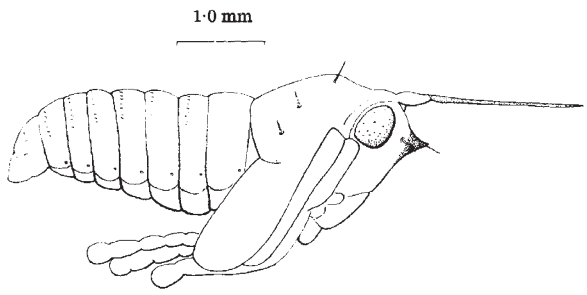

Fig. 2. Right side of the pupa of *Aphrosylus celtiber* Haliday. The plastron-bearing area of the spiracular gill is stippled.

In most climates many terrestrial insects are alternately dry and flooded. When they are covered by water, the water is usually well aerated. To be submerged in water for several hours or even days when it rains heavily is no rare or isolated event but is a normal hazard of their environment. It might therefore be expected that many terrestrial insects are adapted for respiration in water in a manner no less complex than are many aquatic insects. Indeed, it has been shown that a wide variety of terrestrial eggs use the plastron method of respiration when they are flooded[1–6].

The intertidal Tipulidae all belong to the tribe Limoniini. It has previously been noted[7,8] that in all or nearly all Limoniini the epidermal cells which secreted the gill are left behind in the lumen of the gill at the time of the pupal–adult moult. During the pupal–adult moult a thin sheet of cuticle, the basal membrane, is secreted across the opening into the gill, with the result that the tissue in the gill is completely isolated from the haemocoele of the living animal not only by the basal membrane but also by a layer of moulting fluid and the cuticle of the adult. In *Geranomyia unicolor* Haliday, and presumably all other intertidal Limoniini, the epidermis that is shed at this time does not degenerate but remains in good condition throughout the pharate adult period, that is, from the pupal–adult moult to the pupal–adult ecdysis. It has been shown[7] that during this period the epidermis isolated in the gills of *Geranomyia unicolor* is competent to repair damage to the walls of the gill by secreting a plug of sclerotin.

In *Aphrosylus* epidermal cells are isolated in the spiracular gill in the same manner as in the Tipulidae. It may be noted here that epidermal cells are also isolated in the lumen of the respiratory horns of a number of terrestrial Dolichopodidae. The isolation of epidermal cells, or both epidermal and blood cells, in the spiracular gills or in the respiratory horns is now known to occur in about ten families of insects. Only a few of those insects which have evolved the habit of discarding tissue in the appendages of a previous stage exploit some of the attributes of the discarded tissue, which then comes to have a selective value it did not originally possess. The function of the isolated tissue in different spiracular gills has been summarized by me[8]: in some, for example the Simuliidae, it is used to expand the gill after the pupal–adult ecdysis[9], whereas in others it is used both to expand the gill and to maintain its turgidity throughout the life of the pharate adult, as in the Tipulid *Taphrophila vitripennis* Meig[7].

I thank the Cambridge Instrument Co., Ltd., for Fig. 1.

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MICROBIOLOGY

Problem of Absolute and Relative Specificity of Intestinal Microfloras based on Investigations on *Bibio marci* (Diptera) Larvae

The question whether in the intestinal tract of arthropoda and worms, living in the soil and feeding on it, a specific microflora, differing from that of the soil, is existing or not has not yet been clarified. According to some authors the intestinal flora is essentially identical with that of the soil[1,2]. Others have found it different and characteristic of the animal species or group[3–5]. We examined this problem on streptomycetes living in the intestine of *Bibio marci* larvae. The larvae are extremely widespread in numerous soil-types of Middle Europe and live in populations containing 400 to 2,000 individuals (from the same egg-laying). Those examined by us were taken from a rendsina soil, where their food consisted of forest litter and the organic material of the soil. More than 500 larvae were dissected for the examination of the intestinal content, and the microbiological analysis of many excrement masses, which had accumulated under the larva-populations, was also carried out.

