REVIEW ARTICLE

THE CHEMOTHERAPY OF HUMAN VIRUS INFECTIONS

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WITH the possibility in sight of controlling the majority of infectious diseases due to protozoa and bacteria by active chemotherapeutic drugs virus diseases have become of even greater importance although, in any case, viruses are responsible for such widespread and important infections as the common cold, influenza, smallpox, poliomyelitis, and infective hepatitis, not to mention the numerous forms of encephalitis, yellow fever, and dengue. Among animal infections, foot and mouth disease, fowl pest, rinder pest of cattle, distemper and hard pad of dogs still cause enormous losses. Against some viruses it is already possible to produce an effective vaccine more especially when the various virus strains are antigenically closely similar: in other cases as in influenza, foot and mouth disease, and African horse sickness, there are many antigenically distinct strains of virus. Successful immunisation thus becomes difficult because the exact strain causing infection may be absent from the vaccine.

Chemotherapeutic treatment of virus infections, however, meets with difficulties which are not present, at any rate to the same extent, in bacterial diseases. Viruses are obligatory intracellular parasites formed of nucleoproteins. When they parasitise a cell they compete with the cell for metabolites essential for the formation of nucleoproteins and quite possibly use some of the cell's own enzyme systems. They thus enter very closely into the metabolism of the cells which they parasitise and any agent which kills the virus may also very easily damage the cell with which the virus is so closely linked. In addition, many of the signs and symptoms of virus diseases appear only when a considerable amount of cellular destruction has already occurred and by this time the virus itself may have been neutralised by means of specific immune serum formed by the body. Thus in a man infected with yellow fever the virus is successfully neutralised by immune serum within 48 to 72 hours of the onset of symptoms but the patient dies on the 5th to 7th day of illness from the injury done to the liver, kidneys, and heart. The same is true also of smallpox: by the time the characteristic skin rash appears antibodies are already present in the blood stream.

PRINCIPLES OF VIRUS CHEMOTHERAPY

There are, however, points of attack on the virus, and the chemotherapy of virus infections has already scored some successes, more especially with the larger viruses. Thus (1) the virus may be prevented

from reaching susceptible cells as suggested for the action of mucin on viruses by Armstrong[1]; (2) the virus may be destroyed while it is adherent to the surface of the cell but has not yet penetrated into the cell substance. Immune serum coats the surface of the cell so that the virus cannot penetrate into the interior. Removal of specific virus receptors on red cells can be brought about by cholera toxin (Stone[2]) or by sodium *meta*periodate (St. Groth[3], St. Groth and Graham[4]): this whole question has been fully treated by Burnet[5]. (3) Within the cell the multiplication of the virus may be inhibited because certain cellular enzymes essential for the production of essential metabolites are fully occupied by other compounds as in the case of sulphonamides and lymphogranuloma venereum. (4) When the virus is localised to certain small groups of cells these cells can be destroyed together with the virus as in the destruction of warts by podophyllin. (5) Certain virus infections are in themselves very mild, but they permit the multiplication of bacteria which are normally restrained from infecting the tissues. If these bacterial invaders are removed the virus infection itself becomes of minor importance.

The Sulphonamides

Although many folk remedies have been used for long periods very few have proved to be of value. An exception is the milky juice of the spurges. *Euphorbia helioscopia* is an old remedy for warts, described by Cockayne[6] from the Anglo-Saxon leech books; it is still used in France (Michon[7], Hissard[8]). Lyell[9] draws attention to the fact that petty spurge, *Euphorbia peplus,* causes contact dermatitis in gardeners and *E. resinifera* is frequently used as a vesicant in veterinary medicine. It was not, however, until the introduction of the sulphonamides that any controlled chemotherapy of virus infections became possible.

The chemotherapeutic action of sulphonamides is specially noteworthy on the larger viruses comprising the psittacosis-lymphogranuloma venereum group: this group comprises the lymphogranuloma venereum virus, psittacosis and ornithosis viruses, trachoma, inclusion conjunctivitis or inclusion urethritis, mouse pneumonitis of Nigg and Eaton, and cat pneumonia of Baker.

Lymphogranuloma venereum. The sulphonamides were first used by Gjurić[10], in conjunction with stibophen, for the treatment of a case with large inguinal buboes: prompt resolution occurred. Stibophen and other antimonials have no specific action on the virus. These results were confirmed in man by Kubitzki[11] and Montel and Nguyen-Van-Tho[12]. Experiments on guinea-pigs and mice infected with lymphogranuloma venereum were also positive (Levaditi[13], Bär[14], MacCallum and Findlay[15]): however, although symptoms cease the virus can still be obtained from the infected tissues for at least a year. All sulphonamides, as well as the sulphones, are active on the virus of lymphogranuloma venereum but their action is virustatic and not virucidal. In early cases of inguinal bubo in man 6 g. of sulphadiazine on the first day, followed

by 3 g. for 20 days, a total of 66 g., as a rule causes rapid improvement. When rectal stricture and chronic ulceration are present sulphonamides may have to be continued for a longer period and surgical measures may be necessary (Shropshear[16], Woods and Hanlon[17]).

The relative efficiency of a sulphonamide such as sulphathiazole is shown by Wilson[18] who treated varying types of lesion:—

Type of lesion	Total sulphathiazole dosage in gm.	No. of cases	Time of healing in weeks
Male penile lesion without bubo...	18	5	2
Male penile lesion with unilateral bubo... ...	62	10	4·5
Female elephantiasis vulvæ with ulceration ...	70	4	5
Female genito-ano-rectal syndrome	150	2	15*

* Improved but stricture persisting.

Conjunctivitis or uveitis due to lymphogranuloma venereum readily yields to doses of sulphonamides such as 3 g. of sulphadiazine for 10 days (MacNie[19], Oliphant *et al.*[20]). Fever may occur without localising signs but with a positive complement fixation test: Sulphadiazine, 1 g. 4-hourly for 2 weeks, cured the condition (Luger[21]).

