A STUDY OF THE PENETRATION OF MAMMALIAN CELLS BY DEOXYRIBONUCLEIC ACIDS

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ABSTRACT

Tritium-labeled deoxyribonucleic acid (DNA) from pneumococci and from human leukocytes was added to growing cultures of HeLa cells at 37°C. Autoradiography revealed an extensive localization of tritium in the nuclear regions. The label could not be removed by treatment with ribonuclease or dilute perchloric acid, but quantitative removal from the cells could be effected with deoxyribonuclease. Chemical and radioactivity determinations on nucleic acids isolated from the exposed HeLa cells revealed the presence of tritium in all 4 DNA bases. About 12 μg. of tritiated DNA was recovered from 6×10^6 HeLa cells which had been exposed for 24 hours to 240 μg. of the human DNA. From this, it is concluded that the amount of DNA, or its degradation products, taken up by the cells was equivalent to at least 10 per cent of the normal HeLa cell complement.

Hereditary determinants have been permanently acquired by cells by mere exposure to nucleic acids. This has been observed in several bacterial species (1, 2) in the transformation phenomenon experimentally induced by the appropriate DNA isolated either from closely related strains or from different species (3, 4). Although transfer of genetic information may be mediated, in both microbial and mammalian systems, by more elaborate devices such as viral infection, transduction or mating, hereditable transformation of mammalian cells by isolated nucleic acids has not yet been recorded. However, the genetic character of free nucleic acids has been demonstrated with mammalian cells in infectivity experiments with ribonucleic acids, (RNA) (5, 6) and DNA (7), isolated from viruses. In these instances, the invasion of the cells by the nucleic acid was manifested by cytopathological changes and by the appearance of virus particles identical with those from which the nucleic acids were isolated.

In the absence of such obvious biological activities as nucleic acid-mediated genetic transformation or virus induction, it is difficult to know whether penetration of exogenous nucleic acids into cells always constitutes a transfer of genetic information. It may be that the lack of observable biological activities is due to alteration or inactivation of the molecule by the cell following penetration. It may also be that invasive nucleic acids are not integrated into the genome of mammalian cells. In an attempt to gain further insight into the fate of invasive nucleic acids, following penetration, DNA from pneumococci and human leukocytes was labeled with tritium (8) and added to cultures of human (HeLa) cells.

MATERIALS AND METHODS

Nucleic Acids: DNA was isolated from the white cells of a patient with chronic granulocytic (myeloid) leukemia according to the method of Kay, Simmons, and Dounce (9). The preparation, designated 3LD, has been described in greater detail elsewhere (10). The pneumococcal DNA was obtained (11) by lysing

TABLE I

Effect of Exposure of Deoxyribonucleic Acid to Tritium at 0.39 Atmospheres at 27°C.

DNA preparation	Length of exposure to 3 curies of tritium	Radioactivity after reprecipitation*	$S_{50\,\%}$‡	Alkali hyperchromic effect	Relative transforming activity‖
		μc./mg.		*per cent*	*per cent*
Human	No exposure		23.5	36	
Human	2 hours	0.15	21.6	27	
Human	11 days¶	3.8	9.9	28	
Pneumococcal	No exposure		10.7	34	100
Pneumococcal	8 hours	1.75	10.9	33	60
Pneumococcal	1 day	3.9	10.2	36	35
Pneumococcal	2 days	21	7.85	26	10

* Precipitations carried out until constant radioactivity was achieved.

‡ Average sedimentation coefficient, $s_{20,w}$

§ Increase in absorbancy at 260 mμ due to alkalinization of dialyzed specimens to pH ca. 13.5.

‖ Transforming activity to streptomycin resistance relative to original taken as 100 per cent.

¶ Exposed to 2 curies.

the bacteria in 0.2 per cent deoxycholate followed by extraction with 0.15 M NaCl/0.15 M citrate. After centrifugation, the supernatant was precipitated with 1½ volumes of ethanol, and the DNA deproteinized (9). Relative transforming activities to streptomycin resistance (kindly determined by Dr. S. M. Beiser) were measured as previously described (11a).

