CHEMOTHERAPEUTIC EXPERIMENTS WITH GREY LUNG VIRUS.

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GREY lung virus, described by Andrewes and Glover (1945), is an agent pathogenic for mice, cotton-rats and, to a lesser extent, other rodents. Its natural habitat is uncertain; though possibly of rodent origin it does not now exist as a latent infection of our laboratory mice. While many viruses persist for long in recovered animals, grey lung virus exhibits this property *par excellence*: it rarely kills its host, but causes extensive lung lesions which remain indefinitely—at least for eighteen months. Virus, also, persists indefinitely in the lungs and in high titre: neither active immunity nor antibody-production have been demonstrable.

While on a visit to Dr. K. Goodner, of Jefferson Hospital, Philadelphia, in October, 1949, one of us (C. H. A.) was shown a rather similar condition in mice under study there, and was informed that that infection (" Potash virus ") was susceptible to treatment with aureomycin. Accordingly, on returning to England tests were made of the activity of aureomycin against grey lung virus.

EXPERIMENTAL METHODS.

Mice were inoculated intranasally under ether with centrifuged suspensions of infected lungs (0·05 ml.); these were either freshly obtained from mice killed at any time from a week to a year after infection, or were removed from storage in a dry ice (— 76° C.) container. The experimental mice were killed 9 to 13 (usually 10) days after infection and an assessment made of the extent of lung-involvement. In untreated animals this was usually so extensive as to involve all lobes, which were distended, grey and oedematous. So few mice died that mortality was useless as a measure of the effectiveness of treatment. The dosage of virus used was of the order of 10^4 to 10^5 m.i.d.

Aureomycin was suspended in physiological saline containing 5 per cent gum acacia. In most experiments the strength of aureomycin was 2 mg./ml., and 0·5 ml. was given subcutaneously. Such injections produced moderately severe local reactions, so the site of injection was varied at each dose.

RESULTS.

Effect of Aureomycin.

When mice were given 1 mg. aureomycin once or twice daily from the day of inoculation until the tenth day afterwards, lung lesions were wholly suppressed (mice killed on thirteenth day).

The histological structure of the lungs of treated mice was always compared with that of untreated animals inoculated at the same time. The development

TABLE I.—*Effect of Aureomycin on Grey Lung Virus.*

Lesions in		
Control mice.	Mice on 1 daily dose.	Mice on 2 daily doses.
+++ +++ +++	0 0 0 0	0 0 0 0
+++ +++ +++	0 0 0	0 0 n.sp.*

+++, ++, +, 0 indicate degrees of lung involvement.

n.sp. = non-specific lesion: no virus recovered on passage.

* = non-specific death.

of the lesions up to the end of the usual experimental period (10 to 14 days) has already been described (Niven, 1950). At this stage abundant fluid exudate is already present in the alveolar spaces, and considerable areas of consolidation, composed of enlarged desquamated septal cells and varying numbers of polymorphs have formed. Infiltration of mononuclear cells around arteries, and to a lesser extent around bronchioles and bronchi, has also begun, and an increased cellularity of the epithelium of the respiratory tract generally within the lung is apparent.

Following treatment for 10 days, histological examination confirmed the impression gained from naked-eye inspection that the lungs in the treated animals were normal. No cellular accumulations or fluid exudate were found in the alveolar spaces, and the walls of the blood vessels and bronchi were free from infiltrating cells. Only occasionally in the septal capillaries was a slight excess of polymorphs noted. That the causative agent did in fact obtain a foothold in the lungs for a short time before its activity was cut short by aureomycin administration is suggested by the abundance of polymorphs in the sinuses and within phagocytes in the tracheo-bronchial glands.

The suppressive effect of aureomycin was reproduced in many subsequent experiments, but such drastic treatment was not found necessary. Almost complete suppression of lesions was produced by daily doses of 0·25 mg., but the results were less consistent. Only in three out of nine animals of the series were the lungs identical on histological examination with those of the previous series (1·0 mg. per day). In the others, lesions varying from small sub-pleural areas of consolidation to almost complete lobar involvement were found, but the amount of fluid exudate was always much less or absent, polymorphs were much less numerous, and mononuclear infiltration less extensive. Since one daily dose was effective, this routine was used in all later experiments. Good results were obtained and lesions apparently wholly suppressed when beginning of treatment was delayed until five days after infection. Microscopic examination showed, however, that suppression of the lesions was not quite complete. In addition to a few small areas of perivascular infiltration, the capillaries generally showed an excess of polymorphs, and at the periphery of the lung, where areas of consolidation most commonly first appear, the alveolar septa were enlarged and prominent. The alveolar spaces, however, contained neither fluid nor free cells. In each of two tests lesions were suppressed by only two doses of 1 mg., given at the time of inoculation and on the following morning. In one experiment a single dose of 1 mg. given at the time of infection was equally successful, but another similar attempt, against more potent virus, failed. Administration of drug into the stomach, by the aid of a syringe fitted with a wide blunted needle,

