Virus-Mediated Transfer of DNA from Isolated Nuclei to the Cytoplasm and Nucleus of Hamster Fibroblasts in Culture

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Inactivated Sendai virus causes a temporary and temperature-dependent communication between isolated nuclei and cells, resulting in the transfer of small amounts of DNA from nuclei to the cells.

Introduction

Sendai virus-induced fusion of mammalian cell membranes, leading to the formation of multinucleate hybrid cells has been shown to occur between a great variety of different cell types with varying efficiencies [1–4]. The membranes of cells differing in origin or cells artificially altered, e.g. virus-transformed or mutant cells, differ widely in lipid composition, presence of specific enzymes and their functional properties, proteins, mucopolysaccharides, antigens, contact inhibition properties, electrical surface charge, adhesiveness to foreign or similar surfaces, permeability etc. [5–9]. Moreover, modifications to the cell membrane occur at different phases of the cell cycle [10–13]. Furthermore, Sendai virus-induced fusion can occur between homologous or heterologous membranes. The necessary conditions and proposed mechanisms for the phenomenon have been reported by several workers [14–18]. It might be proposed here that correlation of genetic differences between cells and fusion properties could assist in the elucidation or inherited or acquired membrane differences.

Although the external cell surfaces can show differences in different cell types, this is not the case with the nuclear membrane [19], although the importance of this membrane has been stressed [20–25].

In this paper, we report interactions between the surface membrane of cultured mammalian cells with isolated homologous nuclei, under the influence of inactivated Sendai virus. As it was decided to study membrane fusion in connection with the transfer of genetic material, it was thought that autoradiographic methods could give both a quantitative and qualitative interpretation of this phenomenon.

Methods

Cell Lines and Culture Methods

Chinese hamster fibroblasts [26] were cloned in this laboratory and the clone used in these studies was designated CH23, as it is characterized by the presence of 23 chromosomes in 37% of the cell population [27]. Ham's F12 medium [28] supplemented with 10% foetal bovine serum, 50,000 U/l penicillin, (I.C.I.), 50 mg/l streptomycin (Glaxo) and 2 mg/l amphotericin B (Squibb), was used throughout all the experiments, except in the case of relabelling of nuclei with ^{3}H-thymidine, where Eagle's minimum essential medium [29] (MEM) supplemented as above was used, because this medium does not contain thymidine and therefore labelling should be more efficient. Cells were free of contaminations as shown by ^{3}H-thymidine labelling and autoradiography [30].

Isolation and Assay of Labelled Nuclei

CH23 cell monolayers were labelled with ^{3}H-thymidine (sp. act. 25 Ci/m mol, the Radiochemical Centre, Amersham) at a concentration of 0.2 uCi/ml of MEM, for 3 days, corresponding to about 6 cell generations, starting from a cell population with a density of 7×10^3 cells per ml, in Roux bottles. Cells were collected by trypsinization and washed twice with Hank's BSS [31] to remove excess free ^{3}H-thymidine. Nuclei were isolated by the procedure of Chauveau *et al.* [32] slightly modified [33]. 2.5×10^8 cells were suspended in 10 ml of a solution (A) containing 2.2 M sucrose, 0.01 M KCl, 0.002 M $CaCl_2$, 0.002 M $MgCl_2$ and 0.01 M Tris, pH 7.0. The cytoplasmic membranes were disrupted by 3 cycles of freezing-thawing and homogenisation (less than 50 strokes using a glass homogeniser). Lysis was followed by examining acetic orcein-stained drops of the preparation under the microscope. After distruption of nearly 95% of the cells, the homogenate was transferred to 2 × 5 ml plastic centrifuge tubes and centrifuged at 23,100 rpm, at 0° C for 60 min, on the SW 39 rotor in the L-Spinco centrifuge. The top dense layer consisting of lipids, membranes mitochondria and a few intact cells and the middle layer, consisting of soluble cytoplasmic material were removed, and the walls of the tube were cleaned with tissue paper. The pellet, consisting mainly of nuclei, was suspended in 5 ml of fresh solution A by gentle homogenisation and the suspension centrifuged under the same conditions. The pellet obtained,

consisted almost exclusively of nuclei showing a normal appearance when stained with acetic orcein or safranin and examined under the microscope.