Both DNA preparations were stored in the form of air-dried fibrous material. Tritiation of the DNA fibers (8) by the Wilzbach procedure (12) was carried out at room temperature by the New England Nuclear Corporation, Boston 18, Mass. Some of the human DNA specimens were exposed at 0.39 atmospheres to 2 curies of tritium for 11 days while others received 3 curies for 2 hours. The pneumococcal DNA samples were exposed to 3 curies of tritium for 8 hours as well as for 2 days. In order to remove nonspecific radioactivity and impurities, solutions of the nucleic acids (1 mg./ml. 0.002 M NaCl) were first dialyzed in the cold (0–3°C. for 2 days) against 6 changes of 0.002 M NaCl and then precipitated 2 to 4 times with 1½ volumes of ethanol. The precipitates were finally redissolved in 0.002 M NaCl. Constant radioactivity was achieved after the second reprecipitation. Specific radioactivities as determined by scintillation counting are listed in Table I. When examined by paper chromatography employing two different solvent systems (13, 14), the tritiated nucleic acid preparations did not reveal any degradation products under ultraviolet light.

Column Fractionation: A sample of the tritiated human DNA (3LD-H^3) was fractionated as described elsewhere (8, 10) on a column of the anion exchanger ECTEOLA-SF-1. Selected fractions, after dialysis against 0.002 M NaCl to remove excess salts, were concentrated and redialyzed. The DNA was precipitated with alcohol and finally taken up in a small volume of 0.002 M NaCl.

Hyperchromic effects were determined (15) by measuring the increase in absorbancy at 260 mμ after addition of 0.04 ml. of 19 N NaOH to 2.0 ml. of 0.01 mg. DNA/ml. 0.002 M NaCl.

Sedimentation measurements were made with a Spinco Model E ultracentrifuge equipped with ultraviolet optics employing solutions which contained about 0.03 mg. of DNA/ml. 0.2 M NaCl. Average sedimentation coefficients, $s_{50\%}$, were calculated by a modification of the method of Schachman (16).

Viscosity determinations were carried out with a modified Ostwald viscometer (16) which gave a flow time of about 180 sec. with 0.15 M NaCl at 25°. Measurements were made on different dilutions of the DNA ranging in concentration between 0.07 and 0.02 mg./ml. 0.15 M NaCl.

Tissue Culture: Cultures of HeLa cells were grown at 37° in a 5 per cent CO_2 atmosphere in Eagle's defined medium (17) to which penicillin (100 units/ml.), streptomycin (100 μg./ml.), and 20 per cent human serum had been added. The cells were then trypsinized (0.05 per cent) for 5 min. and harvested by centrifugation (18). About 1×10^5 cells were added to Eagle's enriched medium in petri dishes 5 cm. in diameter, each of which held 20×40 mm. coverslips. During the next 2 to 3 days of growth at 37° in the 5 per cent CO_2 atmosphere, the cells became attached to the coverslip to form a monolayer (18). The medium was then withdrawn and the cells washed with Hanks' balanced salt solution. Fresh

medium containing only 10 per cent human serum and the respective tritiated nucleic acid samples were added and the cells permitted to grow for predetermined lengths of time. The medium was then withdrawn, the cells washed 8 times for 10 min. with Hanks' balanced salt solution, fixed for 20 min. with acetic acid-alcohol (1:3) and again washed in several changes of salt solution. The coverslips with the adhering cells were then removed, broken into 5 approximately equal sections and mounted with Kaiser's gelatin on microscope slides which had previously been coated with 1 per cent gelatin. Some of the slides were then either treated with enzymes or extracted with dilute perchloric acid (19) before Kodak AR-10 photographic stripping film was applied for autoradiographic examination (20).

For enzymatic digestions, the slides were placed into 70 per cent alcohol for 10 minutes and then exposed for 2½ hours at 37° to 0.05 per cent solution of pancreatic deoxyribonuclease (DNase; Worthington Biochemical Corp., Freehold, New Jersey, 4 times recrystallized) which contained 0.003 M $MgSO_4$ and was adjusted to pH 6.5 with dilute alkali. The slides were then briefly dipped into 2 per cent perchloric acid (4°) and washed with distilled H_2O.

Other slides were exposed to a solution of 0.03 per cent ribonuclease (RNase, Armour Co., Chicago, twice recrystallized) for 2½ hours at 37°, then briefly dipped into 2 per cent perchloric acid and washed with distilled water. In order to extract acid-soluble material, slides were kept in 2 per cent perchloric acid for 20 min. at 4°.

After thorough rinsing with distilled water, Kodak AR-10 stripping film was applied to treated and untreated preparations. The slides were developed after exposure to the film for times varying between 10 days to 4 weeks, and stained with McNeal's tetrachrome stain (21).

