Elevating the dose of aspirin to 20 and 40 mg/kg resulted only in increasing the variability of the urinary data. Electrolyte excretory patterns were little altered from control or were such to imply renal changes compensatory to possible respiratory stimulation.

Summary and conclusions. Aspirin (10 mg/kg) administered *per os* to influenza-infected and non-infected mice reduced urinary excretion of Na^+, K^+ and water. This evidence indicates that aspirin is not adrenal corticoid in nature. In the infected animal aspirin also decreased the thiocyanate space which thus precludes a direct renal action for the urinary changes observed. Evidently aspirin activates mechanisms favoring expansion of intracellular fluid volume at the expense of extracellular fluid.

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Lipase Activity in the Chick Liver during Development. (27348)

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Fat is considered to be the predominant energy source in avian development. About 60% of the total fatty acids present initially in the egg is oxidized from which the embryo derives over 90% of its caloric requirements (1). Though much work has been done on enzymes during development, either in the whole chick embryo or in the different organs of the embryo, relatively little quantitative information is available on the day-to-day fluctuations in concentrations of the enzyme, lipase, during development. Gavialo (2,4) showed that lipase is present on the first day of development of the chick embryo. Gavialo and Goryukina(3,4) later observed increasing lipase activity in the liver about the 15th day of incubation. Recently George and Iype(5) demonstrated histochemically the occurrence of lipase in the heart of the early chick embryo (40 hr). They also assessed quantitatively the changes in activity of the enzyme in this organ during development and showed that increase of lipase activity paralleled increase in heart rate. This lends further support to the view of Moog(6) that "the differentiation of the enzyme is directly related to the differentiation of function."

Liver is also known to be active during development. Bile secretion is known to appear about the 6th day of incubation(7). Needham(4) reviewing the older literature pointed out that fat metabolism is highest during the last week of incubation. We undertook the present study to find out precisely whether there is a direct relationship between this period of the chick embryo and the lipase activity in the liver.

Materials and methods. White Leghorn eggs of uniform size were used. They were incubated at 37.8 $\pm$ 0.01°C. After the desired period of incubation, the embryos were carefully removed from the yolk. The liver of embryos from 8th day to 19th day of incubation was used for quantitative determination of lipase activity. Up to the 10th day

TABLE I. Lipase Activity in Chick Liver during Development.

Age (days)	Lipase activity* (μl CO_2/mg protein/hr)	Age (days)	Lipase activity* (μl CO_2/mg protein/hr)
8	295.4 ± 36.5†	14	646.0 ± 140.7†
9	378.4 ± 53.8	15	1156.0 ± 180.5
10	562.0 ± 47.4	16	1311.0 ± 186.6
11	574.2 ± 115.6	17	1035.0 ± 72.6
12	630.7 ± 134.8	18	974.0 ± 146.9
13	611.0 ± 109.0	19	1057.0 ± 100.6

* Mean values of 4 to 5 sets of experiments using as many times pooled material or different liver samples when not pooled.
† Mean ± S.D.

of incubation, the tissue from 2 to 4 embryos was pooled for each experiment. Both lobes of the liver were used after carefully removing the gall bladder, since it was observed that gall bladder bile activates the liver lipase of the developing chick. For experiments on embryos after the 10th day of incubation, a liver sample was taken from the left ventral lobe of one embryo. In all cases the excised liver was kept on a piece of filter paper to remove the blood. The tissue after having been quickly weighed was homogenized in distilled water in the cold and diluted with cold distilled water so that each ml of homogenate contained 1.5 to 3 mg wet weight of tissue. The lipolytic activity of the homogenate was determined manometrically in a bicarbonate carbon dioxide buffer system of pH 7.4 at 37°C using the Warburg apparatus (8). The manometers were shaken at 115-120 oscillations per minute with an amplitude of 5 cm. Enzyme activity is expressed as μl CO_2/mg protein/hr. Protein was estimated according to the micro-Kjeldahl steam distillation method(9).

Results and discussion. (Table I). Our results show that lipase activity in the liver increases gradually and steadily up to the 14th day of incubation. The activity of the enzyme then rises suddenly, reaching the peak on the 16th day, and maintains a more or less steady level which is slightly lower than that of the 16th day but considerably higher than that of the 14th day. Since lipase activity is computed per mg protein, changes in protein content of the tissue during development are likely to give a deceptive picture, because, if there is an increase in protein, there may or may not be a simultaneous increase in the enzyme under investigation. There is a nearly 2-fold increase in total protein per g wet weight of the liver at about 14-16 days of incubation(10). Davidson and Leslie(11) have shown that there is a similar 2-fold increase of protein nitrogen per unit weight of desoxyribonucleic acid phosphorus in the liver of the chick embryo at about 13 days of incubation. In spite of the considerable protein increase, the liver lipase activity shows an increase during this period and not a decrease, which means that the increase in enzyme activity is definitely significant.

Romanoff(12) showed that the fat content of the chick embryo increases while the yolk fat decreases at about the 14th day of incubation. Trotti(13,4) observed that infiltration of the liver with neutral fat begins about the 15th day. Lipase activity was shown to increase in the liver at this time (3,4) and in the whole embryo(14,4). The growth curve of the triglyceride fatty acids obtained by Cahn(15,4) for the chick embryo showed a peak at 13-15 days of incubation and a decline in production of fatty acids thereafter. Entenmann *et al.*(16,4) found that the chick hatches with a fatty liver but becomes normal after hatching. Our present study has definitely established that liver lipase reaches the peak of activity on the 16th day of incubation and maintains a steady level for the rest of the embryonic life. It is interesting that while liver fat and lipase activity are both at a high level about the 16th day, in the yolk the fat level is low but lipase is at the maximum(4). These observations lead to the inference that while lipase, known for its reversible action, is actively breaking up fat into fatty acids in the yolk sac, the lipase in the liver is building up the liver fat from fatty acids. It may also be mentioned that the level of blood cholesterol in the chick embryo was shown to be maximum (double the adult value) on the 18th day of incubation, but normal at hatching (15). Feeding of cholesterol is known to induce the deposition of abnormal amounts of fat in the liver(17).

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-

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