EFFECT OF CONTINUOUS MEDIUM CHANGE ON THE BIOSYNTHESIS OF FOLINIC ACID BY L. CASEI*

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Studies on the effects of chemical carcinogens on one carbon metabolism could be greatly aided by an understanding of the metabolic interrelationships of active C_1 units involving members of the folic acid group (Huennekens, et al (1958)). The influence of various chemical and physical agents on the production of folinic acid, citrovorum factor (CF), by both microorganisms and animal tissues have been previously studied (Broquist and Kohler, (1953); Hakala and Welch, (1957); Nichol and Welch, (1950); Chang, (1953); Miller and Bond, (1959)). The usual procedures for the production of CF by microorganisms yield such small quantities of the active principle that isolation and purification of the natural factor(s) have been only partly successful. Because of the relative instability of folinic acid, time is a factor in maintaining a sample of high potency. It was thought desirable to evaluate the effects of modifications in methods on the biosynthesis of CF by L. casei to see if an improvement in total yield of an active principle of high potency could be effected.

In this report, a method (referred to as flow process) for quantity production of CF from L. casei is compared with unstirred and stirred culture techniques.

An apparatus was designed which allowed a continuous flow of

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biosynthesis medium into and out of a flask containing L. casei cells. The apparatus consisted of a biosynthesis medium reservoir which was connected through a stopcock to the reaction vessel containing the cells. The vessel, a 3-neck, 500 ml roundbottom flask was provided with a mechanical stirrer. The withdrawal of medium from the reaction vessel was through a tube attached at a level such that a volume of 200 ml would be maintained in the reaction vessel at all times. A Millipore filter disc (Millipore Filter Corp.) placed in the withdrawal system, prevented removal of cells. A graduated flask, which was connected to an aspirator, was used as a receiving vessel.

The biosynthesis medium (folic acid 30 mg, sodium formate 2 g, ascorbic acid 2 g, glucose 2 g, in one liter of 0.1 M phosphate buffer pH 7) was made up in one batch and divided equally among the reservoir of the above apparatus, a flask for the unstirred culture, and a flask equipped with a mechanical stirrer. The L. casei (ATCC 7469) was prepared for biosynthesis by inoculating 600 ml of the enriched medium (Bond, (1953)) with an actively growing 18 hour culture. At the end of 18 hours, the highly turbid cell suspension was centrifuged, washed and resuspended in a small amount of 0.15 N sterile NaCl solution. One third of this saline suspension was added to each of the three culture vessels. Medium was added to the latter flask from the reservoir to bring the volume to 200 ml.

A sample of the biosynthesis medium was taken prior to inoculation to serve as a medium control. After addition of the cells, samples were taken accordingly to the time schedule given in Fig. 1. A volume of medium equal to the amount collected in the graduated filter flask attached to the flow process was removed from each of the other two culture systems. The samples were

immediately centrifuged for 10 minutes at 0°C and 2500 rpm and stored at 10°C until all collections were made. All samples, including the medium control, were assayed simultaneously for CF activity by conventional methods using P. cerevisiae (ATCC 8081) Bacto CF assay medium (Difco Laboratories), and folinic acid - SF as a standard. The results of a typical six-hour biosynthesis are given in Fig. 1.

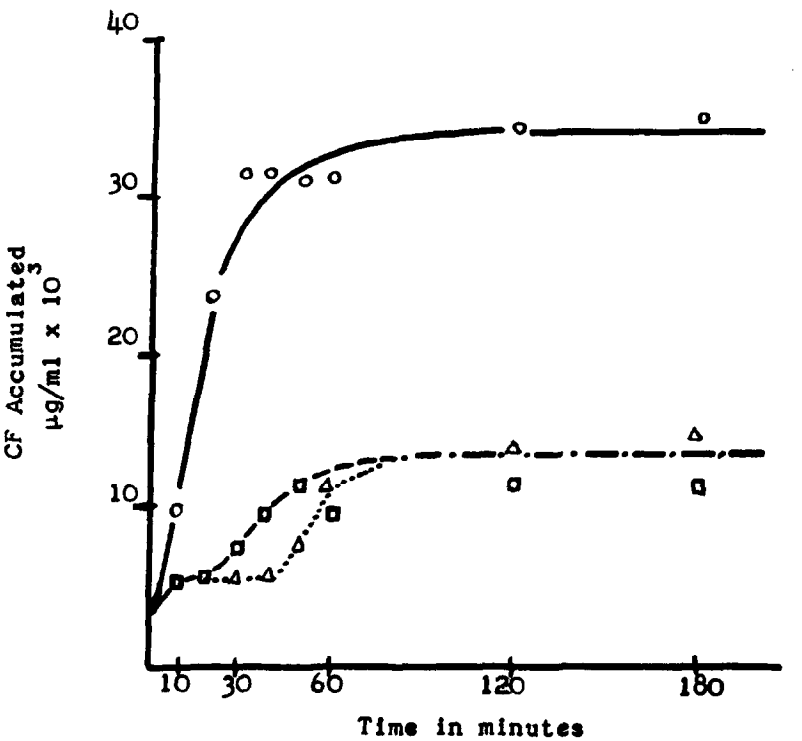

Fig. 1: Effect of variation in biosynthesis methods on the production of folinic acid (-O-, flow process; -Δ-, unstirred process; -□-, stirred process.)

As expected, the stirred culture reduced the lag period observed in the unagitated culture during early phases of biosynthesis. The flow process offers the advantage of rapid as well as increased conversion, making it possible to shorten the time of residence (in the medium) of the biologically active factor. The data indicate that there was no further accumulation of CF

after 2 hours. The active material began to slowly disappear after 15 hours. Since this experiment was designed to study the relative rates of production of CF under three different mechanical processes, an excess of folic acid was employed in each reaction vessel, and efficiency on the basis of percentage yield was not considered.

Paper chromatography, using Whatman No. 1 filter paper strips developed in O.1 M phosphate buffer saturated with isoamyl alcohol by descending technique, followed by bioautography gave Rf values of O.77 for the biologically active zones of the biosynthesized samples compared with a value of O.76 for folinic - SF as a standard. No significant differences in Rf values were noted when active samples produced by each method were compared.

The results indicate that the flow process gives a greater rate of production and as a result, a larger total yield of CF than either of the other two processes. There appears to be no significant difference in CF production between the unagitated and the agitated cultures. This may be explained on the basis that the availability of CF reaches a maximum in these processes, because of the accumulation of product(s); whereas, in the flow process, the products of the biosynthesis were removed from the reaction vessel, thus permitting more CF to be formed on a mass action basis.

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