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# ANTIVIRAL ACTIVITY OF SODIUM FUSIDATE AND RELATED COMPOUNDS

BY

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Several steroid antibiotics (Halsall, Jones, Lowe & Newall, 1966) have been isolated from the fermentation products of various fungi; fusidic acid from *Fusideum coccineum* (Godtfredsen, Roholt & Tybring, 1962); helvolic acid from *Aspergillus fumigatus* (Chain, Florey, Jennings & Williams, 1943) and cephalosporin $P_1$, together with the other two, from *Cephalosporium acremonium* Brotzu (Burton & Abraham, 1951; Anslow & Fletcher, 1965). These compounds (Fig. 1) have a structural resemblance to the steroid hormones

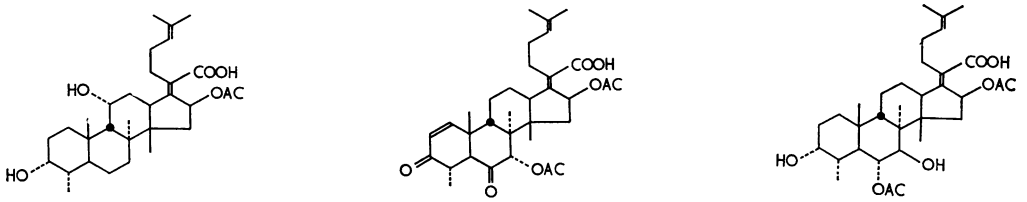



Fig. 1. The structure of fusidic acid, cephalosporin $P_1$ and helvolic acid.

but different stereochemical and biological properties (Johnsen, 1962; Wynn, 1965). In the present studies the antiviral activities in tissue culture of these antibiotics and of some derivatives of fusidic acid have been investigated. Sodium fusidate (Fucidin, Leo Laboratories), which is in general use for the treatment of staphylococcal infections, was also tested in human volunteers.

## METHODS

### *Viruses*

The viruses used were rhinovirus type 1B (B632), rhinovirus type 5 (Norman), Coxsackie virus A21, and adenovirus type 3.

### *Compounds*

Sodium fusidate (Fucidin) and 26 derivatives were supplied by Leo Laboratories, helvolic acid by Glaxo Laboratories and cephalosporin $P_1$ by Boots Pure Drug Company and Glaxo Laboratories.

*Tissue cultures*

Roller tube cultures of human embryonic lung cell strains (H.E.L. cells) were used in most experiments. The cells were grown and maintained in Eagle's medium with, respectively, 10% or 2% calf serum.

*Cytotoxicity tests*

The growth medium in tube cultures was replaced by 1 ml. maintenance medium containing dilutions of the drug. The cultures were incubated at 33° and examined microscopically at intervals for any cytotoxic effect.

*Antiviral activity*

One millilitre aliquots of serial twofold dilutions of drugs in maintenance medium were added to washed tube cultures of H.E.L. cells. These cultures were challenged in one of three ways. In the first type of test the tubes were inoculated with an intended dose of 2, 20 or 100 expected $TCD_{50}$ of the virus, which was titrated in parallel in cells of the same batch, so that the actual challenge doses could be calculated. After 2 or 3 days incubation at the optimal temperature for virus growth (33° or 37°) the drug-treated tubes were examined microscopically and the degree of protection was scored as 100% or 0% ; those markedly, but not completely, protected were assigned a score of 50%.

The second method of challenge, used with rhinoviruses, was to add a known dose of virus to two sets of tubes containing either the test compound or maintenance medium. Microplaques were counted after 2 days and the concentration of drug that reduced the number to 50% was determined.

The third method of challenge was to titrate half logarithm dilutions of a virus seed pool in cultures maintained in a given concentration of drug and in a parallel series of controls. The minimum concentrations of virus required to infect each type of culture were compared.

*Assay of fusidic acid in sera and nasal secretions*

Sera, diluted 1/20 in pH 6 phosphate buffer, were assayed against a standard range of dilutions of sodium fusidate by the agar plate diffusion method, using *Staphylococcus aureus* 3452 in nutrient agar pH 6.8. The mean diameters of the standard inhibition zones plotted against the log dose gave a straight line response from 10 to 1.25 $\mu$g/ml.

Nasal secretions were diluted in pH 6 phosphate buffer to give concentrations within the standard range.

