

ability to inhibit active glucose absorption in the isolated surviving guinea pig intestine. For example, one fraction (at a concentration of 0.1%) stated to be active as a tumor promoter, gave an inhibition of glucose transport of 68% whereas another fraction, stated to be inactive, gave no inhibition of glucose transport at concentrations up to 0.5%. It is intended to proceed further with this work when more of the croton oil fractions become available.

Summary. 1. Croton oil (0.5%), when present in an oxygenated Ringer medium circulating through lumen of isolated surviving guinea pig intestine, inhibits active glucose absorption. It has relatively little effect, under identical experimental conditions, on active galactose absorption. 2. Castor oil has much smaller activity than croton oil. Cod liver oil and olive oil, under same experimental conditions, have little or no effect on glucose absorption by the isolated intestine. 3. Presence of L-phenylalanine in the mucosal solution diminishes inhibition of active glucose absorption by croton oil. D-phenylalanine, under same conditions, has little or no effect. L-phenylalanine when present only in the

serosal solution has little or no effect on croton oil inhibition. Glycine and DL-methionine resemble, but are less effective than, L-phenylalanine in diminishing croton oil inhibition. Results indicate that croton oil inhibition may be mediated through some phase of intestinal amino acid metabolism.

1. Berenblum, I., *Arch. Path.*, 1944, v38, 233.
2. Berenblum, I., Shubik, P., *Brit. J. Cancer*, 1947, v1, 379.
3. Mottram, J. C., *J. Path. Bact.*, 1944, v56, 181.
4. Roe, F. J. C., Salaman, M. H., *Brit. J. Cancer*, 1955, v9, 177.
5. Boutwell, R. K., Rusch, H. P., Bosch, D., *Proc. Am. Assn. Cancer Research*, 1955, v2, 6.
6. Selye, H., *J. Nat. Cancer Inst.*, 1955, v15, 1291.
7. Darlington, W. A., Quastel, J. H., *Arch. Biochem. and Bioph.*, 1953, v43, 194.
8. Fridhandler, L., Quastel, J. H., *ibid.*, 1955, v56, 423.
9. Riklis, E., Haber, B., Quastel, J. H., *Can. J. Biochem. Physiol.*, 1958, v36, 373.
10. Riklis, E., Quastel, J. H., *ibid.*, 1958, v36, 347.
11. Lijinsky, W., *Biochem. J.*, 1958, v70, 5P.
12. Fisher, R. B., Parsons, D. S., *J. Physiol.*, 1953, v119, 224.

Received January 15, 1959. P.S.E.B.M., 1959, v100.

Oxidation of L-glutamic Acid in Relation to Antigenic Composition of *Salmonella typhi*.^{*} (24753)

GOBERT A. COSTA AND GILBERTO G. VILLELA

Divisions of Bacteriology and Biochemistry, Inst. Oswaldo Cruz, Rio de Janeiro, Brazil

Enzymatic activity of various strains (Vi, O,H) of *Salmonella typhi* in relation to their antigenicity has been undertaken by Shrivastava *et al.* (1), who observed a marked difference in oxidative metabolism of glutamic acid and tyrosine of various strains containing Vi antigen in comparison with those devoid of it, maximum oxygen consumption with L-glutamic acid being higher with Vi rich strains. They concluded, therefore, that the Vi antigen is responsible for higher metabolic activity of strains containing this antigen. On the

other hand, Baron, using Vi strains in V forms and W variants, demonstrated that each strain behaved identically in oxidative metabolism of amino acids, and that the difference observed cannot be correlated with presence or absence of the Vi antigen. Consequently, the observed variations in oxidative metabolism may be due to enzymatic differences inherent to each strain (2). In the present study we investigated whether different strains of *S. typhi* with optimally developed Vi antigen, or devoid of it, in comparison with H-91 and O-901 strains showed any difference in metabolic patterns to oxidize

^{*} Abstract was reported at VII Internat. Congr. of Microbiol., Stockholm, 1958.

