Table 1. Activity of γ-aminobutyric transaminase and semialdehyde dehydrogenase (in micromoles per hour per milligram of protein).

Medium*	Activity	
	Dehydrogenase†	Transaminase‡
Pyrrolidine	1.5	2.3
Aminobutyrate	1.3	1.7
Glucose	0.7	0.1
Glutamate	0	0

* Carbon source, 0.5 percent plus the following in grams per liter: K_2HPO_4, 1.5; NaH_2PO_4, 0.5; NH_4NO_3, 1; $MgSO_4 \cdot 7H_2O$, 0.2.
† Assayed by following the formation of DPNH spectrophotometrically (reaction 2) after the addition of cell-free extract to a system containing the following in micromoles per milliliter: potassium phosphate at *p*H 7.3, 100; DPN, 1; succinic semialdehyde, 0.2; mercaptoethanol, 5.
‡ Assayed by following the formation of DPNH spectrophotometrically (sum of reactions 1 and 2) after the addition of cell-free extract to a system containing an excess of the dehydrogenase and the following in micromoles per milliliter: potassium phosphate at *p*H 7.3, 100; DPN, 1; α-ketoglutarate, 1; γ-aminobutyrate, 1.

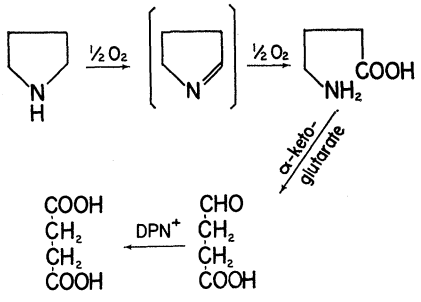
glutamic transaminase (reaction 1) and succinic semialdehyde dehydrogenase (reaction 2):

γ-Aminobutyrate + α-ketoglutarate ⇌ succinic semialdehyde + glutamate (1)

Succinic semialdehyde + TPN^+ ⟶ succinate + TPNH + H^+ (2)

The transaminase has been separated from the dehydrogenase activity and has been partially purified (85-fold) by protamine treatment, by ammonium sulfate and acetone precipitation, and by absorption and elution from calcium phosphate gel. The reaction appeared to be specific in that β-alanine, Δ-aminovaleric acid, or ornithine could not substitute for γ-aminobutyrate in reaction 1; pyruvate, oxalacetate, or α-ketovalerate could not substitute for α-ketoglutarate as amino group acceptor. The semialdehyde dehydrogenase (reaction 2) has been separated from the transaminase and has been partially purified (45-fold) by protamine and gel treatment and by ammonium sulfate and acetone precipitation. The reaction is specific for succinic semialdehyde. TPN is eight times as active as DPN at a concentration of 1 m*M* (*1*); malonic semialdehyde, glyoxalate, glycolaldehyde, and a variety of aliphatic and aromatic aldehydes were inactive. The reaction has not been experimentally reversed. The best preparations of these enzymes under the assay conditions outlined in Table 1 had specific activities of 4.5 and 3 μmole/min per milligram of protein for the transaminase and the dehydrogenase, respectively. A particulate preparation from brain had previously been found to catalyze a similar transamination (*2*). The description of a dehydrogenase from brain which catalyzes the oxidation of succinic semialdehyde is reported in an accompanying manuscript by Albers and Salvador (*3*).

That the formation of these enzymes depends on the carbon source employed in the culture medium is shown by the data presented in Table 1. Thus, in the absence of γ-aminobutyrate or pyrrolidine, little transaminase activity could be detected. Similarly, the dehydrogenase content is increased by growth on γ-aminobutyrate or pyrrolidine. Growth did not occur with 2-pyrrolidinone as the carbon source. The simultaneous induction (see *4*) of the transaminase and the dehydrogenase when growth occurs on pyrrolidine, as well as the apparently analogous primary degradation of proline (*5*) and hydroxyproline (*6*) suggests the following scheme for pyrrolidine catabolism:



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26 February 1958

Late Lower Silurian Fossils from Sillimanite Zone near Claremont, New Hampshire

J. B. Thompson has reported the discovery of fossils in the sillimanite zone of regional metamorphism in the extreme western part of Croydon Township, near Claremont, N.H. (*1*). To the best of our knowledge the Croydon occurrence of generically identifiable fossils in regionally metamorphosed rocks of the sillimanite zone is unique (for a summary of occurrences, see Bucher, *2*). The area has been mapped by C. A. Chapman, and the fossils are in rocks mapped by him as the upper part of the Clough formation (*3–5*). Detailed remapping by Thompson leads to the same stratigraphic interpretation as that arrived at earlier by Chapman but shows that the fossils are preserved in the nose of a major recumbent fold rather than in a homoclinal sequence as indicated by Chapman's geologic map (*4*, plate I).

The fossils are preserved as coarsely crystalline calcite in a matrix containing quartz, diopside, grossularite, and hornblende. Argillaceous rocks of the Littleton formation exposed about ¼ mile to the west contain almandite, staurolite, biotite, kyanite, and locally fibrous sillimanite. About ⅛ mile to the east, argillaceous rocks of the Littleton formation [mapped as Clough formation by Chapman (*4*, plate I)] contain almandite, biotite, and sillimanite. The sillimanite is in part in fibrous knots possibly pseudomorphous after kyanite. Some specimens contain staurolite, but it is less abundant than it is to the west. The fossil locality is thus interpreted as lying in the low-grade part of the sillimanite zone.

The fossils occur as a shell bed that has suffered a certain amount of current action, as is indicated by the disarticulated state of the brachiopods. The cardinalia of all the brachiopods are still well preserved, but fine external striae are barely discernible. Study of the fossils by Boucot shows the presence of the following: *Eospirifer* cf *E. radiatus, Plectambonites, Resserella* (= *Parmorthis*) cf *R. elegantula, Atrypa* cf *A. reticularis, Leptaena* cf *L. "rhomboidalis," Stricklandia* cf *S. lens ultima, Cyrtia* (?), unidentified rostrospiroid brachiopod, unidentified high-spired gastropod, *Porpites* (= *Paleocyclus*) cf *P. porpita* (*6*), unidentified zaphrentid tetracorals, *Heliolites,* and *Favosites.*

The presence of a smooth *Stricklandia* lacking outer plates suggests similarity to Williams' *Stricklandia lens ultima* (*7*, pp. 103–104). Williams records *S. lens ultima* from zones C_4 and C_5 of the Upper Llandovery in the Llandovery region (*7*, p. 129). The presence of *Porpites porpita* suggests a stratigraphic position near the top of the Upper Llandovery—that is, C_6—as is indicated by its occurrence in the lower Visby marl of Gotland (Hede, *8*) together with a plicated *Stricklandia* (*S. lirata*) and in 7_c of the Oslo region (*9*) together with *Stricklandia lirata. Porpites porpita* has not been recorded together with fossils of C_4 to C_5 age elsewhere, whereas unplicated or sparsely plicated species of *Stricklandia* do not occur elsewhere as high as C_6 or its equivalent. *Porpites porpita* is known in New York from the Upper Clinton Schroeppel shale (*10*, p. 350) and in

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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24 March 1958

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