In order to examine whether labelled DNA was excreted from the isolated nuclei into the medium, the labelled nuclei were treated as in the nuclei-cell incubations (Table 1), but the cell sheets on coverslips were omitted and the experiments were carried out in small centrifuge tubes. After incubation, the nuclear suspension was centrifuged at 3,000 rpm at 0° C for 15 min and the supernatant medium removed. The nuclei pellets were dried at 90° C overnight and 0.05 ml distilled water plus 0.3 ml Soluene TM 100 Packard sample solubiliser were added by whirlymixing vigorously with the aid of a Fison's Whirlymixer. The tubes were transferred to a water bath at 30° C and the temperature was gradually increased to 55° C, agitating from time to time. The contents of every tube were transferred to counting vials with the aid of 4 × 2.5 ml proportions of ethanolic scintillator (10 g 2,5-diphenyloxasone, 1 g 1,4-bis-2-(4-methyl-5-phenyl-oxazolyl) benzene, 1,113 ml absolute ethanol in 2.5 litres xylene). The tubes containing the supernatant medium were cooled in an ice bath and each treated as follows: 1 ml of an ice cold solution of calf thymus DNA (100 ug/ml) was added by stirring, followed by 5 ml of ice cold 10 percent trichloroacetic acid (TCA) solution. The mixture was filtered through Whatman paper, grade 3 discs, which retained only the precipitated highly polymerised ^{3}H-labelled DNA. Discs were washed with TCA (25 min), cold ethanol 95 percent (25 min twice) and ether (15 min twice) and then dried using infra-red lamp. The discs were transferred to counting vials with 10 ml of the above scintillator without ethanol.

All vials were counted in a Packard Tricarb Liquid Scintillation Counter model 3320, using 55 percent gain and 50—1000 window setting, where the counting efficiency for tritium was a maximum. Counts per minute (CPM) were recorded and corrected by subtracting the counts given by the corresponding blank (vial plus scintillator). This experiment indicated that the nuclei preparation contains highly polymerised ^{3}H-labelled DNA and no free ^{3}H-thymidine.

Procedure for the Study of Nuclei-cell Communication

CH23 cells were cultured on glass coverslips (22 × 32 mm) in plastic petri dishes (60 cm diameter), using a humidified $-CO_2$ incubator at 37° C, until a non-confluent monolayer was formed and were then used immediately. All dishes were inoculated with the same number of cells by distributing in every 5 ml of a cell suspension containing 8×10^4 cells per ml of medium.

Before use, the nuclei were suspended in 10 ml Ham's F12 medium and centrifuged at 3,000 rpm at 0° C for 15 min to remove the sucrose, and subsequently resuspended in fresh medium at a density of 4×10^6 nuclei per ml.

The old medium of each petri dish was replaced by 2 ml of the above nuclear suspension. Sendai virus, inactivated as described [27] was added (where required) at this stage by means of concentrated suspension in Hanks' BSS, at the final concentrations of 3×10^3 or 10×10^3 HAU per ml of medium. Membrane suspension was also added at this stage, where required. The volume of supernatant medium plus nuclei, over the cell monolayers in every dish was finally adjusted to 2.5 ml by adding medium.

Where cell to cell fusion conditions [34] were used, the period of incubation at 0° C was increased to 40 min. This time was sufficient to permit all the nuclei to collect near the cell monolayer.

After incubation of the dishes at 37° C or 0° C for the appropriate time (Table 1) the coverslips were removed, dipped for a short period in 3 changes of physiological saline and the adhered cells fixed in methanol for 6 min. They were dried and kept in a dessicator under reduced pressure at 5° C prior to autoradiography.

Autoradiography and Microscopy

Each coverslip was mounted on a slide with the cells upwards and then coated with AR10 Kodak autoradiographic film, which was kept at 4° C and developed 18 days later using the D19 Kodak developer [35]. The cells were stained with buffered Giemsa and all the cells (approximately 2×10^4) on every coverslip were examined at the 480 × magnification of the Zeiss light microscope.

Results

Nuclei-cell Communication

The aim of these experiments was to investigate the uptake and fate of labelled nuclei in mammalian cells. However, under all conditions studied, no labelled nuclei phagocytised by cells were found, although the following critical points were noted:

a) The nuclei were heavily labelled with tritiated thymidine and as can be seen in Fig. 1 the number of grains over each nucleus exceeds 200. Isolated nuclei were washed extensively before use to eliminate any free ^{3}H-thymidine and labelled DNA released from broken nuclei during preparation (see Materials and Methods). Autoradiographs of labelled isolated nuclei did not show any background grains.

b) The autoradiographs were developed sucessfully and the film was not moved during the process of development. Several cumps of heavily-labelled nuclei could be seen in many autoradiographs, always absolutely corresponding to the light blue-stained nuclei beneath the grains. Fewer similar clumps were observed when the coverslips were rinsed rigorously with physiological saline before fixation; this leads to the conclusion that nuclei have no special affinity for the glass, at least under the conditions used.

c) The cell monolayer was used for the uptake experiment when it was dense but not confluent.

d) Parallel tests for the detection of any contaminants were negative.