Radioactivity: An internal Geiger-Muller flow counter using helium-isobutane gas was employed for radioactivity determinations of samples eluted from paper chromatograms. Solutions were plated on 10 $cm.^2$ aluminum planchets and counted in triplicate. Standard counting error was less than 5 per cent. *Anti-DNase:* Anti-DNase serum was obtained from rabbits which had been immunized with crystalline pancreatic DNase (We are greatly indebted to Drs. S. Beiser and H. S. Rosenkranz for a sample of the anti-DNase. A detailed description of the properties and characteristics of this preparation will be published elsewhere).

EXPERIMENTAL

Autoradiography Studies

In a typical experiment, 0.2 ml. of a solution of tritiated DNA was added to a petri dish which contained 4.8 ml. of Eagle's enriched medium and a monolayer of HeLa cells grown on a coverslip. The final radioactivity did not exceed 0.025 μc. per milliliter of medium. Samples with different specific activities were tested, and the nucleic acid concentrations ranged between 2 and 100 μg. per milliliter for different experiments. Incubation times at 37° varied between 2 and 24 hours. In some instances, the culture media which contained the tritiated nucleic acid were withdrawn after 24 hours, the cells thoroughly washed with Hanks' salt solution and then exposed to fresh media without radioactive material for an additional 24 hours. In some controls, cells were kept in contact with labeled DNA or tritiated thymidylic acid at 4° for comparable lengths of time.

The various nucleic acid samples tested were: 3LD exposed to 2 curies H^3 for 11 days, 3LD exposed to 3 curies for 2 hours, fractions of these samples eluted from Region I of ECTEOLA chromatograms, pneumococcal DNA exposed to 3 curies for 2 days and 8 hours, respectively, and tritiated thymidylic acid (11 days with 3 curies).

A number of competition studies were carried out in which unlabeled nucleic acids in 10 times larger concentration were added to the radioactive DNA in the culture medium. In these experiments, "cold" 3LD, thymidylic acid and a mixture of equal amounts of deoxyadenylic, thymidylic, deoxyguanylic and deoxycytidylic acids were tested. Grain counts over cytoplasmic and nuclear areas of at least 50 cells were made in order to determine whether incorporation of the radioactive DNA had been affected.

Results: Some properties of the tritiated nucleic acids are listed in Table I. As can be seen, the average sedimentation coefficient for both 3LD and the pneumococcal DNA decreased with the length of exposure to tritium gas. The hyperchromic shifts were somewhat lower than those of the corresponding untreated samples. An intrinsic viscosity of 29 dl./gm. was found for the 3LD preparation which had been tritiated for 2 hours. This can be compared to a viscosity of 39 dl./gm. for the same sample before exposure.

The pneumococcal DNA showed little alteration in either physical or biological properties after 8 hours' exposure to tritium. Further exposure, however, proved to be injurious to both.

Incorporation Studies: HeLa cells exposed for 1½ and 3 hours to tritiated nucleic acid showed little incorporation of radioactivity when examined by autoradiography. When cultures were incubated with tritiated DNA for 6 hours, about 35 per cent of the cells were labeled. After 24 hours, practically all cells contained radioactive material. Although silver grains were found over cytoplasmic and nuclear areas, the nuclear label was decidedly more pronounced. (Fig. 1). It can be seen from Figs. 1, 5, and 6 that the pneumo-

coccal and human DNA as well as a fraction of the human DNA which was eluted from the anion exchanger ECTEOLA-SF-1 were all equally well incorporated.

Since the mitotic cycle of HeLa cells lasts for about 26 to 28 hours, most of the cells probably divided at least once and synthesized DNA during the 24 hour incubation period.

Cells which had been extracted with cold 2 per cent perchloric acid for 20 min. to remove acid-soluble nucleotides still retained the radioactive label (Fig. 7). Retention of silver grains above the nuclei were also still evident after RNase treatment (Fig. 3). Treatment with DNase on the other hand removed most of the radioactivity from the cells (Figs. 2 and 8).

Addition of the anti-DNase to the culture medium apparently did not affect the extent of incorporation of the label. The antiserum was used in the event that some DNase might be released from the cell into the medium and break down the added nucleic acid.

