Summary. The lipase activity in the liver of the embryonic chick from 8 to 19 days of incubation has been determined manometrically. Enzyme activity was found to increase gradually from the 8th to the 14th day then to shoot up suddenly, reaching the peak on the 16th day. This rise in activity has been correlated with the extent of fat metabolism in the embryo during that period.

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Isolation, Growth Requirements, and Pathogenicity for Rabbits of *Leptotrichia dentium.** (27349)

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The genus *Leptotrichia* was ill defined(1), hence it was omitted from Bergey's Manual of *Determinative Bacteriology*(2). The current literature(3-10) contains information sufficient perhaps to merit its recognition. Davis and Baird-Parker(6) suggested that 2 species be recognized in the genus, *L. buccalis* and *L. dentium*. The biochemical and serological reactions of *L. dentium* have been investigated(8). The studies reported here deal with isolation, growth requirements and pathogenicity for rabbits of the same species.

Materials and methods. Cultures. Six *L. dentium* isolates (L-E, L-1, L-13, L-16, L-17, and L-26) were obtained through the courtesy of Dr. Louis R. Sibal, Univ. of Illinois College of Medicine. Additional cultures were isolated in this laboratory from *materia alba* from dental patients. All cultures were maintained on Difco Brain Liver Heart (BLH) agar slants as recommended (8) and in 1 ml ampules containing Brain Heart Infusion (BHI) broth cultures stored at −70°C.

Isolation. BLH agar medium, prepared to contain 1.5% agar and 7 μg/ml polymyxin B (Chas. Pfizer & Co., Lot No. OY139), was poured into plates. The control medium lacked Polymyxin. Each plate received a single drop from a 5-ml pipette of a 1:100 dilution of saline suspension of *materia alba* homogenized for 1 minute in a Teflon tissue grinder. The inoculum was spread with a bent glass rod. Plates were incubated aerobically for 4 days at 37°C. Growth was compared and colonies showing scalloped edge (7) were counted with the aid of a stereomicroscope, and were subcultured for further identification. A total of 12 periodontal patients contributed specimens for this portion of the study.

Growth requirements. A basal medium composed of 12 g Difco vitamin-free Casa-

* Supported by U.S.P.H.S.

mino acids, .1 g cystine, .2 g DL tryptophane, 3 g glucose, and 1000 ml demineralized water was prepared up to 10 times the final concentration, dispensed into 16 × 150 mm test tubes in 1 ml aliquots and sterilized by autoclaving at 120°C for 12 minutes. Appropriate amounts of solutions of samples to be tested were added to the tube, and the final volume brought up to 10 ml by addition of sterile .02 M phosphate buffer (pH 7). The inoculum consisted of cells harvested from 48-hour BHI broth cultures, washed 3 times with the buffer solution by repeated centrifugation, homogenized in a Teflon tissue grinder for one minute, and adjusted to 80 ± 3% transmission at 540 mμ with a Coleman Junior spectrophotometer. Tubes of basal medium were inoculated each with a single drop of the cell suspension, and incubated as before. Assay of growth was at best empirical. Growth in the basal medium was compared with that in BHI broth. Growth equivalent to the optimal was recorded as 4+, increasingly lighter growth as 3+, 2+, +, ±, and no growth as 0.

Pathogenicity. Thirty-one rabbits (1.6-2.1 kg), housed in individual cages, were injected with cultures of L-E, L-1, and L-26. Forty-eight hour BHI broth cultures were homogenized as before, and 1 ml portions each of viable cells, heat killed (80°C) cells, cell-free Seitz-Filtrates, and sterile BHI broth were employed for intravenous (marginal ear vein), and subcutaneous (shaved skin of back) inoculations. The number of cells in these suspensions could not be determined by direct counts or by plating out technics, but was estimated turbidimetrically(11) to be equivalent to 1.5×10^8 cells per ml. All animals were observed daily for 28 days.