The mode of action of sulphonamides on the virus of lymphogranuloma venereum is very similar to that on malaria parasites and bacteria for the virustatic action is counteracted by the simultaneous administration of *p*-aminobenzoic acid (Findlay[22], Levaditi and Perault[23], Mudrow and Bock[24], Rodaniche[25]). Takemori[26] has emphasised the difference in the response of lymphogranuloma venereum virus and typhus rickettsiæ to sulphonamides and *p*-aminobenzoic acid. In tissue cultures, as in mice, sulphonamides inhibit growth and this growth-inhibition can be antagonised by *p*-aminobenzoic acid or procaine. The growth of typhus rickettsiæ is not inhibited by sulphonamides but is inhibited by *p*-aminobenzoic acid. On the other hand, *p*-aminobenzoic acid and its esters accelerate the growth of lymphogranuloma venereum virus. Both organisms grow quite happily together in tissue culture so that there is no interference but by the appropriate drug the growth of one organism can be stimulated, that of the other inhibited.

The virus of lymphogranuloma venereum can be rendered resistant to sulphonamides (Rake[27]).

Psittacosis is very rarely sensitive to sulphonamides either in mice (Rudd and Burnet[28]) or in man (Meiklejohn *et al.*[29], Levinson *et al.*[30]) but Hinshaw[31] cured one patient with sulphapyridine. Some strains of psittacosis, however, can be shown to be susceptible to sulphonamides. Strain 6 BC, for instance, is sensitive both the sulphadiazine and penicillin in mice and chickens (Wiseman *et al.*[32], Meiklejohn *et al.*[33]). Another well-known strain "Gleason" is sulphadiazine-sensitive. Hurst[34] found only very slight increase in the mean survival-time in days when sulphadiazine was given in the food of mice whereas sulphamezathine had no

action. Golub[35] showed that 1 μg. of *p*-aminobenzoic acid antagonises the protective action of 1 mg. of sulphadiazine on 6 BC strain of psittacosis virus. Pteroylglutamic acid is less effective but 0·1 mg. antagonises the action of 1 mg. of sulphadiazine. Pteroylglutamic acid by itself is rather stimulating to the growth of the virus *in vitro*. Morgan[36] similarly showed that *p*-aminobenzoic acid inhibits the action of sulphadiazine on the 6 BC strain of psittacosis. A given dose of pteroylglutamic acid is actively antagonistic for increasingly large doses of sulphadiazine suggesting that, as in the case of certain bacteria which synthesise pteroylglutamic acid, the primary action of sulphadiazine on psittacosis virus is directed against the incorporation of *p*-aminobenzoic acid into pteroylglutanic acid.

Trachoma. For many years copper sulphate has been applied to the conjunctiva and recently (Parlange[37]) the use of bile on the conjunctiva has been again suggested. Bile was formerly recommended for trachoma in Ancient Greece but unfortunately it was thought to be equally efficacious whether it was applied directly to the conjunctiva or merely placed under the pillow at night. The use of the sulphonamides marked an important advance in the treatment of trachoma. The original observations by Heinemann[38] in the Dutch East Indies on the use of prontosil were followed by those of Dik[39], Koperberg[40], and Lian[41] also in the Dutch East Indies, Kirk *et al.*[42] in the Anglo-Egyptian Sudan, Loe[43] and Gradle[44] in the United States and Burnier[45], and Victoria[46] in South America. These early results have been amply confirmed all over the world: results may be gradual or sudden and dramatic. Sulphonamides and sulphones have been given both locally or systemically or by both routes. Systemic administration is more effective than local application. Thygeson[47], Richards *et al.*[48], Poleff[49] and others have demonstrated that the specific Halberstaedter-Prowazek bodies disappear from the conjunctiva after 10 days of treatment in 80 per cent. of cases. Bietti[50] has shown that if massive doses are given this disappearance may take place in 3 days after beginning treatment. Sulphadiazine is probably the most effective of the sulphonamides and it should be given in doses sufficient to produce a blood level of 3 mg. per 100 ml. as long as the cornea shows active signs of disease. All the evidence goes to show that the action of sulphonamides is virustatic and not virucidal, since it is possible to isolate the trachoma virus from apparently cured cases.

Pneumonitis. Sulphonamides inhibit infections in mice with the virus of mouse pneumonitis (Rake *et al.*[51]) but infections with the agents of feline pneumonitis and meningo-pneumonitis are scarcely affected (Rake *et al.*[51], Rake and Hamre[52], Eaton and Hanford[53]). Mudrow and Bock[24] found that *p*-aminobenzoic acid inhibits the action of sulphapyridine on the mouse pneumonitis virus.

Inclusion Conjunctivitis, the virus of which is probably responsible also for one of the forms of abacterial urethritis, is usually considered as having some sensitivity to sulphonamides. McKelvie[54] in Khartoum obtained good results which have been confirmed by others: local treat-

ment is less satisfactory in adults than in children. Thygeson[55] found that the virus is still infective for baboons three but not five days after treatment with sulphonamides. Epidemic kerato-conjunctivitis does not respond to sulphonamide treatment.

There is some evidence to show that viruses of the lymphogranuloma venereum-psittacosis group produce toxins. Rake and Hamre[52] found that sulphamerazine had no effect on the toxins of the viruses of lymphogranuloma venereum, mouse pneumonitis, and feline pneumonitis.

Antibiotics and Viruses of the Lymphogranuloma Venereum-Psittacosis Group

This group of viruses is also susceptible to certain of the antibiotics.

Lymphogranuloma Venereum

Penicillin. Andrewes *et al.*[56] found that penicillin would inhibit the growth of this virus in tissue cultures although they were unable to influence the infection in mice. This was almost certainly due to insufficient dosage, for Levaditi and Vaisman[57,58] and Rake and Jones[59] showed that very large doses, such as 1 million units/kg. of body weight, will not only protect against intracerebral inoculation but will completely destroy the virus in the brain. Penicillin in concentrations of 10 to 100 μg/ml. kept in contact with the virus for 2½ to 3 hours at room temperature does not have any direct lethal action (Barski and Baurin[60]). Provided that large doses of penicillin in oil are given satisfactory results have been obtained in man (Willcox[61]). Parenteral injections are much more satisfactory than the oral route. It is not without interest that Hamre and Rake[62] showed that the purer the penicillin the less active it is on this virus; in addition, penicillin is most active during the period of growth.