The first experiments on the uptake of isolated and purified CH23 labelled nuclei by CH23 cells in monolayer cultures, were carried out at 37° C, for 3 and 5 h, without using Sendai virus. When the autoradiographs were examined under higher magnification, it was observed that a few cells contained several grains but these grains were not so abundant as to consider them belonging to phagocytised nuclei but rather to ^{3}H-labelled macromolecules.

Evidence that the grains represented DNA and not free ^{3}H-thymidine is as follows:

First, the nuclei preparations were washed extensively to remove free ^{3}H-thymidine and only DNA was labelled with ^{3}H-thymidine (see Materials and Methods).

Secondly, when ^{3}H-labelled DNA was isolated from the CH23 nuclei and incubated with CH23 cells, grains were obtained in both the cytoplasm and the nucleus. It has already been reported from this laboratory that mammalian DNA can be taken up in a macromolecular form and integrated into the recipient chromosomes [36, 37].

It became obvious that the origin of these grains over the spread cells could be explained in two ways:

(a) DNA was transferred from nuclei to cells through a kind of connection, or,

(b) DNA was excreted from the labelled nuclei in the medium and then taken up by the cells.

It was decided therefore to study further this peculiar way of transfer of genetic material, which has not been reported before. Because the phenomenon takes place obviously to a very limited extent, the autoradiographic methods employed were superior to liquid scintillation counting by which it seems very doubtful that the very small quantity of radioactivity transferred from nuclei to only a few cells, could be detected. The advantages of autoradiography, although time-consuming, against liquid scintillation counting in studying this phenomenon, were found to be the following:

a) The number of cells carrying grains could be easily found.

b) The number of grains over each cell could be counted.

c) The position and/or distribution of grains over the cells could be observed.

d) Connection between cells and nuclei could possibly be seen, giving a source of explanation of the mechanism of the phenomenon.

The conditions under which the experiments were carried out are shown in Table 1. Parameters which were varied in these experiments were: temperature, time of exposure of the cell monolayers to the labelled nuclei and the presence or absence of different doses of inactivated Sendai virus. Parameters which remained invariable were: the cell density on the glass, the density of nuclear suspension and the time of development of the autoradiographs. In every experiment, all the cells adhered on 3 coverslip cultures showing the same cell density were examined, and the cells with grains were recorded. The number of cells exhibiting grains in every experiment is shown in Table 2. Analytically the results of every experiment are as follows:

Experiments a, b and c: Maintenance of the coverslips carrying the cells and the nuclei at 0° C was the most favourable condition for any supposed communication

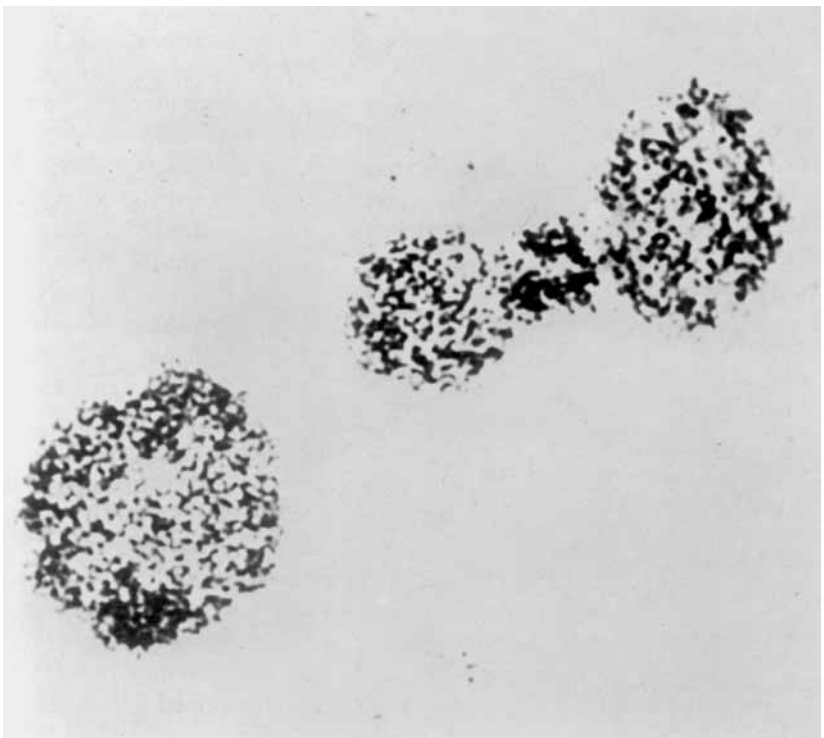

Fig. 1. Autoradiograph of isolated ^{3}H-thymidine labelled CH23-nuclei. Nuclei in interphase and telophase are shown to be heavily labelled Giemsa

