viable cells in control and virus-inoculated cultures.

Cells from inoculated cultures during the growth-inhibitory period showed a considerable increase in size, when compared with uninoculated controls (Fig. 2); a certain amount of cell rounding and clumping was also observed. Staining with May-Grünwald-Giemsa revealed few pyknotic nuclei in virus-inoculated cultures, but many densely stained cells. The growth-inhibitory effect was used to titrate Shope virus in Leighton tubes containing flying coverslips. After May-Grünwald-Giemsa staining, titers were determined by gross comparison of cell populations between inoculated and control cultures, and by microscopic examination of stained cells for qualitative cytopathic effects. Good agreement was obtained among several replicate experiments, with use of the same batch of virus (SPV-C) throughout. The titers obtained (10^{-3}) were identical with those obtained by titration in Indiana cottontail rabbits.

The cytopathic effect described, as well as papilloma formation in rabbits, were both completely inhibited by prior incubation at 37 C for 30 min of the virus inoculum with specific antiserum. Antiserum was added in a final concentration of 10%, the total serum concentration being adjusted with normal serum in every case to 20%. Both hyperimmune serum (having a complement-fixation titer of 1:320) and naturally occurring antiserum (titer, 1:5) were effective in neutralization of virus. None of the growth-inhibitory or growth-stimulatory effects was seen on serial passage of virus-inoculated culture fluids, nor did passage fluids induce papilloma formation after intradermal inoculation in rabbits.

The growth-inhibitory effect was correlated with adsorption of infective virus by P-114 cells; 3 days after virus inoculation, P-114 cells were harvested, washed three times, and inoculated into rabbits for papilloma formation. Papillomas grew in two of four rabbits with an induction period of 27 days, as compared with induction periods of 18 to 21 days for positive control virus.

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COMPARISON OF FLUOROCHROMES FOR THE PREPARATION OF FLUORESCENT-ANTIBODY REAGENTS

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The four dyes used most frequently for production of immunofluorescent conjugates are fluorescein isothiocyanate (FITC), tetramethylrhodamine isothiocyanate (RITC), lissamine rhodamine B (RB-200), and 1-dimethylaminonaphthalene-5-sulfonic acid (DANS). In view of the widespread employment of immunofluorescent procedures for both diagnostic and research purposes, a comparison was undertaken of conjugates prepared from these dyes.

Rabbit antisera to *Corynebacterium diphtheriae* and enteropathogenic *Escherichia coli* O119:B14 were fractionated by addition of ammonium sulfate solution to a final concentration of 1.95 M. The crude γ-globulin fractions were adjusted to 1% protein, and portions were conjugated with FITC (Sylvana Co., Millburn, N.J.) according to the technique recommended by Lewis et al. (Appl. Microbiol. **12**:343, 1964), with RITC (BBL) as described by Smith, Carski, and Griffin (J. Bacteriol. **83**:1358, 1962), and with RB-200 (George T. Gurr Ltd., London, England) and DANS (Calbiochem) according to the directions of Chadwick and Fothergill (Fluorochromes and their conjugation with proteins, p. 21. *In* R. C. Nairn [ed.], *Fluorescent protein tracing*, E & S Livingstone Ltd., London, 1962). Immediately after conjugation, the reagents were freed from unreacted fluorescent material by elution from Sephadex G-25 columns (Pharmacia Ltd., Uppsala, Sweden) with 0.85% saline buffered at pH 7.4 with 0.01 M sodium phosphate. Equal

TABLE 1. *Comparison of fluorescent staining titers of rabbit antiglobulins conjugated with four fluorochromes*

Organism for production of antiglobulin	Organism tested for fluorescent staining	Fluorescent staining titer*			
		FITC	RB-200	RITC	DANS
Corynebacterium diphtheriae strains 5 and 7	*C. diphtheriae* strain 7	1:128	1:64	1:32	1:8
C. diphtheriae strains 5 and 7	*C. diphtheriae* strain 8	1:64	1:16	1:16	1:8
Escherichia coli O119:B14	*E. coli* O119:B14	1:512	1:32	1:16	1:8

* Conjugates for comparison of titer were produced from samples of the same antiserum fractions and were of equal final volumes. Abbreviations: FITC, fluorescein isothiocyanate; RB-200, lissamine rhodamine B; RITC, tetramethylrhodamine isothiocyanate; and DANS, 1-dimethylaminonapthalene-5-sulfonic acid.

volumes of each conjugate to be compared were collected from the columns.

Staining of antigens with the conjugates for *E. coli* was carried out as described by Thomason et al. (Bull. World Health Organ. **25**:137, 1961); the technique of Moody and Jones (J. Bacteriol. **86**:285, 1963) was employed for staining with conjugates for the diptheria bacillus. For microscopic observation of the stained antigens, an Osram HBO-200 light source was used in conjunction with a cardioid dark-ground condenser, a 3 mm BG-12 primary filter, and a 2 mm OG-1 secondary filter.

Results (Table 1) revealed that highest specific staining titers were produced by FITC conjugates, with DANS conjugates exhibiting the lowest titers. Smears from pure cultures of two diptheroids and smears of normal throat flora from six individuals exhibited no troublesome nonspecific staining with any conjugates for the diptheria bacillus. Likewise, all labeled antiglobulins for *E. coli* O119:B14 failed to stain the two heterologous *E. coli* serotypes tested.

Results of this study clearly showed the superiority of FITC for preparation of immunofluorescent conjugates used to stain bacterial smears. The technical conditions for conjugation with each reagent and the optical filters employed were selected on the basis of conditions and optics recommended in the literature. Improvement of conjugation methods and filter combinations for rhodamine and DANS may increase the effectiveness of these dyes.

METHOD TO FACILITATE THE ISOLATION OF *CLOSTRIDIUM BOTULINUM* TYPE E

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During the investigation of three outbreaks of type E botulism in the United States in 1963, difficulty was encountered in obtaining pure culture isolates of the causative organism from foodstuff in which type E toxin was demonstrated.

The spores of *Clostridium botulinum* type E (hereafter referred to as type E spores) are known to be almost as heat-sensitive as the vegetative cells of numerous contaminants, precluding the use of heat to reduce the extraneous microflora. Thus, the isolation of this species from toxic foodstuffs, grossly contaminated with facultative microorganisms, is difficult. Preliminary studies were made to determine whether the chemical resistance of type E spores could be employed to separate them from the contaminating vegetative microflora. A 50% concentration of ethanol was chosen, since it was found to be germicidal for vegetative cells, without adversely affecting type