*Volunteers*

Volunteers were isolated at the Common Cold Research Unit and housed and observed as described by Tyrrell (1963).

*Virus isolations*

Coxsackie virus A21 was isolated by inoculating 0.3 ml. aliquots of nasal washings into H.E.L. cell cultures, which were then rolled at 33°.

*Antibody titrations*

Antibodies against Coxsackie virus A21 were measured by a haemagglutination inhibition technique (Buckland, Bynoe & Tyrrell, 1965).

## RESULTS

### *Cytotoxicity*

When tube cultures of H.E.L. cell strains were incubated at 33° with maintenance medium containing 2,000 and 1,000 $\mu$g/ml. sodium fusidate, the cells became granular and rounded in all tubes within 15 or 30 min respectively. These effects were not reversed

when the drug was removed. 500 $\mu$g/ml. caused only slight toxicity after 24 hr and 100 $\mu$g/ml. gave rise to only slight granularity and rounding of cells in one out of three cultures after 2 days ; these effects, however, became more marked in all three tubes after 5 days. Tubes with concentrations of 50 $\mu$g/ml. or less did not show any toxic effects after 5 days. Less pure preparations of sodium fusidate were cytotoxic at 100 $\mu$g/ml. if the tubes were incubated at 37° but not at 33°. Cephalosporin $P_1$ at 100 $\mu$g/ml. was usually well tolerated by H.E.L. cells for 5 days.

When H.E.L. cells were sub-cultured twice in the presence of either 25 $\mu$g/ml, 50 $\mu$g/ml. or 100 $\mu$g/ml. sodium fusidate, the appearance of the cells in all groups remained normal and approximately the same total number of cells was present in each drug concentration.

*Tissue culture tests*

Sodium fusidate inhibited rhinoviruses and Coxsackie virus A21 in H.E.L. cells and an attenuated poliovirus type 1 (L Sc 2 ab) in HeLa cells ; it was however inactive against a virulent poliovirus type 1 (Bruenders) in primary cynomologous kidney cells, and adenovirus type 3 in H.E.L. cells. Helvolic acid inhibited Coxsackie virus A21 but had no significant effect on rhinoviruses or adenovirus type 3. Cephalosporin $P_1$ was inhibitory to all these viruses except poliovirus (see Table 1).

TABLE 1

ANTIVIRAL ACTIVITY OF THREE STEROID ANTIBIOTICS

Tests were in tissue cultures of human embryo lung cell strains (H.E.L.), HeLa, or primary monkey kidney. + = compound active, 0 = compound inactive, N.T. = not tested.

| Virus | Tissue culture | Sodium fusidate | Helvolic acid | Cephalosporin $P_1$ |
|---|---|---|---|---|
| Rhinovirus type 1B | H.E.L. | + | 0 | + |
| Rhinovirus type 5 | H.E.L. | + | 0 | + |
| Coxsackie virus A21 | H.E.L. | + | + | + |
| Adenovirus type 3 | H.E.L. | 0 | 0 | + |
| Poliovirus type 1 LSc 2 ab | HeLa | + | N.T. | N.T. |
| Poliovirus type 1 Bruenders | Primary monkey kidney | 0 | N.T. | 0 |

The percentage protection of H.E.L. cell cultures by sodium fusidate and cephalosporin $P_1$ in the same experiment, is shown in Table 2. An impure preparation was used in this test. The compounds had similar activities against two rhinoviruses and Coxsackie virus A21.

When half logarithm dilutions of Coxsackie virus A21 were titrated in H.E.L. cells with or without 50 $\mu$g sodium fusidate/ml., the titre of virus in the presence of the drug was reduced by about 50-fold.

The effect of adding sodium fusidate to H.E.L. cell cultures at different times before or after inoculation with a rhinovirus was investigated. If 50 $\mu$g/ml. were added to cultures 18 or 3 hr before, or 6 hr after virus inoculation, there was a 50% reduction in the number of microplaques ; but when the drug, after 18 hr incubation, was removed

TABLE 2

ANTIVIRAL EFFECT OF CEPHALOSPORIN $P_1$ AND SODIUM FUSIDATE IN HUMAN EMBRYONIC LUNG CELLS

Each tube was scored as 100, 50 or no per cent protection. The mean percentage protection in 10 tubes at each compound dilution is recorded.

