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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For assaying the radioactivity of ^{3}H-proteins and ^{3}H-RNA in nuclei, the cells were fed with labelled tetrahymenae at different intervals of time after they were made hybrid. The cells were fed for 12 h with ^{3}H-tetrahymenae and chased for ca. 36 h with unlabelled food, starting from 0 h, i.e., immediately after nuclear transfer. The nuclei of these labelled cells were transplanted into non-radioactive cells according to the method of JEON and LORCH[3] at different intervals of time. Immediately after the transplantation, the cells were placed individually on steel planchets and fixed with acetic ethanol (1:3). The fixed cells were treated with formic acid, dried and counted in a windowless, low-background, gas flow counter. Unincorporated RNA precursors were removed by treating the fixed cells with 5%, ice-cold tricholoroacetic acid before they were assayed for radioactivity. A few cells were also extracted with RNase (Worthington Biochemical Corp., Freehold, N.J., 0.5 mg/ml, pH 6.9) for 6 h at 37 °C. All counts (cpm) were corrected for background.

The controls in both cases consisted of homotransfers, i.e., labelled *Amoeba amazonas* nuclei transplanted into *Amoeba amazonas* and assayed in same fashion as experimental ones. All experiments were conducted only up to 6 days after the amoebae were made hybrids, because hybrid cells are unable to capture food or digest already ingested food beyond this period.

Results and discussion. From the data presented in Table I, it is obvious that the hybrid cells do not show any appreciable change in the incorporation of label into the nucleus specific proteins and presumably the migration of these proteins into the nucleus[4,5] during the period under study. On the other hand, the kinetics of ^{3}H-uridine incorporation in hybrid nuclei shows a pronounced depression (Table II) when compared to the controls. This indicates a continual decrease in the RNA synthesis by the nuclei of the interspecific cells. This inability of the hybrid cells to sustain the nuclear RNA synthesis apparently has little effect on the nuclear protein metabolism. Perhaps this is to be expected due to the possible existence of long-life mRNA(s) in amoebae[6,7].

Most of the interspecific amoeba hybrids are non-viable[8–10]. JEON and LORCH[11,12] have demonstrated the presence of an anti-mitotic and lethal factor in the amoeba hybrids, which inhibits the mitosis in the heterologous nucleus, eventually killing the cell. Whether this factor can be responsible for the observed inhibition of the RNA synthetic activity in the nucleus of the hybrid cell is not known. Recently, FLICKINGER[13] has found gross alterations in the fine structures of nucleus and cytoplasmic organelles in A_nP_c hybrids within a few days after insertion of *A. amazonas* nucleus into *A. proteus* cytoplasm. However, more information is needed for a better understanding of the cause of lethality in the heterospecific amoeba.

Table I. The degree of nuclear radioactivity in control and hybrid amoeba nuclei following labelling of cells with amino acid-^{3}H at different intervals of time

Nucleus	$\bar{X}$ cpm ± S.E.M./nucleus at different h		
	48	96	144
Control (N_1)	81 ± 3.5	74 ± 10.5	79 ± 3.5
Hybrid (N_2)	68 ± 9.0	69 ± 7.0	74 ± 9.0
$\bar{X}$ Ratio (N_1:N_2)	1.18	1.07	1.07

The cells were labelled and chased at 0, 48 and 96 h and the nuclei were isolated and assayed for radioactivity at 48, 96 and 144 h. Each number represents the mean count of 6–10 nuclei.

Table II. A comparison of the rate of ^{3}H-uridine incorporation between control and hybrid amoeba nuclei

Nucleus	$\bar{X}$ cpm ± S.E.M./nucleus at different h		
	48	96	144
Control (N_1)	379 ± 45.5	400 ± 37.0	371 ± 40.5
Hybrid (N_2)	214 ± 16.0	203 ± 50.0	110 ± 12.0
X Ratio (N_1:N_2)	1.17	1.97	3.37

The cells were labelled and chased at 0, 48 and 96 h and the nuclei were isolated and assayed for radioactivity at 48, 96 and 144 h. Each number represents the mean count of 6–10 nuclei.

Zusammenfassung. In heterospezifischen Zellen, welche durch Transplantation des Zellkerns von *Amoeba amazonas* in kernlose Zellen von *Amoeba proteus* erhalten wurden (begrenzt lebensfähig und nicht mehr teilungsfähig), nimmt die intranukleäre RNS-Synthese im Laufe der Versuchsperiode kontinuierlich ab, während sie in Kerntransplantationsversuchen zwischen Zellen derselben Art konstant bleibt. Diese Unfähigkeit der Hybridenzellen, ihre nukleäre RNS-Synthese aufrechtzuerhaltten, ist mit keinem nachweisbaren Effekt auf den Proteinstoffwechsel des Zellkerns verbunden.

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