was also effective, but larger doses (2·5 to 5 mg. twice daily) were necessary, and even then suppression of lesions was less complete. In fact, the range of histological appearances varied from almost complete suppression, as described above, to a condition practically indistinguishable from that seen in the untreated animal.

It was found that aureomycin suspensions boiled for 5 minutes or allowed to stand for several days at room temperature were still effective; from the relevant literature it did not seem that aureomycin should be as stable as this. The question therefore arose as to whether aureomycin itself, or some impurity or breakdown product, was responsible for the therapeutic effect. Careful comparison was therefore made of the activity of aureomycin against grey lung virus and against a staphylococcal culture. (We express our thanks to our colleague Dr. A. T. Fuller for collaborating in these tests.) Aureomycin boiled for 5 minutes retained nearly all its activity against both test objects: but after boiling for one hour its activity against both was reduced to about 10 per cent. of its former value. It was also found that a sample of aureomycin "purified for intravenous use" was as effective as other samples. Histological study confirmed throughout the impressions gained by naked-eye inspection. We found, therefore, no reason to conclude that the activity was due to anything other than aureomycin itself.

Effect of delayed treatment.

Most chemotherapeutic agents are progressively less effective the longer the interval between infection and their administration. We were therefore surprised to find aureomycin so effective against grey lung virus when the beginning of treatment was delayed for five days. Subsequent tests showed that treatment might be postponed for three months and lesions could still be cleared up. Only small numbers of mice were available for these tests, but the results were consistent.

In these experiments 1 mg. doses were given daily over a period of 10 days, and mice were killed at the end of the course of treatment. The ± lesions represent small indefinite whitish areas in the lungs.

When the disease has been in existence for 2 to 3 months complete restoration of normal structure would scarcely be possible, for the usual alveolar pattern of the lungs by that time may well be distorted by the disintegration of alveolar cells and related interalveolar septa (Niven, 1950), and the disappearance of the large encircling cuffs of mononuclears is always followed by perivascular and

TABLE II.—*Effect of Aureomycin on Long-standing Infection* (6 *Experiments*).

Period after infection.	Lesions in untreated mice.	Lesions in treated mice.
3½ months.	+++	0
3½ months.	+++ +++ +++	± ±
3 months.	+++	0 0 0
3 months.	+++ +++ +++ +++	± ± ±
2 months.	+++ +++ ++	0 ± ±
2 months.	+++ ++ 0	0 0 0

peribronchial fibrosis. In addition to these signs of pre-existing infection, all the animals of this series showed some degree of perivascular and peribronchial infiltration, which was, however, very slight in those in which the lungs were macroscopically normal. In the others an assortment of residual lesions was found amongst which areas of resolving consolidation, unaccompanied by invasion of polymorphs, were the most conspicuous. The most interesting feature was the complete disappearance of fluid exudate, except in those lungs in which considerable alveolar consolidation still remained, and even in those cases it was much reduced in amount. The epithelial hyperplasia also had undergone almost complete regression, and excessive mucus secretion had ceased. The surprising thing is, not that clearing-up of lesions was incomplete, but that so much resolution was produced so quickly in lesions of 3 months' standing.

In our earlier studies no evidence was found of development of immunity to grey lung virus in mice. We were now able to test whether mice cured by aureomycin were susceptible to re-infection with the virus ; this proved to be the case. Six mice were infected with grey lung virus, and cured by a course of six 1-mg. doses of aureomycin given between the fifth and eighth days after infection. Three were challenged with a dose of grey lung virus on the forty-first day, and all developed widespread lesions. These apparently developed as quickly and extensively as in animals following primary inoculation, and the appearances were in striking contrast to those of the series which were not re-infected ; two of these had trivial, doubtfully specific lesions, and the third none at all. Histologically, scanty foci of infiltration were noted in two, while in the third, the focus of infiltration found was trivial in comparison with the florid lesions of the reinfected group.

