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EVIDENCE FOR SULFITE AS AN ESSENTIAL METABOLITE FOR HUMAN PERIPHERAL LYMPHOCYTES

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SUMMARY: Sulfite has been identified as an essential metabolite by means of growth studies using a chemically-defined, protein-free medium for culture of human peripheral lymphocytes. Sulfite reduced the amount of cysteine required for optimum growth by at least four-fold. In some subjects, sulfite stimulated growth even in the presence of optimal amounts of cysteine indicating that lymphocytes of some individuals are unable to convert cysteine to sulfite in adequate amounts.

A protein-free, chemically-defined medium (CFBI 1000) which supports short term growth in human lymphocytes (1,2,3) was developed in this laboratory for use in assessing metabolic and nutritional status in a variety of subjects. In this medium, containing only minimal amounts of nutrients essential for optimal growth, fifteen amino acids are included; most of them support maximum growth at less than 0.05 mMolar. Only lysine (0.08 mM), cysteine (0.168 mM), and glutamine (0.4 mM) were required at relatively higher concentrations. Thus, glutamine and cysteine are required at a higher concentration than appeared to be necessary for protein synthesis as judged by the necessary amounts of the other amino acids required. Subsequent investigation revealed that glutamine was spared by addition of an essential metabolite, asparagine, to the medium (4). Efforts were made to determine which metabolic product of cysteine metabolism was limiting growth by testing the effect of including various end products in the media. Since the major pathway of cysteine catabolism involves oxidation to cysteine sulfinic acid, which is converted to pyruvate or alanine and sulfite,

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these end products were tested as possible sparing agents for cysteine.

MATERIALS AND METHODS

The media used for cell culture was the same as described previously (1, 2, 3) with the exception that the cysteine was omitted from the media. Human peripheral lymphocytes were collected from a group of randomly selected patients. Cells were collected, worked as described (1), counted in the Coulter counter, and inoculated at a final concentration of 150,000 cells per ml into wells of plates containing the indicated supplements in 0.2 ml of medium Cysteine hydrochloride and all other media components were obtained from Sigma Chemical Company. After four days incubation at 37°C in a 5% CO_2 atmosphere, the samples were pulsed with [^{3}H]-thymidine (0.29×10^{-6}M final concentration; specific activity 1mCi/mol), incubated, and harvested after twenty-four hours using an Inotech Cell Harvester and Automatic Filter Counting System. Growth was measured by the amount of radioactivity which had been incorporated into the cell. Triplicate samples were run in all experiments.

RESULTS AND DISCUSSION

Cysteine growth response curves obtained from growth studies in the presence of different levels of sulfite disclosed a marked sparing effect on the cysteine requirement as shown in Figure 1. More than a four-fold sparing effect has been demonstrated in each of the more than one hundred fifty subjects tested thus far. This effect was not produced by other antioxidants, including dithiothreitol, glutathione, alpha-tocopherol, and ascorbic acid. Since sulfite is further oxidized to sulfate, the form in which ingested sulphur is excreted, this final product was also tested, but it exerted no effect on the amount of cysteine required by the cells for maximum growth. Neither pyruvate nor alanine had any effect on the requirement for cysteine. Sulfite seems to be unique in its activity as a sparing agent for cysteine for growth of lymphocytes in this media.

In the majority of subjects, sulfite exerted a sparing effect, but did not stimulate growth of the cells when optimal levels of cysteine were included in the medium. However, in a significant number of cases, sulfite stimulated growth at all concentrations of cysteine as shown in Figure 2. Apparently, cells from some subjects were unable to produce enough sulfite to meet their requirement even when cysteine is included at optimal levels.

There are a number of possibilities for the essential functions of sulfite. For example, it may be a necessary factor in the transport of cysteine, and it may be the moiety which can

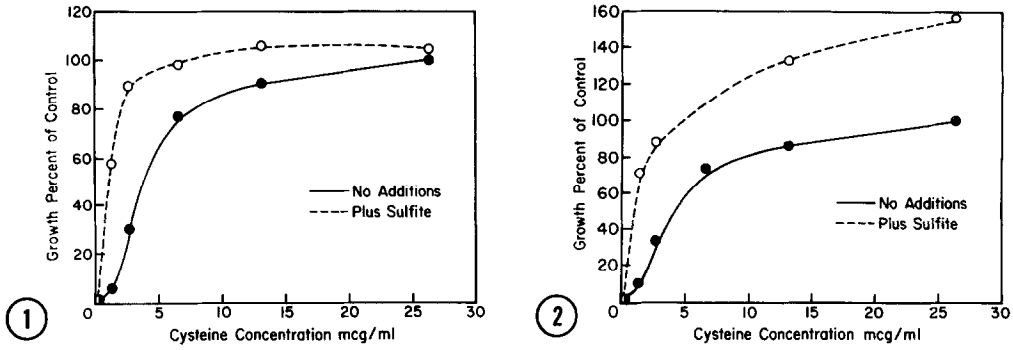

Figure 1. Cysteine Dose Response Curves. Cells were grown and harvested as described in the text. These curves represent data on a single individual. Sodium sulfite was added at a concentration of 6.3 mcg/ml. Growth is expressed as per cent of control with optimal cysteine.

Figure 2. Cysteine Dose Response Curves. Data from a different individual was obtained in the same way as in Figure 1.

be transported across the cell membrane to essential sites before it is converted to sulfate. An essential role for sulfite, which cannot be replaced by sulfate, has been reported for sulfolipid biosynthesis in chloroplasts of Euglena gracilis var bacillaris, which lacks the sulfate activating enzymes (5). On the other hand, utilization of cysteine from external sources could be diverted by sulfite regulation of pathways not essential for growth, thereby diverting cysteine into growth-essential pathways.

Sulfite at a concentration more than ten-fold above the level required for decreasing the cysteine requirement is inhibitory to the growth of human lymphocytes. The detrimental effects cause by sulfite oxidase deficiency indicate that sulfite oxidation to sulfate is an essential process, presumably controlling the level of sulfite. Thus, the lymphocyte growth system may also be useful in studying the nature of the toxicity of sulfite.

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