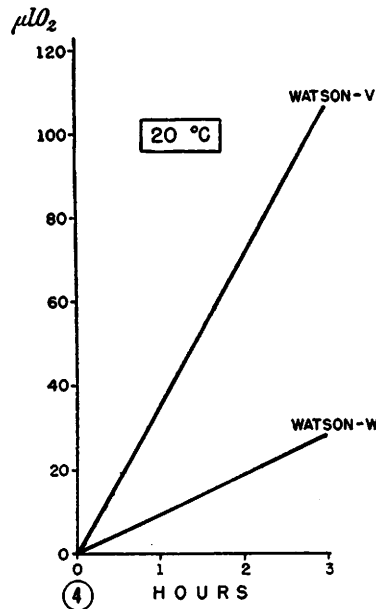
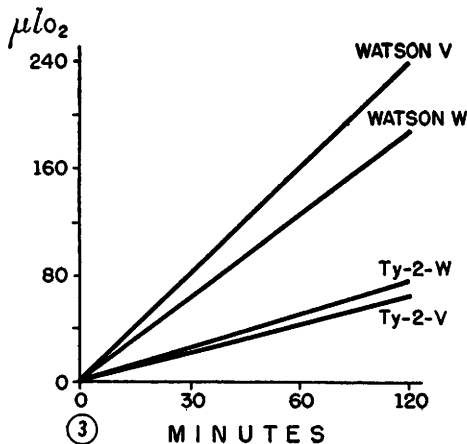
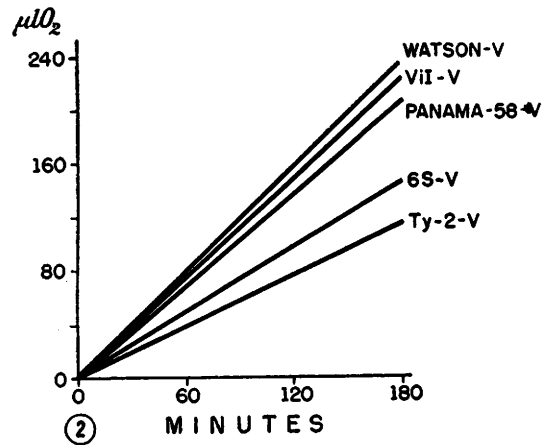
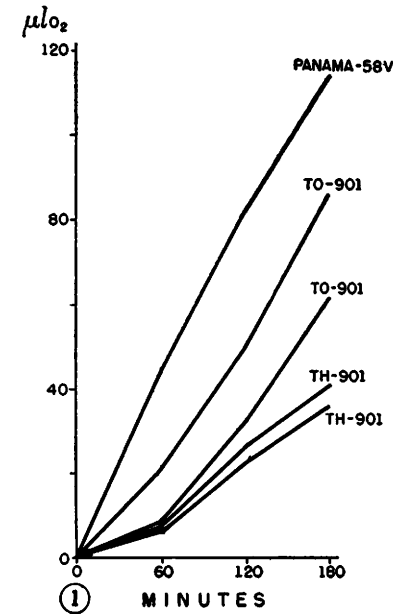

 FIG. 1. Oxidation of L-glutamic acid by different antigenic strains of *Salmonella typhi*.

 FIG. 2. Oxidation of L-glutamic acid by *S. typhi* containing Vi antigen.

 FIG. 3. Oxidation of L-glutamic acid by V and W forms of *S. typhi*.

 FIG. 4. Oxidation of L-glutamic acid with strains V and W of *S. typhi* grown at 20°C.

amino acids. We used strains rich in Vi antigen (Bhatnagar Vi I; Ty 2; Panama 58 V; 6-S) and those containing O and H antigen (O-901; H-901). The V and W variants from Vi strains were obtained through pas-

sages in plate cultures. The presence of each antigen was tested before the experiments, using slide agglutination with specific Vi and somatic sera. Only forms with Vi antigen optimally developed and variants lacking Vi

TABLE 1. Oxidation of L-Glutamic Acid by *S. typhi* Strains Incubated at 37°C.