Two other experiments gave similar results.

Effect of aureomycin in eliminating virus.

Chemotherapy of infections of the psittacosis group with sulphonamides and other drugs may save the lives of mice or prevent lesions, but virus is not eliminated, nor, in the case of parakeets, are carriers cured (Quan, Meyer and Eddie, 1950). With aureomycin treatment of grey lung virus infection, however, virus seems to be extirpated. When mice are cured of infection, they may be killed at any time up to three months later, and the lungs will still be found to be clear : since active immunity seems not to be induced, this in itself indicates that there is no persisting pathogenic virus. We tried to recover virus from lungs of such cured mice, killed after eight and fifteen days : none was obtained, even after two serial passages. Even from a chronically-infected mouse, killed after a course of therapy carried out after infection, no virus was recovered.

Effect of Other Drugs.

Against most bacterial infections, chloromycetin (chloramphenicol) and aureomycin show a similar range of activity. Against grey lung virus, however, chloromycetin seemed quite impotent. Doses up to 5 mg. were given intraperitoneally twice daily from the first to ninth day after infection ; mice so treated showed lesions similar to those of controls.

Penicillin procaine in three divided doses, each of 33, 333 units, and streptomycin twice daily in doses of 0·2 to 0·4 mg., were equally ineffective. So, too,

was sulphamerazine (7·5 mg. daily intraperitoneally); indeed in some experiments we gave sulphamerazine routinely to all experimental animals, to keep under control the mouse pneumonitis (Nigg, 1942) virus which was prevalent in our stock mice. The results were again checked by histological study.

On the other hand, terramycin, in doses of 1 to 2 mg. daily for ten days, completely suppressed grey lung virus lesions; when given subcutaneously the local reactions produced were much less than those which aureomycin caused. Histologically the lungs of treated mice in this series showed a normal structure; a slight excess of polymorphs in the interalveolar septa and sinus catarrh and active phagocytosis of polymorphs in the tracheo-bronchial glands were the only abnormal findings.

It may be noted that Smadel and Jackson (1948) found chloromycetin effective against lymphogranuloma injected intraperitoneally in mice, but not when the virus was given intracerebrally. Aureomycin, however, was found by Wong and Cox (1948) to be active against intracerebrally inoculated lymphogranuloma in mice. We have found that in the case of intranasally inoculated mouse pneumonitis virus—another member of the psittacosis group—chloromycetin (2 mg. daily for one week) was ineffective, while aureomycin (1 mg. daily) inhibited lesions completely.

DISCUSSION.

There is very little evidence for the chemotherapeutic activity of any substance in experimental infections of animals with viruses outside the psittacosis-lymphogranuloma group. Eaton (1950) reports activity of aureomycin, but not chloromycetin, against his " atypical pneumonia virus " in cotton rats—a result which accords with favourable clinical reports concerning aureomycin on " primary atypical pneumonia " in man (Meiklejohn and Shragg, 1949). Activity of a number of substances against viruses in developing eggs is unfortunately not in general accompanied by demonstrable activity in infected mice.

The activity of aureomycin—and the inactivity of chloromycetin—against grey lung virus are therefore of special interest. It does not appear that this virus belongs to the psittacosis-lymphogranuloma group: it behaves differently immunologically; it is unaffected by penicillin or sulphonamides; elementary bodies are not demonstrable. The activity of aureomycin has led us to re-examine the question of whether " grey lung virus " is in truth a virus. So far we have found no evidence to the contrary, but are endeavouring to obtain further information, particularly by microscopical methods.

In a subsequent paper we describe a virus from cotton rats; this is possibly related to grey lung virus, and resembles it in its powers of long persistence and in its sensitivity to aureomycin (Andrewes and Niven, 1950).

We wish to thank Messrs. Lederle & Co., and Pfizer & Co., who made supplies of aureomycin and terramycin respectively available to the Medical Research Council. We also thank Mr. D. Busby, who carried out many routine inoculations of mice.

SUMMARY.

Infection of mice with grey lung virus is remarkably susceptible to therapy with aureomycin. Lung lesions, even chronic ones, are quickly resolved and

virus wholly eliminated. Cured mice are susceptible to re-infection with the virus. Terramycin is also active, but chloromycetin, penicillin, streptomycin and sulphamerazine are therapeutically ineffective.

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