Table 1. Changeable conditions of nuclei-cell communication

Exp.	Temperature	Time Hours	Inactivated Sendai virus HAU/ml$\times 10^{-3}$	Percentage of cells exhibiting silver grains
a	0° C	3	0	0
b	0° C	5	0	0
c	0° C	5	10	0
d	37° C	3	0	0.090
e	37° C	5	0	0.105
f	37° C	3	3	1.082
g	37° C	5	3	1.306
h	37° C	3	10	1.037
i	37° C	5	10	1.410
j	fusion conditions		3	1.270

Table 2. Distribution of grain numbers over the nucleus and the cytoplasm of cells after nuclei-cell communication

Exp.	Location of grains	Number of cells with grains 3–10*	11–30*	= 31*	Total number of cells with grains	Percentage of cells with grains
f	Nucleus	37	11	3	51	
	Cytoplasm	36	42	38	116	
	Both	7	11	31	49	
	Total	80	64	72	216	1.082
g	Nucleus	29	45	16	87	
	Cytoplasm	42	34	23	99	
	Both	16	22	38	76	
	Total	87	101	77	262	1.306
h	Nucleus	18	19	7	44	
	Cytoplasm	29	49	48	126	
	Both	10	5	23	38	
	Total	57	73	78	208	1.037
i	Nucleus	42	16	21	79	
	Cytoplasm	37	55	40	132	
	Both	12	18	41	71	
	Total	91	89	102	282	1.410
j	Nucleus	30	31	5	66	
	Cytoplasm	57	18	27	102	
	Both	24	33	29	86	
	Total	111	82	61	254	1.270

* Number of grains

and transfer between nuclei and cells. After 3 or 5 h, no cell containing silver grains could be recorded under high magnification. Aggregates of heavily labelled nuclei were found, and this is evidence that the autoradiographs were successful. Similarly in experiment c, using 10×10^3 HAU/ml of inactivated Sendai virus, no cell with superimposed silver grains could be located.

Experiment d: Grains were located over 18 cells (0.090 percent) out of about 2×10^4 cells examined in 3 coverslip cultures under high magnification. 16 cells had grains only over the cytoplasm and 2 cells had grains only over the cytoplasm and 2 cells had grains over the cytoplasm and the nucleus.

Experiment e: 5 h after the addition of nuclei to the petri dishes, the autoradiographs showed that 21 cells (0.105 percent) out of about 2×10^4 cells examined as described above, contained grains: 15 over the cytoplasm and 6 over the cytoplasm and the nucleus.

Experiments f to i: These experiments, in which inactivated virus was used, showed that the inactivated Sendai virus causes a 10- to 14-fold increment of the number of cells containing grains, compared to the previous cases where no virus was used. In all these cases more than 200 labelled cells out of about 2×10^4 were observed and the number and position of grains over the cells were recorded. The results are shown in detail in Table 2. From these results the following can be inferred.

a) The percentage of cells with grains is higher in cells which were left to interact with nuclei for 5 hours (experiments g and i) than for 3 h (experiments f and h).

b) The distribution of grain number is divided into 3 groups, containing 3–10, 11–30 and more than 30 grains per cell, over the cytoplasm, the nucleus or both the cytoplasm and nucleus seems to be random, although some observations could prove interesting. The cytoplasm of the labelled cells examined in every case is always the part which carries the majority of grains. Cells having their nuclei labelled with more than 30 grains are very rare after the 3-h treatment, but they become more frequent after 5-h treatment. Also, generally, cells with labelled nuclei are more abundant after the 5-h than after the 3-h treatment. These facts possibly could be explained on the basis that the material which is transferred from the nuclei to the cells is eventually transported from the cytoplasm to the nucleus.

Experiment j: In order to find out whether the phenomenon of nuclei-cell communication, which is clearly demonstrated above, is related to the phenomenon of virus-mediated cell fusion, experiments were carried out under the conditions used during cell-to-cell fusion process [34].

Cell-to-cell fusion requires random contacts between the cells at 4° C for about 15 min. When the cells are in a dense suspension and shaken gently, the possibilities of random cell contacts are obviously many. This random contact would not apply when studying the nuclei-cell interactions. The fusion process was therefore slightly modified by increasing the time of incubation at 4° C from 15 min to 40 min during which period the petri dishes were left undisturbed in order to allow the nuclei to sediment on the cell monolayer and interact by contact with the spread cells. As observed under the microscope, the time required for the nuclei to sediment on the bottom of a dish containing a 0.1–0.2 cm layer of nuclear suspension, is not longer than 15 min.

The efficiency of transfer in experiment j was almost as high as experiment g. This shows that fusion conditions do not facilitate further the communication between nuclei and cells.

