Summary. A strain of psittacosis virus (TTF) developed resistance to 5-flourouracil (FU) after serial passages of the original FU-sensitive virus in tissue culture cells with small concentration of FU in the medium. The FU-resistant strain of TTF virus multiplied and produced more than 10^5 infective units per ml in presence of 2 μg FU/ml, at which level FU suppressed the original wild-type TTF virus completely. Reversion of character from FU-resistance to FU-sensitivity following serial passages in FU-free medium was not observed. This suggests that the acquisition of FU-resistance is due to certain gene mutation(s), rather than to enzymatic adaptation.

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Comparison of Assays for Mengovirus and Reovirus-3.* (26716)

RICHARD M. FRANKLIN (Introduced by Igor Tamm)
The Rockefeller Institute, New York City

Reoviruses were recently recognized as a group distinct from enteroviruses and other well known groups of animal viruses(1). In view of the susceptibility of weanling mice to these viruses(1), it appeared reasonable to determine whether reoviruses would grow and produce cytopathic effects in L-cells, a well defined cell line originally derived from mouse connective tissue. This paper describes the conditions for plaque assay of reovirus-3 and compares these conditions with those for the plaque assay of Mengovirus in L-cells, a virus which has been studied in this laboratory in recent years.

Materials and methods. L-cell, strain 929, originally obtained from Microbiological Associates, Inc., was cured of PPLO infection with kanamycin(2) and maintained free of PPLO by continued use of kanamycin in the cell stock cultures. The medium for stock cultures consisted of Eagle's minimum essential medium (MEM)(3) supplemented with 5% horse serum. Serum from a single selected horse was used, since sera from some horses are cytotoxic. The following antibiotics were used in the stock cultures: penicillin (500 units/ml), streptomycin (0.5 mg/ml), mycostatin (25 units/ml), and kanamycin (0.5 mg/ml). When stock cells were plated for any biological studies or plaque assays, the same nutrient medium was used, but only streptomycin and penicillin were present.

Mengovirus was obtained from Dr. M. Theiler as a frozen mouse brain suspension,

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stored since 1955. This virus was originally isolated by G. W. A. Dick in Uganda(4). Reovirus-3, strain Dearing, was obtained from Dr. H. J. Eggers as the 10th monkey kidney cell passage. This virus was isolated by M. Ramos-Alvarez in Cincinnati(5).

Plaque assays were performed as follows: Virus was diluted in MEM. Monolayers of L-cells grown in 60 mm plastic Petri plates (Falcon Plastic Co.) were washed once with MEM and 0.3 ml of an appropriate virus dilution was added. The virus was allowed to adsorb for an adequate time (see results) at 37°C in 5% CO_2. The inoculum was redistributed over the monolayer every 10 minutes. The agar overlay consisted of an enriched modification of MEM containing 5% horse serum(6). Cells were stained with neutral red agar after several days of incubation.

Results. Reovirus-3 was passaged in monolayers of L-cells, the very first passage resulting in a rapid cytopathic effect. Two more passages were made and then a plaque assay was attempted on monolayers of L-cells. This proved successful and therefore the conditions for optimal plaque formation were examined and compared with the conditions for Mengovirus plaque formation. Table I indicates an increase in plaque number from day 8, when some plaques are just visible, until day 14. No further increase was noted up to day 20. In contrast Mengo plaques are visible 2 or 3 days after inoculation and there is no increase in number of plaques after 4 days. The plaque size of uncloned reovirus stock ranges from 0.1-1.0 mm in diameter on day 9, whereas the plaque size of uncloned Mengovirus ranges from 1.0 to 8.0 mm in diameter on day 8. Large and small plaque variants have been isolated from Mengovirus and a cloned line of moderately uniform plaque size has been isolated from reovirus-3.

TABLE I. Variation of Reovirus Plaque Titer with Time of Incubation. Cells were exposed to 30-100 plaque forming units for 1 hr and overlaid with nutrient agar. After 7 days of incubation the cells were stained with neutral red agar and plaques were counted on subsequent days.

Day after inoc.	Avg PFU/ml
8	1.30×10^8
9	1.33×10^8
10	1.80×10^8
14	2.33×10^8

TABLE II. Comparison of Plaque Assay and Limiting Dilution Assay for Mengovirus and Reovirus-3.

	Plaque assay* (PFU/ml)	Limiting dilution assay† (infectious units/ml)
Mengovirus	1.02×10^8	1.96×10^8
Reovirus-3	1.89×10^8	2.77×10^8

* Plaque assays on L-cell monolayers. Mengovirus plaques were read on 4th day and reovirus plaques on 9th day.

