demonstrated By Fluorescent Antibody Technic. (21021)

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The fluorescent antibody technic was developed by Coons *et al.*(1) and is a method in which antibody conjugated to fluorescein is employed as a specific histochemical stain for the localization of antigen in cells. The technic has been used to demonstrate antigens of rickettsiae and mumps virus(1), pneumococcus(2,3), Friedländer bacillus(4), leptospira(5) and homologous plasma proteins in tissues(6). The method was recently employed to show viral antigen in the inclusion bodies of canine infectious hepatitis(7). This report deals with the application of the technic to cells containing inclusion bodies of canine distemper.

Materials and methods. Fluorescein isocyanate was prepared and conjugated to protein of anti-canine distemper serum* according to the method of Coons and Kaplan(8). Absorption of the conjugate on liver powder for preventing non-specific fluorescence in tissues was not found necessary in the smear preparations which were used. Smears of urinary bladder epithelium from natural cases of canine distemper were used as a source of inclusion bodies. Smears were made on coverslips which were subsequently dried 30 minutes before staining. The staining procedure was as follows: A smear was treated for 20 minutes with the specific conjugate and the excess shaken off. The smear was washed for a few seconds in buffered saline, pH 9, and then gently agitated in fresh buffered saline for 10 minutes. The coverslip was dried and mounted smear surface down over a square aperture in a slide. *Fluorescence* in the smears was produced with ultraviolet light and observed through a standard microscope. The light source was a Leitz, 8 ampere, carbon arc. Filters consisted of 3.2 cm of $CuSO_4$ (25 g/100 ml) in a pyrex cuvette and Corning filter No. 5840 (½ standard thickness) to remove visible light. The filtered ultraviolet radiation practically free of visible light was directed into an ordinary glass substage condensor by means of a polished mirror of aluminum-magnesium alloy. A protecting filter (Wratten gelatin filter No. 2A) was mounted in the ocular of the microscope. Photomicrographs of fluorescence were taken with Eastman Super Panchro-Press, Type B film with 20-minute exposures. After a suitable area of fluorescence was located in the smear and photographed, the slide was re-

* Obtained through courtesy of Dr. Hilary Koprowski, Lederle Laboratories.

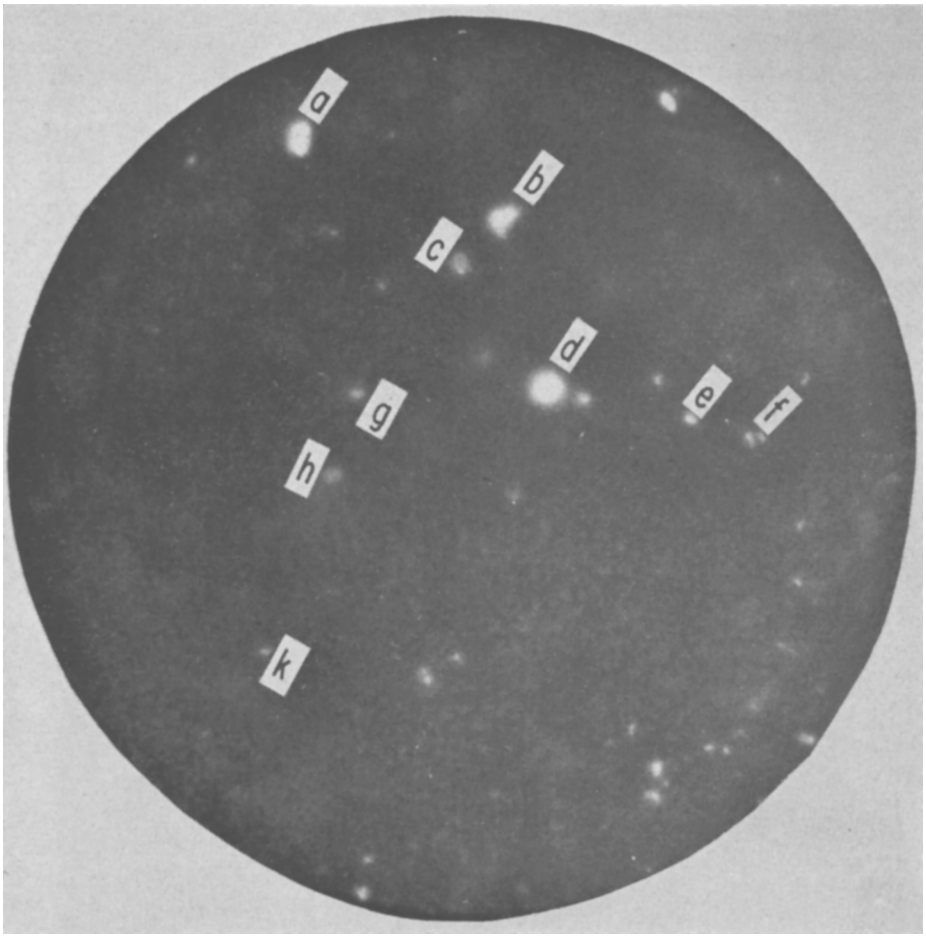

FIG. 1a

moved and stained with Sellers' stain (9). The slide was then replaced in the microscope and a photograph of the same field was taken with visible light. In this manner by comparing photographs, fluorescent objects could be correlated with their color-stained counterparts. *Specificity of staining* was determined by 1) treating bladder smears from infected dogs with normal distemper antiserum for 10-20 minutes prior to staining with conjugate and 2) by staining smears from normal dogs with conjugate.

