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TRANSFORMATION EXPERIMENTS WITH MURINE LYMPHOBLASTIC CELLS (L5178Y) GROWN IN CULTURE*

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Abstract—Experiments are described which were designed to accomplish the transformation of murine leukemic L5178Y cells from the "wild" state of amethopterin-sensitivity to drug-resistant forms by incubation in culture with DNA from resistant lines; evidence of such transformation could not be detected.

The conditions governing the method of assay are considered. The cells do not form an extracellular DNase and possess only rather weak intracellular DNase activity; the latter has an acid pH optimum. The uptake by L5178Y cells of tritium-labeled DNA, derived from L5178Y cells grown in the presence of ^{3}H-thymidine, has been studied.

THE transfer of genetic material from one bacterial cell to another may be accomplished through the mediation of a bacteriophage or by the direct contact or "conjugation" of the donor and the recipient. In some cases, "transformation" may be accomplished by incubating recipient cells with suitable extracts prepared from donor cells; the active constituent of such extracts appears to be a deoxyribonucleic acid (DNA). Transformation has been demonstrated with only a few species of micro-organisms; indeed, with many bacterial species, under similar circumstances, transformation has not been demonstrable.

At least two attempts to obtain transformation in mammalian systems have been reported; each of these involved intact animals[1, 16] and transformation was not demonstrated.

Recent developments in the culture of mammalian cells *in vitro* have led, as Zamenhof predicted three years ago, to attempts to obtain transformation of somatic mammalian cells.[21] The development of media which support the clonal proliferation of murine leukemic lymphoblasts L5178Y in culture[11] has permitted the isolation of numerous mutant strains of this cell line, including a series resistant to the action of various antimetabolites. Thus, step-wise mutational selections have led to the derivation of many new resistant clones which require, for a given degree of inhibition, up to as much as 100,000 times the concentration of amethopterin necessary for comparable inhibition of the sensitive parent line. Some evidence has been obtained which indicates that the genetic material of the cell was the mutational site which conferred

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resistance to amethopterin,[6, 7*] although the possibility that non-genetic mutational sites were affected is not excluded.

MATERIALS AND METHODS

The composition of the medium which supports the clonal proliferation of L5178Y cells has been described[11] and this medium was used throughout these studies. The conditions employed for the growth of the cells have been described previously.[6, 11]

Amethopterin, generously supplied by the American Cyanamid Company, was prepared as a concentrated stock solution, dispensed in ampoules and kept in the frozen state. Calf thymus DNA was prepared according to the method of Chargaff.[3] Tritiated thymidine (172 μC/μmole) was obtained from the New England Nuclear Corporation.

The preparation of DNA from L5178Y cells

For the experiments described herein, a simple method was designed to extract DNA following the disruption of the cells with minimum damage to the nuclei. After harvesting with the aid of a refrigerated centrifuge from large-scale incubations of media, the cells were washed twice with approximately 15 vols of ice-cold solution of sucrose (0·25 M) containing calcium chloride (0·002 M). The washed cells were suspended in 10 vols of the same solution and homogenized for 75 sec at 5000 rev/min in a homogenizer (A. H. Thomas Co., size C) with a Teflon pestle fitted to an aluminum rod. The homogenate was centrifuged at about 900 **g** for 7 min at 0° and the resultant residue was washed 3 times (each washing employed 10 vols of the solution of sucrose-calcium chloride) with intermediate centrifugations for 7 min at 0°. The washed nuclei were stirred with 15 vols of a solution of sodium chloride (2 M) at 2° for 3–4 hr, and the resultant mixture was centrifuged at 20,000 **g** (Spinco; Rotor 30; 15,000 rev/min) for 30 min. The supernatant fluid was poured into 2·5 times its volume of ice-cold ethanol (95%). After collection of the precipitated fibers on a glass rod, these were washed with a solution of ethanol (80%), and then with ethanol (95%). The precipitate was dispersed in 10 vols of a solution of sodium chloride (1 M) and a sufficient amount of a solution of recrystallized sodium dodecyl sulfate (5%) in aqueous ethanol (45%) was added to give a final concentration of 0·5%. After 15 min at room temperature, the mixture was gently homogenized with an equal volume of chloroform:octanol (5:1; v/v). After centrifugation the aquous phase was separated, and the procedure was repeated until no precipitate was formed at the interface. The aqueous phase was poured into 2·5 vols of ice-cold ethanol, the precipitated fibers were washed with ethanol (first with 80%, and then with 95%), blotted dry and dissolved in the same volume of a sterile solution (0·9%) of sodium chloride as that used for the homogenization. Estimation of the DNA content was carried out by the method of Schneider,[19] while analyses for ribonucleic acid (RNA) used the procedure of Zamenhof.[22]

