11272

Utilization of Serine by *Clostridium botulinum.**

C. E. CLIFTON.

From the Department of Bacteriology and Experimental Pathology, Stanford University, California.

A number of amino acids are utilized by washed suspensions of *Clostridium botulinum,* for the most part in coupled reactions between pairs of different amino acids.[1, 2] For example, alanine is deaminatively oxidized to CO_2 and acetic acid by glycine or proline which are reduced to acetic acid or δamino-n-valeric acid, respectively. However, amino acids such as leucine and particularly serine appeared to be deaminated when present singly in washed suspensions of this organism. The utilization of serine has been studied in detail by the technic already described,[3] ammonia being determined by the method of Parnas as described by Niederl and Niederl.[4]

Serine is decarboxylated in the presence of washed suspensions of Types A or B *Cl. botulinum* at a rate (Q_{CO_2} of 7-10) approximately one-third of that observed with pyruvic acid (Q_{CO_2} of 25-30) as the substrate at optimal pH values for both reactions. Typical results of CO_2 production are presented in Fig. 1.

It is apparent that the pH optimum for the utilization of serine by *Cl. botulinum* lies on the alkaline side of neutrality, probably near pH 7.5. This is in marked contrast with the pH optimum of 6.0 observed with pyruvic acid as the substrate. In further studies it was observed that apparently only one optical form of serine is attacked at an appreciable rate, deamination and decarboxylation of *dl*-serine proceeding only approximately 50% to completion.

In semi-macro experiments in Warburg vessels of 40 ml capacity with Clerici fluid in the manometers the fermentation was studied in more detail. Ammonia, CO_2, ethyl alcohol and acetic acid were identified as the chief products of degradation of serine. In these experiments the reaction was not allowed to go to completion due to the slow rate observed by the time approximately one-half of the serine had been utilized. Typical results on the utilization of serine

* Aided in part by a grant from the Rockefeller Fluid Research Fund.

1 Clifton, C. E., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 338.

2 Clifton, C. E., *J. Bact.*, 1940, in press.

3 Clifton, C. E., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 585.

4 Niederl, J. B., and Niederl, V., *Micromethods of Quantitative Organic Elementary Analysis,* 1938, 51-59, John Wiley and Sons, New York.

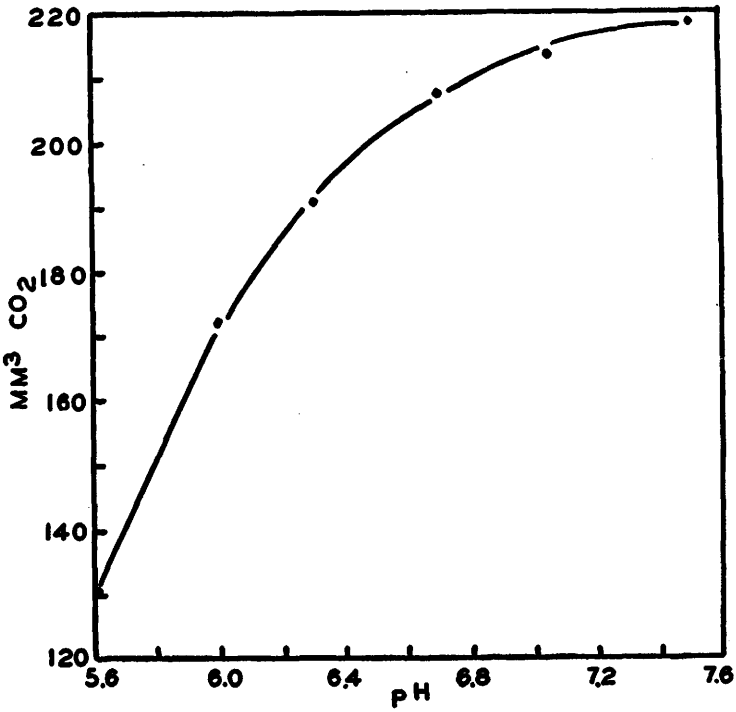

FIG. 1.

Influence of pH on CO_2 production, corrected for bound CO_2, by *Cl. botulinum* from 0.2 ml M/10 *dl*-serine in 90 minutes at 37°C.

by washed suspensions of Type A *Cl. botulinum* in M/15 phosphate buffer of pH 7.2 are recorded in Table I, the fermentation being carried out in an atmosphere of O_2-free H_2. Similar results were obtained in an atmosphere of O_2-free N_2 and with washed suspensions of Type B *Cl. botulinum*. The quantity of serine fermented is based on the NH_3 liberated during the course of the experiment.

The results suggest that serine is deaminated and rearranges to form pyruvic acid which is then fermented, or, that serine is de-

TABLE I.

	mg	Mols/mol serine	mg	Mols/mol serine
Initial serine	52.5		52.5	
Final serine	28.0		32.5	
Serine utilized	24.5		20.0	
Products:				
NH_3	3.98	1.00	3.25	1.00
CO_2	8.98	0.87	7.17	0.86
CH_3CH_2OH	6.15	0.55	5.06	0.57
CH_3COOH	7.20	0.51	6.00	0.53
Total	26.31		21.48	

carboxylated and deaminated at the same time to yield acetaldehyde which is dismuted to form equimolar quantities of ethyl alcohol and acetic acid. If the reaction proceeds by way of pyruvic acid, deamination appears to be the controlling factor of the rate of utilization of serine as evidenced by the marked shift in pH optimum from 6.0 for pyruvic acid to the neighborhood of 7.5 for serine. These results, considering the experimental errors involved, suggest that the degradation of serine by washed suspensions of *Cl. botulinum* may be represented as

$$2\ CH_2OH\ .\ CHNH_2COOH + H_2O = 2\ NH_3 + 2\ CO_2 + CH_3CH_2OH + CH_3COOH$$

The closely related amino acid, alanine, is not attacked directly by *Cl. botulinum* but only when a suitable H-acceptor is also present. Therefore, substitution of an hydroxyl group for a H-atom on the β-carbon of alanine produces a compound that may be employed singly as a source of energy by *Cl. botulinum*. These above results show that *Cl. botulinum* may obtain a portion of its energy requirements by direct utilization of amino acids such as serine as well as through coupled reactions between pairs of different amino acids (Stickland reaction) as previously described.

11273 P

Nasopharyngeal Cultures in Pertussis.*

WILLIAM L. BRADFORD AND BETTY SLAVIN.

From the Departments of Pediatrics and Bacteriology, Rochester School of Medicine and Dentistry, Strong Memorial Hospital, and Rochester Municipal Hospital.

The generally accepted method for the bacteriological diagnosis of pertussis is the cough-plate culture, originally described by Chievitz and Meyer,[1] or some modification of their procedure. The percentage of positive cultures obtained by this technic varies considerably in the hands of different workers and under various conditions.[2]

We have found the cough-plate method satisfactory for older children, but in infants we have obtained positive cultures in only

* Supported by a grant from the Committee on Therapeutic Research of the American Medical Association and the Fluid Research Fund of the University of Rochester School of Medicine and Dentistry.

1 Chievitz, J., and Meyer, A., *Ann. de l'Inst. Pasteur*, 1916, **30**, 503.

2 Sauer, L. W., *J. Ped.*, 1934, **5**, 246.