Autoradiography revealed no intracellular radioactivity when the incubation was carried out with either tritiated DNA or thymidylic acid for 24 hours at 4° (Fig. 4). This suggests that the incorporation of the DNA which took place at 37° was not merely due to exchange of tritium or simple diffusion but that it necessitated an active metabolic participation by the cell. Inhibition of mitosis at the low temperature (22) may also be involved.

Biochemical Studies

A moderately sized tissue culture experiment was carried out in order to study the character of the incorporated nucleic acid. For this purpose, 0.4 ml. of a solution of 3LD-H^3 was added to a 12 ounce rectangular bottle which held a monolayer of about 6×10^6 HeLa cells in 9.6 ml. of Eagle's defined medium. Final concentration of DNA was 24 μg./ml. medium with a radioactivity of 0.022 μc./ml. A culture which received 0.4 ml. 0.002 M NaCl served as a control. The cells were incubated at 37° in a 5 per cent CO_2 atmosphere for 24 hours after which time the media were withdrawn. The cultures were washed with Hanks' balanced salt solution and then harvested by trypsinization in the usual manner (18). An acid-soluble fraction was obtained by extracting the cells with 7 per cent trichloroacetic acid (TCA) at 0 to 4°C. (23). The insoluble tissue sediment was washed successively with 80 and 95 per cent ethanol, and the residue extracted 3 times at 60° for 3 min. with a 3:1 mixture of ethanol-ether and again with cold 7 per cent TCA. The residue was then extracted for 15 min. at 90° with 7 per cent TCA and washed with a small aliquot of cold 7 per cent TCA which was added to the 90° extract. The TCA was removed with ether from the solutions which had been acidified with HCl to a final concentration of 0.01 N. The aqueous extracts were then taken down to dryness *in vacuo* in narrow heavy-walled glass tubes. The samples were hydrolyzed with 88 per cent formic acid at 175° in the sealed tubes (24). The hydrolysates were evaporated to dryness and then taken up in 0.1 N HCl for quantitative paper chromatography using isopropanol-HCl (13).

RESULTS

Paper chromatograms of the hydrolyzed 90° extract of the cells showed 4 ultraviolet-absorbing spots as well as 2 well-circumscribed chalk white

FIGURE 1

Autoradiograph of HeLa cells grown for 24 hours in the presence of tritiated DNA isolated from human leukocytes. McNeal's stain. × 960.

FIGURE 2

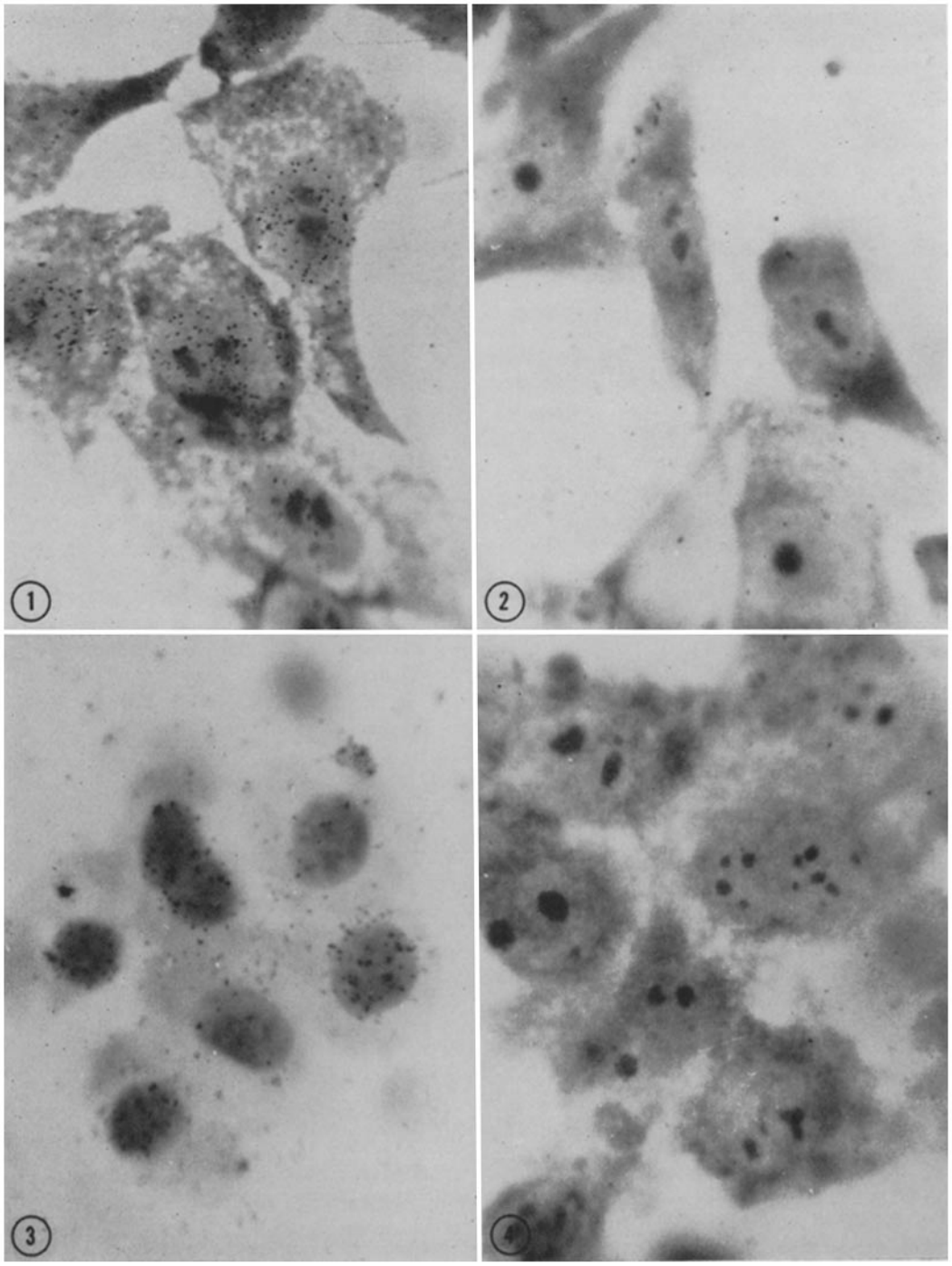
Autoradiograph of HeLa cells grown as above, and incubated for 2½ hours at 37°C. with pancreatic deoxyribonuclease. McNeal's stain. × 960.