For the isolation of streptomycetes, glycerol–arginine[6], glucose–asparagine and glucose–casein agar-media were used. From the soil about 3,000 (ref. 7), and from the intestine and excrement more than 1,000 strains were isolated. The taxonomic examination of the strains was based on the internationally accepted methods of Shirling and Gottlieb[8]. The relationships of the great number of *Streptomyces* varieties, isolated from the intestine of the larvae, were tracked down by the use of the principles of numeric taxonomy, the determination of similarity indexes[9]. Isolates were determined on the basis of collective (parallel) examinations with type or authentic cultures of known species. The results can be summarized as follows:

(1) The percentage occurrence of streptomycetes in the total intestinal microflora of *Bibio marci* larvae fluctuated between 20 and 50 per cent. (2) While the number of *Streptomyces* and *Nocardia* species found in the soil was more than 30, the *Streptomyces*-flora of the intestinal tract was extraordinarily monotonous, only a few species being found, and absolute dominance of one or two species was characteristic. (3) In the intestine of individuals belonging to the same larva-population the species-composition of the *Streptomyces*-flora, more precisely the taxonomic identity of the dominant species (or several species), was the same. (4) Although it appears from point 2 that in the intestine a 'specific *Streptomyces*-flora' is formed, differing essentially from that of the soil, this specificity was not absolute, only relative. In the intestine of individuals of different larva-populations the same species did not consistently dominate. In other words, the local circumstances of the soil, the composition of food, etc., have an effect on the selecting processes taking place in the intestine, which in turn result always in the predominance of one or two species. (5) No *Streptomyces* species could be found which could live and propagate exclusively in the intestine. (6) The *Streptomyces* species found to be dominant in the intestinal tract showed remarkable variability and could be isolated in several varieties. (7) The *Streptomyces* species, dominating in the intestinal tract, preserved their predominance also in the fresh excrement of the animal, where one or two species may give 95 per cent of the *Streptomyces*-flora, and 70 per cent of the total microflora. These species are later gradually repressed, relinquishing their place to the natural multispecies microflora of the soil. (8) The most frequently occurring dominant *Streptomyces* species isolated from the intestine of *Bibio marci* larvae were the following: *Str. finlayi*, *olivaceus*, *antibioticus*, *flaveolus*

and *aureofaciens*. A detailed account of this work will be published elsewhere.

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VIROLOGY

Effect of 'Cephaloridine' on Vaccinia Virus *in vitro*

Experiments in our laboratory have indicated that a new cephalosporin preparation, 'Cephaloridine' (CR), is capable of arresting the development of a trachoma agent at an early stage, when used in concentrations as low as 0·1 μg/ml. of culture medium. In the present communication, some findings on the effect of this antibiotic on the multiplication of vaccinia virus in cell culture will be described.

A dermal strain of vaccinia virus (*V-Led-R*) was used[1]. Details of the preparation and storage of stock virus have been described elsewhere[2]. Cell cultures were prepared from a human kidney line originally obtained from the Laboratory of Hygiene, Department of National Health and Welfare, Ottawa, Canada. Growth medium consisted of *M* 199 containing 10 per cent calf serum. As maintenance medium, a 0·5 per cent solution of lact-albumin hydrolysate in Earle's solution supplemented with 2 per cent calf serum was used. Plaque titrations and experiments were carried out in Leighton tubes, in which confluent cell-sheets were obtained 2–3 days following inoculation with 1-ml. portions of growth medium containing 4×10^5 cells. Details of the plaque technique in liquid medium for the assay of virus and infected cells have already been reported[2,3].

In preliminary experiments, the effect of various concentrations of CR on plaque formation was examined. About 100 plaque-forming units (p.f.u.) of the virus were added simultaneously with the drug to cell cultures. As seen in Table 1, complete plaque suppression was obtained at a concentration of 500 μg/ml. A concentration of 300 μg/ml. reduced the plaque count to less than 50 per cent of that in controls, and the average plaque diameter by about 70 per cent. Plaque count and size in cultures treated with 50 μg/ml. were the same as in control cultures.