Figs. 2, 3 and 4 show the manner of distribution grain numbers over the nucleus, the cytoplasm or both the nucleus and cytoplasm of labelled cells respectively. The number of cells with 5–10, 11–30, and 31–100 grains were counted in every experiment. Cells containing more than about 100 grains were not seen.

From Fig. 4 it can be seen that the mean number of cells labelled with 31–100 grains over the cytoplasm and the nucleus, in all experiments, predominates over the cells with 5–10 or 11–30 grains. This might indicate that when

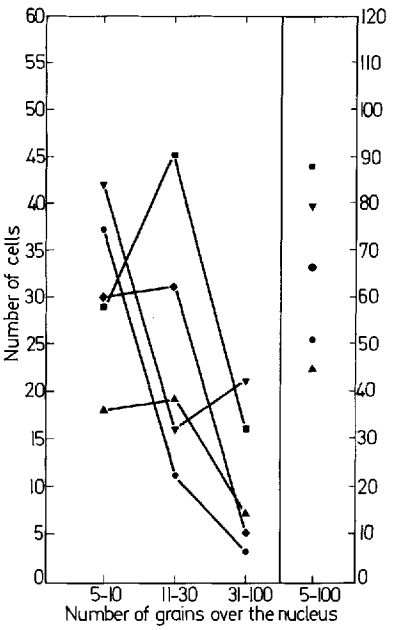

Fig. 2. Distribution of number of grains over the nucleus of recipient CH23 cells after nuclei-cell communication. Nuclei were heavily labelled with ^{3}H-thymidine

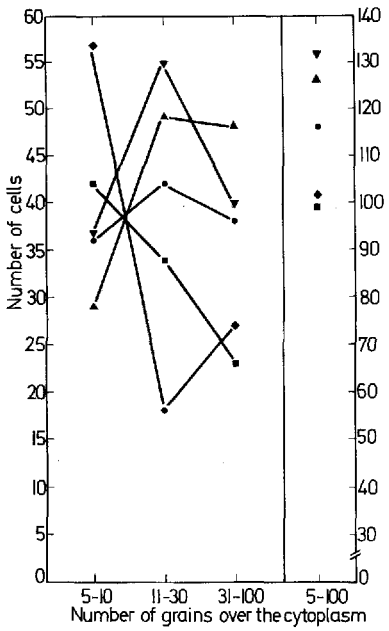

Fig. 3. Distribution of number of grains over the cytoplasm of recipient CH23 cells after nuclei-cell communication

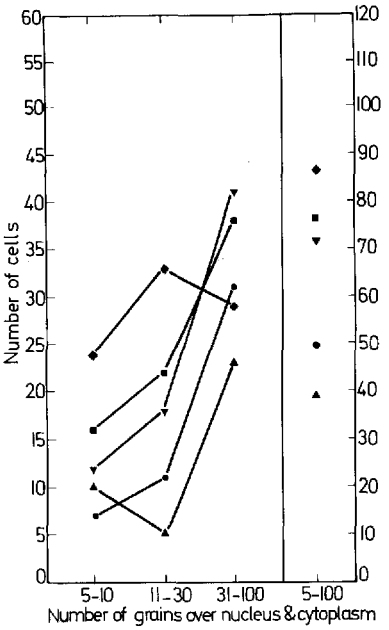

Fig. 4. Distribution of number of grains over nucleus and cytoplasm of recipient CH23 cells after nuclei-cell communication.
Symbols correspond to the changeable conditions of Table 1: ●, f; ■, g; ▲, h; ▼, i; ♦, j

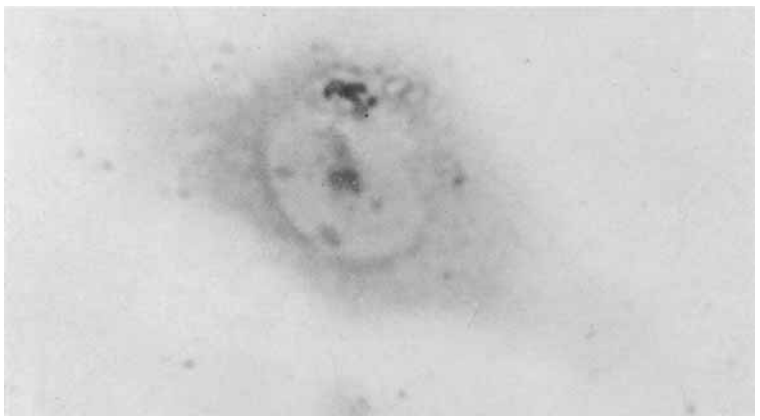

Fig. 5. CH23 cell containing silver grains located over the cytoplasm, after incubation of the cell monolayer with medium containing isolated ^{3}H-labelled CH23 nuclei, for 5 h, under the presence of inactivated Sendai virus. Giemsa

large amounts of labelled material are transferred, after nucleus-cell communication, the labelling of the host-cell nucleus is greater. The distribution of grains over particular regions in the cell could be dependent upon the cell cycle of the recipient cell during the transfer process.