Results. Under ultraviolet light widely scattered, green fluorescing deposits were observed in the smears. Photographs of these fluorescent structures compared with photographs of the same structures stained with Sellers' stain showed that fluorescence was

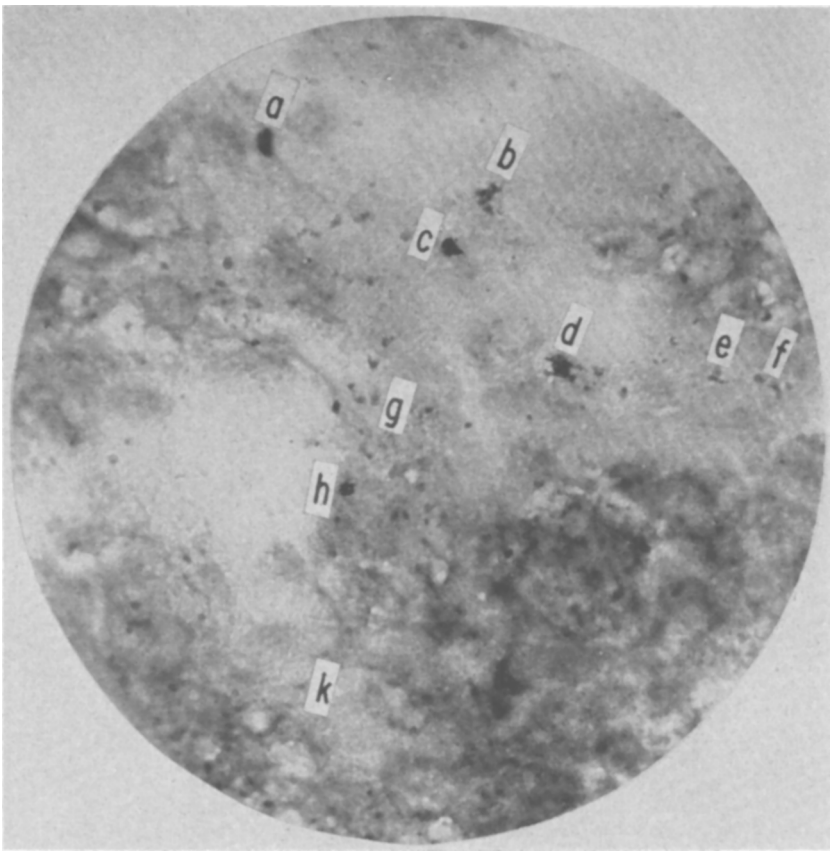

FIG. 1b

FIG. 1. Smear of urinary bladder for comparison of inclusion bodies (a) treated with fluorescent antibody and (b) stained by Sellers' method. Fields are identical. Letters point out corresponding inclusion bodies. × 300.

localized in the inclusion bodies of distemper (Fig. 1). Some distortion was noted in the inclusion bodies after the final staining, but this distortion was not observed in fresh smears of the same material stained immediately with Sellers' (Fig. 2).

When smears containing inclusion bodies were treated with normal antiserum before staining with conjugate, fluorescence was partially inhibited. Smears of normal canine bladder epithelium showed no specific fluorescence when stained with conjugate.

Summary and conclusion. Canine distemper inclusion bodies in urinary bladder epithelium were specifically stained with fluorescein-antibody conjugate. On the basis of this finding it is concluded that these inclusion bodies contain viral antigen.

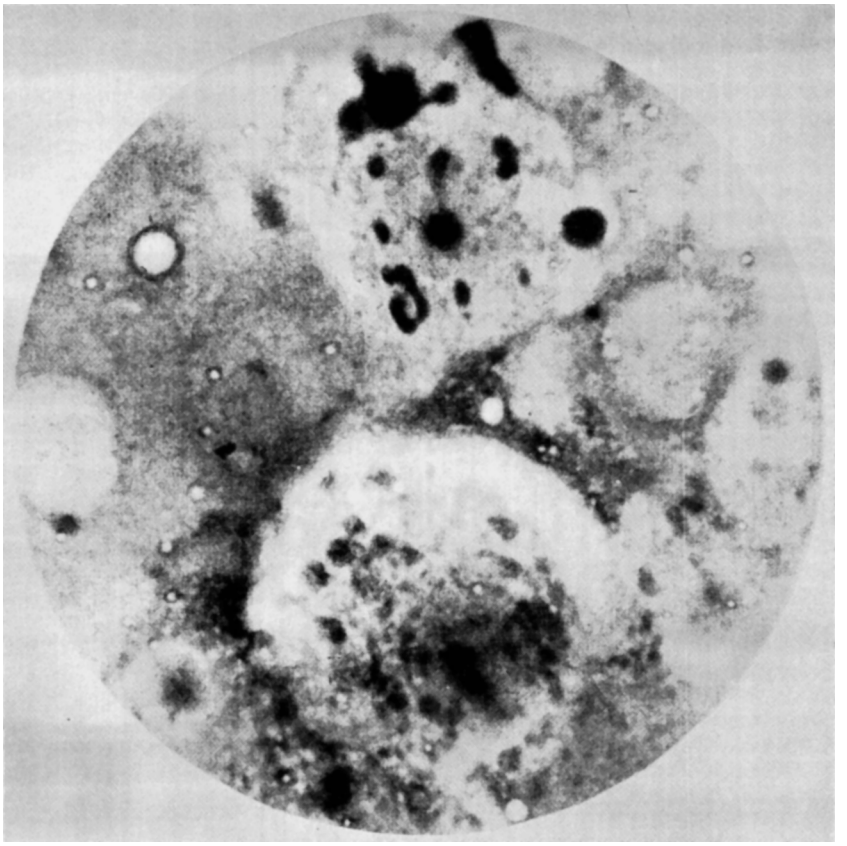

FIG. 2. Fresh smear from same urinary bladder as in Fig. 1 showing epithelial cells filled with inclusion bodies. Sellers' stain. × 900.

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