Streptomycin does not inactivate the virus *in vitro* (Barski and Maurin[60]) and Hamre and Rake[62] were unable to show any activity on the virus *in vivo*. Nevertheless, streptomycin was given by Lambillon[63] to 4 patients with bilateral inguinal buboes in a daily dose of 1 g. for 4 to 6 months. The average total dose was 180 g. and it is concluded that though the action of streptomycin is slow it is remarkably sure. Hirsch and Taggart[64] gave from 5 to 47 g. with good results. Zina[65] treated 2 patients satisfactorily with injections of 0·2 g. every 3 hours: the total doses were 3 and 5 g. In view of its toxicity and the fact that other chemotherapeutic agents are available, there would necessarily be some hesitation in giving this antibiotic to patients with lymphogranuloma venereum.

Aureomycin was found to have no direct action *in vitro* on the virus of lymphogranuloma venereum in concentrations of from 10 to 100 μg. per ml. (Barski and Maurin[60]). Wong and Cox[66] demonstrated that aureomycin was effective in mice infected intracerebrally and a similar result was obtained by Levaditi and Vaisman[67] who showed that a daily dose of 20 mg. by mouth for 6 days was sufficient to prevent infection in mice. Large doses were virucidal *in vitro*. Hurst *et al.*[68] found aureomycin very active in chick embryos and mice, the action being more

marked than that of penicillin but the virus still remains in mouse spleens in from 60 to 100 per cent. of animals. Early accounts of aureomycin treatment in man were enthusiastic. Wright *et al.*[69,70] and Prigot *et al.*[71] reported on the treatment of 49 cases of lymphogranuloma venereum, including 9 with inguinal buboes. A daily intramuscular dose of 10 to 20 mg. was given and all patients showed a reduction in size of the buboes after treatment for 4 days: equally good results were claimed in lymphogranulomatous proctitis, with and without ulceration, and in benign rectal stricture. Benhamou *et al.*[72] gave 750 mg. orally every 8 hours to one case and within 6 days the masses in the groin had disappeared: the electrocardiogram also returned to normal. Dowling *et al.*[73] and Harvey *et al.*[74] likewise claimed good results. Willcox[75] in Rhodesia treated one case with 250 mg. thrice daily by mouth for 2 days and then 250 mg. twice daily for 3 days, a total of 3·0 g. By the fourth day œdema had disappeared and by the fifth day the lymph nodes were no longer palpable. Robinson *et al.*[76] gave aureomycin orally or intramuscularly to 9 patients but the clinical results were not impressive and only 3 were cured. Greenblatt *et al.*[77] had only 4 cures in 13 early patients but in proctitis there was undoubted improvement when treatment was continued for 37 to 60 days. Alergant[78] treated 6 patients with inguinal buboes with 250 mg. every 6 hours to a total of 7 g.: in 4 the clinical results were excellent. Wammock *et al.*[79] believe that results are better in late than in early cases. Among 8 patients with buboes, results were good in 3 and fair in 4 but a relatively good result was obtained in one patient with multiple draining sinuses of 2 years' duration. In 9 of 11 patients with rectal strictures and, or proctitis, results were good in 6 and fair in 3: in 2 cases vomiting necessitated stopping the drug. The minimal effective dose for buboes is, it is suggested, 20 to 30 g. and for proctitis 40 to 50 g. Braley and Sanders[80] gave 100 mg. intramuscularly to a patient with recurrent unilateral uveitis and a positive Frei test. A relapse occurred but after repeating the treatment complete recovery occurred.

Lambillon[63] found aureomycin much superior to streptomycin and in two cases where streptomycin had produced only slow improvement aureomycin caused a very rapid cure. In 7 cases of proctitis and vaginitis with fistulæ and rectal stenosis a 1 per cent. solution in physiological saline was given intramuscularly once, twice or thrice a day. Aureomycin, given intramuscularly, is, of course, very painful. It is doubtful whether aureomycin actually destroys the virus, but Runyan *et al.*[81] failed to obtain virus from the inguinal lymph nodes after 3 days treatment.

Chloramphenicol. There is general agreement that chloramphenicol is less active than aureomycin. At room temperature in concentrations of from 10 to 100 μg./ml. chloramphenicol does not cause any attenuation of the virus but at 37° C. slight destruction occurs after 3 hours' contact (Barski and Maurin[80]). Levaditi and Vaisman[67] found that when the virus was injected intracerebrally into mice and 20μg. was given orally for 6 days all mice survived and their brains had no evidence of lesions due to the virus. Smadel and Jackson[82] failed to cure mice but in developing chick eggs death was delayed by chloramphenicol. McLean

et al.[83] had similar results in chicks, Hurst *et al.*[68] in chicks and mice. Alergant[78] and others have reported failure with chloramphenicol in man.

Terramycin. This antibiotic has as yet received little consideration in the treatment of lymphogranuloma venereum. Hurst *et al.*[68] found terramycin very active in mice and developing chick embryos. Andrea[84], however, reported that it was as satisfactory as aureomycin in treatment of rectal stricture : a total of 25 g. was given in 7 days.

PSITTACOSIS

Penicillin. The curative action of penicillin on ornithosis and psittacosis viruses in mice was first demonstrated by Heilman and Herrell[85,86] and Parker and Diefendorf[87]; it was later confirmed by Bedson and May[88] in mice. Meyer and Eddie[89] reported the successful treatment of infected rice birds and pigeons infected with ornithosis viruses.

Hurst[34] found that penicillin in doses of 500 units per mouse four times daily was more active than either sulphonamides or nitroakridin. Hurst *et al.*[68] found penicillin inferior to terramycin and aureomycin.

In human beings infected with ornithosis virus Turgasen[90], and Ford and Kispert[91] reported excellent results and similar effects were obtained in patients infected with psittacosis virus (Flippin *et al.*[92], Parker[93], Reimann[94], Meyer and Eddie[89], Wolins[95], Goggio[96], Stibbe[97]). There is still some uncertainty as to the best dosage. Rosebury *et al.*[98] reported a dramatic recovery in a patient accidentally infected with the 6 BC strain of psittacosis. Penicillin therapy was begun on the fourth day of illness with 50,000 units intramuscularly every 3 hours: penicillin was continued for 10 days to a total of 3,900,000 units. Goggio[96] noted no action when 100,000 units was given every 3 hours, but as soon as the dose was doubled prompt recovery took place. Meyer and Eddie[89] gave 1,470,000 units in 11 days: treatment was begun on the sixth day of illness and within 24 hours the patient was afebrile.

