lead citrate[12], and viewed under a Phillips 200 E.M. Seeds from the same source were glued to E.M. stubs and coated sequentially with 100 Å thick layer of carbon and gold and viewed with a Cambridge stereoscan S4.

Under both the light microscope and S.E.M., it was very apparent that the taxa investigated fall into 2 groups with respect to their seed coat structure, papillate and smooth. However, within both of these categories it is apparent also that papillation is of 2 types, as is the nature of the so called smooth seeded group.

The 2 types of papillae present are illustrated in Figures 2 and 4. In one the whole of the epidermal cell appears to be modified into a papilla and is substantially thickened on its outer wall. This is noticeable under light microscope sections Figure 8 and also the layered structure is clearly visible under transmission E.M. (Figure 12). The other type of papillation, for convenience referred to as sub-papillate, involves only part of the cell wall (Figure 4). The central region of the outermost part of the epidermal cell is modified into a papilla, but the surface also appears to be foveolate (Figures 3 and 4).

Keys to Figures 1–12

Seed coat structure	Species	Figures
Papillate	*E. glandulosum*	1, 7
	E. platyphyllum Rydb.	2, 8, 12
	E. hornemannii Reichenb.	2, 8, 12
	E. clavatum Trel.	2, 8, 12
Sub-papillate	*E. paniculatum* Nutt.	3, 9
	E. hirsutum	3, 9
	E. anagallidifolium Lam.	3, 9
	E. davuricum Fisch.	4, 8
	E. leptophyllum Raf.	4, 8
	E. palustre L.	4, 8
	E. palustre var. *grammadophyllum*	4, 8
	E. palustre var. *monticolor*	4, 8
Smooth	*E. angustifolium* L.	6, 11
	E. latifolium L.	6, 11
Foveolate	*E. lactiflorum* Hausskn.	5, 10
	E. alpinum var. *alpinum*	5, 10
	E. luteum Pursh	5, 10

In the case of *E. glandulosum*, the papillae are arranged in rows and have 2 almost foveolate cells between them making this taxon particularly distinctive. At the same time, however, the papilla is a complete cell, not just a modified outer wall (Figures 1 and 7).

The smooth or non-papillate seed coat type is of 2 forms (Table, Figures 5 and 6). One as is exemplified by *E. latifolium* and *E. angustifolium* is only superficially sculptured (Figure 6) whereas the other is foveolate, the centre of the cell being sunken and somewhat alveolate in its sculpturing. In sections (Figures 7–11) these seed surfaces, especially the epidermal cells, are very different. The *latifolium-angustifolium* type has unthickened outer wall (Figure 11), whereas the foveolate types have extremely thickened outer wall, almost to the extent that the epidermal cell contents are excluded (Figure 10).

The distinct differences between these seed coat types indicates a possible genetic discontinuity both within and between species. If the genetic relationship between different seed coat morphologies is a simple one, and there is every reason to think so because of the lack of 'intermediate' forms, then it should be possible to demonstrate biochemical affinities between taxa. Such investigations are underway based on the above findings with respect to the flavonoid and isoenzyme profiles of the taxa investigated.

Résumé. Nous avons examiné au MEB et au microscope ordinaire l'épiderme de la graine de 17 formes du genre *Epilobium* provenant de l'Amérique du Nord et relevé l'utilié et la valeur taxonomique et génétique des données obtenues dans le cadre du genre.

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Inhibition of *Neisseria catarrhalis* NE-11 Transformation

The incorporation of antibiotics or other chemical inhibitors into transformational mixtures has been increasingly reported in recent years. These studies have demonstrated the importance of the involvement of cellular processes as energy utilization and protein, DNA, RNA, or cell wall synthesis in the process of transformation.

That DNA uptake is mediated by an energy-requiring process was first demonstrated by Stuy[1] when he showed that *Hemophilus influenzae* transformation could be inhibited with either 2,4-dinitrophenol or sodium arsenate. Strauss[2] found that cyanide, an inhibitor of aerobic energy-yielding reactions and of membrane transport requiring energy, could block transformation of *Bacillus subtilis* apparently by preventing the transport of P^{32} DNA into the competent cell. This led him to believe that transforming DNA in its DNase-insensitive stage was not necessarily inside the cell but may be located in an extramembranal space beneath the cell wall.

Lie[3] and Jyssum[4] agreed that an energy source, as well as a certain level of metabolic activity, was required for the transformation of *Neisseria meningitidis*. Jyssum[4] showed this by the finding that dinitrophenol inhibited *N. meningitidis* transformation.