Results and discussion. Isolation of Leptotrichia from dental specimens is difficult because many other organisms are present in these specimens. Attempts to improve isolation by supplementing media with crystal violet, brilliant green, sulfathiazole, and bacitracin were not successful(10). Howell and Rogosa(4) obtained 42 isolates from a total of 270 specimens (<16%) by plating out on Beef Extract agar, containing soluble starch, basic fuchsin, and Sheep blood. In this study, *L. dentium* was isolated from 5 out of 12 specimens (>40%) by plating out on BLH agar containing 7 μg/ml polymyxin. Polymyxin in this concentration permitted growth of *L. dentium* and was inhibitory for some of the other organisms. However, diphtheroid forms also grew and produced scalloped edge colonies closely resembling those produced by *L. dentium.* Differentiation of the 2 organisms was accomplished by a study of cellular morphology. Further attempts to improve isolation by addition of various concentrations of Gantrisin (Hoffmann-La Roche, Inc., Lot 104) and crystal violets were not successful.

TABLE I. Growth of 6 Strains of *Leptotrichia dentium* in Various Media.

Medium	Growth of strain					
	L-E	L-1	L-13	L-15	L-17	L-26
BHI broth	4+	4+	3+	3+	3+	4+
Defined medium	—	—	—	—	—	—
+.1% starch	—	—	—	—	—	—
+.1% agar	—	—	—	—	—	—
+.1% rabbit serum	±	±	±	±	±	±
+.1% Na thioglycollate	—	—	—	—	—	—
+.3% yeast extract	4+	4+	4+	4+	4+	4+

Some of the results on the growth requirements are summarized in Table I. The basal medium failed to support growth of any of the isolates even from larger inocula. Supplementation of the medium with 0.1% agar, starch, normal rabbit serum, or sodium thioglycollate failed to stimulate growth, whereas addition of yeast extract made growth possible. Yeast extract was replaceable by a solution of 9 vitamins(12). Of these, riboflavin and nicotinic acid were essential since no growth was obtained when either was omitted. Omission of thiamin, calcium pantothenate and biotin greatly reduced growth. The remaining 4 vitamins were dispensable and strains were maintained in the medium made deficient in these vitamins. Omission of glucose slightly reduced growth. Purines and pyrimidines were not required. No difference in results could be detected when the Casamino acids were replaced by some 20 (12) purified amino acids.

A massive dose of approximately 1.5×10^8 viable cells of Leptotrichia was injected intravenously into each of 18 rabbits. All survived for 28 days. *L. dentium* was isolated from the blood of these animals subcultured one hour post-inoculation. Consistently negative cultures were obtained from samples taken 2 hours post-inoculation. Six rabbits which received a similar dose by the subcutaneous route also survived, but developed an ulcerative lesion approximately 14 mm in diameter. The lesions were slightly raised, erythematous, peripherally fluctuant and centrally indurated. Materials expressed from these were whitish and odorless. Subcultures on various media from this material were negative. The lesions appeared within 24 hours post-inoculation and healed in approximately 2 weeks.

Summary. 1. *Leptotrichia dentium* organisms have been isolated from *materia alba* by plating out on Brain Liver Heart agar containing 7 μg/ml polymyxin. The efficacy of this medium resides in its content of polymyxin which permits growth of *L. dentium* and inhibits growth of some of the other forms commonly present in such specimens. 2. A defined medium is described which permits optimal growth of *L. dentium*. The medium is composed of Casamino acids, tryptophane, cystine, glucose and 5 vitamins. Of the vitamins, riboflavin and nicotinic acid are essential, whereas calcium pantothenate, biotin, and nicotinic acid are stimulatory. 3. *L. dentium* appeared to be non-pathogenic for rabbits. Animals given intravenously a massive dose of viable cells (1.5×10^8) survived for 28 days. Animals given equivalent dose by the subcutaneous route also survived but developed a small ulcerative lesion at site of inoculation which healed in approximately 2 weeks.

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Transmission of Mouse Leukemia Virus Through Milk of Virus-Injected C3H Female Mice.* (27350)

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We reported(1) experiments in which C3H mice that had been inoculated with a highly potent leukemic virus (passage A) transmitted this virus to their offspring. All non-treated offspring born to, and raised by, virus-injected mothers developed leukemia, and the disease appeared after a relatively short latency of 4½ to 8 months. It was immaterial whether the father was inoculated, provided that the mother received inoculation of

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