Such preparations contained about 500 μg of DNA and about 50 μg of RNA per ml. Approximately 5 per cent of the uv-absorbing material could be removed by dialysis against saline. The rate of growth of L5178Y cells was not measurably affected by the isolated DNA at levels up to 100 μg per ml of medium.

Preparation of ^{3}H-*DNA*

To 2 l. of medium were added hypoxanthine (12 mg), serine (41 mg), glycine (20 mg), ^{3}H-thymidine (3·4 mg; 680 μC), amethopterin (final concentration, 3×10^{-8}

* G. A. Fischer, unpublished results.

molar), and an inoculum of 4×10^7 L5178Y cells. After 70 hr the cell count was $3{\cdot}1 \times 10^5$/ml and the cells had an unhealthy appearance, an effect attributable to the intense beta-radiation released within the cells by the tritium. The cells were harvested (wet weight, 530 mg) and the DNA was extracted by the method previously outlined. After deproteinization and reprecipitation, the DNA was dissolved in 3% (w/v) sodium chloride (10 ml) and dialysed in the cold against 15 vols of a 3% solution of sodium chloride. The dialysate was stirred for 4 hr with activated charcoal (0·3 g). The charcoal was removed by centrifugation and the supernatant fraction was dialysed overnight against 50 vols of a 3% solution of sodium chloride (two changes). The dialysed fraction was poured into ice-cold 95% alcohol, the DNA fibers were washed with 80% and then with 95% alcohol, and dissolved in sterile physiological saline (6 ml). The DNA-content was 540 μg, as determined by the optical density at 260 mμ, assuming that a 0·1% (w/v) solution of DNA has an O.D. of 22·6 at this wave length. The total radioactivity of the DNA, obtained as determined by liquid scintillation counting, was $1{\cdot}53 \times 10^6$ cpm, and $9{\cdot}06 \times 10^6$ cpm by counting on planchets in a gas-flow counter. The radioactive material was precipitated completely by adjusting the sample to 5% with respect to trichloroacetic acid (TCA) in the presence of added unlabeled carrier DNA.

Preliminary experiments showed that if L5178Y cells were incubated for 2 hr in medium at 37°, under a gas mixture composed of 5% CO_2 and 95% air, at a cell concentration of 1×10^7/ml, the pH fell from 7·5 to $7{\cdot}1 \pm 0{\cdot}05$ within 15 min, and then remained constant. At the end of the incubation period dilution experiments indicated that essentially all cells were viable.

The assay system

A prerequisite for success in transformation experiments is the development of a satisfactory assay system that will detect a small number of transformants in the presence of an overwhelming majority of sensitive cells. The highly resistant lines, used as a source of the DNA, arose from cells in which a number of independent mutations had occurred and with which there were required from 100-fold to 40,000-fold the concentration of amethopterin for equivalent inhibition, as compared to the sensitive line. The preponderance of mutational steps conferred a low level of resistance (from 2- to 8-fold).[7]* Evidence from bacterial systems indicates that when drug resistance is acquired in this stepwise fashion, it is transferred to recipient cells in single steps as well.[5] Consequently, it is necessary to use a level of amethopterin just sufficient to inhibit the reproduction of the sensitive cells, while permitting the reproduction of a single-step transformant. The assay for amethopterin-resistant mutants is complicated by the fact that, under these conditions, a certain rate of spontaneous mutation (approximately once during a doubling of 5×10^6 cells) to drug resistance occurs, and also the progeny of spontaneously resistant mutants will be present in the batch of sensitive cells used as the recipients of the DNA*

It is to be anticipated that only a small percentage of the recipient cells would be transformed. Consequently, incubation must be continued for many days. Under the conditions chosen for the assay, each of 20 tubes contained approximately 40,000 cells in 5 ml of medium which was $2{\cdot}5 \times 10^{-8}$ molar with respect to amethopterin. After incubation for 5 days, the medium was changed by centrifuging the tubes at

* G. A. Fischer, unpublished results.

approximately 7000 **g**, decanting the old medium and replacing it with fresh medium; in some experiments a further change was made on the tenth day. Under these circumstances, no growth occurred in control tubes containing sensitive cells during the first 5 days and, usually, only 10–20 per cent of the tubes had grown appreciably after 10–14 days, suggesting a frequency of occurrence of mutants of about 1 per 100,000 cells.

