

Pennsylvania from the uppermost Rose Hill shale (zone of *Mastigobolbina typus*) of upper Clinton age (11, p. 362). It is concluded that the upper Clough formation of Croydon Township is of C₅ to C₆ (= Upper Clinton) age, but that, pending further information regarding the lower range of *Porpites porpita* and the upper range of unspliced species of *Sticklandia*, it is not possible to arrive at a more definite conclusion regarding its age.

The Croydon Township occurrence of uppermost Lower Silurian strata containing marine fossils provides an eastern limit for the nonmarine sedimentation of Clinton age in eastern New York, plus adjacent parts of New Jersey and Pennsylvania. In New York the transition from marine to nonmarine strata of Clinton age takes place near Utica (10, pp. 339–340) and south-southward in Pennsylvania (12, Fig. 2) near the Delaware Water Gap. Therefore, the maximum width of the region formerly occupied by nonmarine, late Lower Silurian strata is about 140 miles.

Strata of Silurian age (13) occur near Bernardston, Mass., in what appears on a lithologic basis to be the same stratigraphic position as the Croydon occurrence. A stratigraphic sequence similar to that occurring in the Croydon area appears to extend south through central Massachusetts (14, plate X) and into Connecticut (15).

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Adaptation of Tissue Culture Cells to a Serum-Free Medium

Many attempts have been made to develop simplified tissue-culture media. The main difficulty encountered has been replacement of the serum component. Recent developments include media which contain various additives or serum fractions in addition to a basal constituent (1) and those directed toward a medium which is chemically defined (2). This paper reports the adaptation of a line of mouse lung cells to a serum-free medium.

The mouse lung cells used in this work were isolated from lung tissue of newborn Swiss mice (NIH strain). The usual trypsinization methods were used for isolation. Prior to adaptation, the cells were in their 110th passage on a medium consisting of 10 percent horse serum and 90 percent medium 199 (3). Penicillin and streptomycin were each added in a concentration of 50 units per milliliter. Morphologically, the cells appeared fibroblast-like, although small numbers of epithelioid cells were in evidence. These cells were routinely passed every 4 days by trypsanizing, washing, and inoculating (hemocytometer count) approximately 3×10^5 cells/ml in T-30 flasks, the total medium volume being 5 ml.

The medium which proved best for adaptation consisted of 99 percent medium 199, 1 percent Difco Bacto Peptone, 100 mg percent glucose, and the usual 50 units each of penicillin and streptomycin per milliliter. The cells passed on this medium were removed from the glass surface by scraping with a bent glass rod and were further separated by repeated pipetting. They were inoculated into T-30 flasks at a concentration of 6×10^5 cells per milliliter. If trypsin was used to remove the cells, no growth occurred on the serum-free medium. The fact that growth occurs on serum-containing media when this enzyme is used is probably due to the "detoxifying effect" of the serum on the trypsin carried over with the cells.

Growth of the first four passages on the Bacto Peptone medium was slow, requiring several weeks before the bottom of the flask was covered. Subsequent growth was more rapid, allowing transfers to be made every week. At the present time the cells are in their 27th serum-free passage and are being passed every 4 days at a concentration of 4×10^5 cells per milliliter. Primary-growth studies indicate that there is a three-fold increase in cells in 4 days. Aliquots of various passages passed on medium 199 plus 100 mg percent glucose without Bacto Peptone have failed to show a demonstrable increase in cell number and routinely do not survive more than three or four passages.

Attempts to adapt human liver and

HeLa cells to this type of medium have failed, but a line of cells isolated from guinea pig lung tissue has survived early passage and may adapt.

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Transmission of Pasteurella tularensis among Desert Rodents through Infective Carcasses

Although Ussov in 1937 (1) mentioned the "phenomenon of carnivorousism among rodents" as being important in the propagation of tularemia epizootics, a review of available literature reveals no supporting experimental evidence. However, Quan in 1954 (2) demonstrated oral transmission of *Pasteurella tularensis* to laboratory animals by feeding on infective flesh. Also, in a preliminary experiment conducted by one of us (E. D. V.), it was found that five species of desert rodents contracted tularemia by feeding on the infective flesh of native deer mice (3). The present study was conducted in an attempt to determine the extent to which desert rodents may feed on animal matter and the potential importance of ingestion of infective flesh as a means of transmission of tularemia among desert rodent populations (4).

Eleven species of rodents native to the Great Salt Lake Desert in Utah were used in this experiment (Table 1). The deer mice and grasshopper mice were laboratory-reared; the other species were live-trapped in the field and held in quarantine for a minimum of 30 days before use. During the course of the experiment, each animal was maintained in a separate cage.

The Schu A strain (5) of *P. tularensis* cultured in a modified casein partial hydrolyzate liquid medium (6) was selected as the infectious agent. The LD₁₀₀ for deer mice was determined to be 1 to 10 organisms.

Healthy deer mice inoculated intraperitoneally with approximately 1000 organisms were held until they were moribund or dead of tularemia. A moribund or dead deer mouse was then