| Incubation temperature (°C) | Virus | Challenge $TCD_{50}$ X | Per cent protection at 48 hr: Sodium fusidate (μg/ml.) 100·0 | 50·0 | 25·0 | 12·5 | 6·25 | Cephalosporin $P_1$ (μg/ml.) 100·0 | 50·0 | 25·0 | 12·5 | 6·25 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 33 | Rhinovirus type 1B | 229·0 | 70 | 50 | 15 | 0 | 0 | 100 | 70 | 35 | 10 | 0 |
| | | 45·8 | 100 | 70 | 40 | 15 | 10 | 100 | 95 | 50 | 50 | 15 |
| 33 | Rhinovirus type 5 | 467·0 | 50 | 50 | 35 | 5 | 0 | 100 | 80 | 50 | 45 | 0 |
| | | 93·4 | 85 | 55 | 35 | 30 | 0 | 100 | 95 | 50 | 50 | 10 |
| 37 | Coxsackie virus A21 | 38·0 | Toxic | 60 | 45 | 10 | 10 | 90 | 75 | 40 | 20 | 5 |
| | | 7·6 | Toxic | 90 | 50 | 50 | 25 | 100 | 85 | 50 | 50 | 25 |

immediately before inoculation, there was no decrease in the number of microplaques. The antiviral effect of sodium fusidate, therefore, does not persist once it has been removed from cells.

The *in vitro* activity of sodium fusidate against staphylococci is greater at pH 6 or 7 than at pH 8.3 (Godtfredsen *et al.*, 1962) but the effect on rhinovirus type 5 was found to be similar in H.E.L. cell cultures held at a mean pH of 6.9 or 7.8.

*Tissue culture studies with analogues of fusidic acid*

Twenty-six derivatives of fusidic acid were available. The variations were in the two carbon-carbon double bonds and the functional groups at C-3, C-11 and C-21; their structures are shown in Table 3. Helvolic acid, which has antibacterial activity and is structurally related to fusidic acid, was compared with the analogues.

It was hoped that an *in vitro* study of these compounds might reveal a correlation of antiviral activity with molecular structure, and that one of the derivatives might prove more active than sodium fusidate or cephalosporin $P_1$.

Cytotoxicity for H.E.L. cell strain cultures was tested as described above. Five compounds were toxic at 25 μg/ml. in less than 48 hr and were not studied further (WG 936, WG 786, EG 568, WG 646, WG 926). The toxicities of the remaining compounds are shown in Table 3.

The activity of the compounds against rhinovirus type 5 was tested by determining the minimum concentration which caused a 50% reduction in the number of microplaques. The results are shown in Table 3. Six compounds besides sodium fusidate were active at 50 μg/ml. Two were slightly active at 100 μg/ml. and the remaining 13, together with helvolic acid, showed no activity at 100 μg/ml.

Cephalosporin $P_1$ was the only compound tested that was more active than sodium fusidate, causing 50% inhibition of microplaques at a concentration of 25 μg/ml.

The effect of the compounds on adenovirus type 3 was tested by observing the inhibition of the cytopathic effect 2 and 6 days after virus inoculation in six or eight tube cultures of H.E.L. cells. The cytopathic effect took the form of small foci of round cells which

TABLE 3

THE STRUCTURE OF DERIVATIVES OF FUSIDIC ACID, THEIR CYTOTOXICITY FOR H.E.L. CELLS AND PROTECTION OF H.E.L. CULTURES CHALLENGED WITH RHINOVIRUS TYPE 5

The $ED_{50}$/ml. was measured as the minimum concentration giving 50% reduction of microplaques

