

FACTORS RELATED TO THE GROWTH OF PSITTACOSIS VIRUS
(STRAIN 6BC)

II. PURINES, PYRIMIDINES, AND OTHER COMPONENTS RELATED TO NUCLEIC
ACID*

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A number of substances other than *p*-aminobenzoic acid (PABA) and pteroylglutamic acid (PGA) will reverse the inhibitory action of sulfonamides on some bacteria, when employed in the proper mixtures (1). Among these are certain purines and pyrimidines, and this has led to the assumption that they are products either directly or indirectly of the utilization of PABA by the cell and that sulfonamides interfere with the synthesis of nucleic acid and its derivatives (1). Since a similar situation exists with reference to sulfonamides and PABA in the case of psittacosis virus (6BC) (2), the effects of various purines and pyrimidines on the inhibition of virus growth by sodium sulfadiazine (NaSD) were investigated to see if evidence could be obtained concerning relationships of nucleic acid synthesis to virus growth.

Since analogues are now available for a number of purines, it was possible to examine the role of purines in the growth of psittacosis virus (6BC) by testing the effects of these analogues on the multiplication of virus in tissue culture, as already briefly noted in preliminary experiments (3).

Materials and Methods

Virus.—The egg-adapted strain of psittacosis virus (6BC) described in previous studies was used as therein described (4).

Compounds.—The purine analogues, 2,6-diaminopurine (5), 8-aza-2,6-diaminopurine, 8-azaxanthine (6), and 8-azaguanine (6), were furnished by Dr. Harold Petering of the Upjohn Company, who also prepared and tested, by the methods previously reported (7), the various pteridine derivatives used in these studies.

Experiments with Eggs.—The compounds to be tested and the virus inoculum were injected into the yolk sacs of 7-day-old embryonated eggs and the average day of death of the embryos was calculated and used as an index of the rapidity of virus multiplication (4).

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Experiments with Tissue Cultures.—Cultures of minced chick embryo tissues were prepared in Erlenmeyer flasks, using a cellophane disc as a substrate with a nutrient solution composed of Hanks's balanced salt solution and ox serum ultrafiltrate as described in the companion study (4). After addition of the virus inoculum to the cultures, they were incubated for 24 hours and the fluid removed and replaced with fresh nutrient solution. The nutrient fluid was subsequently removed at 4 day intervals and the amount of virus released into the fluid was determined by the egg titration method (8). The test substances were introduced into the nutrient fluid and their effects on subsequent virus growth noted. Toxicity tests with chick embryo heart tissue were carried out, as previously described (4), with each compound studied.

EXPERIMENTAL

Sulfonamide Antagonism by Certain Substances Alone and in Admixtures.—Using NaSD in doses of 0.5 to 2.5 mg. which gave 90 to 100 per cent survival of eggs injected with 10,000 LD₅₀ of psittacosis virus at 10 days, a number of purines and pyrimidines used in maximum tolerated dosage for chick embryos (*i.e.*, 5 to 20 mg.) alone or in various mixtures failed to show any sulfonamide antagonism. The nucleic acid derivatives tested and admixtures used were as follows: thymine; thymine, adenine; thymine, xanthine, hypoxanthine; thymine, adenine, guanine, and cytidylic acid. Thymidine (10 mg.) and an enzymatic digest of deoxyribonucleic acid (20 mg.) also showed no effect.

Since methionine also has been shown to exert sulfonamide antagonism under certain conditions with a number of bacteria (1), it was tested. The addition of 7.5 mg. of methionine to 20 mg. of thymine, adenine, or guanine failed to produce any sulfonamide antagonism.

It is apparent either that the sulfonamide virus inhibition system in eggs is not suitable for investigation of the role of nucleic acid derivatives and certain other materials in virus growth or that these substances are not related to the mechanism of virus inhibition by NaSD or that the proper amount and admixtures of these derivatives have not yet been subject to test.

Similar experiments with these nucleic acid derivatives and 4-aminopteroylaspartic acid in eggs gave no evidence that these derivatives would produce reversal of the inhibitory action of the analogue on virus growth.

Inhibition of Virus Growth by Purine Analogues.—When benzimidazole and 8-azaguanine were tested at the maximum tolerated dosage in embryonated eggs, benzimidazole (1 mg.) had no effect on virus growth, while 8-azaguanine (0.1 mg.) produced slight inhibition.