FIGURE 3

Autoradiograph of HeLa cells grown as above, and incubated for 2½ hours at 37°C. with ribonuclease. McNeal's stain. × 960.

FIGURE 4

Autoradiograph of HeLa cells exposed to tritiated human DNA for 24 hours at 4°C. McNeal's stain. × 960.



areas which were easily distinguishable from the background paper in visible light. Identical results were obtained with material isolated from the control culture which had not been exposed to 3LD-H^3. The mobilities (R_F) of the ultraviolet-absorbing spots were the same as those obtained with adenine, guanine, cytosine and thymine standards. One of the chalk white areas coincided in R_F (0.71) to that of a standard of uridylic acid or deoxyuridylic acid whereas the other white spot (R_F 0.49) remained unidentified. The bases were eluted from the paper and analyzed for concentration and radioactivity. The results are summarized on Table II.

The molar base ratios listed are not to be construed as an accurate reflection of the true base composition of HeLa cell DNA, since the hot acid extract contained RNA. However, since the thymine to adenine ratio in human DNA (25, 26) is near unity, the thymine values given in Table II permit the estimate that about 40 per cent of the isolated adenine had been derived from RNA. Similarly, using the thymine values (Table II) and the amounts of guanine and cytosine relative to the thymine of human DNA, it can be estimated that roughly half of the isolated guanine and cytosine were of RNA origin. No attempt was made to separate the cellular RNA and DNA, or to isolate a highly purified DNA from the cells, since only 240 μg. of 3LD-H^3 was added to the cultures and only a fraction of this was taken up. The problem of a quantitative isolation is aggravated by the fact that the total HeLa cell sample was small (6×10^6 cells). Furthermore, unavoidable losses by adsorption on surfaces are to be expected when solutions of tiny amounts of tritiated macromolecules are put through several manipulations in glassware. This has been observed previously with tritiated insulin in a study deliberately designed to test this point (27). Sudden losses of radioactive phosphorus during washing and manipulation of labeled HeLa cells have also been observed (28). It must therefore be stressed that the recovery of radioactivity in the identified bases (Table II) represents a minimum value. A loss of radioactivity also results during the step of the extraction of the tritiated nucleic acids from the cells with TCA for 15 minutes at 90°.