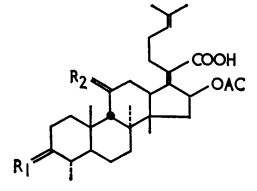
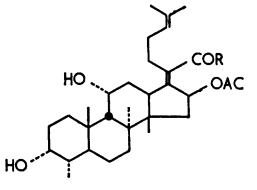
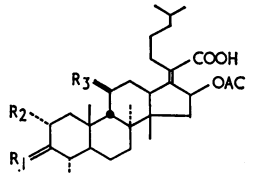
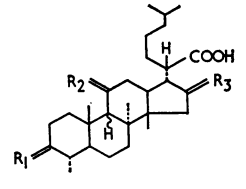
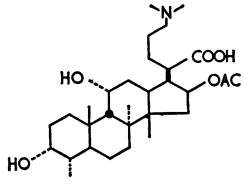
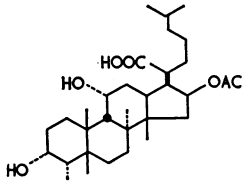
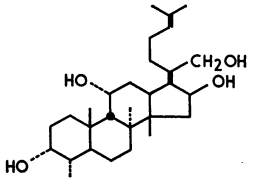
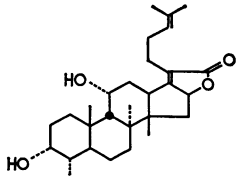
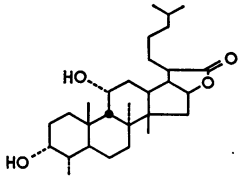
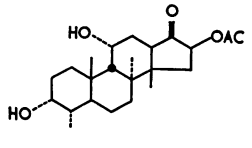
| Derivative | Structural formula | Radicals | Maximum non-cytotoxic dose ($\mu$g/ml.) | $ED_{50}$/ml. against rhinovirus type 5 |
|---|---|---|---|---|
| Fusidic acid |  | $R_1$=H, $\alpha$–OH; $R_2$=H, $\alpha$–OH | | |
| WG 931 | | $R_1$=H, $\alpha$–OH; $R_2$=H, $\beta$–OH | ⩾100 | >100 |
| WG 555 | | $R_1$=H, $\alpha$–AcO; $R_2$=H, $\alpha$–OH | ⩾100 | 50 |
| WG 646 | | $R_1$=0; $R_2$=H, $\alpha$–OH | <25 | Not tested |
| WG 885 | | $R_1$=0; $R_2$=0 | 100 | >100 |
| VD 719 |  | R=$NH_2$ | 50 | 50 |
| VD 728 | | R=NH–OH | ⩾100 | >100 |
| VD 718 | | R=NH–$NH_2$ | 50 | 50 |
| EG 568 | | R=O–$CH_2$–$CH_2$–$N(C_2H_5)_2$ | <25 | Not tested |
| WG 596 |  | $R_1$=H, $\beta$–OH; $R_2$=H; $R_3$=H, $\alpha$–OH | ⩾100 | >100 |
| WG 786 | | $R_1$=O; $R_2$=Br; $R_3$=O | <25 | Not tested |
| WG 935 | | $R_1$=O; $R_2$=H; $R_3$=H, $\alpha$–OH; 4,5-dehydro | ⩾100 | 100 |
| WG 936 | | $R_1$=O; $R_2$=H; $R_3$=H, $\alpha$–OH; 1-2-dehydro | <25 | Not tested |
| WG 792 | | $R_1$=O; $R_2$=H; $R_3$=O; 4,5-dehydro | 100 | >50 |
| CA 546 | | $R_1$=H, $OPO_3H_2$; $R_2$=H; $R_3$=H, $\alpha$–OH | ⩾100 | >100 |
| CA 547 | | $R_1$=H, $\alpha$–$OOC(CH_2)_2COOH$; $R_2$=H; $R_3$=H, $\alpha$–OH | ⩾100 | >100 |
| WG 926 | | $R_1$=O; $R_2$=H; $R_3$=O; 1,2-dehydro | <25 | Not tested |
| WG 553 |  | $R_1$=H, $\alpha$–OH; $R_2$=H, $\alpha$–OH; $R_3$=H, $\beta$–OAc, 9$\beta$–H | 50 | 50 |
| WG 579 | | $R_1$=O; $R_2$=O; $R_3$=H, $\beta$–OH; 9$\alpha$–H | ⩾100 | 100 |
| WG 602 | | $R_1$=O; $R_2$=O; $R_3$=H, $\beta$–OH; 9$\beta$–H | 100 | >100 |
| WG 603 | | $R_1$=O; $R_2$=O; $R_3$=O; 9$\alpha$–H | 50 | >50 |
| WG 604 | | $R_1$=O; $R_2$=O; $R_3$=O; 9$\beta$–H | ⩾100 | >100 |
| VD 760 |  | | ⩾100 | >100 |

TABLE 3 Contd.

| Derivative | Structural formula | Radicals | Maximum non-cytotoxic dose (μg/ml.) | $ED_{50}$/ml. agpinst rhinovirus type 5 |
|---|---|---|---|---|
| WG 618 |  | | ⩾100 | 50 |
| WG 564 |  | | ⩾100 | 50 |
| WG 554 |  | | 25 | >25 |
| WG 568 |  | | <25 | Not tested |
| WG 762 |  | | ⩾100 | >100 |

increased in size and finally coalesced. The percentage of tubes protected at 2 days was calculated. By 6 days there was no difference between treated and control tubes.