Fig. 2 shows that heavy nuclear labelling (31—100 grains) of the recipient cell is less favoured when only the nucleus of the cell is labelled.

Fig. 3 shows that the number of grains over the cytoplasm of the recipient cells is very randomly distributed; this fact indicates that a variable amount of labelled material, within the limits indicated by the minimum (5) and maximum (100) of counted silver grains, can be transferred from the nuclei to the cells.

In all the slide-autoradiographs examined in the series of experiments described above, no kind of connection between labelled nuclei and cells was observed; this indicated that either (a) the connections and communications were temporary, or (b) there was no connection and the cells were taking up labelled material excreted from the nuclei to the medium. Figs. 5, 6 and 7 show cases of labelled cells after nuclei-cell communication.

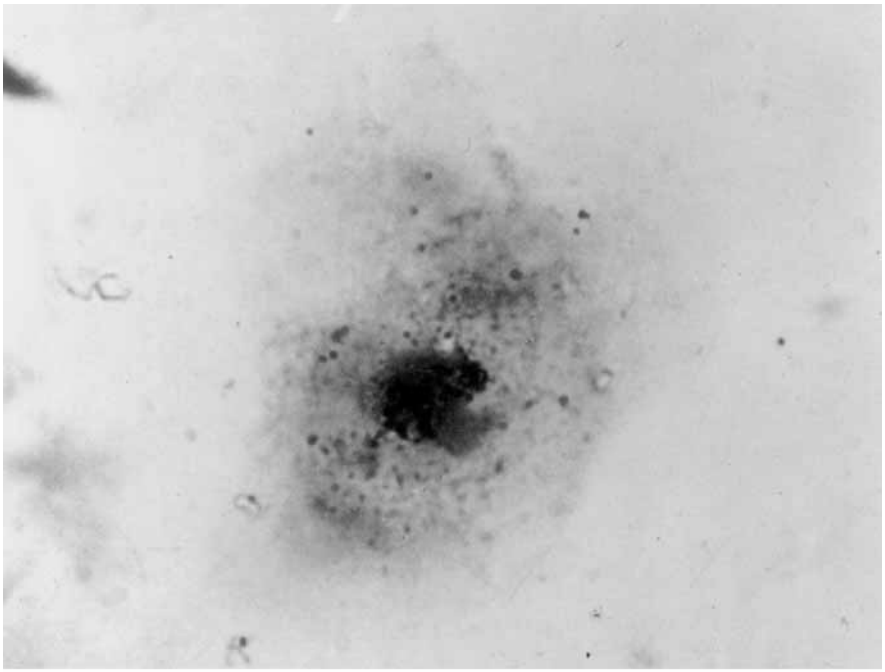

Fig. 6. CH23 cell containing silver grains, located over the nucleus, after incubation of the cell monolayer with medium containing isolated ^{3}H-labelled CH23 nuclei, for 5 h, under the presence of inactivated Sendai virus. Giemsa

