necessary, at −20 °C. DNA and whole RNA (w-RNA) isolation procedure, method for DNA labelling and hybridization procedures were mentioned elsewhere[7].

Results and discussion. Transcription pattern of normal and treated conidia revealed negligible amount of self (DNA-DNA) reassociation (0.5–1.0%) when w-RNA or unlabelled DNA was present much in excess of ^{32}P DNA. In different DNA-RNA reactions, ^{32}P DNA C_0t (C_0: moles ml^{-1}, t: time of reassociation) varied from 1.0×10^{-3} to 1.2×10^{-3} and R_0t (R_0: RNA moles ml^{-1}, t: time) varied from 8.5×10^4 to 8.6×10^4. Results indicate that in nongerminating normal conidia true DNA-RNA hybrid, as analyzed by hydroxyapatite chromatography, represents 14.0% of total mycelial genome. Dutta and Chaudhuri[8] reported that conidial and mycelial genome are identical in base composition. In germinating conidia transcription percent went up gradually and reached a value of 33%, i.e., a relative increase of 2.35 of *N. crassa* genome within 24 h. After EG treatment, mycelial development was found to be arrested, as expected, and w-RNA transcripts were found to increase slightly, and w-RNA transcripts were found to increase slightly, a relative increase of 1.20, which is 20% increase of 0 h transcripts. This signifies that after EG treatments some macromolecular synthesis takes place. To ascertain whether EG affects genome structure, DNA-DNA hybridizations were carried out with treated and untreated DNAs, and it was noticed that EG did not alter DNA base sequences significantly. About 92–93% of *N. crassa* genome was found to react with unlabelled DNAs from both the sources. Reassociation experiments were run upto saturation level. From the above data it appears that EG suppresses the usual RNA production in *N. crassa* conidia, though DNA structure is perhaps not affected. Slight increase in w-RNA production (about 20% or original level) in treated conidia may be due to ribosomal RNA production, and this perhaps explains the original observation of Bates and Wilson[5] that nuclear size, nuclear number and ribosome production increased after EG treatments.

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Relationship between regeneration of cell surface glycoproteins in trypsin-treated chick embryo fibroblasts and cell adhesion to the substratum

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Summary The ability of cells to adhere to a substratum was altered by treatment with trypsin but was restored after a 1.5-h culture. A concomitant incorporation of [^{3}H] leucine and [^{14}C] glucosamine in the trypsin-sensitive cell surface glycoproteins was observed and almost reached a plateau within 1.50 h following the treatment with trypsin.

Cell surface glycoproteins are involved in the control of cell growth, cell differentiation and cell adhesivity[2,3]. The study of cell surface glycoproteins has been carried out following their release by trypsin and has already been well documented[4]. The present report describes the consequences of the treatment with trypsin of 8-day chick embryo fibroblasts on cell adhesion to the substratum, cell morphology in relation to the regeneration of cell surface glycoproteins.

Material and methods. Cells: 8-Day chick embryo fibroblasts were obtained as previously described[5]. Cell cultures: Cells were cultured in Falcon flasks in Eagle's medium (MEM) containing 10% foetal calf serum, 5% glutamine and 5% antibiotics. Cultures were grown in humidified air with 5% CO_2 at 37 °C. The initial seeding concentration was 10^6 cells/ml. After 48 h, the cultures reached subconfluency and they were used for the following experiments. Treatment of cells with trypsin: Cultures were washed 3 times with phosphate buffer saline (PBS) (pH 7.4) and then treated with trypsin (TPCK; 50 µg/ml; 25 °C). After 10 min of treatment, the effect of trypsin was blocked using the soybean trypsin inhibitor (100 µg/ml). Determination of cell adhesion to the substratum: After the treatment of cells with trypsin, the cells were seeded in 50 mm petri dishes in 3 ml of MEM (10^6 cells/ml) and the number of adhered cells was determined using a hemocytometer after the cells had been harvested from tissue culture dishes by trypsin. Cell viability was greater than 95% as estimated by trypan blue exclusion. The morphology of adhered cells was observed in tissue culture dishes by microscopic analyses using an inverted microscope. Incorporation of [^{3}H] leucine and [^{14}C] glucosamine: After the treatment of cells with trypsin, the cells were seeded in 50 mm petri dishes in 3 ml of MEM (10^6 cells/ml) containing 1 µCi/ml of [^{3}H] leucine (sp. act. 55 Ci/mmole, Radiochemical Centre Amersham) and 0.85 µCi/ml of [^{14}C] glucosamine (sp. act. 52 mCi/mmole, Radiochemical Centre Amersham). At various intervals, the cells were washed 3 times with PBS (pH 7.4) and were again treated with trypsin (50 µg/ml). The radioactivity incorporated with the material released by trypsin-treatment and into the trypsin-treated cells was conted in 10 ml of PCS (Amersham). The cellular protein content was estimated by the method of Lowry et al.[6].