The activity recovered from the nucleic acid bases of the cells amounted to about 1 per cent of the total radioactivity added to the culture medium. Since about 20 per cent of the activity of the added DNA is associated with the base moieties (8), the recovered bases therefore represent about 5 per cent of the added radioactivity. The total amount of DNA added to the cultures was 240 μg., and the amount estimated to be present in 6×10^6 HeLa cells in culture is about 120 μg. (29, 30). Thus, despite the losses involved in the several steps, it appears that DNA equivalent in mass to about at least 10 per cent of the cellular DNA had been taken up in the cells.

The possible upper limit for the amount of

FIGURE 5

Autoradiograph of HeLa cells grown for 24 hours in the presence of a fraction of tritiated human DNA eluted from the anion exchanger ECTEOLA-SF-1 with a NaCl gradient of 0–0.5 M. McNeal's stain. × 960.

FIGURE 6

Autoradiograph of HeLa cells grown for 24 hours in the presence of tritiated pneumococcal transforming DNA. McNeal's stain. × 960.

FIGURE 7

Autoradiograph of HeLa cells exposed to tritiated pneumococcal transforming DNA as above, and extracted for 20 min. with cold 2 per cent perchloric acid. McNeal's stain. × 960.

FIGURE 8

Autoradiograph of HeLa cells grown as above, and incubated with deoxyribonuclease for 2½ hours at 37°C. McNeal's stain. × 960.