In mice and birds successfully treated with penicillin during an acute attack the virus is not eliminated but they become immune carriers of the virus (Hurst *et al.*[68]). In the same way Quan *et al.*[99] have found that neither penicillin nor aureomycin successfully eliminates the virus from the abdominal organs of parakeets which have become chronic carriers.

Few comparisons have been made of the relative effectiveness of sulphonamides and penicillin. Against the strain 6 BC, which in mice and chick embryos reacts both to penicillin and sulphadiazine, Early and Morgan[100,101] found both drugs equally effective when psittacosis virus was injected into mice by the intravenous or intraperitoneal routes: if the virus was given intracerebrally or intranasally, penicillin was less effective than sulphadiazine. Stibbe[97] in Holland found that three patients infected with an ornithosis virus from pigeons failed to improve on sulphonamides but promptly recovered when treated with penicillin.

Streptomycin has no effect on the 6 BC strain of psittacosis nor has *p*-aminobenzoic acid.

Chloramphenicol has been shown to be effective against the psittacosis virus when injected into the yolk sac of developing hens' eggs prior to

infection and in mice when 0·75 mg. is given four times daily intraperitoneally (McLean *et al.*[83]). However, it is inferior to penicillin, aureomycin and terramycin.

Aureomycin. The activity of aureomycin against psittacosis in mice was demonstrated by Wong and Cox[66]: Wagner[102] similarly found that injections of 0·1 or 0·5 mg. of aureomycin into mice significantly lengthened the life of infected mice as compared with controls. In eggs infected with five pathogenic strains of psittacosis virus the virus in high dilution was almost completely masked by the presence of the drug. It is noticeable that living virus was recovered from the brains of treated surviving mice 21 days after inoculation of virus. Hurst *et al.*[68] showed that, despite its activity, it caused a high carrier rate in mice.

Since then aureomycin has been used in the treatment of a number of human cases. Brainerd *et al.*[103] noted favourable results in one proved and in two probable cases. Woodward[104] observed a favourable effect in one patient while Green[105] similarly had good results with a patient accidentally infected with the 6 BC strain. The dosage was 0·75 g. every 6 hours, beginning on the fourth day of illness: after 48 hours the clinical condition had greatly improved and in 4 days the temperature was normal. Complement-fixing antibodies developed normally despite the success in overcoming the clinical symptoms. Schalm *et al.*[106] used aureomycin and penicillin in a patient infected with ornithosis virus. The action of terramycin requires investigation.

Trachoma

Antibiotics have been tested against trachoma. In Russia, *tyrothricin* in the form of gramicidin S has been largely employed but the general consensus of opinion is that it acts on the secondary bacteria rather than on the virus itself (Freyche[107]). Similar conclusions have been reached in Sardinia and France (Pasca[108], Sedan and Sedan-Bauby[109]). Netaf[110] prefers tyrothricin for use, as a local agent, to penicillin, in conjunction with systemic sulphonamides.

Penicillin has now been extensively used in trachoma but the conclusions which have been drawn as to its value are extremely contradictory. Bietti[50] treated some 150 cases with penicillin using a collyrium containing 1000 units/g.; he found that all the bacteria which accompany the virus disappear rapidly after local application renewed every 2 hours night and day: the epithelial inclusion bodies are affected more slowly and finally disappear in about 72 hours: but when treatment is stopped recurrence occurs. Focosi[111] claims good results by subconjunctival instillation or injection while Long *et al.*[112] believe that penicillin G is the drug of choice for local treatment. Jébéjian[113] found that regression went on after the cessation of treatment. In 5 cases there was complete clinical cure, 7 showed marked improvement and 5 were unaffected. Penicillin lamellæ containing 250 units were inserted twice daily for 5 days, followed by 2 days' rest: this has continued for 2 months. On the other hand many, like Fornes Peris[114], consider that penicillin is far inferior to

systemic sulphonamides when combined with the classical local therapy by copper sulphate.

Streptomycin has been little used in the treatment of trachoma and in view of the toxicity and the rapidity with which organisms become drug-resistant it is doubtful whether it is of any great value. Bietti[115] collected records of some 40 cases treated by a collyrium containing 10,000 to 100,000 μg. per g. of excipient. Bacteria disappear, somewhat more slowly than with penicillin, and the Halberstaedter-Prowazek bodies seem to persist in the majority of cases. Sometimes corneal ulceration heals but more frequently the corneal lesions remain although the action on the conjunctival secretion is rapid, the œdema and hyperæmia disappear and the papillary hypertrophy diminishes (Alberstadt and Price[116], Epstein[117], Focosi and Scalfi[118], Grignolo[119], Panzardi and Pasca[120]).

Aureomycin has now been used in trachoma with what appears to be considerable success. Braley and Sanders[80] treated one long-standing case of trachoma with an 0·5 per cent. solution of sodium borate locally: the corneal infiltration and conjunctival symptoms disappeared rapidly. In Portugal Moutinho and his colleagues[121,122,123] have described the results in 39 cases: an anhydrous 0·5 per cent. ointment was first applied every 3 to 4 hours, followed later by 1·0 per cent. ointment every 6 hours. Of 28 cases in Stages I and II 19 were cured or showed improvement: of 11 cases in Stages III and IV 8 showed cure or improvement.

Corneal complications such as keratitis and ulceration of the cornea respond readily: pannus disappeared macroscopically in a week and the trachoma follicles reacted irregularly, generally healing without scars. Inclusions disappeared in a few days. Lopes d'Andrade and Ribeiro Breda[124] gave 250 mg. by mouth every 4 hours on the first day and every 6 hours on the following days; in addition a collyrium containing 25 mg. of aureomycin hydrochloride per 5 ml. of sodium chloride and sodium borate solution was instilled locally every 2 hours. In the 6 patients treated the secondary infections disappeared rapidly but granulations and inclusion bodies persisted. Bellows *et al.*[125] instilled an 0·5 per cent. solution of aureomycin borate every 3 to 4 hours: more frequent instillation causes damage to the cornea: in one case of long standing trachoma results were remarkably good. Trope[126] in South Africa treated 8 cases in different stages with aureomycin in castor oil (5 mg. in 10 ml.). All acute signs disappeared in from 1 to 2 weeks. Desvignes and Morault[127] also report good results with collyria instilled 4-hourly: all acute signs disappeared within 5 days. Pagès[128] in Morocco finds penicillin and aureomycin equal in activity. Duke-Elder *et al.*[129] in Uganda also instilled aureomycin every 3 to 4 hours: 36 cases in all were treated and clinical cure occurred on the average in 8 to 10 days. In Italy, Bietti and his colleagues (Freyche[107]) concluded that aureomycin was about equal to penicillin. Lippi[130], it may be noted, finds that aureomycin and sulphonamides cannot be given together since aureomycin is inactivated. It is as yet too early to make any definite statement of the value of

aureomycin instilled into the conjunctival sac but with certain strains of the virus, more especially in Africa south of the Sahara, the results are very promising. Shah[131] in Pakistan had good results in 75 cases so far as acute symptoms were concerned but pannus was not affected.