EXPERIMENTAL AND DISCUSSION

Transformation experiments

Numerous attempts to achieve transformation have been made, with minor modifications of the conditions described. In these experiments, L5178Y cells were employed as the recipients.

L5178Y cells were harvested in the logarithmic phase of growth in culture, washed once and diluted to 90 ml, in order to obtain an inoculum of 2×10^5 cells per ml; these cells were distributed equally among 6 culture tubes. After incubation for 3 hr at 37°, 0·15 ml of solution containing DNA (approximately 100 μg) prepared from amethopterin-resistant cells was added to one culture, and 0·015 ml was added to another. A solution of DNA (0·15 ml and 0·015 ml, respectively), prepared from sensitive cells, was added to a second pair of cultures. The remaining pair served as controls. After incubation for 20 hr, 10 ml of each culture was taken, the cells were harvested by centrifugation, and resuspended in 10 ml of medium. After 5 min at room temperature, the cells were collected and the washing was repeated. To detect transformants, assays were conducted as described above, using 20 cultures for each experimental condition. No evidence for transformation was obtained, variations in estimates of mutant frequency being no greater than might be expected from random distribution of pre-existing resistant mutants.

Many reasons might be advanced to explain our failure to obtain transformation. The actual mechanism of this phenomenon in the bacterial systems, in which success has been achieved, is largely a matter of conjecture. It is believed to be a replacement of a genetic determinant which is part of the recipient cell by a homologue, or allelic form, present in the transforming solution and possibly this involves a recombination mechanism or a copy error.[17]

With near-diploid somatic mammalian cells in which each hereditary property is determined by a pair of genes, this genetic alteration may be correspondingly more difficult, and it is possible that the expression of transformation is complicated by questions of dominance. Accordingly, the conditions which we have employed may be unsuitable. Two other possible causes for failure which were considered are (1) destruction of the potential transforming factor by deoxyribonucleosidases (DNases) of the cells, and (2) the DNA might not have been taken up by L5178Y under the conditions used. These two possibilities have been examined.

Extracellular DNase

For the investigation of extracellular DNases, a turbidimetric method of assay was employed, because the high optical density of the medium excluded the possibility of observing changes at 260 mμ. The assay was a modification of the method of McCarty.[15] The stock substrate solution was a solution of calf thymus DNA (5 mg/ml) in a solution of sodium chloride (0·9%). Before use this was diluted (1 : 10) with the

saline solution or with sodium acetate–acetic acid buffer (pH 5·4, 0·1 M). The diluted DNA solution (5 ml) was incubated at 37° with 5 ml of the solution under investigation. These included: (a) the growth medium which contains 10 per cent horse serum; (b) medium containing L5178Y cells which had grown from an inoculum of from 2×10^4 to $5{\cdot}7 \times 10^5$ in 60 hr; and (c) the same as (b), except that cells were removed by centrifugation. At zero-time, and at regular intervals thereafter up to 6 hr, 1 ml was removed from each incubation mixture and quickly frozen in a bath of acetone and solid carbon dioxide. At the end of the experiment, all samples were thawed, immediately acidified by the addition of 1 ml of a solution of hydrochloric acid (1 N), and the optical density was read at 425 mμ in the Beckman spectrophotometer using 1-cm cuvettes. No change was observed, with (a), (b) or (c), in periods up to 6 hr in several different experiments at pH-values of 7·2 or 5·4. It may be concluded that there is no DNase activity associated with the samples of horse serum that we have tested, nor does L5178Y possess an extracellular DNase.

Although the method of assay for DNase employed in these experiments with L5178Y lymphoblasts is not a sensitive one, and while the results clearly demonstrated that an active DNase is absent from the solutions that have been tested, the possibility remained that enzymes are present which attack only a very limited number of internucleotide linkages near the center of the molecules of DNA, or split off one or more essential nucleotides from the ends of the DNA chains. In either case, no change would be observed in the turbidity in the assay, but transforming activity might be lost. Other experiments, in which DNA containing ^{3}H-thymidine was used, are in agreement with these conclusions and indicate that one of the possibilities mentioned above, i.e., the existence of an exoDNase, is unlikely.

