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The Influence of Thymidine on the Blastoid Transformation of Human Lymphocytes (37932)

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(Introduced by T. F. Dougherty)

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Recent investigations have demonstrated that exogenous thymidine stimulates thymidine kinase in regenerating rat liver (1, 2), and Canellakis *et al.* (3) postulated that thymidine kinase may be a regulator of DNA synthesis. These reports indicate that thymidine may be involved in the regulation of cellular proliferation. The first experimental demonstration of such an effect on cellular proliferation was submitted by Greulich *et al.* (4), who found a significant increase in the mitotic index of epithelial cells in duodenal crypts of mice treated with exogenous thymidine. A similar effect also was found in the rat by Magrot *et al.* (5). This paper reports that the blastogenic response of normal human peripheral lymphocytes induced by phytohemagglutinin (PHA) in culture can be altered by the presence of various concentrations of thymidine.

Materials and Methods. Two hundred milliliters of fresh, heparinized blood (10 units heparin/ml) was taken from three normal volunteers and allowed to sediment at room temperature for 60 min. The leukocyte rich plasma was removed, and cell counts were performed. Cultures were initiated by placing 1×10^6 leukocytes into 1 ml of Eagle's medium (Grand Island Biological Company) supplemented with 10% heat-inactivated calf serum (56° for 60 min), 100 IU penicillin, and 100 μg of streptomycin per ml. Phytohemagglutinin[1] and thymidine were added immediately after initiation of the cultures. The concentrations of thymidine used were from 0.0002 to 20 m*M*. Each culture had its own control without thymidine. All cultures were harvested after incubation at 37° for 72 hr. At the end of the experimental period, the cells and supernatants were separated by centrifugation at 1500*g*. The viability of the lymphocytes was not impaired as determined by the trypan blue exclusion technique (6). The cell pellet was resuspended in medium and spread on slides, which were then fixed in a mixture of absolute methanol and glacial acetic acid (3:1) and then stained with Giemsa. One thousand cells on each slide were examined, and the percentage of typical mature lymphocytes and cytologically transformed cells was determined.

Results and Discussion. Stimulation of lymphocyte blastogenesis was significantly increased as compared with controls in cultures in which the thymidine concentrations varied from 0.02 to 0.2 m*M* (Fig. 1). Concentrations of less than 0.02 m*M* had no significant effect. Thymidine concentrations of 2 m*M* and above inhibited the blastoid transformation. The maximal inhibition of these effects was obtained with 8 m*M* of the nucleoside, but larger amounts of thymidine in the medium did not produce greater inhibition. The optimal thymidine concentration required to increase the blastogenic effect of PHA was between 0.02 and 0.2 m*M*.

This experiment, therefore, demonstrates that the lymphocyte blastoid transformation

[1] PHA-M, 0.1 ml/culture (DIFCO Laboratories).

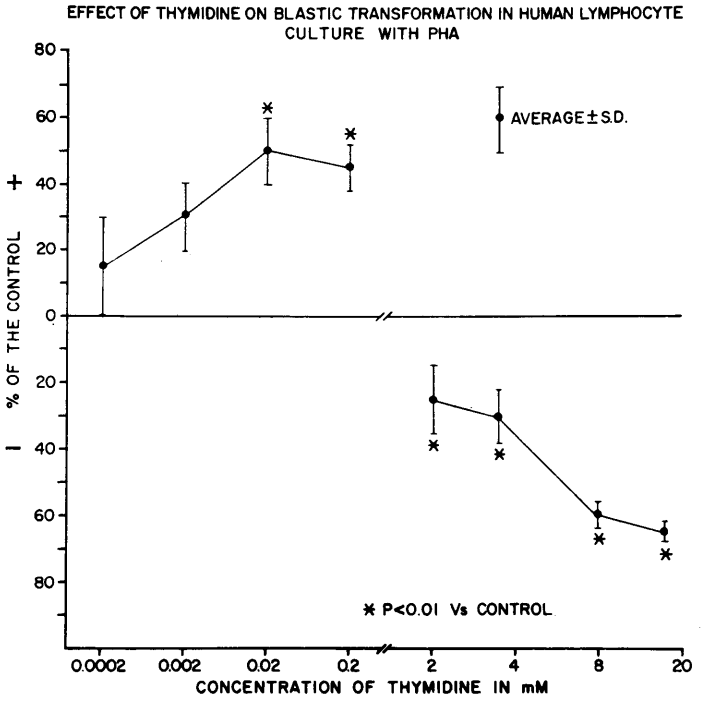

FIG. 1. Each point represents the average ± standard deviation of duplicate determinations from three separate experiments expressed as percentage of its control.

is increased by the presence of thymidine in the PHA incubation medium. Xerox (7) showed that cell growth was inhibited by the addition of thymidine and that cells were synchronized following the removal of thymidine. It is well established that thymidine at 7.5 m*M* concentrations in tissue culture produces optimal inhibition of DNA synthesis (8). This effect is achieved by the prevention of the reduction of ribonucleotides to deoxyribonucleotides (7). The stimulatory effect of thymidine is probably related to the fact that thymidine stimulates thymidine kinase (3) to increase the phosphorylation of thymidine nucleotides, which are necessary for normal DNA synthesis.

Summary. The percentage of human lymphocytes undergoing blastoid transformation induced by PHA was significantly increased by the presence of thymidine at concentrations between 0.02 m*M* and 0.2 m*M*. Concentrations under 0.002 m*M* were ineffective, whereas concentrations between 2 and 20 m*M* inhibited the blastogenic effect of PHA. Maximal inhibition was obtained with 8 m*M* of the nucleoside.

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