Chloramphenicol. A few reports have been published of the use of chloramphenicol in trachoma. Magnol[132] using 1·25 mg. twice daily: the drug is placed in the conjunctival sac and the eye is kept closed for 10 minutes. In all 45 cases were treated: in early cases there was complete disappearance of all objective signs and symptoms inside a week. Florid complications such as large granulations disappear in 7 to 8 days and photophobia and lacrimation in 3 to 4 days. In 2 cases with ulcers healing took place in 4 days. Old chronic cases with firm granulations do not show improvement unless hard scraping is first carried out. Leo[133] treated 8 cases with ointments and 2·5 per cent. collyrium. In 2 cases with numerous inclusions these disappeared only when chloramphenicol had been given by mouth. Bietti (Freyche[107]) has also obtained good results. The effect of terramycin requires study.

While the sulphonamides thus remain the first choice for the treatment of trachoma there are three antibiotics which are undoubtedly active though they are probably virustatic rather than virucidal.

Inclusion Conjunctivitis

Inclusion conjunctivitis occurs for the most part in infants, infection being acquired from the genital tract of the mother. The evidence now available suggests that the virus of inclusion conjunctivitis is responsible at any rate for some forms of abacterial urethritis.

Penicillin was used by Sorsby[134] locally in 44 of 63 cases with excellent results: one patient relapsed but was subsequently cured by a further course.

Aureomycin was shown by Braley and Sanders[80] to have an action on 6 cases of inclusion conjunctivitis: the purulent discharge had disappeared in 24 hours as had the typical inclusion bodies but the conjunctival lesions required from 3 to 7 days to heal. Duke-Elder *et al.*[129] also reported that aureomycin was active in inclusion conjunctivitis.

In cases of inclusion urethritis there is evidence that aureomycin and terramycin are of considerable value: chloramphenicol is also active though relapses may occur (Findlay, Willcox and Howard[135]). Thiers and Pinet[136] found dihydrostreptomycin active in a single case where aureomycin had failed.

Epidemic kerato-conjunctivitis. Patel[137] believed that a combination of "sulphatriad" tablets and 300,000 units of procaine penicillin were responsible for the prompt care of six cases of this infection. Jacobson and Levin[138] had previously reported similar results with penicillin and succinylsulphathiazole. Holmes[139] in Hawaii cured 58 cases with aureomycin and, in Italy, Capalbi[140] obtained excellent results by means of a collyrium containing 25 mg. in 5 ml. of fluid. The effects of chloramphenicol and terramycin do not appear to have been studied.

Bellows *et al.*[125] found that 0·5 per cent. solution of aureomycin borate reduced the period of morbidity to from 4 to 8 days. Corneal infiltration is not prevented.

Pneumonitis Viruses

It is now recognised that there are a number of viruses in mice, cats, and cotton rats. In addition, atypical pneumonia in man is most probably due to a virus although only on rare occasions has a virus been isolated. Some of the animal pneumonitis viruses are susceptible to sulphonamides, but Rake and Hamre[52] showed that sulphamerazine had no effect on the toxins of the viruses of mouse pneumonitis or feline pneumonitis. Manire and Meyer[141] similarly found that neither penicillin nor aureomycin has any effect on the toxin titres of the Lousiana and feline pneumonitis viruses, that is there was no effect on the first phase deaths, believed to be due to the action of toxins. Similarly, treatment of mice with penicillin or aureomycin has no effect on the toxic action of a human pneumonitis virus. There was some evidence that the toxins of some avian ornithosis viruses were inhibited by aureomycin.

Eaton[142] showed that in the developing chick embryo mouse pneumonitis is inhibited by 100 units of penicillin 24 hours after infection. Cat-pneumonitis requires 5000 units, given 2, 72, and 144 hours after infection. Hamre and Rake[62] also found feline pneumonitis to be slightly susceptible to penicillin. *In vitro* pure penicillin G has no effect in a dose of 2000 units on mouse pneumonitis virus but impure penicillin is virucidal *in vitro*. Chloramphenicol has but a slight action on feline pneumonitis (Kneeland and Price[143]).

Human Pneumonitis

Human pneumonitis or atypical pneumonia has become comparatively common in America and in Great Britain cases appear to be on the increase. In addition to the physical signs it is common to obtain high titres for cold and for Streptococcus MG agglutinins. A number of observers (Schoenbach and Bryer[144], Kneeland *et al.*[145], Finland *et al.*[146]), showed that aureomycin was extremely effective in atypical penumonia. Meiklejohn and Shragg[147] noted that aureomycin is far more effective than penicillin when parallel series of cases were treated during the same seasonal outbreak, in the same area, and among the same military population. Schoenbach and Bryer[144] recommended 100 to 250 mg. every hour for 3 doses and then every 2 hours till the patient is afebrile: thereafter aureomycin is given every 4 to 6 hours for 2 to 5 days in a dose of 15 to 20 mg./kg. of bodyweight. Of 13 patients thus treated 9 were afebrile in 24 hours after the institution of aureomycin therapy, 3 became afebrile in from 24 to 48 hours and 1 became afebrile in 72 hours after beginning treatment. Fever fell by lysis. Blodgett *et al.*[148] believe that smaller doses are equally effective; 12 patients received 250 mg. at 6 hour intervals and 2 at 4 hour intervals: 10 patients were afebrile at the end of

48 hours, 3 at the end of 72 hours and 1, after an initial response, had a low grade fever for 4 days. No relapse occurred and intestinal symptoms due to aureomycin were very slight. Thompson and Spector[149] found aureomycin of value in abacterial bronchiolitis. It is perhaps fortunate that aureomycin is as effective against pneumococcal as against atypical pneumonia.