Intracellular DNase

Transformation involves penetration of the cell wall of the recipient cell by a molecule of DNA and, subsequently, its incorporation into the genetic apparatus of the cell. During the interval between these two events, the DNA might be exposed to attack by intracellular DNases. Experiments designed to indicate whether such attack occurred were carried out. Two preparations from the cells were investigated. The first was an acetone powder prepared by homogenizing washed cells with 20 vols of acetone which had been cooled to −20°. The homogenate was centrifuged at approximately 700 **g** in a refrigerated centrifuge and the precipitate was washed twice with cold acetone. The last traces of acetone were removed under vacuum and the powder was stored at −10°. For assays, the powder was stirred with 10 vols of a solution of sodium chloride (0·9%) at room temperature for 10 min. The extract was spun at about 900 **g** for 10 min. The second preparation was obtained by disrupting the cells by shaking at high speed for 30 sec with glass beads (Type Z/120), using water as the suspending fluid. The beads and cell debris were removed by centrifugation, washed with a small volume of water and the combined supernatant fluid and washings were stored at 4° under a layer of toluene. DNase activity was assayed by a modification of the method of Kay.[12]

The incubation mixture consisted of 2 ml of cellular extract (equivalent to approximately $1{\cdot}5 \times 10^8$ cells) and 5 ml of calf thymus DNA (0·1 per cent (w/v) in a solution of sodium chloride (0·9%)); the mixture was adjusted to the appropriate pH, and 1 ml of buffer (either sodium acetate-acetic acid (pH 5·4, 0·1 M) or Tris/HCl (pH 7·4, 0·2 M)

was added). Incubation was carried out at 37°. Aliquots (1 ml) were removed from the mixture at regular intervals and acidified by the addition of 0·25 N sulphuric acid (3·0 ml). After 15 min at 0° the mixture was spun at 1200 g for 10 min and the optical density at 260 mμ of the supernatant fluid was determined.

Neither preparation had any activity at pH 7·4, whereas at pH 5·4 a slight, but detectable, hydrolysis of the DNA to acid-soluble material occurred. The weights of wet cells used and the final total volumes of both enzymic preparations were approximately the same, and it appeared that the glass-bead homogenate contained a higher level of enzyme activity. Webb has reported that an unspecified mouse leukemic tissue has a cytoplasmic DNase with an acid-pH optimum, the activity of which appears after storage at 4°.[20] Thus, it would seem that the transforming DNA is not exposed to any great hazard of enzymic degradation in our system. It remained to determine if DNA could be taken up by L5178Y cells.

Uptake of ^{3}H-*DNA by L5178Y cells*

The results of studies on the uptake of ^{32}P-labeled DNA by cells of strains of *Hemophilus* and *Pneumococcus*[10, 14, 18] indicate that the DNA is first bound in an intermediate form to sites, probably on the cell wall. From these sites it may either return to the medium or pass irreversibly to the interior of the cell. A similar conclusion has been reached by Fox and Hotchkiss[8] from a study of the kinetics of transformation.

L5178Y cells, harvested from a culture during the logarithmic phase of growth (levels of 3–6 × 10^5/ml), were washed twice, and added to 9 ml of growth medium to give a concentration of 1·1 × 10^7 cells per ml. ^{3}H-DNA in sterile saline (1 ml, 90 μg/ml) was added and the suspension incubated at 37° for 2 hr under an atmosphere of 5% CO_2 and 95% air in a Dubnoff shaker. In order to maintain the cells in the logarithmic phase of growth, all operations were carried out at 37° (using medium and glassware that had been pre-heated to 37°). After incubation for 2 hr the suspension was poured into a centrifuge tube and the incubation vessel was washed with medium; the washings were added to the suspension which was then centrifuged. The supernatant fluid was decanted, and the last drops were wiped from the tube with a tissue. The sedimented cells were resuspended in cold medium (2·5 ml), transferred to a clean tube, and, after 5 min in an ice-bath, the cells were spun down. The washing was repeated twice in this manner, except that the solution for the third wash contained 50 μg of unlabeled DNA prepared from L5178Y cells. The volume used for the fourth wash was 5 ml and, at this stage, the cell suspension was divided into two approximately equal portions. One-half was given a further cold wash (cold washed cells). The other half was incubated for 15 min at 37° in medium (2·5 ml), the cells were collected by centrifugation and rewashed (incubated cells). Water (0·4 ml) was added to both samples of cells and the mixture was plated for counting. Because of difficulties arising from self-absorption, the plated cells were triturated with ice-cold 5% TCA (3 × 2 ml); the residue was extracted for 20 min at 90° with 5% TCA (2 × 3 ml). TCA was removed from the extracts by repeated washing with ether. The counts from the various fractions are shown in Table 1. The pattern of uptake of ^{3}H-DNA by the cells was very similar when serum was omitted from the medium. The method of counting the activity in the cells in this experiment, i.e., plating out, followed by extraction of the plated cells with TCA, is inefficient and some

