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

virus development, plaque formation is suppressed or impaired, depending on the drug concentration used.

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PSYCHOLOGY

Behavioural Correlates of the Oestrous Cycle in the Rat

CHANGES in certain behavioural functions including learning and exploratory behaviour during the oestrous cycle of the intact rat have been reported[1,2]. Gray and Levine[3] recently re-opened the question of the extent to which sex differences in such essentially non-sexual behaviour could be determined by the altered behaviour of females during oestrus (heat). They induced oestrus in rats of the Maudsley reactive (*MR*) and non-reactive (*MNR*) strains by injections of oestrogen and progesterone and observed that emotionality as defined by emotional elimination and ambulatory activity in the open-field test was reduced. Our purpose is to repeat this work using natural as opposed to induced oestrus and to extend it both by the use of other inbred strains and measures of learning as well as emotionality.

The oestrous cycle was examined in 62 female rats, commencing at a mean age of 130·1 ± (*S.D.*) 31 days, in three highly inbred strains, *B*, *F* and *MNR* (*LAC* Nos. 163*b*, 163*c* and 163*g* respectively[4]), reared and maintained under carefully controlled and standardized conditions[5]. Mandl's technique[6] of daily vaginal smearing was used, though only four phases were distinguished: early oestrous, oestrous, early dioestrous and dioestrous. After an average of 37 days, 53 subjects which showed regular oestrous cycles were assigned at random to four groups representing the four phases distinguished, and each of these groups began behavioural testing in a different phase of the cycle as shown in Table 1. The lordosis response to manual stimulation of the vulvar region[7] was used throughout to confirm the presence of behavioural oestrus (heat) in rats showing an early oestrous smear.

Experiments were carried out in two phases. In the first, each subject was exposed in the open field[5] on five successive days to the mild stress of constant light (165 ft.-candles) and sound (78 dB above standard reference intensity) for precisely 2 min. Two scores were taken—the number of faecal boluses deposited, giving a measure of defaecation, and the number of compartments marked on the floor and traversed by the subject, giving, on conversion to a metric distance, the ambulation score. In the second phase, 12–36 days later, 44 subjects still showing regular cycles were given 40 trials each on a single day in a fully automated shuttle-box[8]. The rat learns to avoid an unconditioned stimulus shock of 0·2 m.amp by crossing to the other side during a conditioned stimulus (buzzer), the onset of which always preceded shock by 8 sec. The total number of these conditioned avoidance responses (*CAR*), their mean latency and the number of trials required to reach various criteria were all used as measures of learning. All testing was carried out in darkness after 9 p.m. (using red light for identification of subjects) in order to maximize the chances of detecting heat[9].

The results of both tests showed variation attributable to stage of oestrus. Open-field test results for a complete oestrous cycle were analysed separately for each strain because of the strain differences detected in defaecation and ambulation scores, which, incidentally, confirm those previously reported[10]. Because the *B* strain showed very regular four-day cycles, analysis of their ambulation scores was confined to the results for the first four days. The marked oestrual variation found throughout is shown in Table 1, in which the increment for rats in the early oestrous phase, irrespective of the day of its occurrence, is apparent. Analysis of variance of all scores grouped together for each stage, irrespective of day of testing, yielded a statistically significant *F*-ratio (5 per cent level). Subsequent *t*-tests showed that two of the three groups not in heat were significantly less active than the early oestrous group (mean score 6·00 ± 1·45 m). These were early dioestrous (3·93 ± 2·33) and dioestrous (3·84 ± 1·86). The results for the *F* strain were similar, though failing to show statistical significance, nevertheless supporting the view that heat facilitates ambulatory activity in the open field. The results for the third strain, the *MNR*, which had been tested 62·79 ± 5·31 days previously in the open field, suggested that this previous experience abolished both the oestral and daily variation in ambulation scores shown by the *B* strain (Table 1).

Table 1. MEAN AMBULATION (METRES RUN) ± *S.D.* IN OPEN FIELD ACCORDING TO PHASE OF OESTROUS CYCLE (*B* STRAIN)

Oestrous phase on test day 1	No. of subjects	Test day 1	2	3	4	Oestrous phase on test day 4
Oestrous	3	6·26 ± 2·06	1·66 ± 0·54	2·01 ± 1·81	*5·83 ± 1·63*	Early oestrous
Early Dioestrous	5	6·82 ± 2·08	4·19 ± 1·71	*5·74 ± 1·47*	7·99 ± 2·81	Oestrous
Dioestrous	5	5·27 ± 0·78	*5·44 ± 1·38*	4·02 ± 0·76	2·72 ± 1·31	Early dioestrous
Early oestrous	5	*6·95 ± 1·40*	3·76 ± 1·12	3·63 ± 0·11	3·15 ± 1·93	Dioestrous
Total (all phases)	18	6·33 ± 1·63	3·99 ± 1·76	4·05 ± 1·72	4·82 ± 2·95	

Note: Results for groups tested when in early oestrous phase, irrespective of phase on test day 1, are in italics.

The defaecation scores for the *B* and *F* strains showed only a suggestion of a decrement during heat, in contrast with previous findings[3]. The *MNR* strain typically gave zero defaecation scores, having been negatively selected against this trait.

In the shuttle-box, rats of all strains in the early oestrous group consistently gave lower conditioned avoidance responses as compared with the other three groups. Analysis of variance for total number of *CAR*s out of the 40 possible yielded an *F*-ratio for oestrous stage significant at the 5 per cent level. The early oestrous group differed significantly (0·1–5 per cent by the *t*-test) from each of the other three groups. Mean *CAR* scores were 18·80 ± 3·98, for early oestrous group; 25·00 ± 5·97, for oestrous; 24·76 ± 4·77, early dioestrous; and 24·35 ± 5·49, dioestrous. The same significant differentiation of the early oestrous group from the other three groups was revealed in the avoidance latency scores (mean scores (sec): 3·61 ± 1·88, 2·60 ± 2·39, 2·99 ± 1·26, 2·92 ± 2·14 respectively). In addition, four criterial measures, based on the number of trials taken to achieve one, three, five and ten consecutive *CAR*s, lent supportive evidence. These results therefore indicate that during heat *CAR* acquisition was depressed.

It will be noted that the pattern we have detected during the early oestrous phase of the female rat's cycle is one of lowered indices of emotional responsiveness in the open field (increased ambulation and decreased defaecation) together with retarded acquisition of conditioned avoidance responses. This retardation has, however, been characteristically associated with greater emotional responsiveness in comparisons between the two Maudsley strains of rats[8]. On the other hand, the greater adrenal sensitivity to stress known to occur during oestrus[11] may well have occasioned less-efficient learning based on shock while still allowing expression of the gross activity differences which oestrus is known to occasion[2,12]. In the open field these might be seen in