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Failure of L-Phenylalanine to Reverse Growth Inhibitory Effects of Chloramphenicol on McCoy Cell Cultures. (31431)

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In a recent report(1) it was suggested that the non-fatal toxic effects of chloramphenicol on the bone marrow(2) might be reversed by administering L-phenylalanine to patients receiving this antibiotic. If this agent could prevent bone marrow toxicity it is conceivable that it might also prevent the rare, fatal aplastic anemia caused by chloramphenicol (3).

The present report describes the inability of L-phenylalanine to reverse the growth inhibitory and toxic effects of chloramphenicol on a strain of cultured cells of human origin.

Materials and methods. The McCoy strain (4) of cells derived from human synovial fluid was used. Since the cells had been maintained for some time in a penicillin-streptomycin-containing medium, they were propagated for 6 passages in the presence of kanamycin sulfate (100 μg/ml) to minimize the possibility of latent mycoplasma (PPLO) contamination(5). Thereafter, the cells were maintained in an antibiotic-free medium. Periodic culturing of cells and fluids, both before and after kanamycin treatment, on Difco PPLO agar and in a modified PPLO medium(6) has disclosed no PPLO.

The cells were grown at 37°C in screw capped tubes containing 1 ml amounts of Eagle's basal medium supplemented with 10% calf serum. The medium was changed every 3-4 days and a pH of 6.8-7.4 was maintained by addition of sodium bicarbonate solution. Chloramphenicol* and L-phenylalanine were sterilized by filtration and incorporated in the medium to give the final concentrations noted. Test cultures were prepared in triplicate by inoculating 15,000-25,000 cells/tube of a 7- to 10-day culture contained in 0.05 ml of Hanks' basic salt solution. Morphologic observations were made directly of the living cells and also by examination of Wright stained cover slips on which the cells had grown in a replicate series of culture tubes. Direct cell counts were determined by counting at least 400 cells in a haemocytometer after removal of the cells with 0.05% trypsin. Each experiment was repeated 2-3 times.

Results. Chloramphenicol was employed at 10^{-5}M (3.23 μg/ml), 3×10^{-5}M (9.69 μg/ml) and 10^{-4}M (32.3 μg/ml) because these concentrations approximate the usual serum levels in patients treated with this drug. It is evident from the results shown in Fig. 1 that inhibition of cell growth was directly related to the concentration of chloramphenicol. Morphologic changes, indicative of cell toxicity, were produced by higher

* Kindly provided by Parke-Davis & Co.

concentrations of the antibiotic. These consisted of increased granulation of cytoplasm, rounding-up and detachment of cells from the glass, and bipolar appearance of the fibroblast-like cells. To eliminate the possibility that the chloramphenicol was simply interfering with the attachment of the cells to the glass at time of inoculation, similar studies were carried out in which addition of the antibiotic to the growth medium was delayed until the fourth day after inoculation of the cells. In these experiments the degree of inhibition of growth of the cells was comparable with the inhibition shown in Fig. 1.

As noted in Table I, L-phenylalanine in concentrations of 3×10^{-5}M to 3×10^{-3}M failed to reverse the growth inhibitory effect on the cells of 3×10^{-5}M chloramphenicol. In addition, L-phenylalanine also failed to alter the morphologic effects of chloramphenicol on the cells.

Discussion. Woolley(7) suggested an antimetabolite-metabolite relationship for chloramphenicol and phenylalanine as an explanation for the antimicrobial action of chloramphenicol but no definitive bacteriologic data have appeared in support of this hypothesis. The results obtained in the present study offer no indication of such an antimetabolite-metabolite relationship. Since the growth inhibiting and toxic effects of chloramphenicol on McCoy cells could not be reversed with phenylalanine when a molar ratio of antibiotic to metabolite of approximately 1:100 was employed, it appears unlikely that a higher ratio of phenylalanine would have produced a significant reversal action.

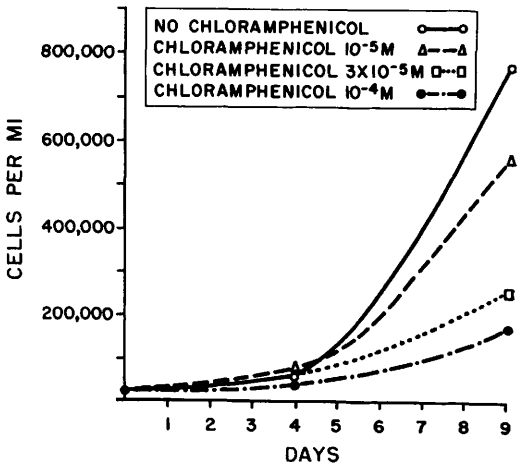

FIG. 1. Effect of chloramphenicol on growth of McCoy cells in culture.

