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Effects of Some Metabolic Analogs on Growth of Mumps and Influenza Viruses in Tissue Cultures.* (19423)

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The inhibition of viral multiplication by various metabolic antagonists in tissue cultures has been studied by several investigators, using a number of viruses(1-4). Indeed, this technic is a promising avenue of approach for studying the growth requirements of many viruses. Using this method, certain vitamin, amino acid, and purine analogs were investigated to determine if they would inhibit effectively multiplication of mumps and influenza viruses in cultures of chick embryonic tissue without serious toxic effects on the host tissue.

Methods. Cultures of 9- to 11-day-old chick embryonic tissue were prepared, employing a method similar to that of Earle(5). Perforated cellophane discs cut to size were placed in the bottoms of 10 ml Erlenmeyer flasks which were then autoclaved. Using whole embryo tissue for the cultures to be inoculated with influenza virus, and amniotic membrane tissue for those cultures to be infected with mumps virus, the tissues were minced with scissors in Hanks's(6) balanced salt solution without added sodium bicarbonate. The tissue fragments were then planted in the flasks underneath the cellophane discs. Two ml of nutrient solution composed of 2 volumes of Hanks's solution with sodium bicarbonate and one volume of ox serum ultrafiltrate, to which the virus was added, were then placed into each flask. The viruses employed were strains of mumps and influenza (PR8) viruses which had been cultivated in the allantoic sacs of embryonated eggs. Incubation of the tissue cultures was at 36°C. After 24 hours the infected nutrient

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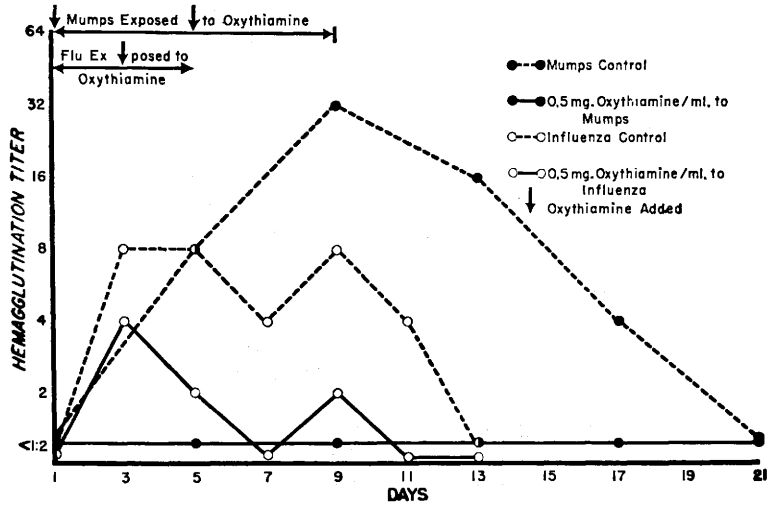

FIG. 1. Inhibition of growth of mumps and influenza (PR8) viruses in tissue culture by oxythiamine.

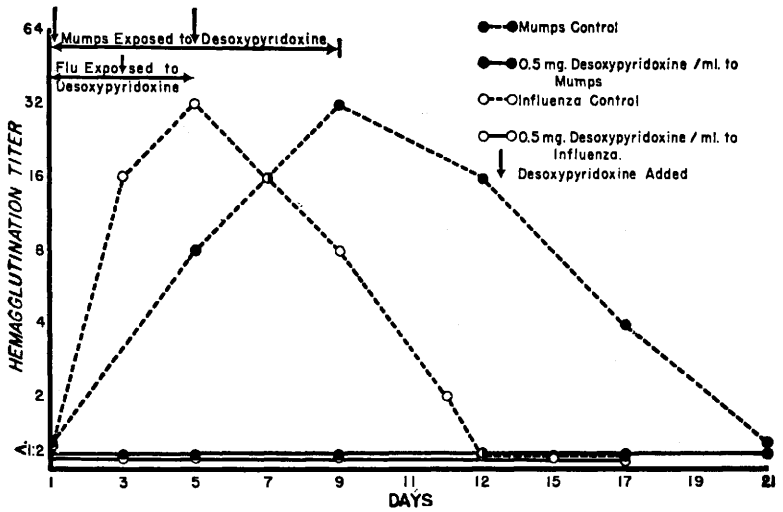

FIG. 2. Desoxypyridoxine suppression of multiplication of mumps and influenza (PR8) viruses in tissue cultures.

fluid was withdrawn and replaced with 2 ml of fresh nutrient solution containing one of the analogs. The analog was added to the cultures for 2 successive fluid changes, after which they were maintained on normal nutrient solution. Fluid changes were made every 48 hours on the influenza cultures, every 96 hours on the mumps cultures, and the fluid removed was tested promptly for virus content by hemagglutination titrations with hen erythrocytes. Duplicate flasks were prepared for each concentration of each analog, and appropriate control cultures were maintained for the duration of each experiment. The experiments were continued until the control cultures showed greatly diminished virus yield. Approximately 100 units of penicillin and 100 μg of streptomycin/ml were added to the nutrient fluid to protect against bacterial contamination.

To study the possible toxic effects of the test analogs on chick embryo tissue, fragments of heart tissue from 12- to 14-day-old embryos were planted in plasma clots in Carrel

TABLE I. Effect of Various Analogs on Growth of Mumps and Influenza (PR8) Viruses in Tissue Cultures.