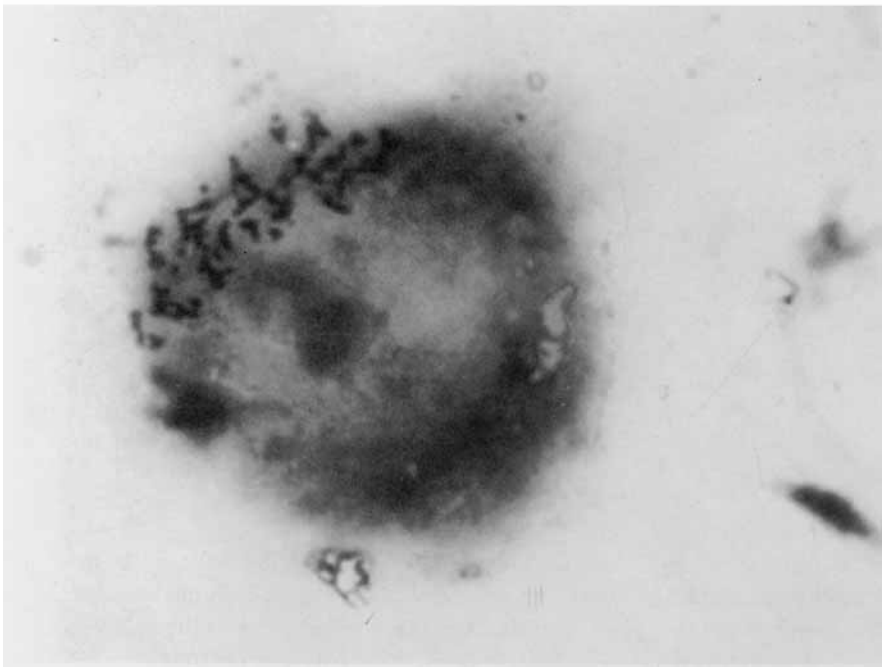

Fig. 7. CH23 cell showing extensive labelling with silver grains, located over a limited district of the nucleus, after incubation of the cell monolayer with medium containing isolated ^{3}H-labelled CH23 nuclei and inactivated Sendai virus for 5 h. Giemsa

Effect of Isolated Membranes on the Phenomenon of Nuclei-cell Communication

The phenomenon of transfer of labelled material from isolated nuclei to cells adhered to the glass provides the basis for the further study of the relationships and interactions between the nuclear and the cellular membranes. Unfortunately, the literature does not provide any data on interaction between these two membrane types so far. It was decided, therefore, to examine whether isolated cellu-

lar membranes had any effect on the nuclei-cell communication or on the DNA excretion from the isolated nuclei to the medium.

The membrane fraction isolated (as described in experimental procedures) was used without any further purification. Membranes were added as an extra component in a new series of dishes prepared (see experimental procedures) which were intended for use under the conditions of experiments b, d, e, h and i, shown in Table 1. An aliquot (0.2 ml) of a membrane suspension (prepared by dispersing the membranes isolated from 2×10^8 non-labelled CH23 cell in 2 ml of Ham's medium) were mixed with the contents of every dish, forming a cloudy mixture.

The results of these experiments were developed in a similar way to those in which no membranes were used.

The isolated membranes did not affect the transfer in any way, under the conditions used: the efficiency of transfer of labelled material from nuclei to cells was within the limits which would be expected if membranes were not used.

In the experiments of DNA excretion by the nuclei, the membranes also did not show any particular effect.

From these results it might be inferred that the fraction of membranes used was unable to modify the nuclear membrane in a way that would increase the communication between cells or would allow the macromolecules such as DNA to pass more freely from the inside to outside of the nucleus. These results also confirm that the inactivated Sendai virus is an external factor which can actively increase the efficiency of the phenomenon, independently of the presence of membrane fragments.

DNA Excretion from Isolated Nuclei

These experiments were carried out in order to ascertain whether or not the nuclei-cell interaction was a result of nuclei-cell contact. The counts per minute for the nuclear pellet (CPMn) varied from 653 to 721 and for the supernatant medium (CPMm) from 9 to 15. Corrections of the original counts by subtracting the background counts given by the corresponding vial plus scintillator were made. The counts given by the precipitate produced after TCA treatment were always very low compared to the counts given by the nuclei. The corrected excretion rate was recorded by plotting the values of $(CPM_n : CPM_m) \times 10^{-1}$ against the time (Fig. 8). From this Figure the following observations are made:

a) At 0° C, no excretion can be determined and the DNA excreted from the nuclei varies from 1.30 to 1.44 percent at different time intervals from 1 to 20 h (Fig. 8a).

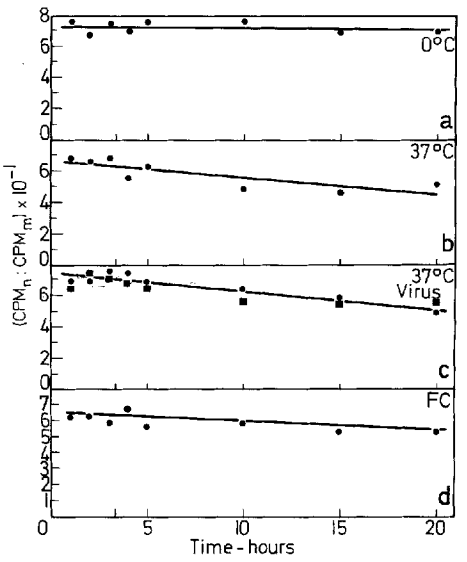

Fig. 8. Time course of DNA excretion from isolated CH23 nuclei. The conditions are described in Materials and Methods. Abbreviations: FC: fusion conditions (Table 1)

b) At 37° C the variation of the % DNA excreted from the nuclei is textended from 1.42 to 2.22 at different time intervals (Fig. 8b). The increase in excretion is more perceptible after the fifth hour, and it can be considered not to occur at all during the initial three hours.

c) The virus effect on the excretion is shown in Figs. 8c and 8d. Apparently the presence of the virus did not cause any alteration to the rate of excretion, during the 20 h of the experiments at both doses (3×10^3 and 10×10^3 HAU per ml) tested.

