containing 10 μmoles of neutralized cysteine hydrochloride per ml. Cell-free extracts were prepared using a sonic oscillator followed by centrifugation at 11,000 rev/min for 20 min at 4 C to remove cell debris.

Malate synthetase and isocitritase activities were determined spectrophotometrically by the method described elsewhere (Reeves and Ajl, J. Bacteriol., **79,** 341, 1960). Protein concentrations were estimated by the method of Warburg and Christian (Biochem. Z., **310,** 384, 1941).

The data presented in table 1 show the specific activities of malate synthetase and isocitritase in *T. pyriformis*. It can be noted that the specific activity of malate synthetase is nearly 10-fold that of isocitritase. This finding is in accord with our earlier data concerning the specific activities of these two enzymes in *Escherichia coli* (Reeves and Ajl, J. Bacteriol., **79,** 341, 1960). Figure 1 presents the details of the isocitritase determination. It is clear that this enzyme and not isocitric dehydrogenase, determined the rate of the coupled reaction of curve 2.

Although the role of both malate synthetase and isocitritase is well established for the aerobic growth of bacteria on such $C_2$-compounds as acetate, their function in Tetrahymena grown in complex medium remains to be elucidated.

## PREPARATION OF STABLE *LISTERIA MONOCYTOGENES* O ANTIGEN

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It is well known that boiled suspensions of *Listeria monocytogenes* generally used for the detection of O antibodies in human and animal sera are quite unstable. Paterson (J. Pathol. Bacteriol., **51,** 427, 1940) was not able to test some strains because of failure in obtaining stable O suspensions. Similar observations can be found also in the recent literature (Hood, Am. J. Clin. Pathol., **28,** 18, 1957). Seeliger (*Listeriose*, 2nd ed., J. A. Barth, Leipzig, 1958) emphasized the use of strongly buffered glucose agar for cultivation, buffered saline for harvesting the bacterial growth, and the treating of the boiled suspensions in the ultrasonator for the dispersion of clumps observed after heating.

Preliminary experiments in our laboratory indicated that we regularly obtained unstable suspensions when employing different media for cultivation and when using unbuffered or buffered saline solutions for washing off the growth. On the basis of these results it was decided to try the cultivation of Listeria on a cellophane layer which excluded the possibility of direct contact between the culture medium and the bacterial cell surface, and to use distilled water instead of saline for harvesting the bacteria.

Blood agar plates, with 5 per cent human blood, covered with a sterile cellophane membrane, and dried in the incubator at 37 C, were inoculated with different Listeria strains. Plates were incubated at 37 C for 48 hr, and the bacteria were washed off with sterile distilled water, pH 7.4. After harvesting, the suspensions were boiled for 2 hr and stored in the refrigerator for 6 months. A number of suspensions were stored in saline solutions: 0.2, 0.4, and 0.85 per cent, pH 7.4. Some lots were preserved by 0.01 per cent merthiolate.

Fifty Listeria strains were tested by the above method. They were obtained from human or animal infections from various parts of the world, among them some recently isolated strains from Hungary. The strains represented all Listeria serotypes and subtypes hitherto recognized. All strains developed well on blood agar covered with cellophane membrane and yielded stable suspensions in every case after harvesting the growth by distilled water. The stability of the suspensions was not altered by boiling for 2 hr, and the boiled suspensions showed no tendency of clumping during the whole period of storage, whether the suspending fluid was distilled water or 0.2 to 0.85 per cent saline.

Agglutination experiments showed that these O antigens reacted easily and specifically with animal and human sera containing Listeria O antibodies. Similar results were obtained with hyperimmune rabbit sera produced by injecting boiled Listeria suspensions of serotypes 1, 2, 3, 4a, 4b, 4c, 4d, and 4e. The O antigens were

employed in physiological saline; we increased the salt content of the suspending fluid to 0.85 per cent by adding small amounts of a concentrated NaCl solution to the antigen suspensions. The results were read after incubation for 4 hr at 50 C and overnight in the refrigerator at 4 C. The suspensions remained stable during storage for at least 6 months and do not contain the components of the medium. End points of the titrations are clear-cut in contrast to those using O suspensions prepared by the older methods, in which the presence of fine clumps often interferes.

# PHAGOCYTOSIS OF STAPHYLOCOCCI BY MOUSE LEUKOCYTES IN THE PRESENCE OF BOTULINUM TOXIN[1]

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Minervin et al. (Zhur. Mikrobiol. Epidemiol. Immunobiol., **5,** 48, 1955) reported that type A and B toxin of *Clostridium botulinum* sharply reduced the phagocytic activity in vitro of leukocytes, and Savin (Zhur. Mikrobiol. Epidemiol. Immunobiol., **8,** 44, 1955) stated that he could detect small amounts of this toxin in foods by means of changes in the phagocytic index. Shevdov (J. Microbiol. Epidemiol. Immunobiol. (U. S. S. R.) **30,** 95, 1959) considered the depression of phagocytosis a more sensitive test than the classical animal intoxication test. The toxin used by these investigators was a crude culture filtrate, and small changes in the phagocytic indices were considered significant.

In an attempt to repeat the work of Minervin, the following technique was used. Pooled heparinized mouse blood was centrifuged, and the plasma and buffy-coat layer were removed separately by means of a capillary tube. The buffy-coat layer was resuspended in plasma to give 20,000 leukocytes per $mm^3$, and 0.4 ml of this suspension was added to 0.2 ml of crystalline type A botulinum toxin diluted in gelatin-phosphate buffer, pH 6.3, to give a mouse $LD_{50}$ titer of $2 \times 10^6$ per ml. A frozen washed suspension of coagulase positive staphylococci, concentrated in normal saline solution, was thawed prior to use, diluted with gelatin-phosphate buffer to give a final concentration of $2 \times 10^8$ bacteria per ml, and 0.2 ml of this suspension was added to the toxin and leukocyte mixture. Exposure of the leukocytes to the toxin prior to addition of the bacteria varied from 0 to 60 min. The mixture was slowly rotated horizontally by means of a mechanical drum in a 37 C incubator for 20 min. Normal saline solution, gelatin-phosphate buffer, and thermally inactivated toxin were used as controls. Smears were made quickly on glass slides, fixed with methanol, and stained with Giemsa's stain. The phagocytic index was expressed as the average number of bacteria engulfed per polymorphonuclear leukocyte out of 100 randomly selected leukocytes examined.

TABLE 1

*Effect of botulinum toxin on phagocytosis of staphylococci by mouse leukocytes*

| Materials | Phagocytic Indices | | | | | Average Index |
|---|---|---|---|---|---|---|
| Gelatin-phosphate buffer* | | | 19.3 | 21.6 | 22.9 | 21.2 |
| Heated toxin | 28.0 | 23.0 | 18.4 | 19.5 | 23.2 | 22.4 |
| Unaltered toxin | 26.1 | 23.2 | 23.0 | 20.2 | 21.2 | 22.7 |

* This buffer is commonly employed in making titrations of botulinal toxin since it seems to prevent detoxification due to experimental manipulations, and in our experience is not harmful to the determination of the phagocytic index.

The moderately high concentration of crystalline type A botulinum toxin used in this study did not significantly decrease the phagocytic activity of mouse leukocytes, in that the indices obtained

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