Discussion. Après l'action de la pepsine, on constate une réduction générale du contraste de la préparation par rapport au témoin. Sur les coupes traitées il se produit une altération progressive et différentielle de certains éléments du flagelle; dans le spermatozoïde néanmoins, à l'exception du noyau, dont les altérations subies feront objet d'une étude ultérieure, les autres éléments cellulaires ne sont pas sensiblement touchés.

Dans le cylindre central du flagelle, une distinction semble exister entre l'élément dense axial le plus sensible de tous à l'action enzymatique, et la gaine corticale, la plus résistante. Les rayons semblent être associés intimement avec celle-ci, puisqu'ils y restent toujours liés et ont la même résistance et densité, tel que le suggèrent aussi d'autres études par microscopie électronique[3]. Rien ne peut être déduit à l'égard de la zone intermédiaire du cylindre, car elle n'est pas contrastée par l'acétate d'uranyle et le plomb[3].

Dès que la pepsine a une action très spécifique sous les conditions utilisées ici[1], il est possible d'affirmer que l'élément axial du cylindre central du flagelle «9 + 1» et les fibrilles périphériques sont de nature protéique, ce qui est d'accord avec les résultats de GIBBONS[5] et de RENAUD et coll.[6] pour les fibrilles isolées des cils de *Tetrahymena*. D'autres études sont encore nécessaires pour déceler la nature de la gaine corticale de ce flagelle et des rayons qui restent presque insensibles au traitement utilisé. BURTON[7] a aussi noté pour le trématode *Haematoloechus* une plus grande résistance du cylindre central que des autres éléments du flagelle, vers le mercaptoéthanol, la colchicine et l'urée.

Bien qu'une certaine similitude morphologique soit évidente entre fibrilles périphériques du flagelle et microtubules du corps de la cellule[8], l'action variable de la pepsine indique que d'autres différences existent entre ces 2 constituants[9].

Summary. Enzymatic extraction with pepsin was carried out on ultrathin sections of the '9 + 1' flagella of *Dugesia tigrina*, using the method of MONNERON and BERNHARD. The 9 peripheral fibrils and the axial zone of its unique central cylinder become altered within 40 min exposure; following 1 h of incubation these same elements are badly damaged, whereas the cortical sheath and associated radial spokes remain fairly well preserved inside the flagellar membrane. Control sections do not show any sensible alteration. The protein nature of peripheral doublets and axial element of the central cylinder seems therefore indicated; the cortical sheath and the spokes have a very similar, if not identical nature, though their chemical constitution still remains to be investigated.

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On the Association Between Lysogeny and Carcinogenicity in Nitroquinolines and Related Compounds

Since the demonstration of phage induction in bacteria by LWOFF[1,2], lysogeny, i.e. induced lysis in bacteria carrying an ordinarily harmless temperate phage, has been shown to be associated with radiomimetic and anti-tumor activities[3,4]. Interruption of DNA synthesis appears prerequisite for inducing activity[5]. Phage induction has been demonstrated for 4-nitroquinoline-1-oxide (4-NQO), a potent water soluble carcinogen, and its reduction product and the presumed proximate carcinogen, 4-hydroxyaminoquinoline-1-oxide (4-HAQO)[6], whose properties also include mutagenicity[7], DNA binding[8,9], production of high toxicity[10], malignant transformation[11], nuclear inclusions[12] and chromosomal aberrations[13] in cultured mammalian cells; 4-NQO is also photodynamically active[14]. We report here the results of lysogenic tests in a series of nitroquinolines and related compounds of known carcinogenicity.

A series of 16 nitroquinolines and hydroxyaminoquinolines, of known carcinogenicity and photodynamic activity[14] (Table), were tested from fresh stock solutions in acetone or 95% alcohol, at concentrations ranging from 100–0.1 μg/ml. Stock cultures of *Escherichia coli* C-600 (λ) and C-600, the lysogenic and indicator strains respectively, were cultured for 24 h at 37 °C on nutrient agar slants and then maintained for 1 week at 4 °C. *E. coli* C-600 (λ) cultures were incubated, in 250-ml flasks containing 100 ml of nutrient broth, for 18 h on a shaker (45 rpm) at 37 °C, washed twice and resuspended in nutrient broth to give an O.D. of 0.2 at 625 nm. Compounds in 0.1 ml volume were added to 0.9 ml cell suspension in screw cap