Analog	Concentration, mg/ml	Metabolite	Virus inhibition Flu	Virus inhibition Mumps
Desoxypyridoxine	.1	Pyridoxine	0	0
	.5		+	+
Oxythiamine	.1	Thiamine	0	+
	.5		+	+
Pyridine-3-sulfonic acid	.5	Nicotinic acid	0	0
2-Amino-4-hydroxy-6-formylpterine	.2	Xanthine	0	0
Salicyl-β-alanide	.5	Pantothenic acid	0	0
2,6-Diaminopurine	.1	Adenine	0	0
8-Azaguanine	.5	Guanine	0	0
β-2-thienylalanine	.5	Phenylalanine	0	0

flasks. Nutrient fluids containing the analogs were added, and these cultures were incubated for 4 days at 36°C. Daily examinations were made to observe the ability of the fragments to pulsate and to note the outgrowth of fibroblasts at the periphery of the explants.

Results. Two of the vitamin analogs, oxythiamine and desoxypyridoxine, demonstrated a definite inhibitory effect on the multiplication of both mumps and influenza (PR8) viruses, as shown in the growth curves (Fig. 1 and 2). Of the two viruses, mumps appears to be the most sensitive to oxythiamine. Mumps virus growth was markedly inhibited, within the limits of hemagglutination measurement, when exposed to 0.1 mg/ml of oxythiamine, while influenza virus multiplication was only moderately inhibited at 0.5 mg/ml of this analog. Both viruses were markedly inhibited by desoxypyridoxine at a concentration of 0.5 mg/ml but not at 0.1 mg/ml. In the toxicity tests these two compounds, at the concentrations employed, did not produce obvious toxic effects on the chick embryonic heart tissue as measured by contractility and fibroblastic proliferation.

The remaining analogs, pyridine-3-sulfonic acid, salicyl-β-alanide, 2-amino-4-hydroxy-6-formylpterine, 2,6-diaminopurine, 8-azaguanine, and β-2-thienylalanine, produced no significant inhibitory effect on the growth of either virus at the concentrations used (Table I).

Discussion. Since sufficient time (24 hr) for the inoculated virus to invade the host tissue elapsed before the analogs were introduced, it would seem that the effects of these analogs were exerted on intracellular virus rather than by blocking the attachment of the virus to the cells.

Ackermann(7) has presented evidence that the functioning of the Krebs cycle of carbohydrate metabolism is essential for the multiplication of influenza (PR8) virus in tissue cultures. It may be that oxythiamine blocks pyruvate oxidation and entry of pyruvate into the Krebs cycle of the intermediary metabolism of the tissue cells sufficiently to inhibit multiplication of the virus. Using cell preparations containing tyrosine decarboxylase as their test system, Umbreit and Waddell(8) have concluded that desoxypyridoxine exerts its antagonistic effect after being phosphorylated by competing with pyridoxal phosphate for the apoenzyme. It might be postulated that phases of amino acid metabolism, such as transamination in which pyridoxine functions as a coenzyme, are essential to viral multiplication.

The inhibitory effect of oxythiamine and desoxypyridoxine on mumps and influenza (PR8) virus growth was not reversed significantly by the withdrawal of these analogs, at least as measured by the hemagglutination technic. It should be noted, however, that the rapid growth phase of the viruses in the control cultures generally had passed its peak by the time the analogs were removed from the test cultures. Hence, it is not clearly demonstrated that the inhibition was irreversible.

Summary. The vitamin analogs oxythiamine and desoxypyridoxine inhibit the multi-

plication of mumps and influenza (PR8) viruses in tissue cultures at concentrations shown to be without observable toxic effects on the tissue itself. Other analogs tested, including pyridine-3-sulfonic acid, salicyl-β-alanide, 2-amino-4-hydroxy-6-formylpterine, 2,6-diaminopurine, 8-azaguanine, and β-2-thienylalanine, demonstrated no significant inhibitory effects on the growth of these viruses under the conditions of these experiments.

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Effect of "Benemid" and Carinamide on Penicillin Therapy of Experimental Pneumococcic Infections in Mice. (19424)

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Carinamide (4'-carboxyphenylmethanesulfonanilide) is a compound capable of inhibiting the excretion of penicillin by the renal tubules(1). Therefore, higher and more prolonged plasma concentrations of penicillin are produced when carinamide is administered during penicillin therapy(2). The oral administration of carinamide was found to enhance the therapeutic effectiveness of penicillin administered intramuscularly 6 to 16-fold in the treatment of experimental pneumococcic infections in mice(3). The enhanced chemotherapeutic effect demonstrated in these experiments has been confirmed clinically(4), but relatively large doses of carinamide were required in order to maintain the blood level of this compound necessary to suppress the tubular excretion of penicillin.

In the continuing search for compounds capable of suppressing more efficiently the tubular excretion of penicillin, 'Benemid',† [p(di-n propylsulfamyl) benzoic acid], was found to be effective in dogs, was well absorbed when given by mouth, and was excreted so slowly that its renal clearance could not be estimated(5). These results suggested the comparative evaluation of carinamide and 'Benemid' for the inhibition of penicillin excretion using dogs, and the results have been reported(5). In addition to these strictly pharmacologic studies, it was considered advisable to compare these two compounds quantitatively in terms of their effects upon the actual chemotherapeutic activity of penicillin in the treatment of an experimental infection in mice. In the experiments to be described, carinamide and 'Benemid' have been compared with respect to their penicillin-enhancing effects and with respect to the time during which the two compounds are able to increase the therapeutic effect of penicillin through the suppression of its tubular excretion. In addition, determinations were made of the blood level curves resulting from the selected doses of carinamide and 'Benemid' so that the potentiating actions of these two

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† 'Benemid' is the trademark applied to p-(di-n propylsulfamyl) benzoic acid by Sharp and Dohme. The tentative nonproprietary name of this compound is probenecid.