reservations must be held concerning accuracy. However, from the data obtained, it would appear that ^{3}H-DNA is bound to the cells in a form which is not removable by washing either in the cold or at 37°. The last washes contained small amounts of radioactivity. This may be explained in a number of ways. There may be a slow release of activity from the cells into the medium; the activity may be associated with a very small number of cells remaining unsedimented; or there may be damage to a few cells (during the resuspension and washing procedure) with release of bound DNA. A portion of the supernatant fraction was cooled to 0°, TCA was added to give a final concentration of 5% and the suspension was centrifuged. All the radioactivity had been precipitated, confirming the conclusion derived from previous experiments that there is no extracellular DNase which acts on the DNA in the medium.

TABLE 1. UPTAKE OF ^{3}H-DNA BY L5178Y CELLS*

	Gas flow counter cpm	Scintillation counter cpm
Supernatant fraction	1,549,200	216,960
1st wash	7250	710
2nd wash	1080	199
3rd wash	579	100
4th wash	960	166
Cold washed cells		
5th wash (0°)	272	58
Cold TCA extract	55	Neg.†
Hot TCA extract	1868	215
Residue	877	
Incubated cells		
5th wash (37°)	350	(0)
6th wash	35	11
Cold TCA extract	12	30
Hot TCA extract	1261	128
Residue	415	

* The cells (1×10^8) were incubated in a medium (10 ml) containing ^{3}H-DNA (9·0 μg per ml). After incubation for a period 2 hr at 37°, the cells were washed and the levels of radioactivity in the cold acid-soluble and hot acid-soluble extractives were determined.

† "Neg." indicates insignificant difference from average background count.

Uptake of ^{3}H-DNA as a function of its concentration

A suspension containing $1·5 \times 10^8$ L5178Y cells was treated as described above. The suspension was divided into 3 equal portions and a solution of ^{3}H-DNA was added to each; (a) 45 μg in 0·5 ml; (b) 4·5 μg in 0·5 ml; and (c) 0·45 μg in 0·5 ml. The suspensions were incubated at 37° for 2 hr under the usual gas mixture (5% CO_2: 95% air) in a Dubnoff shaker. At the end of the incubation period the cells were harvested by centrifugation and washed five times with ice-cold medium (2·5 ml), as in the previous experiment. The third wash contained 20 μg of unlabeled L5178Y-DNA per ml. The cells were lysed by the addition of water (0·3 ml) followed by ice-cold 5% TCA (2·2 ml). The insoluble material was washed once with ice-cold 5% TCA, and extracted with 5% TCA ($2 \times 2·5$ ml) at 90° for 20 min. TCA was removed from the extracts by washing with ether. The results are shown in Table 2.

With DNA concentrations of less than 1 μg per ml, the binding to the cells was roughly proportional to the concentration and the DNA was taken from the medium by the cells. The results suggest that DNA can be bound to only a limited number of sites, and that, with DNA concentrations greater than 1 μg/ml, these sites are largely saturated.