Sodium fusidate and five analogues (WG 618, WG 792, VD 760, CA 547 and WG 931) at 100 $\mu$g/ml, had no detectable effect against 2.7–4.6 $TCD_{50}$ of the virus. The most active compound appeared to be VD 719, which at 50 $\mu$g/ml. gave over 50% protection. Cephalosporin $P_1$ at 100 $\mu$g/ml. gave 50% protection. All other compounds were inactive at their maximum non-cytotoxic dose. This group of compounds, therefore, does not have a marked inhibitory effect on adenovirus type 3.

*Investigation of sodium fusidate in Coxsackie virus A21 infections in human volunteers*

Oral doses of sodium fusidate give high and cumulative concentrations in the blood (Godtfredsen *et al.*, 1962), although up to 90% may be reversibly bound to serum protein. The sera from three volunteers given 1 g three times daily for 3 days contained over 100 $\mu$g/ml. by the third day, a concentration shown to inhibit 10–100 $TCD_{50}$ of rhinoviruses and Coxsackie virus A21 in tissue culture. The prophylactic effect of oral doses against Coxsackie virus A21 in man was therefore studied.

Eight volunteers took four 250 mg capsules of sodium fusidate three times daily during meals for 8 days, and four volunteers took the same number of identical capsules containing glucose. On the third day after commencing to take the capsules, the volunteers were inoculated with a total dose of approximately 30 $TCD_{50}$ of Coxsackie virus A21 applied as a drop (about 0.025 ml.) on the anterior part of each side of the nasal septum. To determine whether the drug caused toxic symptoms, two of the volunteers taking sodium fusidate received diluent without virus.

All volunteers were examined daily for common cold symptoms and, after inoculation, nasal washings were collected daily and tested for the presence of Coxsackie virus A21. The results (Table 4) showed that colds occurred in five out of six volunteers taking

TABLE 4

INCIDENCE OF COLDS AND THE ISOLATION OF COXSACKIE VIRUS A21 AFTER ORAL DOSES OF SODIUM FUSIDATE OR PLACEBO

| Treatment | | | Nasal washes: Virus titre log10 TCD50/0·3 ml. Days after inoculation | | | | | Clinical cold | Toxic symptoms |
|---|---|---|---|---|---|---|---|---|---|
| Drug | Virus | Volunteer | 1 | 2 | 3 | 4 | 5 | | |
| Sodium fusidate | Coxsackie virus A21 | B.G.W. | 0 | 2·0 | 4·5 | 4·0 | 2·5 | + | + |
| | | D.R. | 0 | 2·5 | 3·0 | 4·0 | 2·5 | 0 | + |
| | | F.R.S. | 0 | 3·0 | 5 | 4·0 | 4·0 | + | + |
| | | F.M.S. | 0 | 1·0 | 3·5 | 4·0 | 3·5 | + | + |
| | | C.R. | 0 | 2·5 | 3·5 | 3·0 | 0 | + | 0 |
| | | C.F. | 0 | 3·0 | 2·5 | 2·0 | 3·0 | + | + |
| | | | | Geometric mean titre | | | | Total colds | |
| | | | 0 | 2·3 | 3·75 | 3·5 | 2·6 | 5/6 | |
| Glucose =placebo | Coxsackie virus A21 | A.F. | + | 2·5 | 4·5 | 4·0 | 4·0 | + | 0 |
| | | V.V. | 0 | + | 2·5 | 1·5 | 2·5 | 0 | 0 |
| | | P.E.M.B. | 0 | 2·5 | 2·0 | 3·0 | 2·0 | 0 | 0 |
| | | P.D. | 0 | 3·0 | 3·5 | 3·0 | 3·5 | 0 | 0 |
| | | | | Geometric mean titre | | | | Total colds | |
| | | | | 2·3 | 3·3 | 2·9 | 3·0 | 1/4 | |
| Sodium fusidate | None, diluent only | B.O'C. | | | | | | 0 | + |
| | | L.W. | | | | | | 0 | + |
| | | | | | | | | Total colds | |
| | | | | | | | | 0/2 | |

sodium fusidate and in one out of four taking glucose. Virus was isolated from all volunteers, titres being similar in both groups. Sodium fusidate therefore caused no reduction in the incidence of either clinical colds or infection with virus. Gastrointestinal symptoms occurred in all but one of the treated volunteers, including two not inoculated with virus. Symptoms included malaise, headache, myalgia, dyspepsia, foul taste and anorexia. Three volunteers (F.R.S., F.M.S. and C.F) had nausea and vomiting and two (D.R. and C.F.) abdominal pain and diarrhoea. One volunteer (C.F.) had faintness and stopped taking the drug. No higher dosage was therefore used.