Control experiments indicated that a concentration of 1,000 μg/ml. of CR was toxic to cultures, causing the cells to slough off the glass. Since the dose causing complete plaque suppression was so close to the toxic dose, experiments were carried out to examine its effect on cells in the monolayer. It was found that exposure of cultures to 500 μg/ml. of the drug for 48 h had no damaging effect on the cells as judged by microscopic examination, uptake of neutral red, and growth on sub-culture. Moreover, following removal of the drug after 48 h, the cultures were as competent as unexposed controls in adsorbing and synthesizing virus, forming plaques of the same number and size and yielding the same amount of virus. All further experiments were therefore carried out with a CR concentration of 500 μg/ml.

Table 1. Effect of Various Concentrations of 'Cephaloridine' on the Development of Vaccinia Plaques in Cultures of a Human Kidney Cell Line*

'Cephaloridine' concentration (μg/ml.)	Average plaque No. experiments 1	2	3	Average plaque diam. (mm)
0	78	112	151	1·5
50	81	110	154	1·5
100	64	84	109	1·5
200	46	65	83	1·0
300	36	50	71	0·5
400	10	12	14	0·5
500	0	0	0	—

* CR was added simultaneously with virus, and the number of plaques and diameter were recorded after 48 h.

In order to determine whether the suppression of plaque formation was due to total or partial inhibition of virus multiplication, the increase in virus yield and infected cell count was determined in cultures treated with CR after they had been allowed to adsorb virus. As seen in Table 2, which summarizes a representative experiment, both values showed an increase in the CR-treated cultures which was, however, much smaller than that in the untreated controls. The virus yield after 24 h was only 1/50, and the infected cell count only a sixth of the corresponding values in control cultures. During the following 24 h, virus yield increased more than sixty-fold in the control, and less than two-fold in the treated cultures.

Table 2. Effect of 'Cephaloridine' on the Yield of Virus and Infected Cells in Cultures of a Human Kidney Cell Line*

Time (h)	Concentration of virus in p.f.u. per Control culture	CR-treated culture	Concentration of infected cells per Control culture	CR-treated culture
0	25	25	25	25
24	$1·2 \times 10^4$	$2·4 \times 10^2$	$1·2 \times 10^3$	2×10^3
48	$7·5 \times 10^5$	$3·5 \times 10^2$	ND†	ND

* Each culture was allowed to adsorb 25 p.f.u. of vaccinia virus, as determined from the average number of plaques in 3 control cultures inoculated at the same time and stained after 48 h. 500 μg CR were added to the appropriate cultures 2 h after the termination of a 1-h adsorption period.
† Not done.

A few experiments were carried out to examine the effect of the drug during various stages of virus-cell interaction. A reduction in plaque count of up to about 50 per cent was obtained when the drug was present during the adsorption period only. A similar reduction was noted when CR was incubated with the virus *in vitro* and the mixture was then diluted beyond an inhibitory drug concentration prior to inoculation into cultures. It seems, therefore, that a direct interaction between virus and drug can occur. In order to obtain information on the effect of the drug at different stages of the growth cycle of the virus, cultures were first inoculated at a multiplicity of 10–20 p.f.u. of virus per cell to ensure simultaneous infection of all the cells. After an adsorption period of 1 h, they were exposed to CR for various time intervals. As seen in Table 3, virus yields decreased with increasing periods of contact between drug and cells. No significant difference in yield was noted when treatment was confined to either the first or the last third of the 24-h period of the experiments.

Table 3. Effect of the Presence of 'Cephaloridine' for Various Time Intervals on the Multiplication of Vaccinia Virus in Cultures of a Human Kidney Cell Line*

'Cephaloridine' present (h)	Virus yield: control (per cent) Experiments 1	2	3
0–24	15	13	12
2–24	21	20	25
4–24	28	30	34
6–24	46	44	41
8–24	51	54	59
0– 8	67	65	68
16–24	74	70	71

* Control and CR-treated cultures were inoculated at a multiplicity of 10–20 p.f.u./cell, and virus yields were determined after 24 h.

On the basis of these findings, it would seem that there are two aspects to the action of CR. When it is present during the adsorption period only, it lowers the efficiency of the virus to adsorb to the cells or to penetrate and initiate infection. When it is added after the termination of the adsorption period, it causes a reduction in the amount of virus synthesized. As a result of this reduction, which does not seem to be related to a particular stage in