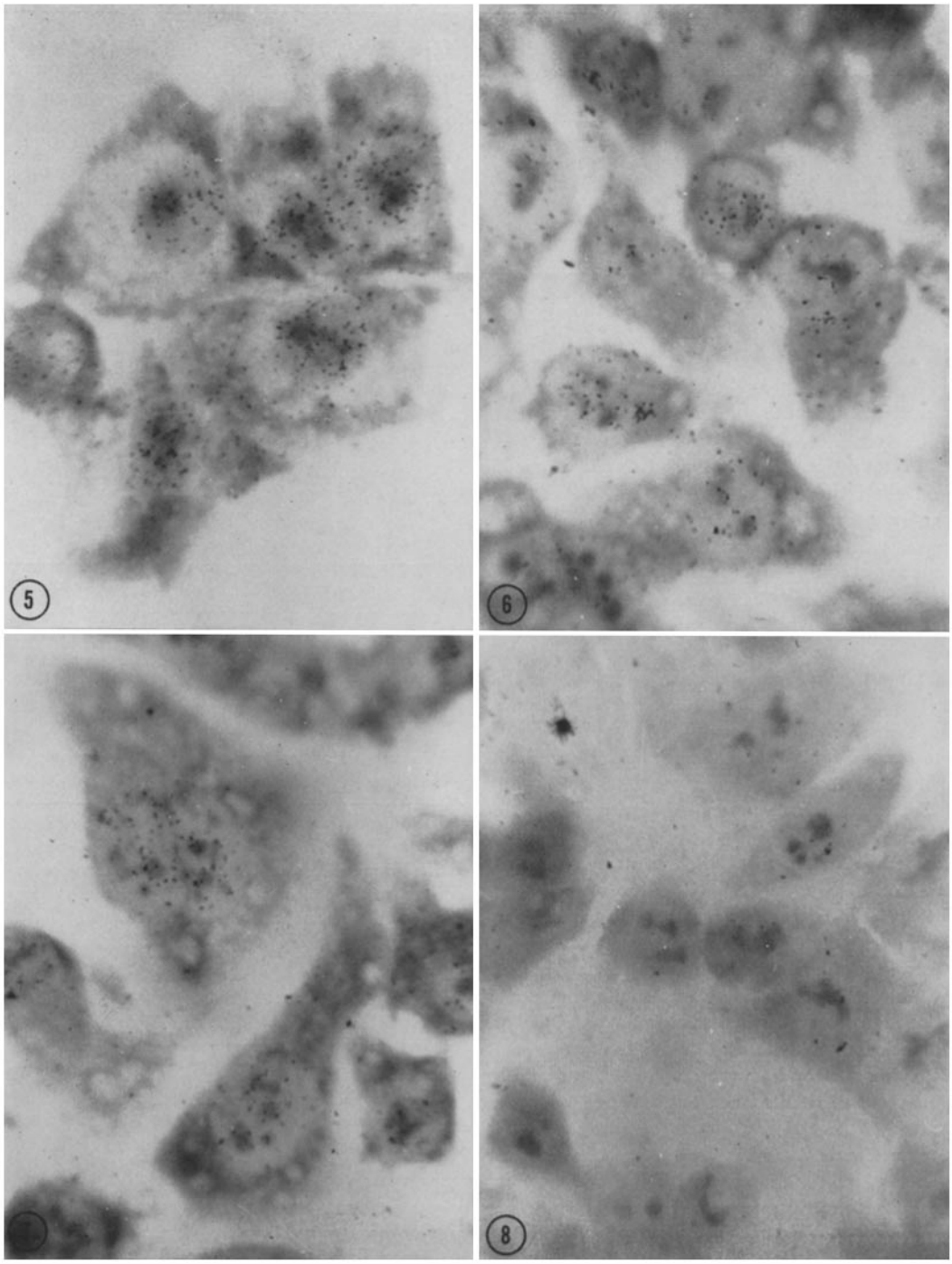


TABLE II

Nucleic Acid Bases Isolated from HeLa Cells Before and After Exposure to Tritiated DNA*

Bases	Before exposure			After exposure‖				
	R_F‡	Recovery	Molar ratio§	R_F‡	Recovery	Molar ratio§	Radio-activity,	Specific radioactivity
		micromoles			*micromoles*		*c.p.m.*	*c.p.m./μM.*
Guanine	0.32	0.046	1.31	0.33	0.038	1.12	520	1.4×10^4
Adenine	0.42	0.035	1.00	0.43	0.034	1.00	1,060	3.2×10^4
Cytosine	0.53	0.020	0.57	0.54	0.020	0.59	560	2.8×10^4
Thymine	0.74	0.020	0.57	0.76	0.022	0.64	1,600	6.8×10^4
Spot below cytosine	0.49			0.50			540	
Spot below thymine	0.71			0.73			790	

* These results were obtained with 6×10^6 cells after prior removal of acid-soluble and lipid-soluble fractions.

‡ In an isopropanol-HCl solvent system (13).

§ Adenine taken as 1.00.

‖ Final concentration of DNA (3LD-H^3) in 10 ml. of culture medium was 24 μg./ml.; total radioactivity was 3.6×10^5 counts/min.; exposure, 24 hours.

DNA taken up by the cells can be estimated from the recovery data in Table III. Whereas 50 per cent of the initial radioactivity could be accounted for by the fractions isolated from the cells and medium, 40 per cent was recovered from the medium. Hence, *at most*, 60 per cent of the added radioactivity could have been absorbed by the cells, assuming no losses. On this basis, this maximum value corresponds to about 1.2 HeLa-cell equivalents of DNA. However, this upper limit is undoubtedly much too high because of the unavoidable adsorption to glass surfaces mentioned above.

Some preliminary attempts were made to see whether inhibition of uptake of the label could be effected. When a 10-fold concentration of unlabeled 3LD was added to the cultures containing 6.4 μg. 3LD-H^3 per ml. and the cells exposed for 24 hours, there was approximately a 3-fold decrease in the nuclear grain count as determined by autoradiography. However, similar decreases were also observed when analogous quantities of unlabeled thymidylic acid or a mixture of the 4 deoxyribonucleotides were employed. Interpretation of these results must await detailed kinetic studies of the relative uptakes of nucleotide and polynucleotide materials by the cells as well as studies of the conditions which affect uptake.

TABLE III

*Distribution of Radioactivity (in c.p.m.) in Cell Fractions and Culture Medium Following Exposure of HeLa Cells to Tritiated DNA**

Fraction	Cells	Medium
Acid-precipitable‡	20,000	95,000
Acid-soluble,§ retained on Dowex-1	3,100	16,000
Acid-soluble,§ not retained on Dowex-1	10,300	32,000
Total	33,400	143,000

* 6×10^6 cells were exposed for 24 hours to 240 μg of 3LD-H^3, 360,000 c.p.m.

‡ Insoluble in 7 per cent TCA at 0–4°C. The radioactivity of the cell fraction includes the white spots above cytosine and thymine (see Table II) and is estimated from the fact that 20 per cent of the radioactivity of 3LD-H3 (8) resides in the bases.

§ Fraction soluble in 7 per cent TCA at 0–4°C.