Chloramphenicol, an antibiotic which inhibits the transfer of amino acids from aminoacyl transfer RNA to a growing polypeptide chain[5], has been shown by many investigators to inhibit the development of competence. This resulted in a reduced frequency transformation in

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Table I. Effect of chloramphenicol[a] on intraspecific STR transformation of *Neisseria catarrhalis* NE-11

Final concentration of chloramphenicol (μg/ml)	T without chloramphenicol (%)[b]	T with chloramphenicol (%)	Loss of T (%)	Average T loss (%)
0.10	1.37	0.96	29.9	18.0
0.10	1.15	0.96	16.5	
0.10	0.26	0.24	7.7	
1.0	1.71	1.05	38.6	38.6
1.0	2.13	1.31	38.5	
2.5	1.93	0.83	57.0	60.9
2.5	0.21	0.11	47.6	
2.5	0.21	0.07	66.7	
2.5	1.15	0.50	56.5	
2.5	0.26	0.06	76.9	
5.0	0.21	0.03	85.7	87.6
5.0	1.37	0.18	86.9	
5.0	1.82	0.18	90.1	

[a] The minimal inhibition concentration of chloramphenicol for *Neisseria catarrhalis* NE-11 was found to be 0.09 μg/ml. [b] T, transformation (%)

pneumococcus[1,6], *H. influenzae*[1,7-9], streptococci[10,11], *B. subtilis*[12-14], and *N. meningitidis*[4].

The function of the cell wall in the transformation process has also been examined. D-cycloserine, an antibiotic, is known to act as an antimetabolite causing the interference of early reactions in the formation of cell wall glycopeptide[5]. RANHAND and LICHSTEIN[8] found that D-cycloserine in concentrations not affecting cell viability has no inhibitory effect on competence development of *H. influenzae* but at concentrations greater than 5 μg/ml did inhibit growth. Since D-cycloserine inhibits the DL-alanine racemase and D-alanyl-D-alanine synthetase enzymes involved in cell wall synthesis, they postulated that the biochemical processes in *H. influenzae* necessary for competence did not require these enzymes nor reactions requiring pyrimidine nucleotide as biosynthetic carrier molecules.

RANHAND and LICHSTEIN[15] reported that periodate greatly reduced transformation of *H. influenzae* and prevented the development of competence. This compound did not effect the transforming DNA itself and had no effect when added after exposure of competent cells to DNA. They concluded that periodate somehow alters DNA binding site(s) or prebinding(s) so that the cell can not irreversibly bind transforming DNA.

This paper concerns itself with the study of inhibition of intraspecific streptomycin transformation using various metabolic inhibitors against protein and cell wall synthesis

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Table II. Effect of sodium periodate[a] ($NaIO_4$) on intraspecific STR transformation of *Neisseria catarrhalis* NE-11

Final concentration of $NaIO_4$ (*M*)	T without $NaIO_4$ (%)[b]	T with $NaIO_4$ (%)	Loss of T (%)	Average T loss (%)
0.001	1.08	1.63	0	0
0.001	0.14	0.36	0	
0.001	0.57	1.08	0	
0.002	0.90	0.43	52.2	70.3
0.002	2.05	0.24	88.3	
0.005	2.42	0.29	88.0	80.7
0.005	2.42	0.24	90.1	
0.005	1.43	0.61	57.3	
0.005	1.86	0.45	75.8	
0.005	2.05	0.16	92.2	
0.01	1.07	0.12	88.8	85.1
0.01	0.14	0.03	78.6	
0.01	0.50	0.06	88.0	
0.02	1.86	0.0	100.0	100.0
0.02	0.90	0.0	100.0	

[a] The minimal inhibition concentration of sodium periodate for *Neisseria catarrhalis* NE-11 was found to be 2.5×10^{-4} *M*. [b] T, transformation (%).

as well as energy mediated processes. The results obtained in this research has been compared to other well known transformational systems to determine if differences could be detected.

Materials and methods. The procedure used for the preparation and isolation of streptomycin (STR) resistant mutant was similar to other investigators[16,17]. Transformational experimentation has been described elsewhere with modification for the STR marker[18]. Serum was not employed in the development of competent cells and trypticase soy broth with added yeast extract (0.35%) was always used in the transformaion mixture with STR DNA. The minimal inhibitory concentration of chloramphenicol, sodium periodate, and D-cycloserine were determined according to the method of GROOVE and RANDALL[19]. Competent cells were exposed to these compounds (chloramphenicol, D-cycloserine, and sodium periodate) for only 30 min during the latter part of the log phase. From this point, the transformation was completed as described[18]. For the dinitrophenol experiment, the compound at a prescribed concentration was added directly to the transformational mixture.

