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Antiviral Activity of 3,5 Diiodo-4-Hydroxybenzenesulfonamide in Chick Embryo Lung Tissue Culture.* (29268)

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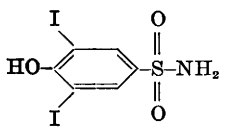
Analogues of thyroxine, as well as thyroxine itself, have been tested with varying success for possible chemotherapeutic activity against the growth of viruses(1-6). While several such derivatives have been shown to be ineffective against the influenza virus in tissue culture(1), 3,5 diiodo-4-hydroxybenzenesulfonamide§ (DHS) was found to possess virus-inhibiting properties.



Methods. For the present experiments growth of the WS strain of influenza A virus was determined by means of hemagglutination titrations. The chick embryo lung tissue culture technique, preparation and administration of test compounds, hemagglutination procedures, as well as tests for the effect of the compound on virus inhibition, virus inactivation and virus growth in eggs and mice, have been described(1).

Results. DHS was found to be effective in inhibiting the growth of influenza A virus in chick embryo lung roller tube tissue culture. Using a virus inoculum of 10^3 50% egg-infectious doses (EID_{50}) DHS was non-toxic to tissue in concentrations up to 0.5 mg/ml, yet it inhibited virus growth in concentrations down to 0.03 mg/ml, giving a therapeutic index of 16.

To determine whether the antiviral activity of DHS was due to its structural resemblance to normal metabolites, several compounds were tested for their ability to reverse the DHS-induced inhibition. Thyroxine, tyrosine, phenylalanine, p-aminobenzoic acid, 3,5 diiodo-1-tyrosine and 3,5 diiodo-4-hydroxybenzoic acid were ineffective in this regard.

Of some 28 other sulfonamides, sulfones or other sulfur bearing compounds, and 100 benzoyl and phenyl compounds, only 4‖ were effective in inhibiting the growth of influenza virus, implying that those structural moieties were not related to DHS viral inhibition. These 4 compounds were not as inhibitory as DHS (therapeutic indices of 8,4,4,4 respectively).

The influence of DHS at 0.12 mg/ml on the rate of virus growth in tissue culture at different time intervals was ascertained (Fig. 1). From the twelfth hour onward DHS depressed the virus infectivity titer about 100-fold until hour 44 when, perhaps because of exhaustion of DHS, the titer began to approach that of the control tubes. At no time did the virus titer reach that of the controls.

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‖ Aniline, benzal dicarboxylic acid, 2-acetoxy-5-bromobenzoic acid and p-nitrobenzoic acid.

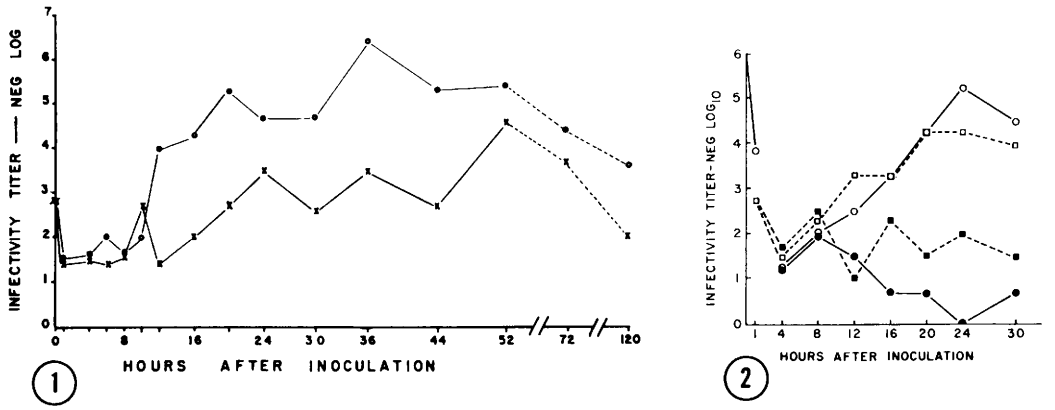

FIG. 1. Effect of 3,5 diiodo-4-hydroxybenzenesulfonamide on growth of influenza virus in chick embryo lung tissue culture. Each point represents growth of virus in a different tube. Titers measured in eggs. ×, 3,5 diiodo-4-hydroxybenzenesulfonamide added, 0.12 mg/ml culture; ○, control culture.

FIG. 2. Comparison of intracellular and extracellular virus titers in chick embryo lung tissue culture. Tubes infected with virus at time zero. At hour 1 fluids removed, cells washed, fresh media with or without DHS 0.25 mg/ml added. Titers of control tubes; ——○—— supernates and — —□— — cells. Titers of tubes receiving DHS; ——●—— supernates and — —■— — cells.