DISCUSSION

The penetration of macromolecular substances such as hen's ovalbumin, human γ-globulin and bovine plasma into mammalian cells has been observed by means of the fluorescein-labeled antibody technique (31). Although these antigens were found largely in the cytoplasm, some could be traced into the nucleus. Enzymes such as ribonuclease have been observed to enter amebae (32), Ehrlich carcinoma (33) and ascites cells (34). In a recent study (35) it was concluded that several, but not all, species of mammalian cells including HeLa can take up rabbit globulin, antimyosin and bovine albumin by the process of

pinocytosis. This process of "cell drinking," first described by Lewis (36), involves invagination of the cell membrane and ultimate pinching off of an intracellular vacuole containing the engulfed macromolecular particle. A striking example of this is the uptake of colloidal particles of gold (mean diameter, 14 mμ) by HeLa cells. These particles could be seen with the electron microscope in inclusion droplets exclusively in the cytoplasm (37). Electron microscope studies (38) have revealed invaginations, many of which may possibly be continuous with vesicles of the endoplasmic reticulum which traverse the cytoplasm to the nuclear membrane. The feasability of an interaction between cytoplasmic and nuclear material was established by the observations of Afzelius (39) and Watson (40, 41) that the double membranes which form the nuclear envelope contain pores 500 to 1000 A in diameter. From these pores, cylindrical channels about 1200 A in diameter lead through the nucleoplasm toward the center of the nucleus. Passage of macromolecular material such as nucleic acid through the cytoplasmic matrix into the nucleus by way of the nuclear pores would thus be possible.

There have been a few experiments (42–44) dealing with the incorporation of nucleic acids into bacteria. There have also been studies of absorption of biosynthetically labeled nucleic acids (45, 46) by mammalian cells. Although evidence of uptake of the DNA was obtained in those experiments, the nature of the absorbed material was not elucidated.

It is known that the complete integrity of DNA involved in genetic determination in bacterial transformation is not necessary for the demonstration of this phenomenon. For example, DNA active in the transformation of pneumococcus to antibiotic resistance can be cut to at least one-fourth its original molecular size and still exhibit this activity (47). Furthermore, the transforming activity of DNA extensively damaged by deliberate high-temperature treatment can be largely restored by subsequent heating at intermediate temperatures (48). However, more extensive damage than that mentioned above can result in irreversible inactivation. Hence, when a nucleic acid penetrates a cell and causes virus production or induces genetic transformation, the biologically and chemically significant portion of the molecule must have survived even though other portions might have been lost or severely damaged.

The experiments reported in this paper indicate that the radioactive label in tritiated DNA can penetrate the human cell (HeLa) in culture. The radioautographic data reveal that the label within the cells is removable by DNase but not RNase treatment. The washing of the cells with dilute perchloric acid also did not result in removal of the label. It can therefore be inferred that the label within the cell is associated with macromolecules with properties expected from DNA. The DNA bases isolated from these cells were all labeled as would be expected from an incorporation of the original intact molecules. The autoradiographic results show that the human leukocytic and the pneumococcal DNA behave in a similar fashion. Both specimens of labeled DNA show about the same degree of preferential labeling of the nuclear regions of the HeLa cells.

These findings can be interpreted in at least two ways. It could be that some or *all* the invading DNA molecules had been broken down by the cellular nucleases and then reassembled before deposition in the nuclear regions. Or, the same findings could have arisen from maintenance of the intact structures during the adsorption, transfer and deposition phases. It would appear that a selection between these alternatives might be possible by exposing mammalian cells to pneumococcal transforming DNA followed by reisolation of active nucleic acid. Such an experiment is now in progress.

The radioactivity data (Table III) suggest that DNA or its components equivalent in mass at least to about 10 per cent of the cellular DNA is taken up by the human cells when they are exposed for 24 hours to a medium containing about twice the DNA content of the cells in the medium. It will be of interest to learn whether such uptake constitutes acquisition by the cell of new genetic information or genetic determinants.

This is not necessarily an academic question since the genetics of strains of *Neisseria* can be permanently altered by the DNA occurring in the normal slimy exudate of heterogenetic strains of *Neisseria* (49, 50). Hotchkiss has speculated (51) that this type of mutation might occur naturally. Whether this type of phenomenon takes place with mammalian cells is not yet known. The closest analogy in mammalian systems is the infection of mouse cells in culture by a DNA or

at least a DNase-sensitive component of the polyoma virus (7). In this instance, as in the several examples of infective ribonucleic acids, formation of a specific virus was a direct result of the infection. It is not yet known whether the infection was mediated by an *intact* molecule of nucleic acid. But those observations as well as the data derived from the present experiments suggest that extracellular DNA, or at least a complex form thereof, can penetrate mammalian cells.

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