Results and discussion. Varying concentrations of chloramphenicol were added to *N. catarrhalis* NE-11 cells growing in the competence development phase (log phase) because this phase is the one most affected by inhibitors of protein synthesis[1,8,10,12,13,20]. DNA was then added after 30 min exposure to the inhibitor. The results of independent experiments of various concentrations of chloramphenical are shown in Table I. Concentrations of chloramphenicol ranging from 0.10 to 5 µg/ml caused increasing inhibition in transformation frequency from 18.0 to 87.6% respectively without loss in cell viability. These results show that *N. catarrhalis* NE-11 STR transformation is dependent on protein synthesis.

Log phase cells were exposed to varying concentrations of sodium periodate in a similar manner as the chloramphenicol experiment to determine if cell wall alteration would affect transformational frequency of *N. catarrhalis* NE-11. The results of independent studies that sodium periodate concentrations ranging from 2×10^{-3} *M* to 2×10^{-2} *M* caused a marked decrease in the number of transformants (Table II) without loss in viability. Apparently there are sites present on the cell wall surface of *N. catarrhalis* NE-11 which necessitate transformation as have been shown with other systems[15,21,22].

D-cycloserine inhibition was conducted similar to the chloramphenicol experiment to determine if an antimetabolite which interferes with the formation of glycopeptides within the cell wall would interfere with *N. catarrhalis* NE-11 intraspecific transformation. Only 1 concentration of the antibiotic was used (5000 µg/ml). An average of 3 distinct experiments showed 66.5% reduction of transformation without any loss of cell viability.

In the dinitrophenol experiment, the inhibitor was added directly to the transforming mixture. As in the D-cycloserine experiment, only 1 concentration of dinitrophenol was used (0.03 *M*). A 100% inhibition of transformation was observed without loss of cell viability. This indicates that possibly the DNA which has become DNAse insensitive during transformation has not passed the cell membrane but may be trapped in the extramembrane space under the cell wall[1,2].

Zusammenfassung. Nachweis, dass die Transformation von *Neisseria catarrhalis* sich mit Antibiotika und Chemikalien hemmen lässt, die ihrerseits die Eiweiss- und Zellwandsynthese hemmen. Daraus wird geschlossen, dass Eiweiss- und Zellwandsynthese zur Transformation nötig sind.

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Nuclear RNA and Protein Metabolism in Lethal Heterospecific Amoeba

The heterospecific cells obtained by the nuclear transplantation between *Amoeba proteus* (P) and *Amoeba amazonas* (A) are non-viable. In the heterotransfers, P nucleus introduced into the enucleate A cells (P_nA_c) always result in the death of such cells within 24 h. On the other hand, when A nucleus is implanted into an enucleate P cell (A_nP_c), the hybrids die within 15–20 days without any division. These A_nP_c cells exhibit normal, visible cell activities for 6–8 days after they are made hybrids but gradually fail to capture food organisms after that period. Thus it seemed of interest to investigate whether there are any changes in the synthetic activity of nuclear proteins and RNA in these interspecific amoebae.

This report is concerned with the determination of the labelled nuclear protein content and the kinetics of ^{3}H-uridine incorporation in the nuclei of A_nP_c hybrids at different periods of time.

Materials and methods. *Amoeba proteus* and *Amoeba amazonas* were cultured according to the method of PRESCOTT and CARRIER[1]. *Amoeba amazonas* was collected by Dr. D.M. PRESCOTT from the River Amazon. All the cultures were maintained at 22 ± 1 °C.

Tetrahymena pyriformis were grown either on 2% proteose-peptone containing 25µc/ml of ^{3}H-lysine (L-lysine 4 H^3; 7.0 Ci/m*M*, Schwarz Bioresearch Inc., Orangeburg, N.Y.) and 25 µCi/ml of ^{3}H-leucine (L-leucine 4, 5–H^3; 45 Ci/m*M*, New England Nuclear Corp., Boston, Mass.) or on synthetic medium[2] containing 50 µCi/ml of tritiated uridine (29 Ci/m*M*, Radiochemical Centre, Amersham).

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