Strain	Oxygen consumption (μ l O ₂)		
	Time in min.		
	60	120	180
58-V	39.4	185.8	201.3
6 S-V	78.4	145.0	
Vi 1	28.2	223.2	
TO	21.3	49.2	85.4
TH	9.8	25.3	40.8
Ty-2-V	22.5	41.7	69.2
-W	34.7	63.9	73.7
Watson-V	42.2	208.3	239.3
-W	89.4	168.2	177.5

antigen were used for metabolic tests.

Methods. *S. typhi* strains were grown on meat extract agar in Roux bottles for 18 hours at 37°C and harvested, washed and resuspended in 0.85% NaCl solution. Suspensions were adjusted to optical density of 7 (10 x 7) in Lumetron photocolimeter with 650 m μ filter, corresponding to 24 mg dry cells/1 ml. Oxidative activity was measured in conventional Warburg respirometer, the final volume being 3 ml (1 ml cells and 1 ml phosphate buffer pH 7.4 in main compartment, 1 ml M/50 L-glutamic acid as substrate in side arm and 0.2 ml 20% KOH in central well). Oxygen consumption was measured for 2 and 3 hours at 37°C.

Results. The Vi rich strains showed a marked oxidation toward L-glutamic acid whereas those containing no Vi antigen metabolized this amino acid to a lesser degree. Fig. 1 gives typical experiments. However, when several strains containing Vi antigen were studied it appeared that oxygen consumption varied greatly (Fig. 2). The V forms and W variants obtained from Vi strains when cultured separately oxidized L-glutamic acid with a variable activity not related to Vi content (Fig. 3). To be certain of purity of the antigenic constitution, agglutination tests were performed in the same suspensions of V and W variants used for oxidation in the manometer.

In Table 1 typical results of the different experiments are summarized.

Kozinski *et al.* (3) demonstrated that when *S. typhi* is cultivated at 18°C, no synthesis of Vi antigen in cells occurred, although the enzyme responsible for synthesis of the antigen was present. We undertook experiments growing *S. typhi* strains (V and W forms) at 18 and 20°C and measuring thereafter the oxidative metabolism of L-glutamic acid. Serological analysis of these strains showed that the suspensions of V culture were devoid of Vi antigen, confirming the results of Nicolle *et al.* (4). It was observed that oxidative activity of the V form was much higher than of the W form although no Vi antigen could be detected serologically in this strain (Fig. 4). Therefore, production of the Vi antigen seems to be independent of the enzyme system oxidizing the amino acid.

Summary. It was shown that differences in oxidative metabolism of L-glutamic acid observed with V and W variants of *S. typhi* are related to enzymatic activities of each strain as reported by Baron. Results obtained with one strain varied greatly and the differences were only detectable when experiments were carried out with pure V and W forms of each strain. Variants V and W of *S. typhi* when cultured at 18-20°C showed higher oxidative activity toward L-glutamic acid than with V and W forms even when no Vi antigen could be detected.

1. Shrivastava, G. C., Agarwala, S. C., Bhatnagar, S. S., *Enzymologia*, 1954, v17, 41; Shrivastava, G. C., Arora, K. L., Bhatnagar, S. S., *Experientia*, 1954, v10, 493.

2. Baren, L. S., *ibid.*, 1955, v11, 268.

3. Kozinski, A. W., Opara, Z., Kraft, Z., *Nature*, 1957, v179, 201.

4. Nicolle, P., Jude, A., Diverneau, G., *Ann. Inst. Pasteur*, 1953, v84, 27.

Received January 16, 1959. P.S.E.B.M., 1959, v100.