In experiments using tissue cultures, three purine analogues were found to produce a marked inhibition of virus growth. Benzimidazole (1 mg./ml.) caused a decrease in virus titer from 10⁻⁶ to less than 10⁻¹, and virus reappeared in the culture fluids following removal of the analogue, indicating that the host cells had suffered no irreversible toxic damage due to the analogue.

The adenine analogue, 2,6-diaminopurine, suppressed virus growth in tissue cultures at concentrations of from 0.025 to 0.1 mg./ml. (Fig. 1) with virus reappearing on removal of the analogue. The specific nature of this inhibitory action was indicated by its ready reversal with the addition of adenine (Fig. 2) and the

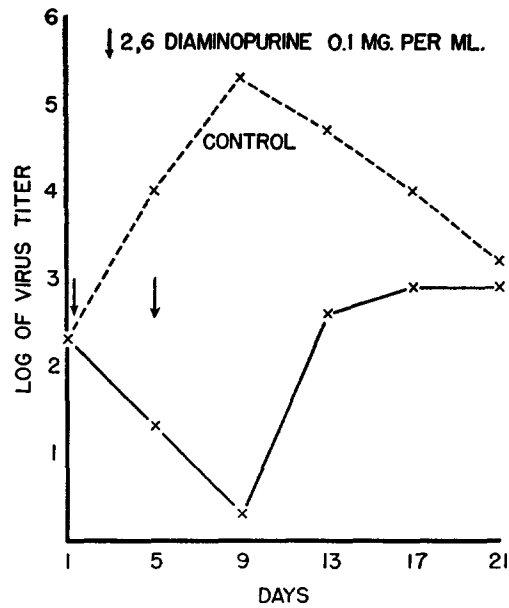


FIG. 1. Suppression of psittacosis virus (6BC) growth in tissue culture by 2,6-diaminopurine.

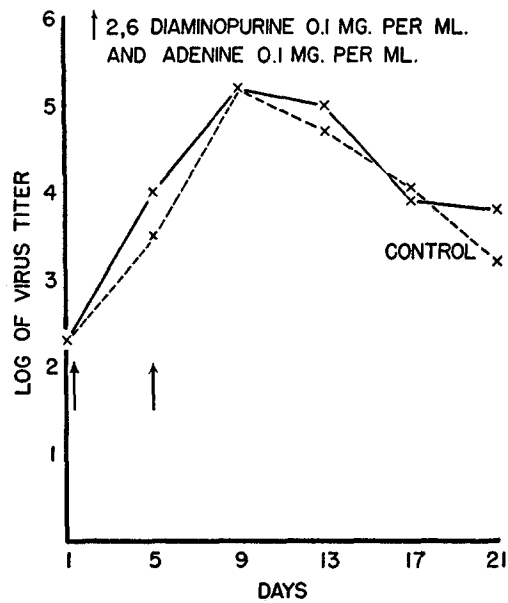


FIG. 2. Adenine reversal of the viral inhibitory action of 2,6-diaminopurine.

failure of a similar quantity of guanine to have any influence on its inhibitory effects. In other experiments it was found that the virus inhibitory action of

0.1 mg./ml. of 2,6-diaminopurine was more readily overcome by 0.1 mg./ml. of adenine than by 0.14 mg./ml. of adenosine, 0.25 mg./ml. of adenosine triphosphate, or 0.2 mg./ml. of adenylic acid.

The virus inhibitory action of 2,6-diaminopurine in tissue culture was exhibited at a concentration of 0.1 mg./ml. This concentration possessed no serious toxic activity for chick embryo heart tissue cultured in Carrel flasks, since the tissue explants continued to beat for at least 4 days and outgrowths of fibroblasts appeared.

The compound 8-aza-2,6-diaminopurine had no effect on the growth of psittacosis virus (6BC) in concentrations up to 0.1 mg./ml.