TABLE 2. UPTAKE OF ^{3}H-DNA AS A FUNCTION OF ITS CONCENTRATION*

	DNA 9·0 μg/ml		DNA 0·9 μg/ml		DNA 0·09 μg/ml	
	Gas cpm	Scintillation cpm	Gas cpm	Scintillation cpm	Gas cpm	Scintillation cpm
Supernatant	456,000	80,000	23,630	5625	1895	342
1st wash	1350	597				
2nd wash	150	62				
3rd wash	148	67				
4th wash		43				
5th wash	50	44	14	10	Neg.†	11
Cold TCA	20		Neg.†		Neg.†	
Hot TCA	3880	380	2380	140	280	12
Residue	340		180		102	

* For each concentration of ^{3}H-DNA, 5 × 10^7 cells were incubated in a medium volume of 5·0 ml and for a period of 2 hr at 37°. The cells were washed with cold medium (2·5 ml), the third wash containing 20 μg of unlabeled DNA per ml. The levels of radioactivity in the cold acid-soluble and hot acid-soluble extractives were determined.

† "Neg." indicates insignificant difference from average background count.

L5178Y cells (1·5 × 10^8), harvested at 4 × 10^5/ml, were suspended in medium (13·5 ml) and ^{3}H-DNA solution (90 μg/ml, 1·5 ml) was added. Of the suspension, 5 ml was immediately removed and placed in an ice-bath with occasional shaking for 2 hr. The cells were harvested, washed and extracted with TCA in the usual manner. The remaining portion (10 ml) of the suspension was incubated, under sterile conditions and with shaking, for 2 hr at 37°, in an atmosphere of 5% CO_2 and 95% air. The cells were harvested and washed as in the previous experiments. After the last wash the cells were suspended in the medium (5 ml). The cells were harvested from 2·5 ml of the suspension and extracted with TCA. The remaining 2·5 ml of the suspension were diluted to 100 ml with growth medium (the count was 2·05 × 10^5/ml) and incubated for 22 hr (count 4·4 × 10^5/ml). The cells kept at 0° were harvested, washed (2 × 2·5 ml) and extracted in the usual manner. The counts are recorded in Table 3.

It will be observed that considerable uptake of ^{3}H-DNA occurred at low temperatures. This process may have a low energy requirement, and be partly explained by ionic binding. The cells incubated at 37° had a much greater ^{3}H-DNA content, indicating the participation of an additional process with a higher energy requirement for binding DNA. A considerable proportion of the radioactivity bound to the cells at 37° was retained by the cells during rather more than one division. Thus, the bound DNA did not equilibrate with the medium during growth and cell division.

There is strong evidence that DNA is taken up intact by L5178Y. Great care was taken to ensure that all the radioactivity in the DNA preparations was in the form of material of high molecular weight. All the radioactivity present in the medium after

incubation (the supernatant fraction) was precipated on the addition of acid, and only a negligible proportion of the radioactivity that became associated with the cells was soluble in cold TCA. This rules out the possibility that the DNA was degraded to compounds of low molecular weight that diffused readily into the cells and then were resynthesized into DNA. The finding that DNA was taken up by the cells even at 0° confirms this view, since it is most unlikely that there would be catabolism followed by anabolism at this temperature.

TABLE 3. TEMPERATURE DEPENDENCE OF ^{3}H-DNA-UPTAKE BY L5178Y CELLS AND RETENTION OF ^{3}H-DNA DURING SUBSEQUENT REPRODUCTION*

	Uptake at 37°		Uptake at 0°		Retention during division	
	Gas cpm	Scintillation cpm	Gas cpm	Scintillation cpm	Gas cpm	Scintillation cpm
Cold TCA	30	Neg.†	Neg.†		Neg.†	
Hot TCA	6480	348	1994	240	1645	181
Residue	145		94		7	

* For each condition, 5×10^7 cells were incubated in a medium volume of 5 ml containing 6 μg/ml for a period of 2 hr. After washing, as in the previous experiments, the radioactivity of the cold acid-soluble and hot acid-soluble fractions was determined in a gas-flow counter and in a liquid scintillation counter. The washed cells (an aliquot of those incubated at 37°) which had undergone slightly more than one doubling in number at 37° were harvested, washed again, and the radioactivity of the DNA-containing fraction was determined.

† "Neg." indicates insignificant difference from average background count.

These experiments demonstrate that L5178Y cells can take up homologous DNA from the medium. Several reports have been published which indicate that exogenous DNA may enter mammalian cells.[2, 4, 9, 13] The data presented concerning the association of homologous DNA with L5178Y cells do not give any information about the intracellular location of the bound DNA, although the fact that it is retained during cell division indicates that this site is probably within the cell wall. Taken in conjunction with the information about the DNases of these cells, they augur well for eventual success in transforming these leukemic lymphoblasts.

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