Terramycin has been found by Kneeland *et al.*[150] to give highly satisfactory results in doses of 2 g. daily for 6 or 7 days.

Other Compounds in Treatment of Viruses of the Lymphogranuloma-Psittacosis Group

Brief reference may be made to the treatment of this group of virus infections by other compounds.

Shah and Awan[151] have recently revived the use of intramuscular injections of benzyl cinnamate for trachoma: this method of treatment was originally recommended by Jacobson[152], who gave a series of 12 intramuscular injections and claimed that 56 per cent. of 244 cases showed marked improvement.

Mauer[153] found that 50 per cent. of mice infected with psittacosis virus can be saved by treatment with acriflavine. Later, Eaton *et al.*[154] showed that growth of the viruses of lymphogranuloma venereum, feline pneumonitis and meningopneumonitis was inhibited in eggs by acriflavine, 3-nitro-6,7-dimethoxy-9-(2-phenyl-4-diethylaminobutylamino)acridine and 3-nitro-6,7-dimethoxy-9-2(2-hydroxy-3-diethylaminopropylamino) acridine. The first two compounds were less active against the virus of mouse pneumonitis: the relative resistance of the mouse pnuemonitis virus to acriflavine and to two of the nitroacridines is of interest in view of the fact that it is quite susceptible to sulphonamides and to penicillin. Feline pneumonitis and meningopneumonitis on the other hand are relatively resistant to penicillin and sulphonamides but are easily inhibited in chick embryos by acriflavine and the nitroacridines. It is thus possible that penicillin, sulphonamides and acridines employ different virucidal mechanisms on the viruses of the lymphogranuloma-psittacosis group: only the sulphonamides are inhibited by *p*-aminobenzoic acid.

Hurst[34] found that nitroacridine 3582 possesses some activity against the virus of psittacosis in mice, being more active than sulphonamides but less efficient than penicillin when given in a daily dosage for a 20 g. mouse of 0·25 mg., intraperitoneally, the dose being dissolved in 0·5 ml. of sterile distilled water. If very small doses of virus are injected there is a tendency for many mice to die about a week after the cessation of treatment, so that the final mortality is as great as in controls. The same phenomenon does not occur if large doses of virus are given. It would seem that with larger doses of virus an immune body response occurs so that any virus particles not destroyed by the drug are subsequently held in check by the antibodies formed: with very small doses of virus no such antibody response occurs. Peterson and Fox[155] noted the same phenomenon in tsutsugamushi disease treated with methylene blue for

there also treatment could be discontinued sooner after a massive infecting dose than after a smaller one.

Proflavine in a concentration of 0·001 M and mepacrine in the concentration inactivate psittacosis virus when incubated *in vitro* at 37°C. *p*-Chloromercuribenzoate under the same conditions causes complete inhibition in a concentration of 0·0001 M. Glutathione and cysteine prevent or reverse the inactivating action of *p*-chloromercuribenzoate. Sulphydryl groups are therefore probably present in the virus (Burney and Golub[156]).

Variola-Vaccinia Viruses

Although the variola-vaccinia group of viruses almost equals in size the lymphogranuloma-psittacosis group no successful antiviral chemotherapy has yet been devised. The smallpox virus first multiplies in the reticulo-endothelial system of the internal organs, no symptoms are then present and before virus reaches the reticulo-endothelial system there is a symptomless period of primary viræmia when virus is passing from the naso-pharynx to the internal organs. At the time of onset of symptoms and before the rash there is a second period of viræmia. When the typical skin lesions appear, antibodies have already been produced but a bacterial infection of the pustular lesions then takes place with hæmolytic staphylococci and streptococci. If virus could be attacked while in the blood stream, symptoms of the disease might well be aborted: this is the rationale for the intravenous injection of 1 in 1000 potassium permanganate solution in a daily dose of not more than 10 ml. for adults, a proceeding of doubtful value. Antibacterial chemotherapy, however, is of great importance, once the rash has come out, although even if the bacterial infection is overcome the patient may die from toxæmia, the result of the cellular damage which has been caused by virus action.

Sulphonamides do not have any direct action on variola virus but in large doses they may help to overcome bacterial infection. Sulphadiazine is probably the most satisfactory compound (Osborne[157]) but its action on the kidneys must be remembered. Pure penicillin seems to have little or no action but there is some evidence that impure penicillin has an action on experimental vaccinial infection (Groupé and Rake[158]): there is some uncertainty as to the antiviral substance in crude penicillin; possibly it is *o*-hydroxyphenylacetic acid (Fischbach *et al.*[159]). There is no doubt that when the rash has appeared penicillin should be given in large doses of at least 2 mlilion units daily. Johnstone and Fluker[160] suggest that when the vesicles become umbilicated or their contents turgid intramuscular injections of streptomycin should also be given and continued for 14 days or until the lesions have dried up: in severe cases the penicillin dosage should be increased to 2·5 million units daily. In discrete or modified cases the injection of streptomycin is probably unnecessary. Chloramphenicol has no direct action on vaccinial or variola infection in the developing hen's egg (McLean *et al.*[83]) nor it seems have aureomycin or terramycin. Perry and Martineau[161], however,

believed that aureomycin was of value in curing a vaccinial infection accidentally acquired. Felsenfeld *et al.*[162] found neomycin of no value in preventing vaccinial infection of the rabbit's cornea. When, however, 500 units of bacitracin were applied to the cornea, not later than 6 hours after infection and repeated 24 and 48 hours later, infection was prevented. When vaccinia virus was injected intracerebrally into mice and 200 units per kg. of bacitracin were injected, infection was completely aborted: an injection of 10 mg./kg. of neomycin showed a favourable action in only half the mice. In addition to an action on the 8th cranial nerve neomycin is toxic for the kidneys.

