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Studies of Listerellosis. VI. Isolation of *Listerella monocytogenes* from Liver of Pig.

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In 15 natural outbreaks of ovine and bovine listerellosis in Illinois¹ including 7 in sheep and 8 in cattle, characterized by encephalitic syndrome, *Listerella monocytogenes* was not encountered in the tissues of affected animals other than the central nervous system. In one instance *Listerella monocytogenes* was isolated from the spleen of a heavily parasitized chicken² showing clinical symptoms of coryza. A heavy mortality had occurred in this flock but the primary nature of the disease as well as the cause of the loss was not established. In another instance, *Listerella monocytogenes* was isolated from the stomach contents of an aborted bovine foetus.³ This strain apparently induced abortion following intravenous injection of an experimental pregnant heifer.⁴ The present report concerns the isolation of *Listerella monocytogenes* from the liver of a pig (specimen G89876). This animal did not display clinical symptoms indicative of a central nervous system involvement and represents the third occasion in this laboratory in which *Listerella* has been isolated from tissue other than the central nervous system.

The pig yielding *Listerella monocytogenes* was one of a litter raised on a stock farm in central Illinois and at the time of examination weighed approximately 30 pounds. According to the owner, this animal had exhibited symptoms of intermittent diarrhea and failed to gain in weight as compared to litter mates. On arrival at the laboratory the animal appeared listless and dull but history and clinical symp-

toms failed to suggest the nature of the illness. All other animals in the herd were apparently healthy.

Postmortem examination of the pig failed to reveal gross pathological changes other than a small number of grayish foci of match-head size on the surface of the liver. The character of these foci was not determined. The heart blood, liver, and spleen were cultured on blood agar and MacConkey's agar plates. Following 48 hours incubation at 37°C, the liver blood agar plate cultures yielded pure and abundant growth of a Gram positive rod, possessing biochemical characters indistinguishable from *Listerella*. Fermentation reactions in differential sugars including dextrose, lactose, maltose, sucrose, arabinose, rhamnose, xylose, galactose, levulose, trehalose, raffinose, inulin, dulcitol, mannitol, sorbitol, inositol, salicin, and changes in litmus milk were similar to those of 2 *Listerella* strains, isolated from the central nervous system of naturally affected sheep.

Unilateral supraconjunctival inoculation of the culture into three guinea pigs produced purulent conjunctivitis and keratitis within 4 days. One of 2 rabbits exposed in a similar manner proved resistant. *Listerella monocytogenes* was isolated from the conjunctiva of the 3 experimentally exposed guinea pigs. Agglutination of a suspension of the *Listerella monocytogenes* isolated from the pig liver (G89876) occurred in a dilution of 1:50 in tube antigen and in approximately 1:200 in the rapid plate test using a *Listerella* immune equine serum. There was no agglutination with normal bovine serums in 1:12.5 in the tube and 1:25 on the plate.

Evidence suggesting the pathogenesis of *Listerella monocytogenes* for swine was previously noted following experimental exposure of healthy pigs but the natural disease accompanied by encephalitic symptoms had not been observed in swine in Illinois. However, Biester

¹ Graham, Robert, Levine, N. D., and Morrill, C. C., *Bull.* 499, University of Illinois Agricultural Experiment Station.

² Hurt, H. R., Levine, N. D., and Graham, Robert, *Am. J. Vet. Res.*, 1941, **2**, 279.

³ Graham, Robert, Hester, H. R., and Levine, N. D., *Science*, 1939, **90**, 336.

⁴ Graham, Robert, Hester, H. R., and Levine, N. D., *J. Infect. Dis.* 1940A, **66**, 91.

and Schwarte⁵ reported natural outbreaks of *Listerellosis* in swine and the isolation of *Listerella monocytogenes* from the central nervous system of naturally affected pigs showing clinical symptoms of a central nervous system disorder including incoordination,

⁵ Biester, H. E., and Schwarte, L. H., *Am. J. Vet. Med. Assn.*, 1940, **92**, 339.

stilted gait, trembling, and paralysis of posterior extremities.

Summary. Isolation of *Listerella monocytogenes* from the liver of a pig unaccompanied by encephalitic symptoms is described. The presence of *Listerella monocytogenes* in the liver raises the question of its pathologic significance in non-encephalitic syndromes swine.

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Biological Activity of Synthetic d,l-Desthiobiotin.

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Optically active desthiobiotin was first obtained by du Vigneaud, Folkers and coworkers^{1,2} as a degradation product of natural biotin. The Cornell group made the interesting observations that desthiobiotin is equally as effective as biotin in stimulating the growth of *Saccharomyces cerevisiae*,² but has an anti-biotin effect for *Lactobacillus casei*. They further provided evidence³ to show that the growth effect on the yeast is probably due to the conversion of desthiobiotin to biotin by the yeast cell. Lilly and Leonian⁴ and Stokes and Gunness⁵ have demonstrated the ability of desthiobiotin to replace biotin in the nutrition of various microorganisms.

Biological data on the activity of desthiobiotin for rats have, however, been lacking. In a recent report on the biological activity of synthetic biotin and several intermediates, Emerson⁶ remarked that "desthiobiotin shows a low order of activity in biotin-depleted rats.

However, the response was irregular and the slight activity of this compound may have been due to the presence of traces of biotin, since the compound was prepared from biotin." Since synthetic d,l-desthiobiotin⁷ known to be free of biotin, has been available to us for some time, we have examined its activity for microorganisms and biotin-deficient rats in some detail.*

Experimental. The various samples of d,l-desthiobiotin tested melted at 163-164°C (corr.). The substance was dissolved for the assays in one of two ways: (1) When dilute solutions were required, as for the microbiological experiments or for some of the bioassays, it was dissolved in water, in which the solubility is about 0.1%, giving a solution of pH 3.5; (2) when more concentrated solutions were needed, as in bioassay dosage at higher levels, the compound was dissolved in somewhat less than the stoichiometric amount of sodium hydroxide solution to give a pH of 6-7.

Microbiological assays. The effect of d,l-desthiobiotin on the growth of *Saccharomyces cerevisiae* No. 139 was examined with 2 different media, that of Snell, Eakin and

¹ du Vigneaud, V., Melville, D. B., Folkers, K., Wolf, D. E., Mozingo, R., Keresztesy, J. C., and Harris, S. A., *J. Biol. Chem.*, 1942, **146**, 475.

² Melville, D. B., Dittmer, K., Brown, G. W., and du Vigneaud, V., *Science*, 1943, **98**, 497.

³ Dittmer, K., Melville, D. B., and du Vigneaud, V., *Science*, 1944, **99**, 203.

⁴ Lilly, V. G., and Leonian, L. H., *Science*, 1944, **99**, 205.

⁵ Stokes, J. L., and Gunness, M., *J. Biol. Chem.*, 1945, **157**, 121.

⁶ Emerson, G. A., *J. Biol. Chem.*, 1945, **157**, 127.

⁷ Duschinsky, R., and Dolan, L. A., to be published.

* The samples of d,l-desthiobiotin were made available to us by Dr. R. Duschinsky.

⁸ Snell, E. E., Eakin, R. E., and Williams, R. J., *J. Am. Chem. Soc.*, 1940, **62**, 175.