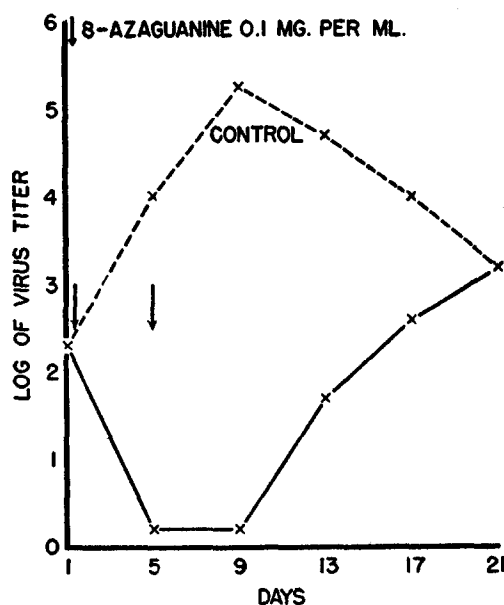


FIG. 3. Inhibition of the multiplication of psittacosis virus (6BC) by 8-azaguanine.

8-Azaguanine produced a marked suppression of virus growth in tissue cultures at concentrations of from 0.05 to 0.1 mg./ml. (Fig. 3). At these concentrations, 8-azaguanine had no serious toxicity for chick embryo heart tissue. This inhibitory effect of 8-azaguanine was partially overcome by the simultaneous addition of 1.0 mg./ml. of guanine (Fig. 4) but not by 0.5 mg./ml. of adenine. In another series of experiments, it was found that inhibition of virus by 0.05 mg./ml. of 8-azaguanine was completely overcome by the addition of 0.5 mg./ml. of guanine, while the reversing effects of 1.9 mg./ml. of guanosine and 2 mg./ml. of guanylic acid were less striking.

Some inhibition of virus growth was obtained with 0.5 mg./ml. of 8-azaxanthine, but it was not comparable to the effects seen with the lower concentrations of 8-azaguanine or 2,6-diaminopurine.

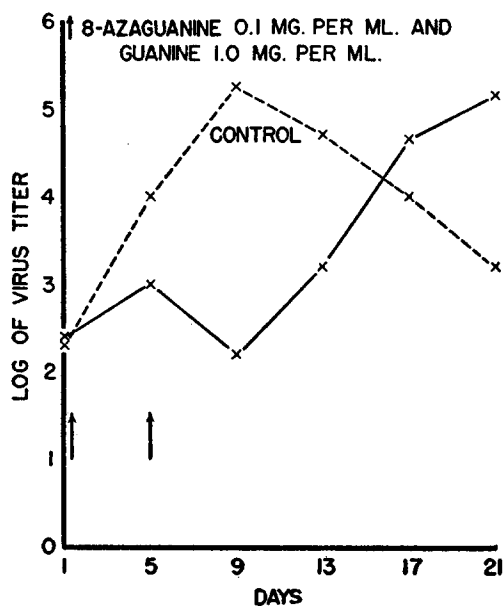


FIG. 4. Reversal of viral growth inhibition produced by 8-azaguanine on the addition of guanine.

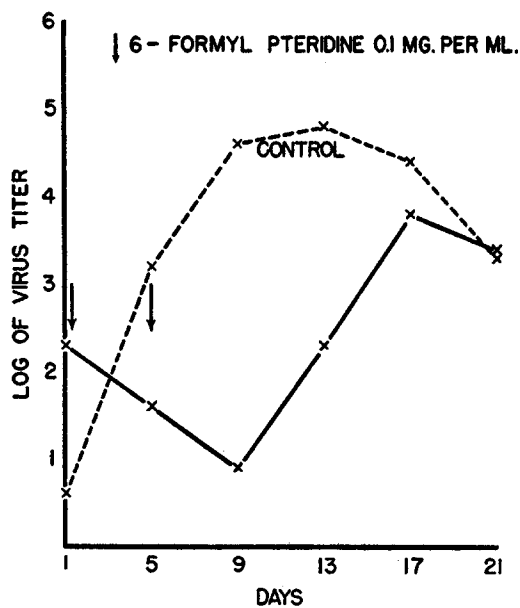


FIG. 5. Inhibition of growth of psittacosis virus (6BC) by 6-formylpteridine (2-amino-4-hydroxy-6-formylpteridine).