Certain other unrelated compounds have been found active against vaccinia either in the mouse, the chick embryo or in tissue cultures. Hamre *et al.*[163], for instance, showed that two thiosemicarbazones, *p*-aminobenzaldehyde-3-thiosemicarbazone and *p*-acetamido-benzaldehyde thiosemicarbazone, delayed the death and allowed the survival of a small percentage of mice and of chick embryos infected with vaccinia virus. These compounds are inactive against swine influenza virus and the meningopneumonitis virus. Thompson *et al.*[164,165] found that amides of 5-aminouracil inhibit the multiplication of vaccinia virus in tissue culture. Based on antagonisms demonstrable when using *Lactobacillus casei*, it was suggested that the most active member of the series would be 5-chloroacetamidouracil or amides of 5-aminouracil with other acid pK_a values near that of chloracetic acids. Actually the activity of chloroacetamidouracil was greater than the predicted value. A high degree of antiviral activity was found to be a function of all the chloro- and bromoacylamides and dichloracetamides, including chloroacetamide. 5-Dichloroamidouracil is inhibitory whereas 5-fluoroacetamidouracil is inactive.

It may be noted that chloramphenicol is related to 5-chloroacetamidouracil, 5-dichloroacetamidouracil and α-chloro-*p*-nitroacetanilide. If the virucidal activity of chloramphenicol resides in the dichloroacetamide linkage it is possible that all haloacylamides are potentially virucidal.

Influenza

Up to the present no chemotherapeutic agents have been found sufficiently active to warrant their use in influenzal infections in man.

Green *et al.*[166] found that 2,3-dimethoxy-6-nitro-9(diethyl-aminooxypropyl)aminoacridine dihydrochloride, or nitroacridine 3582, was capable of inhibiting or delaying the development of from 1 to 10,000 minimal infected doses of influenza virus B (Lee strain) in developing chick embryos. The same compound had a similar but less pronounced effect on influenza A (PR 8 strain) in developing chick embryos. Although nitroacridine 3582 had some virucidal action *in vitro*, this is insufficient to account for the degree of inhibition observed *in vivo* (Rasmussen *et al*[167]).

The effect of some 24 antibiotic lactones and their analogues on the PR 8 strain of influenza A virus in mice was studies by Rubin and

Giarman[168]. Some of the furan series were active and, in addition, the following compounds had some action: γ-butyrolactone; 6-methoxy-8-(2,5,dimethylpyrryl-1)-quinoline; 3-phenyl-2-butene-1,4-olide; 3-methyl-5-carboxy-2-pentene-1,4-olide; parasorbic acid, and *iso*clavacin. These compounds were not administered until the disease had reached a fairly advanced stage in mice. Fleischer[169] observed that certain dyes in the quinone-imide group had an effect on the virus *in vitro*. Of these dyes the only ones which were effective *in vivo* were Janus green B and safranine-pyrazalon-sulphonamide. Janus green B was the more effective but the *in vivo* effect is evident only when the chemical is injected into mice intravenously in a dosage close to the LD50. Both dyes had a more pronounced effect against the PR 8 strain of influenza virus than against other strains. Hoyle[170] finds that certain basic dyes of the triphenyl-methane group, especially dahlia violet and crystal violet, can in suitable doses retard and reduce the intracellular growth of the influenza virus, as measured by the production of complement-fixing soluble antigen in the chorio-allantoic membrane of eggs inoculated with a large dose of virus. It is suggested that these dyes interfere with the metabolism of ribonucleic acid in infected cells but unfortunately they are more toxic than other dyes to the embryo so that it is unlikely that they would be of any chemotherapeutic value in influenza. Green[171,172] has shown that tannic acid or a commercial sample of black tea will inhibit multiplication of influenza A virus in embryonated eggs while Wagner and Stacy[173] find that *in vitro* sodium periodate, *p*-benzoquinone and potassium permanganate all inactivate influenza A virus up to 50,000 infective doses. Inactivation is associated with dehydrogenation. Klein and Perez[174], Perez *et al.*[175] and Klein *et al.*[176] have shown that influenza A virus can be inactivated by mercurials such as mercuric chloride. Sodium thioglycollate and dimercaprol in a dilution of 1 in 400 are able to reactivate the virus in the chick embryo if given 3 to 5 hours before and from 15 minutes to 2 hours after the injection of inactivated virus. Similarly in mice the injection intramuscularly of 0·1 ml. of a 1 in 200 aqueous solution of dimercaprol some 5 to 15 minutes before the intranasal instillation of virus inactivated by bichloride of mercury can reactivate the virus: 30 minutes after injection of inactivated virus dimercaprol has no effect in bringing about reactivation. Thus though a chemical agent may be active *in vivo*, with a direct action on the virus, the adsorption of virus on to the cells of the host will protect it against the action of the chemical agent. Aureomycin is of little value except where secondary bacterial infection is present (Thalmann *et al.*[177]).

The possibility of preventing infection by destruction or removal of the specific virus receptors has already been mentioned. The receptors are probably complex muco-polysaccharides, since they are destroyed by periodate (Hirst[178]). Green and Woolley[179] find that complex carbohydrates such as gum acacia, flax seed mucilage, citrus pectin, apple pectin and blood group A substance inhibit the agglutination of influenza virus by fowl red cells. Apple pectin not only inhibits hæm-agglutination but also prevents multiplication of the influenza virus. It

seems probable that this is an example of biological competition between apple pectin and the receptor substance for influenza virus. Ginsberg and Horsfall[180] and Hurst and Stacy[181] have further investigated and discussed this question in relation to influenza while Horsfall and McCarty[182] and Ginsberg and Horsfall[183] found that polysaccharides of bacterial origin, as well as from other sources are capable of modifying the course of infection by the pneumonitis virus.

Although successful chemotherapy of influenza has not been attained, Andrewes and Niven[184] have found that grey-lung virus in the mouse, a virus which does not belong to the lymphogranuloma-psittacosis group, is readily inhibited by aureomycin and terramycin but not by chloramphenicol, penicillin or sulphonamides. Eaton[185], it may be noted, found that aureomycin, but not chloramphenicol, was active against his "atypical pneumonia virus" in cotton rats.