Sera collected before virus inoculation and 3 weeks later were titrated for haemagglutination-inhibiting antibody using human foetal O cells. None of the volunteers possessed antibody before inoculation so all were presumably equally susceptible. Antibody rises after virus inoculation occurred in three out of five volunteers given sodium fusidate and in two out of two given glucose. As these numbers are small, the sera from another 14 volunteers who had been given the same dose of the same Coxsackie virus pool were tested. Nine of these volunteers had antibody rises and the final titres were similar to those in volunteers given sodium fusidate. We concluded therefore that the drug had no effect on antibody production.

The concentration of sodium fusidate in the sera collected on the morning of the day of inoculation (that is after 3 days of dosing) and 5 days later is shown in Table 5.

TABLE 5

SODIUM FUSIDATE (μG/ML.) IN SERUM AND NASAL SECRETION

All volunteers took sodium fusidate for 8 days except C.F. whose treatment stopped on the 7th day

| | Volunteers given 3 g sodium fusidate daily | | | | | | Mean drug concn. |
|---|---|---|---|---|---|---|---|
| Specimen | B.G.W. | D.R. | F.R.S. | F.M.S. | C.R. | C.F. | |
| Serum, day 3 | 63 | 106 | 124 | 98 | 88 | 112 | 99·8 |
| Serum, day 8 | 10 | 42 | 104 | 70 | 11 | 42 | 64·5 |
| Nasal secretion, day 8 | | | 1–2 | 4–5 | | | |

These concentrations would have been expected to have had some effect on the growth of Coxsackie virus A21 and another explanation for the failure of sodium fusidate was sought.

It is known that, *in vitro*, bacteria readily develop antibiotic resistance (Barber & Waterworth, 1962) but the virus strains isolated from volunteers 5 days after inoculation were found to be as sensitive to sodium fusidate as the original strain.

Nasal secretions collected from two volunteers on the fifth day after virus inoculation, by allowing their noses to drip into containers, assayed at 1–2 and 4–5 μg sodium fusidate/ml., the dose response being non-linear. The sera from these volunteers at the same time showed 104 and 70 μg/ml. (Table 5). The low concentration of the antibiotic at the site of virus multiplication seems a likely explanation for its failure to prevent infection.

*The effect of sodium fusidate taken as a snuff on Coxsackie virus A21 infection*

As a result of the foregoing experiment, an attempt was made to get a high concentration of the drug in contact with the nasal epithelium. Four volunteers took a snuff, composed of 1% pure sodium fusidate in lactose, from a measured spoon at 2 hr

intervals throughout the day. The amount taken up into both nostrils was about 50 mg; if it is assumed that the nose holds 1 ml. of nasal secretion, the maximum concentration of sodium fusidate would have been in the region of 50 mg/ml. Volunteers started taking snuff early in the morning and were inoculated with 30 $TCD_{50}$ of Coxsackie virus A21 in the afternoon; they continued to take snuff for the next 4 days.

All four volunteers developed colds and Coxsackie virus A21 was isolated from each of them, so sodium fusidate taken in this way did not prevent infection.

Nasal drips collected from two volunteers during the night and morning after they had stopped taking snuff assayed at 61 and 70 $\mu$g sodium fusidate/ml. These levels are at least 10 times greater than those achieved when the dose was taken orally, but they may not represent the concentration in the cells infected with virus, especially between doses.

Two volunteers had blood spotting on their handkerchiefs, so the dose of sodium fusidate by this route could not be increased.

It is evident that a satisfactory way of administering sodium fusidate to prevent infection with Coxsackie virus A21 has not been found. It may well be that it is unsuitable for use against respiratory infections.

## DISCUSSION

Although sodium fusidate did not prevent respiratory illness in volunteers infected with Coxsackie virus A21, the trials in man illustrate some of the problems likely to be encountered in the chemotherapy of respiratory infections.