Therefore it is evident that the 10—14 fold increases in the number of labelled cells when using inactivated Sendai virus during cell-nuclei interaction, is not connected with or corresponding to an increased DNA excretion from the labelled nuclei. The two phenomena take place separately and independently. It might be said that the effect of the virus on the transfer phenomenon is rather more profound on the cellular than the nuclear membrane. The characteristic affinity of the inactivated Sendai virus for the cellular membrane is also becoming evident from the cell to cell fusion experiments and from the previously published electron micrographs showing viral particles associated with the cellular membrane, or viral particles associated with the cellular membrane, or viral particles within intercellular connections [15].

Experiments were also carried out to find out whether the membranes had any effect on the excretion of DNA from nuclei. The results are quite similar to those obtained from the experiments where membranes were not used, and are presented in Fig. 9. It is therefore concluded that

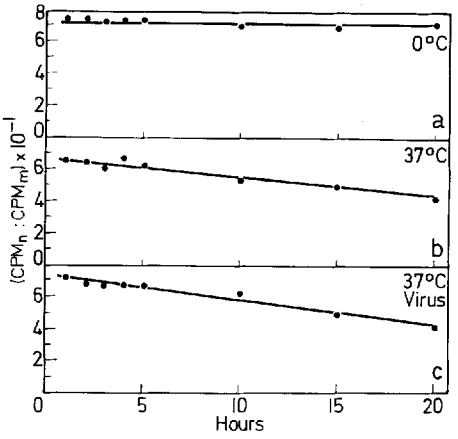

Fig. 9. Time course of DNA excretion from isolated CH23 nuclei in the presence of isolated cellular membranes. The conditions are described in Materials and Methods

cellular membranes do not cause any modifications to the nuclear membranes resulting in an altered pattern of DNA excretion with time.

Discussion

Phagocytosis has been shown to be a characteristic property of several types of cultured mammalian cells [38–40]. Fusion between the phagocytising cell membrane and the phagocytised membran-ecovered material does not seem to be essential during the process of phagocytosis and it is believed that all the phagocytised material is included in special vacuoles, the lysosomes, where it is degraded [39].

In this study, attempting to introduce homologous nuclei into the hamster fibroblasts, under the influence of inactivated Sendai virus, it was found that Sendai virus gives rise to a communication very different from phagocytosis or membrane fusion between nuclei and cells. This phenomenon was named nuclei-cell communication and was characterised by the following:

a) It is temperature-dependent. At 0° C no communication was observed. This might be considered as being related to the temperature-dependent factor of inactivated Sendai virus, inducing cell fusion [41–43], although a significant differences was found: fusion conditions (that is maintenance at 4° C and then a rise in temperature to 37° C) did not enhance the efficiency although this happens in cell fusion.

b) The phenomenon is temporary and does not lead to permanent fusion of the cellular with the nuclear membrane, as happens in cell fusion [2]. The contacts between cells and nuclei seem to be random and no special affinity to the nuclei for the adhered cells was determined. On the contrary, it has been shown that special affinity of the P388F-36 mouse lymphoma cells for the CH23 cells exists during fusion [44]. Therefore, inactivated Sendai virus causes aggregation of cells but does not cause aggregation of nuclei and cells.

c) During the temporary contact, a rather small amount of genetic material (DNA) is transferred from the nuclei to the cells. RNA or nuclear proteins associated with DNA may also be transferred. It is not known whether any transfer from the cytoplasm of the cell to the adhered nucleus occurs. The transferred material was located everywhere in the cell and its distribution seems to be random. However, the location of the transferred material over the nucleus was more frequent after 5 h of incubation; this might suggest that the transferred DNA in a degraded or macromolecular form is guided into the nucleus very possibly during the phase of DNA synthesis [36].

It has been shown that DNA can be taken up into the nucleus of recipient cells in a macromolecular form [37, 45, 46]. The problem in the direct transfer of isolated DNA was always its protection from degradation during isolation and inside the cell. Under the conditions of nuclei-cell communication it is obvious that the DNA in the isolated nuclei is intact, undegraded, in its natural environment and as shown biologically active [47]. The DNA is transferred directly from the nucleus to the cell and it is certain that at the first stage of transfer it exists in its natural macromolecular form and biologically active. Further investigation of the fate and activities of this DNA in the recipient cell should be suggested here; particularly after the findings of transport of nuclear DNA into the cytoplasm in cultured cells [48] and its association with the plasma membranes [49]. The function and significance of this membrane-associated DNA are not clear at the moment. Techniques of sub-nuclear fractionation [25] and studies on the interactions between purified isolated fractions of nuclear membranes, DNA and plasma membranes [21, 47, 50] are now being developed.

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