Warts

Countless methods of treatment of warts have been employed, including pure magic and psychotherapy. Many of the treatments recommended such as sulpharsphenamine or bismuth have failed to take into account the fact that warts is a self-limiting disease (Miller and Delaney[186], Paulosky and Leider[187]). In 1942 Kaplan[188] showed that 25 per cent. podophyllin in mineral oil caused rapid cure of soft warts. This result has been confirmed by numerous studies throughout the world. For many years resin of podophyllin was used as a folk-remedy for genital warts in New Orleans. The active principle is a complex of resinous substances obtained from the dried rhizome and roots of the may apple *Podophyllum peltatum* L, a perennial plant in the middle and northern United States. Sullivan and King[189] bring forward evidence to show that solutions of resin of podophyllum are inert when treated with sodium or potassium hydroxide: podophyllotoxin which is destroyed by such treatment is therefore the active substance. Resin of podophyllum is a very active cutaneous sensitiser and is intensely irritating to mucous membranes; thus in the male with genital warts it may only too easily cause extensive balanitis. Podophyllotoxin is far more active than podophylloresin or picropodophyllin (Sullivan *et al.*[190]). In place of podophyllum in oil Alechinsky[191] and Sullivan and King[189] have found a 20 per cent. solution of podophyllum in alcohol (95 per cent.) far less irritating. Common warts are usually not affected by podophyllum in oil because, owing to their horny exterior, the drug does not penetrate. With the alcoholic solution there is better penetration and 15 out of 100 cases of common warts were readily cured. Kurtin and Yontef[192] apply podophyllin to plantar warts by paring the upper corneous layer with a small electric hand drill till bleeding begins. The skin surrounding the wart is painted with collodion and a drop of podophyllum suspension is put into the small excavation: a strip of well-flamed adhesive plaster is then fixed over the lesion. The plaster is removed on the eighth day and the grey friable mass is scooped out. Warts on other parts of the body can be similarly treated. Superficial warts in cattle and dogs have

also been treated but the venereal sarcomata of dogs, though they may show some retrogression, do not entirely disappear (Ingram[193], Alechinsky[191], Collet and Vellut[194]). It has also been suggested that podophyllum should be used in papilloma of the bladder, which may or may not be due to a virus (Garzón and Firstater[195,196], Semple[197], Jarrams[198]). This proceeding, however, is not without danger for, in addition to damaging the bladder mucosa the podophyllum may be absorbed causing diarrhœa or the resin may form a calculus in the bladder.

Both podophyllum and colchicine have been used in the treatment of superficial new growths on the skin of man where there is no evidence of virus infection. Podophyllum and colchicine appear to exert a dual action on cells. In the nucleus, mitosis is stimulated so that there is an increase in the number of mitotic figures but these are arrested in metaphase so that there is a complete absence of anaphase and telophase figures: in addition, there is an effect on the cytoplasm through interference with ribonucleic acid metabolism (Sullivan and Wechsler[199], King and Cauldwell[200]).

In connection with the chemotherapy of the virus tumours it is interesting to note that pteroylglutamic acid is apparently an essential growth factor for the cells of the Rous sarcoma: by substituting analogues of pteroylglutamic acid such as 4-amino-*N*-methyl-pteroylglutamic acid, 4-amino-pteroylaspartic acid and 4-aminopteroyl-D(-)glutamic acid it is possible to inhibit the tumour growth (Little *et al.*[201]). Use of these analogues in human leukæmia has not yet met with great success.

Other Viruses

Many other virus infections have been treated with chemotherapeutic agents.

One of the most promising results has been in zoster. Finland *et al.*[202] treated 24 cases with 1 g. of aureomycin four times daily. They claim rapid healing within 24 hours, usually complete in 7 or 8 days. In 4 cases, however, new lesions broke out during the first 2 days of therapy. In all cases the pain was greatly reduced in from 4 to 5 days. Binder and Stubbs[203] obtained similar results, as did Jessen and Mosegaard[204] in 2 cases. On the other hand, Braley and Sanders[80] saw no effect in two cases of keratitis due to zoster. Philip[205] treated 8 cases: in 4 the pain had gone within 12 hours but the eruption was unchanged, in the other four pain continued but in 72 hours the eruption had healed. One patient who was given aureomycin ointment locally had no pain after 36 hours and in 4 others with local and systemic treatment pain had gone in 72 hours though the eruption took three weeks to heal. Other observations show that zoster has actually broken out in patients who were taking aureomycin, and in varicella when Mazursky *et al.*[206] treated 41 cases with aureomycin they could see no difference between the treated and the control cases. Incidentally, some of the treated patients developed mumps. Dawson and Simon[207] and St. John[208] claim good results with chloramphenicol. It must be remembered that zoster is a self-limiting

disease with symptoms of varying severity. Obviously careful investigations must be made in a long series of cases before the value of these antibiotics can be correctly assessed.

The same criticism can be levelled against the reports on the treatment of simple herpes, made by Braley and Alexander[209], Zeller and O'Connor[210], Braley[211], and Everett[212]. Braley and Alexander[209] believe that aureomycin has some retarding effect on herpes simplex virus when injected intracerebrally in mice: incidentally, in the discussion of their paper various speakers mentioned 17 cases in which only 6 appear to have benefited. Thygeson and Hogan[213] treated 24 cases of herpes of the cornea with drops of 0·5 per cent. aureomycin borate: 14 cases responded, but they confess that these results are not superior to iodising the cornea. Zelman and O'Neil[214] found that aureomycin cured a very intractable herpetic ulceration of the buccal mucosa but in rabbits experimentally infected on the cornea and in 12 cases of labial herpes the results were doubtful.

Baer and Miller[215], Bereston and Carliner[216], Borrie[217], Bookman[218], and Rook and Upjohn[219] report a total of 8 cases of Kaposi's varicelliform eruption due to herpetic infection. New vesicles continue to appear after the administration of aureomycin and any benefit is due to control of secondary bacterial infection.

Aureomycin has no action on vaccinial keratitis (Bellows *et al.*[125]).

Incidentally, when aureomycin is to be applied to skin lesions it is advantageous to coat the area with a film of aureomycin in methyl cellulose (Kalz *et al.*[220]).

There is as yet no specific treatment for colds, and antihistamines are useless. Good results are claimed in mumps with chloramphenicol Ghalioungui[221]).

Levaditi and Vaisman[222] claim that guinea pigs infected with foot and mouth disease and given aureomycin 100 mg./kg. of body weight daily for 2 days are protected from infection: this dose, however, is toxic for guinea pigs.

There are many other possibilities in regard to the suppression of virus infections. Absence of certain food factors may reduce the virulence of an infection while the injection of a non-pathogenic virus before a virulent one may interfere with the virulence of the latter virus. These factors are discussed by Findlay[223] and Henle[224]. Evidence brought forward by Isaacs and Edney[225] suggest that interference is an intracellular and not a surface phenomenon. The next few years will undoubtedly see many fresh advances in the chemotherapy of virus infections.

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