The concentration of sodium fusidate found in the blood after oral doses could have been expected, from the *in vitro* tests, to have some effect, but the concentration in the nasal secretion was 10 to 100-fold lower, and probably insufficient drug reached the epithelial cells to alter their sensitivity to the virus. In the experiment in which the drug was administered in the form of snuff the nasal secretion contained 60–70 $\mu$g/ml. but the treatment was again ineffective; this could be due to poor distribution over the nasal epithelium or poor cellular absorption. Alternatively the failure of sodium fusidate in human volunteers may mean that inhibition of virus cytopathic effect in tissue culture at one-half to one-eighth the cytotoxic dose is not necessarily significant for protection *in vivo*.

An interesting aspect of the trial was the frequency of side effects which occurred in seven out of eight volunteers who took the drug orally. Some gastrointestinal symptoms have been reported in patients taking courses of sodium fusidate (Crosbie, 1963) but they are not usually as widespread as in this trial, where the dose used, 3 g daily, was higher than that generally recommended (1–2 g). Local application of sodium fusidate in a snuff caused mild nasal bleeding.

The antiviral activity of some steroid derivatives has been described by Cavallini, Massarani & Nardi (1964), whose test systems were influenza A (PR8 strain) in chick embryos, vaccinia in tissue culture and mouse hepatitis virus in mice. Fusidic acid also has a cyclopentanoperhydro-phenanthrene ring system and modification of the functional groups at C-3, C-11, C-16 and C-21 and in the two C=C double bonds has been shown

to alter the antibacterial activity (Godtfredsen, von Daehne, Tybring & Vangedal, 1966), but none of the derivatives tested was more active than fusidic acid and many were inactive. A similar situation was found when the derivatives were tested against viruses. However, the antibacterial and antiviral activities did not parallel each other—for example, the minimum inhibitory concentration of WG 564 for *Staphylococcus aureus* was greater than 100 $\mu$g/ml., while for rhinoviruses it was 50 $\mu$g/ml. ; and the corresponding figures for WG 885 were 5 $\mu$g/ml. for *Staphylococcus aureus* and greater than 100 $\mu$g/ml. for rhinoviruses. Cephalosporin $P_1$, which is less active than sodium fusidate against bacteria (Barber & Waterworth, 1962), had greater activity against rhinoviruses and adenovirus type 3.

There was also a difference in the effect of the derivatives on adenovirus type 3 and rhinovirus type 5. Compounds such as fusidic acid and WG 618 inhibited the rhinovirus at 50 $\mu$g/ml. but they were inactive against the adenovirus even at 100 $\mu$g/ml. This may reflect the differences in the sites of multiplication of the two viruses or in the composition of the viruses themselves. There was, however, no apparent correlation between the molecular structure and the antiviral activity of the compounds.

The mode of action of fusidic acid against bacteria has been studied by Yamaki (1965) and Harvey, Knight & Sih (1966). The primary action was found to be an immediate inhibition of protein syntheses. RNA and DNA syntheses cease later and are regarded as secondary effects. It was also shown that the incorporation of $C^{14}$-lysine into the mucopeptide fraction of the cell wall is not affected.

Preliminary experiments showed that the effect of sodium fusidate on rhinoviruses is not a direct action on viral infectivity, and the compound did not prevent the adsorption of rhinoviruses to H.E.L. cells. It must, therefore, act later in the multiplication cycle ; perhaps as with bacteria it is an effect on protein synthesis, but at the time of these studies it was not possible to define the time or mode of action further.

SUMMARY

1. In tests of antiviral activity in tissue cultures of human embryo lung cell strains sodium fusidate, cephalosporin $P_1$ and helvolic acid inhibited Coxsackie virus A21. Cephalosporin $P_1$ and three derivatives of fusidic acid were active against rhinovirus type 5 and adenovirus type 3 ; fusidic acid and five derivatives inhibited only the rhinovirus, and three others only the adenovirus. Fifteen derivatives of fusidic acid were inactive or cytotoxic.

2. An attempt to prevent Coxsackie virus A21 colds in volunteers by daily prophylactic doses of 3 g sodium fusidate was unsuccessful. The drug was found in low concentration in nasal secretion and there was no evidence of any reduction in the severity of symptoms or the extent of virus multiplication. A further experiment in which snuff was given locally was also unsuccessful.

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