Effects of Pteridines on Virus Growth.—Recent investigations of some pteridines (7) have shown that certain of these compounds will inhibit the enzymatic

oxidation of xanthine by xanthine oxidase. Since this metabolic system may be of significance in the metabolism of purines (7), the effect of certain pteridines on growth of psittacosis virus (6BC) in tissue culture was studied. The compound 2-amino-4-hydroxy-6-formylpteridine (7) inhibited growth of virus in tissue culture at concentrations of 0.1 mg./ml. (Fig. 5), but showed no activity at 0.025 mg./ml. The inhibitory action of 2-amino-4-hydroxy-6-formylpteridine was reversed by 0.2 mg./ml. of xanthine. 2-Amino-4-hydroxy-6-formylpteridine had been shown to be a potent inhibitor of xanthine oxidase (7), but another pteridine, 2-amino-4-hydroxy-6-hydroxymethylpteridine which showed the same capacity to inhibit xanthine oxidase (7) was without effect on growth of the virus at concentrations of 0.2 to 0.5 mg./ml. On the other hand, xanthopterin, which has a low order of xanthine oxidase inhibitory activity (7), produced a striking inhibition of virus growth at 0.1 mg./ml. There is, therefore, no correlation between the inhibitory activity of these compounds on xanthine oxidase and on the multiplication of the virus.

DISCUSSION

The failure of a number of purines and pyrimidines alone or together and with methionine added to produce sulfonamide antagonism with psittacosis virus (6BC), as they do with many bacteria, is at first consideration paradoxical. Since sulfonamide antagonism by PABA and PGA is readily obtained with the virus, as it is with many bacteria, it was expected that a similar situation would apply to sulfonamide antagonism by purines and pyrimidines. On further examination, however, it has been shown that the situation with reference to sulfonamide antagonism even among various bacterial species is very complex. A given substance may be active with one organism in sulfonamide antagonism and with another inactive (or even synergistic with sulfonamides) (1). Of course, in dealing with a virus whose growth is intracellular, the compound must get into the cell before it can exert its effect on virus growth. Therefore, since the purines and pyrimidines will enter the cells lining the yolk sac selectively and in concentrations determined by the properties of the cell, and these compounds may not be present intracellularly in the combinations or concentrations required to exert sulfonamide antagonism. It is also possible that failure to demonstrate sulfonamide antagonism in these experiments may be due to the fact that the proper quantity or mixture of these substances was not selected for test.

Inhibition of virus growth by the purine analogues of adenine and guanine and the ready reversal of their inhibitory action by the corresponding purines in the several instances tested provides direct evidence of the importance of these substances for the growth of virus. Since psittacosis virus (6BC) contains large amounts of desoxyribonucleic acid, these purines are probably utilized in the synthesis of desoxyribonucleic acid during virus multiplication.

The observed effects of various pteridines on virus growth present some especially interesting aspects. In the first place, the difference in the inhibitory activity of the various compounds tested shows that minor variations in the chemical structure affect their activity in a striking manner. From the observations on virus inhibitory effects as compared with xanthine oxidase inhibition, it appears unlikely that these compounds interfere with virus growth through their influence on xanthine oxidase. Another mode of action for these pteridines may be interference with the utilization of PGA, since other studies (9) have shown that a large number of pteridines possess this capacity.

These experiments present additional illustrations of the advantages of the tissue culture system for investigations of the sort undertaken. Compounds whose toxicity for the chick embryo renders studies in embryonated eggs impossible can be examined in tissue cultures for their influence on virus growth.

SUMMARY

In various amounts and mixtures, adenine, guanine, xanthine, hypoxanthine, thymine, thymidine, cytidylic acid, and an enzymatic digest of desoxyribonucleic acid all failed to influence the inhibition by sulfadiazine of the growth of psittacosis virus (6BC) in embryonated eggs.

A number of purine analogues, including benzimidazole, 2,6-diaminopurine, and 8-azaguanine, inhibited the growth of psittacosis virus (6BC) in tissue cultures at concentrations which had no obvious toxic effects on the host tissues. The virus inhibitory action of 2,6-diaminopurine was reversed by addition of adenine and that of 8-azaguanine by guanine.

The growth of psittacosis virus (6BC) was inhibited by the pteridine compounds 2-amino-4-hydroxy-6-formylpteridine and xanthopterin, while other related substances had little or no inhibitory activity. Xanthine reversed the inhibitory effects of 2-amino-4-hydroxy-6-formylpteridine. There was no correlation between the inhibitory activity of the pteridines on xanthine oxidase and multiplication of the virus.

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