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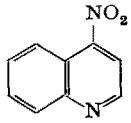
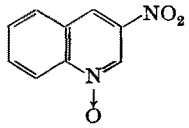
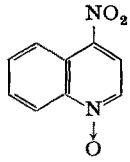
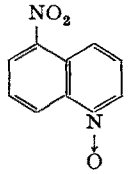
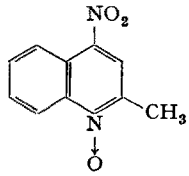
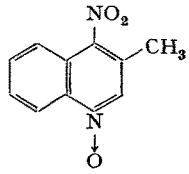
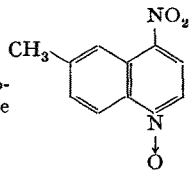
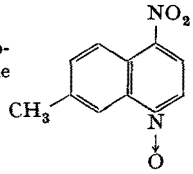
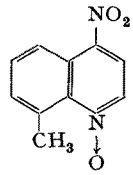
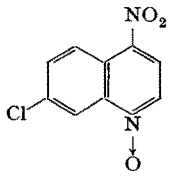
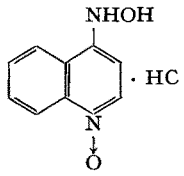
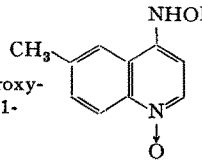
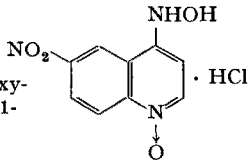
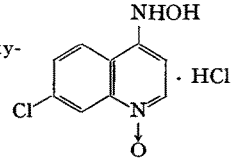
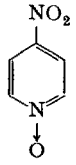
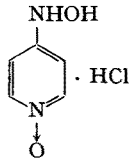
Phage induction in *E. coli* C-600 λ by nitroquinolines and related compounds of known carcinogenic photodynamic and mutagenic activities

Compound	Formula	Relative photo-dynamic potency[14]	Carcino-genicity[14]	Mutagen-icity[15]	Phage yield/plate at specified concentrations (μg/ml)					Minimal inducing concentration (μg/ml)
					100	10	1	0.1	0	
(A) Nitroquinolines										
1. 4-Nitroquinoline		1.7	–	+	< 1	4	8	10	15	> 100
2. 3-Nitroquinoline-1-oxide		< 0.1	–	–	< 1	9	10	10	18	> 100
3. 4-Nitroquinoline-1-oxide		11.1	+	+	20	142	28	12	19	1.8
4. 5-Nitroquinoline-1-oxide		< 0.1	–	–	6	10	6	7	23	> 100
5. 2-Methyl-4-nitro-quinoline-1-oxide		2.9	+	+	117	41	16	8	18	17
6. 3-Methyl-4-nitro-quinoline-1-oxide		< 1.0	–	–	28	12	11	8	11	> 100
7. 6-Methyl-4-nitro-quinoline-1-oxide		1.3	+	+	496	89	13	9	16	4.8
8. 7-Methyl-4-nitro-quinoline-1-oxide		7.1	+	+	14	> 1000	84	14	14	0.40
9. 8-Methyl-4-nitro-quinoline-1-oxide		2.4	+	+	106	106	19	10	10	1.8

Compound	Formula	Relative photodynamic potency[14]	Carcinogenicity[14]	Mutagenicity[15]	Phage yield/plate at specified concentrations (μg/ml) 100	10	1	0.1	0	Minimal inducing concentration (μg/ml)
10. 7-Chloro-4-nitroquinoline-1-oxide		1.1	+	+	37	12	10	10	12	94
(B) Hydroxyaminoquinolines										
11. 4-Hydroxyaminoquinoline-1-oxide·HCl		< 0.1	+	+	> 1000	50	19	19	14	8.0
12. 6-Methyl-4-hydroxyaminoquinoline-1-oxide		< 0.1	+	no data	> 1000	76	10	7	10	5.1
13. 6-Nitro-4-hydroxyaminoquinoline-1-oxide·HCl		< 0.1	+	–	19	16	12	12	27	> 100
14. 7-Chloro-4-hydroxyaminoquinoline-1-oxide·HCl		< 0.1	+	–	80	46	21	18	14	7.9
(C) Hydroxyaminopyridines										
15. 4-Nitropyridine-1-oxide		< 1.0	–	–	5	11	13	16	13	> 100
16. 4-Hydroxyaminopyridine-1-oxide·HCl		< 0.1	–	–	5	9	9	10	14	> 100

tubes (20 × 125 mm), giving final concentrations of 100 to 0.1 μg/ml. Solvent controls apart, mitomycin C at 20 μg/ml was used as a positive control. Cultures were incubated on a shaker (70 rpm) for 10 min in the dark to allow phage induction, which was then terminated by diluting 10-fold with media. Cultures were re-incubated for 2.5 h under the same conditions, to permit cell growth and phage liberation, and then diluted $^1/_{10,000}$ in 4.5 ml of media to give approximately 300 cells/ml. Subsequently, 1 ml of *E. coli* C-600 suspension was added to each tube of lysogenic cells, well mixed and 0.1 ml of the mixture was plated in triplicate on nutrient agar in plastic plates (15 × 60 mm). Plaques were counted on each plate after 24 h incubation in the dark at 37 °C and the minimal concentration of compound inducing a 3-fold increase in the average phage yield was derived graphically as a measure of lysogenic potency.

