Comparison of Virulence and Activity of some Enzymes of *Listeria monocytogenes*

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Received May 11, 1962

**ABSTRACT**

The virulence of microorganisms and the activity of some enzymes were compared on 17 strains of *Listeria monocytogenes*, including 5 Patterson's strains, 4 strains maintained for longer periods on nutrient media and 8 strains freshly or recently isolated from animals in our field material.

The LD₅₀ and invasivity index were determined as indicators of virulence. The activity of catalase, glucose and lactic acid dehydrogenases and glutamic-oxaloacetic transaminase were determined quantitatively. Close agreement was found between the values of both indicators of virulence and the activity of catalase. The quantitative assay of catalase and to some extent of some dehydrogenases, as well as glutamic-oxaloacetic transaminase thus represents a simple and satisfactory method of assessing the virulence of *Listeria monocytogenes*.

It is known that the occurrence of clinical listeriosis in domestic animals and in man is only sporadic and predominates in young individuals. In addition to other mechanisms the relative virulence of the microorganisms is of importance. In investigating listeriosis of animals we were therefore concerned with the relative virulence of the *Listeria* strains isolated. In view of the high requirements posed by the determination of the virulence factors or components we set out to establish whether in Listeria there is a relationship between virulence and the activity of some easily assayed enzymes, in particular of catalase, glutamic-oxaloacetic transaminase and some dehydrogenases. In some microorganisms catalase has been found to be a satisfactory indicator of virulence, e.g. in *Mycobacterium tuberculosis* and *Brucella abortus* (Schierholz & Jeder, 1957; Vischer & Tirunarayan, 1957). According to Rolle and Meyer (1958) the LD₅₀ of Listerias for white mice is 10⁷ germs on i.v. injection. Patočka and Schindler (1958) determined the LD₅₀ for young rats and found that females are considerably more susceptible than males. Pregnant animals are also more susceptible. On passage through animals a considerable increase in the virulence of listerias can be attained. For an infection to succeed the method of introduction is of importance in addition to the number of microorganisms involved (Seeliger, 1958).

**MATERIALS AND METHODS**

*Cultures.* Seventeen strains of *Listeria monocytogenes* were used, among them 5 standard Patterson's strains, 1 strain from the Microbiological Institute of the Medical Faculty of Charles University, isolated from man. The remaining strains were isolated from sheep in the Bacteriological Department of the State Veterinary Institute in the years 1955—1959. The cultures were maintained on Albimi agar with a buffer and 0.5% glucose and regularly transferred. Finally, three strains...
were freshly isolated from sheep in the course of the experiment. Each strain was passaged once through a mouse before using it for the experiment, with the exception of the directly isolated strains. They were all in the S form. White mouse males used for the experiments formed a homogeneous set weighing 14—16 g. each.

**Determination of LD₅₀.** Listerias were isolated from an infected mouse 48 hrs. after injection, on glucose agar with a buffer, for 20 hrs. at 37°C. Peptone water of the following composition was used for rinsing and dilution: Bacto-Peptone Difco 2.0 g.; NaCl 0.5 g.; distilled water added to 100 ml., pH adjusted to 7.4 and a phosphate buffer added. In contrast to the physiological saline in which about 50% listerias were found to perish within 60 min., peptone water brought about practically no decrease in viability. The density of the basic suspension was set optically to E = 0.60—0.75 (Pulfrich photometer, 1 cm. cuvette, filter S-66). One ml. of this suspension contained 3.48 × 10⁸ ± 20% microorganisms. For each dilution sets of 4 mice were used. At the same time, the number of germs for the individual dilutions was calculated by the plate method (after 48 hrs. of incubation). The mice were inoculated with 0.5 ml. i.p. and observed for 7 days after infection. The results were evaluated according to Reed-Muench.

**Determination of invasivity.** In determining the invasivity the standard suspension (prepared as for the LD₅₀ estimations) was diluted 10⁴ to 10⁸ times and 0.2 ml. doses of these dilutions were injected s.c. to sets of 4 mice. After 18 and 24 hrs. the mice were killed. The spleen was aseptically removed, weighed and homogenized in a mortar with 0.2 g. marine sand and 2.5 ml. glucose broth. After filtration (about 3 min.) 1 ml. spleen suspension in broth was diluted with broth 10⁴ to 10⁸ times. The plate method was used for calculating the number of growing colonies (after 48 hrs. of incubation) from concentrated and dilute suspensions and the average number of colonies was referred to 1 g. of spleen. The ratio between the number of germs grown from 1 g. spleen after 18 and 24 hrs. and the total number of inoculated germs was used as a measure of invasivity. The increase or decrease in the number of microorganisms in the spleen between the 18th and 24th hours was recorded (the number of microorganisms in the inoculum is thus cancelled).

**Determination of catalase.** Catalase was determined manometrically. Five ml. of listeria suspension were mixed with 10 ml. of hydrogen peroxide solution, three concentrations of which were used: 3%, 1.5% and 0.75%. At the beginning and then every 3 min., 2 ml. were removed from the mixture and after adding 5 ml. 20% H₂SO₄ the amount of intact hydrogen peroxide was determined. Finally, hydrogen peroxide was determined after 30 min. The logarithm of the ratio of the initial concentration and the concentration during each time interval, kₜ = log cₜ/c₀, was evaluated graphically.

**Determination of the activity of glutamic-oxaloacetic transaminase.** Two ml. of substrate for the glutamic-oxaloacetic transaminase according to Cabaud, Leeper and Wróblewski (1956) was added to 2 ml. of a standard listeria suspension. The increment of pyruvic acid content was determined after 24 hrs. in 1 ml. of the mixture according to Cabaud (Dubach, 1957; Krasánek & Okape, 1958). The determination was carried out on a Pulfrich photometer (S-53 filter, 0.5 cm. cuvette).

**Determination of glucose and lactic acid dehydrogenases.** Two ml. standard suspension of listerias was added to 0.1 ml. 1% triphenyltetrazolium chloride and 0.5 ml. 2% substrate in a phosphate buffer of pH 8.2. After thorough mixing, the test-tubes were placed in a water-bath at 37°C. After 60 min. of incubation the reading was carried out, + standing for a pink colour of the reaction mixture,