TABLE I. Effect of L-Phenylalanine on Chloramphenicol Growth Inhibition of McCoy Cells.

L-phenylalanine*	Cells per ml after 10 days growth: No chloramphenicol	3×10^{-5}M chloramphenicol
0	880,000	94,000
3×10^{-5}M	746,000	89,000
3×10^{-4}M	870,000	98,000
3×10^{-3}M	830,000	95,000

* In addition to the 10^{-6}M L-phenylalanine contained in basal medium.

Except for its association with rare fatal cases of aplastic anemia chloramphenicol has been considered relatively non-toxic for man as well as for mammalian cells in culture(8). Despite this belief, some earlier experimental observations indicated the antibiotic may exhibit toxicity for human and animal cells in culture(9,10,11) at concentrations necessary for antibacterial action. More recently chloramphenicol in concentrations of 5 to 50 μg/ml was reported to inhibit growth of a strain of cells derived from human bone marrow(12) and to inhibit antibody synthesis by rabbit lymph node(13) or rabbit spleen(14) cells *in vitro*. In addition to temporary bone marrow dysfunction(2) suppression of the immune response(15) in man by doses of 2 to 4 g daily of chloramphenicol has also been described. Thus, the evidence is good that chloramphenicol, in concentrations found in patients under treatment with the drug, alters body cellular function, perhaps by interfering with protein synthesis at the messenger RNA level(16). The importance of this toxic effect in relation to the development of fatal aplastic anemia, wound healing, phagocytosis(17) or the immune response bears further investigation.

Summary. Chloramphenicol, in concentrations found in patients under treatment with the drug, markedly inhibited the growth of the McCoy strain of human cells in culture. The growth inhibition was not overcome by L-phenylalanine in amounts up to approxi-

mately 100 times the growth inhibitory concentration of chloramphenicol.

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Influence of Vehicle of Administration on Intestinal Absorption, Fat Storage, and Biological Activity of Ethynylestradiol (EE) and its 3-Cyclopentyl Ether (EECPE) in Rats. (31432)

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Ethynylestradiol-3-cyclopentyl ether (EECPE)* is more potent orally than its parent compound, ethynylestradiol (EE)† as a uterotrophic agent and as a gonadotrophin inhibitor in laboratory animals(1-5). Unlike EE, EECPE is stored in body fat and brain (6,7) and sustained release from these depots may account for its enhanced potency. The greater affinity for lipids of EECPE as compared with EE may also influence route of absorption from the gut. Thus, when administered in sesame oil rather than aqueous suspension, a significant amount of EECPE but not EE is transported through the lymphatic system(8).

The above findings suggest fundamental differences in the interaction of EECPE and EE with lipids, both as constituents of sesame oil and of body fat. Present experiments show that intestinal absorption of EECPE from sesame oil is qualitatively and quantitatively different from that of EE and that vehicle of administration greatly affects storage of EECPE in body fat and brain.

Materials and methods. (A) *Preparation of radioactive steroid doses.* Ethynylestradiol-6,7-H^3-3-cyclopentyl ether, specific activity (s.a.) 0.78 $\mu c/\mu g$ and ethynylestradiol-3-cyclopentyl-1-C^{14} ether, s.a. 0.392 $\mu c/\mu g$ were mixed to give an H^3 to C^{14} ratio of approximately 10:1 and a s.a. of approximately 0.26 μc H^3 and 0.026 μc $C^{14}/\mu g$ EECPE. Ethynylestradiol-6,7-H^3, s.a. 0.93 $\mu c/\mu g$ was diluted with unlabeled EE to give a s.a. approximately the same as that of EECPE (*i.e.*, 0.26 $\mu c/\mu g$). Doses for administration (approx. 5 μc or 19.1 μg of steroid) were either dissolved in sesame oil or suspended in an aqueous vehicle (0.9% NaCl, 0.4% Tween 80, 0.5% carboxymethylcellulose, 0.9% ben-

* 3-cyclopentyloxy-19-nor-17 α-pregna-1,3,5(10)-trien-20-yn-17 β-ol.

† 19-nor-17 α-pregna-1,3,5(10)-trien-20-yne-3,17 β-diol.