† Mengovirus was assayed in 6-8 wk old mice by standard intracerebral inoculation. Reovirus was assayed in primary cultures of rhesus monkey kidney. LD_{50} or $TCID_{50}$ was determined by probit analysis and infectious units calculated by multiplication of the LD_{50} or $TCID_{50}$ by 0.69.

A second variation in plaque assays is the length of time allowed for adsorption of virus. This was studied by exposing monolayers to 50-100 PFU of virus in 0.3 ml MEM at 37°C in 5% CO_2 for varying periods of time, after which the inoculum was removed and the cells washed twice with MEM before addition of nutrient agar. The results shown in Fig. 1 indicate that maximum amount of

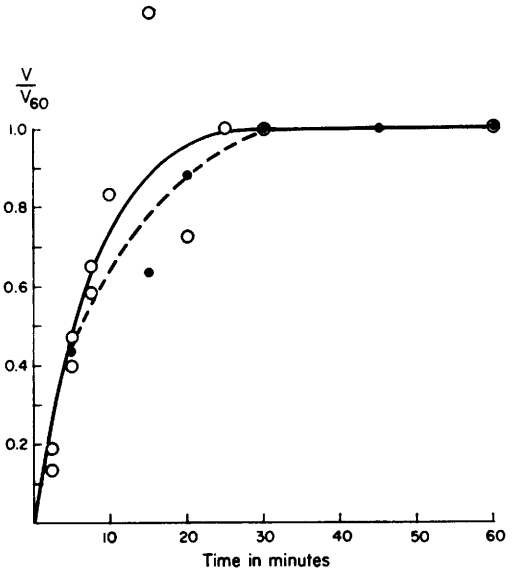

FIG. 1. Adsorption of Mengovirus (○) and reovirus-3 (●) to monolayers of L-cells at 37°C. The fraction of maximum amount of PFU adsorbed is plotted *vs* time. This fraction is calculated from ratio of PFU at time t to that at 60 min. (V/V_{60}).

each virus adsorbed in 30 minutes. This time has therefore been adopted in assays of both viruses.

The plaque assay of Mengovirus was compared with the assay in mice and the plaque assay of reovirus with the 50% infective endpoint titration in monkey kidney cultures. The plaque-forming units of both viruses are very similar in number to the infectious units in the ID_{50} assays. Reovirus plaques were read on the 9th day; corrected to the 14th day gives a titer of 3.3×10^8 PFU/ml. Thus it appears that plaque assays of both Mengovirus and reovirus-3 are as sensitive as ID_{50} assays and that repeated passage in L-cells does not result in loss of virulence for mice in the case of Mengovirus or for monkey kidney cells in the case of reovirus.

Summary. Reovirus-3 was passaged in L-cells where it grew and produced a cytopathic effect. A plaque assay in L-cells is described for this virus and compared with the plaque assay for Mengovirus.

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Relationships of Testicular Cholesterol to Hormonal Activity and Behavior in the Starling.* (26717)

FREDERICK K. HILTON† (Introduced by William B. Atkinson)
The Johns Hopkins School of Hygiene and Public Health, Baltimore, Md., and Department of Anatomy, University of Louisville School of Medicine, Louisville, Ky.

Androgenic hormones are known to influence very dramatically behavior associated with courtship and breeding in many birds (1). In the British starling Bullough showed that these behaviors were greatly intensified during the period of most rapid growth of the testes(2). Witschi and Miller found that the color of the bill in both sexes of the starling is controlled by testosterone (3). As the gonads increase in mass the bill changes from black to yellow, and as the testes decrease in weight the bill returns to the black condition.

Cholesterol has been shown to be an important precursor in the biogenesis of the steroids of adrenals and testes(4,5,6). Marshall and others have observed histochemically changes which occur in the cholesterol-positive lipoids of avian testes during the annual cycylic activity(7). The present study conducted on starlings correlates the cyclic changes in mass of the testes with quantitative changes in concentration of cholesterol present in the organ. An hypothesis is advanced concerning the relationship of cholesterol levels to steroid hormone production. Correlations between behavioral activity of the starlings and general histological condition of the testes are also reported.

Material and methods. Starlings from a known stage in their behavioral cycle were killed with a shotgun. They were autopsied immediately and the testes were removed, blotted and weighed on a Roller Smith balance, and frozen in glass vials imbedded in dry ice. Cholesterol content of the testes was determined by the method of Knobil, *et al.*(8) and reported as total cholesterol since the free and esterified forms were not sepa-

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† Present address, Univ. of Louisville, Ky.