To determine whether antiviral activity was taking place intracellularly, infectivity titers of supernatants and cells were compared, using a larger viral inoculum (10^6 EID_{50}) and a heavier drug concentration (0.25 mg/ml). Virus infected chick embryo lung culture tubes were incubated in a roller drum for an hour. Fluids were removed. The tubes were washed twice with saline. Fresh media containing DHS were added. Control tubes received fresh media without DHS. After further incubation pairs of tubes were periodically removed. Supernatant fluids were saved. To determine intracellular virus content cells were washed twice with saline, frozen and thawed twice and brought back to volume with fresh media. The infectivity of the supernates and the disrupted cell suspensions were ascertained in embryonated eggs. Results are shown in Fig. 2.

In control tubes the amounts of virus in cells and supernates increased in roughly similar increments from the eighth hour onward. By the 24th hour infectivity of the supernates appeared to exceed that of the cells. There was no comparable rate of viral growth in the presence of DHS. The intracellular infectivity titer remained essentially unchanged. The amount of extracellular virus slowly decreased however. From the 16th hour onward titers were consistently lower.

The virucidal nature of DHS was indicated when 12.5 mg of the drug was added to one ml of diluent containing $10^{6.5}$ EID_{50} of virus and the mixture incubated at 37°C. After 6 hours no living virus was detectable in the fluid, while virus in a drug-free tube had an EID_{50} titer of 10^5. In other tests the drug had no effect on the adsorption of virus to susceptible cells, and failed to inhibit growth of influenza virus in fertile hens' eggs and in mice. The compound inhibited the Lee strain of influenza B and the PR8 strain of influenza A to the same extent.

Discussion. It is not clear whether the apparently static intracellular infectivity titer of DHS treated culture tubes reflects a decrease in rate of infection of susceptible cells (due perhaps to extracellular inactivation of virus), an intracellular inhibition of viral synthesis, a direct inactivation of intracellular virus or a combination of these. Nevertheless DHS is an effective antiviral agent in a tissue culture system as indicated by its selective effect on influenza virus.

Although the antiviral activity of the related compound 3,5 diiodo-4-hydroxybenzoate may be due to its ability to uncouple phosphorylation from oxidation(2) the virucidal capacities of the iodine moiety itself, either in free or bound form(7-9), may also be responsible. The antiviral activity of DHS does

not appear to be related to that shown by 5-iodo-2-deoxyuridine (IDU). IDU acts as a thymidine antagonist and is infective against RNA containing viruses(10).

Summary. 3,5 diiodo-4-hydroxybenzenesulfonamide was found to inhibit the growth of the influenza virus in chick embryo lung tissue culture. The compound appeared to exert a selective direct effect upon the extracellular virus particle.

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Effects of Dietary Phenylalanine on Activity of Phenylalanine Hydroxylase from Rat Liver.* (29269)

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Conflicting reports exist concerning the effects of dietary phenylalanine and tyrosine on levels of phenylalanine hydroxylase from rat liver, especially as such effects are related to experimental phenylketonuria. Knox and Behrman concluded that a marked depression of the liver hydroxylase was demonstrated by feeding a diet high in tyrosine to young rats, but a diet also rich in phenylalanine does not affect the level of this enzyme(1). Contrary to this review, the data of Auerbach *et al,* whose work was cited, show a severe decrease in activity of phenylalanine hydroxylase in animals which received a diet to which 5% DL-phenylalanine was added as well as 2.5% each of DL-phenylalanine plus L-tyrosine or 5% L-tyrosine alone(2). These authors presumed that these amino acids cause a decreased production of the enzyme. To complicate matters further, a succeeding report by Freedland *et al* claimed a more moderate decrease of the hydroxylase occurs upon feeding L-phenylalanine, and that L-tyrosine feeding causes an increase in activity of the enzyme(3). Huang *et al* reported only a slight decrease in activity of the liver hydroxylase after feeding 3.75% L-tyrosine plus 2.75% DL-phenylalanine for up to 9 weeks. However, when only 2.5% DL-phenylalanine was added to the diet for 6½ or 9 weeks, no significant decrease in activity was noted(4). More recent investigations by Wang and Waisman show a 40 to 60% depression of phenylalanine hydroxylase levels in liver upon feeding L-phenylalanine to weanling male rats for 2 and 3 weeks(5). The depression elicited by the highest amount of phenylalanine fed (7%) was no greater than the lowest amount fed (3.5%), so that quantitation of the effect has not been adequately described. Moreover, no evidence has been presented which indicates whether decreases in measured activities of phenylalanine hydroxylase are real or apparent.

The present investigation was undertaken to clarify previous reports on the effects of dietary phenylalanine on activity of phenylalanine hydroxylase from rat liver, to quantitate more exactly the amount of phenylalanine necessary to produce a given lowering in hydroxylase activity, and to ascertain

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