As can be seen (Table), the inducing activity of these compounds falls into 3 groups: highly active (inducing concentration, < 2 μg/ml), 7-methyl-4-nitroquinoline-1-oxide, 4-nitroquinoline-1-oxide, and 8-methyl-4-nitroquinoline-1-oxide; moderately active (inducing concen-

tration, 2–20 μg/ml), 6-methyl-4-nitroquinoline-1-oxide, 6-methyl-4-hydroxyaminoquinoline-1-oxide, 7-chloro-4-hydroxyaminoquinoline-1-oxide·HCl, 4-hydroxyaminoquinoline-1-oxide·HCl, and 2-methyl-4-nitroquinoline-1-oxide; low activity (inducing concentration 20–100 μg/ml), 7-chloro-4-nitroquinoline-1-oxide; inactive (inducing concentration, > 100 μg/ml), 3-methyl-4-nitroquinoline-1-oxide, 4-nitroquinoline, 4-hydroxyaminopyridine-1-oxide·HCl, 4-nitropyridine-1-oxide, 3-nitroquinoline-1-oxide, 6-nitro-4-hydroxyaminoquinoline-1-oxide·HCl, and 5-nitroquinoline-1-oxide.

These results indicate a clear positive association between phage induction and carcinogenicity. All 6 non-carcinogens were also non-lysogenic and of the 10 carcinogens, all were lysogenic with the exception of 6-nitro-4-hydroxyaminoquinoline-1-oxide. It is of additional interest that of the 15 compounds for which mutagenicity data is available[15], this property is closely associated with lysogeny with 2 exceptions (Nos. 1 and 14)[16].

Zusammenfassung. In einer Serie von 16 Nitroquinolinen und verwandten Substanzen wurde eine gute Übereinstimmung zwischen Phageninduktion in *Escherichia coli* C-600 und Karzinogenese nachgewiesen.

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[15] S. S. EPSTEIN and J. A. ST. PIERRE, unpublished data.

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Excessive Proliferation of Epithelium of the Rabbit Cornea[1]

Epithelial growth into the damaged cornea is not a common phenomenon, although it is recognized that proliferation and movement of epithelial cells is responsible for closure of any gap in the corneal surface[2]. The usual limit of ingrowth appears to be several layers of cells, as in the case of mucopurulent conjunctivitis in man reported by SIHOTA[3], or in the epithelial thickening after injury of the rabbit cornea reported by BERENS et al.[4]. In the course of studies of the production of collagenous tissue in the rabbit cornea by injection of the polysaccharide carrageenan[5], uncontrolled epithelialization was observed in 13 out of 90 eyes, in both experimental and control rabbits. In several of these the excessive proliferation of epithelial cells obliterated the corneal stroma. We have failed to find corneal epithelialization of this magnitude described in the literature.

Methods. Details of the materials used and of the animal experiments have already been described in a previous publication[6]. Injections of 0.05 ml 1% λ carrageenan (Marine Colloids, Inc.) in 0.9% NaCl, or of 0.9% NaCl alone, were made into the centre of the cornea, using a 0.25-ml syringe and 30 gauge needle. No further treatment was applied to the cornea until the animals were killed for histological analysis at intervals up to 2 months postinjection.

Results and discussion. Microscopic examination of sections from the area into which material had been injected revealed a marked proliferation of epithelial cells in 5 out of 20 control (saline injected) corneas and in 8 out of 58 carrageenan injected corneas. The cells which participated in the epithelialization process stained like normal epithelial cells, viz. blue with hematoxylin and eosin, dark purple with Masson's trichrome, yellow with the von Gieson method, green with dialyzed iron, brick red in the periodic acid Schiff reaction and orthochromatically with toluidine blue. The corneas which exhibited this phenomenon were taken from corneas sacrificed 3–49 days after the original injection. Although 15 of 58 carrageenan injected corneas became ulcerated, the epithelialization process was not associated with this complication, as epithelialization occurred in none of these.

The epithelialization penetrated the stroma at various places and was not uniform at its various levels of penetration. The increasing mass of epithelial cells encroached upon the stroma to varying extents, as shown in Figures 1 and 2, sections from the same cornea. The proliferation was usually 30–40 cells thick, but in 2 cases it was approximately 75 cells thick and completely obliterated the stroma. One of these cases is illustrated in Figure 3. In a few cases, corneal endothelial cells also divided and invaded the stroma.

Only a few mitotic figures were observed in normal basal cells of the epithelium, and none at all were seen in the proliferating epithelial cells. To ascertain whether they had been missed because of a diurnal rhythm in mitotic activity with a maximum at night, reported in other species[6,7], 6 additional rabbits were injected and killed 11 and 14 days later at 23.00, 03.00 and 07.00 with entirely negative results.

The significance of the usual restriction of the number of layers of epithelial cells in the normal cornea is really not understood. The observations of SIHOTA[3] and BERENS et al.[4] have already been mentioned. Even in rabbit corneas cultured in vitro, this restriction appears to be operative[8]. On the other hand, KNOWLES[9] found hyperplasia and downgrowth of epithelial cells 18–30 cell layers thick in rabbit corneas which had ulcerated after insertion

[1] This research was supported in part by grants from the National Research Council (Canada) and the National Cancer Institute of Canada.

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