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 C_5 to C_6 (= Upper Clinton) age, but that, pending further information regarding the lower range of *Porpites porpita* and the upper range of unplicated 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-southwestward 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 epitheliod 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 milliter. 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 carnivorism 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 anmials 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 LD_{100} 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

Table 1. Tularemia in rodents after ingestion of infective carcasses. All fed animals became infected.

Species	Common name	No. used	No. fed	Day of death or sacrifice
Onychomys leucogaster	Northern grasshopper mouse	16	16	3–4
Citellus leucurus	Antelope ground squirrel	16	15	3–4
Eutamias minimus	Least chipmunk	6	5	3–7
Reithrodontomys megalotis	Western harvest mouse	8	5	3–6
Peromyscus maniculatus	Deer mouse	16	13	3–5
Peromyscus crinitus	Canyon mouse	15	10	3–4
Peromyscus truei	Pinyon mouse	16	15	3–6
**Dipodomys microps*	Chisel-toothed kangaroo rat	16	15	3–6
**Dipodomys ordii*	Ord kangaroo rat	16	11	3–7
**Neotoma lepida*	Desert wood rat	16	10	4–7
**Perognathus parvus*	Great Basin pocket mouse	16	4	5–6

* Required starvation periods of 48 hours before carcasses were introduced.

placed with each of the experimental animals, from which food and water had been withheld for 24 or 48 hours. At the end of 24 hours the carcasses or remains were removed, and food and water were restored to the test animals.

When the exposed animals died, or when they exhibited acute symptoms of infection, they were autopsied, or killed and autopsied, and liver and spleen homogenates were cultured on glucose cysteine blood agar. If *P. tularensis* was not isolated by culture, 0.2-ml aliquots of the liver and spleen homogenates which had been kept frozen at −28°C were inoculated intraperitoneally into healthy deer mice in a further attempt to isolate the organism. Those animals that survived exposure to infective carcasses were held for 14 days, after which they were killed and autopsied. The tissues were examined for *P. tularensis* as outlined above. In all cases definitive identification of the isolated organisms was made by specific slide agglutination tests.

Under the experimental conditions described, the first seven species listed in Table 1 showed little reluctance to feed on deer mouse carcasses. Most of the ground squirrels and chipmunks readily consumed the entire carcass, while the other rodents generally ate only the anterior part of each carcass. The pocket mice, wood rats, and kangaroo rats were reluctant to eat the dead flesh unless regular food supplies were withheld for 48 hours.

In all cases, every rodent that ingested infective flesh contracted tularemia; those that did not ingest infective flesh did not contract the disease.

Although the extent to which wild rodents supplement their natural diet with flesh has not yet been determined, these results indicate that carnivorism among desert rodents may occur and that it may be of importance not only in the maintenance of this disease in nature, but also in the propagation of tularemia epizootics. The first seven species listed in Table 1 must be considered to be potentially capable of contributing to the maintenance and dissemination of tularemia among desert rodent populations. Because of their reluctance to feed on dead flesh, the other four species must be considered of somewhat less importance in the transmisison of this disease.

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Radio-frequency Resistive Impedance Pulsations over the Heart, Lungs, and Abdomen

The accurate timing of the major volumetric events in the atria, ventricles, lungs, and periphery of the human body may easily be accomplished without blood letting by surface study of the electrical resistive impedance changes around or over a given region of the human subject. Figure 1 illustrates the relative amplitude and direction of the pulsatile vertical impedance changes observed during held respiration over a 5 by 5 cm ventral surface area. These changes were studied in progressive areas in the left mid-clavicular line downward from the clavicle to the region below the umbilicus. Pertinent landmarks in the transition are identified together with the distance in centimeters below or above the sternal angle of our normal adult male subject Control electrocardiogram and deltoid-chest-deltoid tetrapolar electrical resistive impedance plethysmogram (*1–4*) serve to line up the corresponding tracings from each level. A decrease in electrical resistive impedance records upward in the trace and corresponds to an increase in volume pulsations. The paper speed is 50 mm/sec.

The study shows that with constant instrumental sensitivities the largest segmental impedance pulsations are found directly over the heart and apical regions 10 to 15 cm below the level of the sternal angle. The minimal impedance pulsations occur in the abdomen at the level of the umbilicus or lower.

An increasing volume pulse is present above the sternal angle and corresponds in its systolic upstroke with the control impedance systolic pulse of the upper torso body segment. These events begin about 0.11 to 0.12 second after the onset of the ventricular QRS of the electrocardiogram. These result from expansion of the great vessels, lungs, and chest segments with blood.

Just below the sternal angle level, we observe a transitional resistive impedance curve having a sigmoid-shaped downward deflection beginning 0.02 to 0.04 second after the onset of QRS in the electrocardiogram and 0.08 to 0.10 second before the control impedance pulse. This event is probably due to contraction of the atrial blood pool, which produces a decreased electrical conductance. A similar early change occurs at −10 and −15 cm below the sternal angle.

The initial ventricular emptying is best seen at −10 and −15 cm (reduced to 1/5 scale) in this case. Like the atrial tracing, it is also directed downward in the record. It is also sigmoid in shape and begins simultaneously with the positive pulse in the control tracing at 0.10 to 0.12 second after the onset of QRS deflection. The ventricular refilling at the −15-cm segment corresponds to the completion of the T wave of the electrocardiogram at 0.36 second after the onset of QRS deflection. A distinct notching or inflection occurs simultaneously with the onset of the U wave of the electrocardiogram in the record at 0.40 second. Since we did not take a simultaneous phonocardiogram, we cannot ascertain in this study whether it is signaled by dynamic events in the heart valves.

Figure 2 illustrates our observations, in the same subject, of resistive imped-