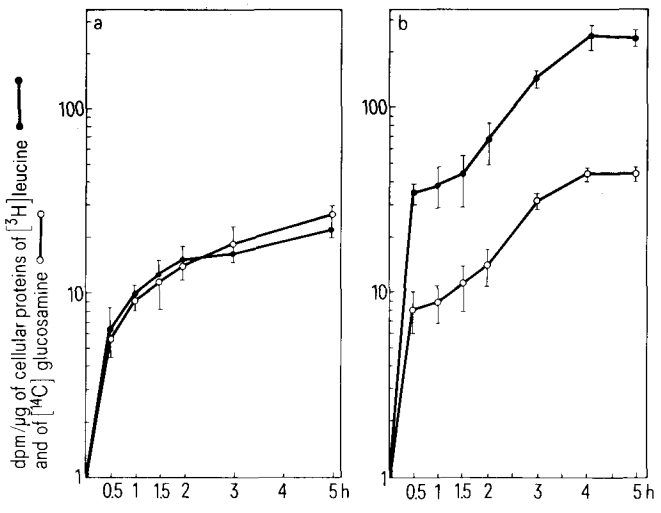
The adhesion of cells to the substratum and the morphology of 8-day chick embryo fibroblasts following trypsin treatment

Time in h	Number of attached cells* × 10^6/dish		Cell morphology
0.5	1.2	(40%)	Most cells were spherical, a few were elongated
1	1.2	(40%)	Increase in number of cells which were elongated
1.5	2	(66%)	Fibroblastic morphology began to be restored
3	2	(66%)	Fibroblastic morphology began to be restored
4	2.2	(73%)	Complete morphology was restored to all cells

* The initial seeding concentration was 3×10^6 cells/dish. Values given in parentheses represent the percentage of attached cells as compared to the initial seeding concentration.

Results and discussion. The cultured fibroblasts, on reaching subconfluency, were elongated and had adhered to the substratum. Trypsin treatment altered these characteristics. Although the cell viability was not affected, the cells were spherical and did not adhere to the substratum immediatly following their treatment by trypsin. After a 30 min culture, 40% of the cells were attached to the substratum. With time this percentage increased, reaching 66 and 73% after a 1.50-h and a 4-h culture respectively (table). It was observed that, as the percentage of cells attached to the substratum increased with the time of culture, there was also a concomitant increase in cell elongation, complete fibroblastic morphology being restored to all cells after a 4-h culture (table).

Cell morphology appeared to be dependent on the adhesion of cells to the substratum. These results are in agreement with those of Maroudas[7] and Martin and Rubin[8], who established that the cell adhesion of chick and mouse fibroblasts affects their morphology and proliferative capacity. Parallel to the adhesion of cells, the incorporation of [^{3}H] leucine and [^{14}C] glucosamine in trypsin-sensitive glycoproteins increased markedly from a 30-min to a 1-h culture. This incorporation was then slight between a 1.50-h and a 4-h culture, almost reaching a plateau (figure, A). These results suggest that the regeneration of trypsin-sensitive glycoproteins was reached within 1.50 h following the trypsin treatment of 8-day fibroblasts. This time appeared to be short in comparison with that observed by other workers[9] using different cells possessing low proliferative capacities. The time involved in the regeneration of cell surface glycoproteins seemed to be dependent on the cell type. However, our results are in agreement with those of Moreau and Bourrillon[10] obtained using Zajdela's tumor cells in primary cultures, suggesting that the proliferative capacity of cells may be associated with the velocity of glycoprotein regeneration. On the other hand, the incorporation of [^{3}H] leucine and [^{14}C] glucosamine in trypsin-treated cells reached a plateau after a 3-h culture (figure, B). This may be due to their incorporation into intracellular glycoproteins and/or cell surface glycoproteins, insensitive to the trypsin action. These results suggest that the regeneration of cell surface glycoproteins following the trypsin-treatment, may be due to their in situ synthesis. Therefore, the restoration of cell adhesivity and the synthesis of the cell surface glycoproteins occured in parallel after culturing for 1.50 h following the treatment of cells with trypsin. In addition, cell morphology appeared to be dependent on these 2 properties, since it was completely restored within 4 h following the trypsin treatment.



Incorporation of [^{3}H] leucine and [^{14}C] glucosamine in *A* The trypsin-sensitive cell surface glycoproteins and *B* in the 8-day chick embryo fibroblasts following trypsin-treatment. Each point represents the value obtained from 4 separate experiments.

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A promoting action of cyclic GMP on contractions of guinea-pig vas deferens[1]

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Summary. The effect of dbc-GMP has been studied in ductus deferens of the guinea-pig. The nucleotide potentiated the contractions induced by electrical field stimulation and by adrenergic agonists. The site of action was probably in the smooth muscle cell, since the release of excitatory transmittor was not influenced.

Earlier observations have indicated that cholinergic drugs, and other agents which stimulate contraction, also enhance the accumulation of cyclic GMP of isolated smooth muscle preparations[2-5]. It has been suggested that cyclic GMP may be causally involved in the contractile response. In the vas deferens of the rat it was observed that norepinephrine and phenylephrine increased the cyclic GMP level about 2fold after 3 min[6]. Recently Schultz et al.[7] demonstrated that some relaxing agents, such as nitroprusside, also increased the cyclic GMP level of vas deferens. The authors suggested that their results support the hypothesis that cyclic GMP may act as a negative feedback inhibitor of hormonally stimulated calcium influx into cytoplasm. Earlier studies performed on rabbit colon smooth muscle in our laboratory[5,8] are not consistent with this assumption. Our data are more consistent with the suggestion that cyclic GMP may act as a comediator with calcium to promote contraction.

In this work we present some data from vas deferens of guinea-pig according to our earlier suggestions. The dibutyryl derivative of cyclic GMP has been tested on vas deferens of guinea-pigs. For these tests, vasa deferentia were removed and dissected free from adjacent tissue. The