parties de l'explant où la vie est le mieux entretenue, soit la périphérie, tandis qu'ils s'estompent au centre du fragment, dès qu'apparaissent des signes de nécrose (Figures 2 et 3).

Discussion. Les mucopolysaccharides acides du néphron correspondent bien à une activité cellulaire locale et non à la sédimentation de colloïdes transportés par l'urine en voie de concentration et modification de pH car, si tel était le cas – peut-on ajouter aux observations précédentes – ils disparaîtraient avec prédilection des régions externes que le milieu de culture, toujours en mouvement, perfuse au maximum et seraient relativement préservés au centre du tissu. C'est le contraire que l'on observe.

D'autre part, les quelques cylindres demeurés in loco, lesquels résultent indiscutablement d'une précipitation de glycoprotéines urinaires et possèdent, entre autres, les affinités tinctoriales des mucopolysaccharides acides, conservent ces propriétés dans les néphrons centraux en dégénérescence, tandis que les mucopolysaccharides qui revêtent l'épithélium environnant ne donnent plus aucune réaction.

Quel est leur rôle ? Des expériences à publier nous permettent d'avancer qu'au niveau de la macula densa où ils abondent, leur quantité peut varier selon l'activité de l'appareil juxtaglomérulaire.

D'autre part, certaines tendances physico-chimiques reconnues aux polymères de leur type suggèrent que, dans l'ensemble, ils pourraient jouer le rôle de transporteurs ou de membranes échangeuses d'ions, les complexant et leur faisant franchir l'épithélium. GINETZINSKI[8], confirmé par DICKERS et EGGLETON[9], paraît avoir montré, en corrélation, qu'une activation tubulaire d'hyaluronidase est en rapport direct avec l'antidiurèse localisée aux segments collecteurs et distaux[10]. Conséquemment, on pourrait attribuer à l'affinité et à la disponibilité de valences non saturées ou de radicaux physiquement avides appartenant aux mucopolysaccharides acides décrits, entre autres, les réabsorptions hydrominérales qui relèvent justement de leur topographie[10] et que stimulerainent donc, par libération de nouvelles fonctions, scission de chaînes et perméabilisation de la surface cellulaire, une hydrolyse et une dépolymérisation traduites par l'élaboration adaptée d'hyaluronidase endorénale[8,9].

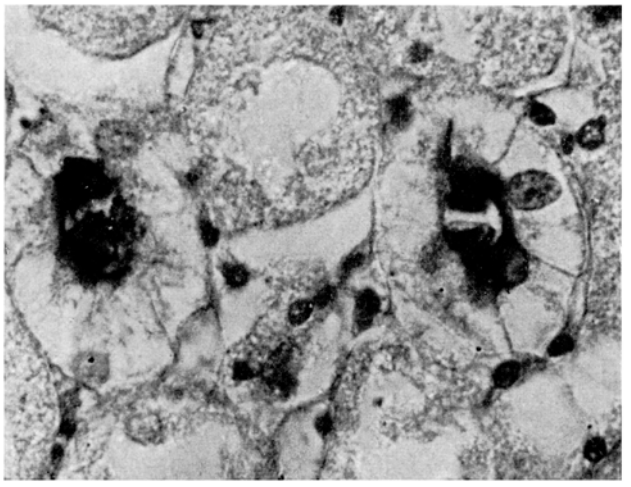

Fig. 3. Deux tubes collecteurs d'un explant rénal de cobaye maintenu en vie pendant cinq jours. Coloration selon Müller-Mowry, aggr. 1650 fois, filtre rouge. Les mucopolysaccharides acides, visibles en noir, emplissent l'apex des cellules épithéliales et tapissent le pourtour de la lumière. Au centre, zone de nécrose, sans réaction visible.

Summary. The tissue culture of renal explants proves that the acid mucopolysaccharides, previously described in the distal convolution and the collecting tubule, do not result from urinary colloid precipitation, but from local epithelial secretion. Their physiological significance is discussed.

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The Effect of Proflavine on the Infection of *Phaseolus vulgaris* L. by Tobacco Necrosis Virus

It is well known that proflavine and other photodynamically active dyes such as acridine orange and neutral red, have an inhibitory effect on bacterial[1] and animal[2] viruses. The action of proflavine has been considered to be due to its positively charged molecule combining with the nucleic acid and inhibiting the proper aggregation of the nucleic acid with its protein coat[3].

Comparatively few studies have been made on the effect of acridine dyes on plant viruses. Acriflavine has no marked effect on the multiplication of the rod-shaped tobacco mosaic virus (TMV)[4], but CHESSIN[5] reported that acridine orange causes loss of activity of infectious TMV nucleic acid. The following is an account of studies on the action of proflavine on the polyhedral tobacco necrosis virus (TNV).

