TABLE 1. *Assimilation of hydrocarbons by yeasts*

Yeast	Substrate*									
	Decane	Dodecane	Tetradecane	Hexadecane	Octadecane	1-Decene	1-Dodecene	1-Tetradecene	1-Hexadecene	1-Octadecene
Candida lipolytica	+	+	+	+	+	+	+	+	+	+
C. pulcherrima	+	+	+	+	+	+	+	+	+	+
C. reukaufii	+	+	+	±	±	−	−	±	−	−
Cryptococcus laurentii	−	−	−	−	−	−	−	±	−	−
Debaryomyces kloeckeri	−	±	+	±	−	−	−	+	−	−
D. membranaefaciens	−	−	±	−	−	−	−	±	−	−
Hansenula anomala	−	−	+	−	−	−	−	+	−	−
H. saturnus	−	−	±	−	−	−	−	±	−	−
Rhodotorula glutinis	−	±	+	±	−	−	−	+	±	−
R. gracilis	+	+	+	+	+	−	+	+	+	±
R. mucilaginosa	−	±	+	±	±	−	±	+	±	−
Schizoblastosporion starkeyi-henricii	−	−	−	−	−	−	−	±	−	−
Trichosporon capitatum	−	−	+	−	−	−	−	+	−	−

* Symbols: + = growth; − = no growth; ± = questionable growth.

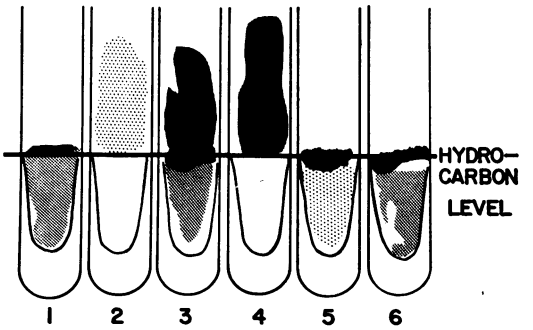

FIG. 1. *Growth of yeasts on hydrocarbons in Wickerham nitrogen base medium solidified with purified agar. Tube 1, Rhodotorula glutinis (tetra decane); tube 2, R. glutinis (tetradecene-1); tube 3, R. gracilis (tetradecane); tube 4, R. gracilis (tetradecene-1); tube 5, R. mucilaginosa (tetradecane); tube 6, R. mucilaginosa (tetradecene-1).*

211, Cambridge University Press, New York, 1962). Perhaps the major taxonomic significance of yeast alkane and alkene assimilation lies in the recognition that both paraffin and olefin oxidations are mediated by some type of oxygenase system (Stewart et al., J. Bacteriol. **78**:441, 1959; Ishikura and Foster, Nature **192**:4805, 1961; Hayashi, *Oxygenases*, Academic Press, Inc., New York, 1962); growth of yeasts on hydrocarbons, therefore, may be taken as evidence for these enzymes in such yeasts.

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BEHAVIOR OF COXSACKIE A6 VIRUS IN MURINE CELLS: FAILURE OF NEWER TECHNIQUES TO DETECT MULTIPLICATION IN VITRO

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The six "herpanginal" serotypes of group A Coxsackie viruses, i.e., 2, 4, 5, 6, 8, and 10, have to date consistently escaped facile in vitro detection in cell cultural systems, although each serotype can be recovered by inoculation of suckling mice. Only laboratory strains of types 8 and 10 have produced cytopathic effects in human amnion cell cultures (Lenahan and Wenner, Proc. Soc. Exptl. Biol. Med. **107**:544, 1961; Dunnebacke and Mattern, Proc. Soc. Exptl. Biol. Med. **105**:553, 1961); how wild strains of these two serotypes behave in amnion cells is not known. Types 2 and 4 have undergone in vitro replication in mouse and chick tissue cultures, but the nonmonolayer nature of the cultural systems did not permit microscopic examination for cytopathological changes (Fujita et al., Biken's J. **2**: 295, 1959; Shaw, Proc. Soc. Exptl. Biol. Med. **79**:718, 1952; Slater and Syverton, Proc. Soc. Exptl. Biol. Med. **74**:509, 1950).

Extrapolation from in vivo suckling mouse pathogenicity to in vitro susceptibility of cultured cells from this host would seem reasonable for Coxsackie viruses and, indeed, has been observed for a group B type 1 strain which, concurrent with multiplication, induced a cytopathic change in fibroblastic cell cultures of suckling mouse adipose tissue and skeletal muscle (Stulberg et al., Proc. Soc. Exptl. Biol. Med. **81**:642, 1952; J. Immunol. **72**:107, 1954). Although search for in vitro murine cell cytotropism with group A Coxsackie viruses has been discouraging (Lenahan and Wenner, 1961; Stulberg et al., 1954), further experiments seemed justified in an attempt to detect either cytopathic effects, subtle cytolysis as evidenced by altered rates of host cell replication (Dubbs and Scherer, Federation Proc. **19**:386, 1960) or (by in vivo assay of inoculated cell cultures or virus interference, or both) evidence for multiplication of a group A virus serotype in primary embryonic and infant mouse renal cells and established murine cell cultures.

In the following experiments, Coxsackie A6 virus, strain Israel, was prepared as a 10% suspension of infected mouse torso, and was used at dilutions appropriate to circumvent nonspecific cell toxicity. Virus assays were carried out in litters of 1-day-old white Swiss mice by intraperitoneal inoculation of 0.05-ml volumes of test fluid, and calculation of the suckling mouse (SM) LD_{50}. The maintenance medium for murine cells consisted of Hanks' balanced salt solution supplemented with 10% inactivated horse serum and either 0.1% yeast extract or Eagle's amino acid and vitamin mixture. The incubation temperature was 37 C.

An estimated multiplicity of 0.1 SM LD_{50} of Coxsackie A6 virus per cell was inoculated in 0.1-ml volumes onto replicate monolayers of primary embryonic mouse cells grown in slanted culture tubes. Cytopathic effect was not observed, and supernatant fluids and cells harvested daily for 5 consecutive days revealed no evidence of replication of mouse pathogenic virus upon subsequent assay in vivo. Challenge of cultures on the fifth day with 100 $TCID_{50}$ per 0.1 ml of herpes simplex virus failed to demonstrate the presence of noncytopathic interfering Coxsackie A6 virus; such an approach was very successful in the detection of rubella virus (Parkman et al., Proc. Soc. Exptl. Biol. Med. **111**:225, 1962).

An essentially identical in vitro experiment was carried out in primary renal cells obtained from 24-hr-old white Swiss mice. A search for cytopathology or replication, or both, by in vivo assay of culture fluids and cells and the interference phenomenon also yielded negative results.

The growth rate, in replicate slanted-tube cultures, of established strain L cells exposed to Coxsackie A6 virus at a multiplicity of 1.0 was compared with appropriate control cultures for a 21-day interval. During this period, neither cytopathic effect nor subtle cytolysis was detected; growth curves of cells from both experimental and control cultures were essentially identical. In vivo assay of supernatant fluid harvested on the eighth and fourteenth days of the experiment revealed no evidence for replication of virus.

Thus, although capable of lethal multiplication in the intact mouse host, this strain of Coxsackie A6 virus failed to multiply in either primary or established murine cell cultures. This observation may be explained by the alteration of virus receptor sites or the disruption of virus-synthesizing mechanisms upon in vitro cultivation of murine cells. Data to support these postulates are neither available nor likely to accrue for mouse-locked "herpanginal" serotypes without a more precise method for experimental assay than suckling mouse lethality.

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