Results. When solutions of proflavine hemisulphate were rubbed with a camel-hair brush on the upper surface of the primary leaves of French bean seedlings (*Phaseolus vulgaris* L. 'Prince') at concentrations greater than 0.6 g/l distilled water they were usually phytotoxic. However, at concentrations of 0.5 g or 0.2 g/l proflavine had no deleterious effects on the leaves and reduced the infectivity of TNV (Table I). Proflavine had no apparent effect on virus infection when diluted to a strength of 0.02 g/l. The dye was inhibitory to virus infection when rubbed on the primary leaves up to 24 h before or after inoculation. In

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[4] D. E. SCHLEGEL and T. E. RAWLINS, J. Bacteriol. *67*, 103 (1954).
[5] M. CHESSIN, Science *132*, 1840 (1960).

all instances, excess proflavine and inoculum were washed off the leaves with distilled water within 5 min of application.

These results could mean that proflavine either acts directly on the virus or that it alters the metabolism of the host cells in such a way that they do not support virus replication. In this respect, the fact that applications delayed until 24 h after inoculation reduced the number of lesions suggests that proflavine can effect the multiplying stage, as well as the initial stage, of infection.

Proflavine was added to buffer solutions at pH 5, 7 and 9 to a strength of 0.3 g/l and rubbed with a partially purified preparation of TNV on an equal number of right and left-hand halves of the primary leaves of French bean seedlings. As a control, the other half of each leaf was rubbed with buffer solution and a comparable quantity of inoculum. The number of lesions for the proflavine treatments, expressed as a percentage of the appropriate controls, was 25, 66 and 15% for pH 5, 7 and 9 respectively. It seems possible that more molecules of the dye are attached to the virus particle in an alkaline or an acid medium than in a neutral one.

When 1 ml aliquots of a 0.1% solution of partially purified TNV were added to 3 ml aliquots of 4 g, 1 g, 0.5 g and 0.1 g/l solutions of proflavine and dialysed in cellophane sacs against distilled water for 24 h at 5°C, all the excess proflavine was removed. An appropriate control with water instead of proflavine was set up and the residual solutions in the sacs either undiluted, or on the addition of 9 ml distilled water for each ml of mixture all produced fewer numbers of local lesions compared to the control, when rubbed on the half leaves of French bean seedlings (Table II).

The degree of inhibition of virus activity was similar whether the original concentration of proflavine was 0.5 or 4 g/l; and none of the solutions was phytotoxic although undialysed solutions of proflavine at concentrations of 1 or 4 g/l normally are. These results suggest that only a certain quantity of proflavine can become attached to the protein coat of the TNV particle. The dye may not destroy the particle but merely render it non-infectious due to the formation of a complex with the protein coat. Neither prolonged dialysis, nor any other physical or chemical treatment was effective in separating the proflavine from the virus which suggests that this attachment is irreversible.

When an infectious nucleic acid solution of TNV was prepared, using the method of SCHLEGEL[6], and mixed with a solution of proflavine (at a concentration of 0.3 g/l) it was found to produce comparatively fewer lesions than a comparable mixture of intact virus and proflavine (Table III).

The evidence from our experiments, to date, indicates that proflavine may act in a number of ways to inhibit TNV infection. While it seems that it may act on virus nucleic acid, it appears (unlike with poliovirus[7]) that it can attach to mature TNV particles, and it is possible that the attachment may render these particles non-infectious. However, the possibility cannot be eliminated that the inhibitory effect of proflavine is indirect, due to its action on host cell metabolism.

Table I. The effect of proflavine on the infectivity of TNV on the primary leaves of French bean seedlings

Concentration of proflavine (g/l)	Time of application of proflavine				
	24 h before inoculation	0 h	1 h after inoculation	3 h after inoculation	24 h after inoculation
1.0	–	phytotoxic	4	–	–
0.5	–	14	12	–	–
0.2	16[a]	15	–	29	30
0.1	22	26	24	33	39
0.05	–	59	53	–	–
0.01	–	72	42	42	–
0 (water)	44	70	46	40	58

[a] Each figure = mean no. of lesions per half leaf (12 half leaves per treatment).

Table II. The effect of dialysis on a mixture of proflavine and TNV prior to inoculation on the primary leaves of French bean seedlings

Original concentration of proflavine (g/l)	No. of lesions from dialysed mixture	No. of lesions from dialysed mixture (dil. 1/10)
0.1	40.6[a]	22.5
0.5	21.1	6.7
1.0	19.3	3.6
4.0	20.5	3.8
0 (water)	82.9	49.0

[a] Each figure = mean no. of lesions per half leaf (12 half leaves per treatment).

Table III. The effect of proflavine on the infectivity of TNV and TNV nucleic acid on the primary leaves of French bean seedlings

Inoculum	Experiment 1	Experiment 2
TNV	55.5[a]	60.0
TNV + proflavine	26.0	21.3
TNV nucleic acid	42.7	45.4
TNV nucleic acid + proflavine	6.8	8.1

[a] Each figure = mean no. of lesions per half leaf (10 half leaves per treatment).

Zusammenfassung. Proflavin hemmt die Infektion von *Phaseolus vulgaris* L. durch Tabaknekrosevirus, wenn es vor oder nach dem Virus auf die Blätter aufgepinselt wird. Die Versuche lassen vermuten, dass Proflavin sowohl mit dem intakten Virus als auch mit der infektiösen Nukleinsäure eine Verbindung eingeht.

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