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Phenotypic Differences Between Tumor Cells Derived From Different Stages of Neoplastic Growth*

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Abstract—*Under conditions employed in our laboratory, tumors which are induced by avian sarcoma virus (ASV) usually grow progressively for several weeks and then regress. In order to further understand the basis for tumor regression in this model, we compared avian sarcoma cells which were cultured from tumors at different stages of development in terms of various phenotypic properties. The results indicate that tumor cells which are derived from progressively-growing sarcomas are rapidly growing, produce large quantities of the enzyme plasminogen activator, and have much in common generally with chicken embryo fibroblast (CEF) cells that have been transformed by ASV. In contrast, tumor cells that are obtained from regressors have elevated levels of hexose transport, grow very slowly, are greatly enlarged and display properties that are characteristic of senescent cells in culture.*

INTRODUCTION

THE INOCULATION into chickens of avian sarcoma viruses (ASV) leads to the development of tumors which frequently regress [1]. It has been shown that regression in this model is dependent on the development of a strong, cell-mediated, anti-viral and anti-tumor immune response [2, 3]. Thus, animals which are unable to mount such a response, such as hosts which have been neonatally thymectomized, almost invariably die of progressive neoplastic disease [2, 4]. On the other hand, bursectomized birds behave like normal controls with regard to the outcome of the host–tumor confrontation [4, 5].

Previous research from our laboratory has shown that the tumor cells which can be cultured from regressing neoplasms differ from those which can be cultured from progressors in one important respect. Namely, while the latter produce progeny transforming virus that appears wild-type in every respect, the former are only able to synthesize glycoprotein-deficient, non-infectious particles [6]. It is known that avian sarcomas generally grow as a consequence of recruitment of newly-transformed cells into the tumor mass, rather than mitosis of previously infected cells [7]. The inability of regressing tumor cells to produce infectious virus, coupled with a successful immune response directed against virus-producers, therefore explains the failure of avian sarcomas to enlarge progressively. What remains unexplained, however, is the inability of those tumor cells which are cultured from regressors to continue to grow *in vivo*.

The present research was initiated to determine whether progressively-growing avian sarcoma cells possess phenotypic properties similar to those of virus-transformed chick embryo fibroblast (CEF) cells, and whether these in turn might differ from regressing tumor cells. These various cell types have been compared in terms of *in vitro* growth, sugar transport, approximate size, total protein and DNA content, and production of the enzyme plasminogen activator. The results indicate that avian sarcoma cells which are cultured from progressively growing neoplasms have much in common with ASV-transformed CEF cells in terms of these various criteria. On the other hand, tumor cells which are derived from regressors tend to be greatly enlarged, grow very slowly and display a number of properties characteristic of senescent cells in culture [8, 9].

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MATERIALS AND METHODS

Virus

The following strains of avian sarcoma virus (ASV), including Rous sarcoma virus (RSV), were used in these experiments: Prague strain, subgroup A (PrA), kindly provided by Dr. P. Vogt, University of Southern California; and B_{77}, subgroup C, kindly supplied by Dr. J. Perdue, Lady Davis Institute for Medical Research, Montreal, Canada. Viruses were propagated in cultures of CEF cells according to a previously published procedure [10]. Supernatant fluids, usually containing about 10^5 focus-forming units (ffu)/ml of virus, were collected after 24 hr from cultures of almost completely transformed cells, clarified by low-speed centrifugation and frozen at −70°C until use.

Eggs, chickens and tumor induction

COFAL-negative [11], leukosis-free C/E eggs and chickens were purchased from the breeding colonies of the Institut Armand Frappier, Laval, Quebec. Both male and female animals, 8–12 weeks of age, were injected in the right wing webs with transformed culture supernatant fluid containing approximately 10^4 ffu of virus. In some few instances, younger birds (1–2 weeks old) were employed for virus inoculation. After approximately 3 or 4 weeks in the case of the older animals and 1 week in the case of the younger chickens, by which time sizeable neoplasms (at least 2 cm in diameter) had developed, pieces of tumor tissue were removed surgically under anesthetic conditions and prepared for growth in tissue culture as explained below.

Cell cultivation

Normal CEF cells were secondary cultures (2–3 days old) derived from 10 to 11-day-old embryos and grown in Temin-modified minimum essential medium (MEM) supplemented with 4% fetal calf serum (FCS), 100 IU/ml penicillin and 100 μg/ml streptomycin as previously described [6]. ASV-transformed CEF were secondary cultures derived from the same embryos, and were maintained in the same way. In the case of avian sarcoma cells, pieces of tumor tissue which had been excised aseptically from ether-anesthetized birds were cut up into 2 mm^3 slivers, subjected to trypsinization and plated for growth in tissue culture as described for normal and transformed CEF cells. Avian tumor cells were passaged once in tissue culture before use.

Viable growth studies

Avian sarcoma cells as well as normal and transformed CEF cells were grown on 35-mm Petri dishes in supplemented MEM as described. Fresh medium was added daily. When indicated, the number of viable cells in a preparation was determined by the trypan blue exclusion technique. After a 10-min incubation period in 0.1% trypan blue in phosphate-buffered saline (PBS), cell monolayers were washed three times in Hanks' balanced salt solution (BSS) before detachment with 0.1% trypsin in 10^{-3} M disodium ethylenediamine tetraacetic acid (EDTA) in PBS. A single cell suspension was achieved by gentle pipetting and unstained cells were counted in a hemocytometer. For the purpose of protein and DNA determinations, cells which were at or near confluence were washed twice with BSS and then gently scraped from vessel surfaces. They were re-suspended in serum-free MEM and pelleted. Protein determinations on pelleted samples were performed according to published procedure [12]. Total DNA was estimated on the basis of reactivity in a diphenylamine assay [13].

Flow cytophotometry

In order to determine approximate cell sizes we employed a CYTOGRAF 6300 A flow cytometer (Biophysics Instruments, Mahopac, NY). This apparatus includes a multichannel distribution analyzer, which permits size distinctions to be made among various cell types. The distribution frequency for cells of various dimensions, which had been gently trypsinized from vessel surfaces, was recorded automatically. Approximate cell diameters were determined by including lycopodium, pecan and corn pollens of known dimensions, i.e. 26.7, 40 and 77.2 μm respectively (Coulter Electronics, Hialeah, FL), as standards.

Fibrinolytic assays

These experiments were performed for the determination of plasminogen activator (PA) activity according to published procedure [14]. Briefly, bovine fibrinogen (Miles Laboratories, Elkhart, IN) was iodinated with [^{125}I] and diluted in PBS, pH 7.2, to a desired final radioactivity. Aliquots of 0.1 ml of labelled fibrinogen were added to Petri dishes (35 × 10 mm) (Flow Laboratories) to deliver about 50,000 counts/min per plate. The plates were allowed to dry and then incubated at 37°C for 2.5 hr with 1 ml of MEM supplemented with

2.5% FCS, to convert fibrinogen to fibrin. The plates were washed with 0.1 M Tris buffer, pH 8.1, both thereafter and immediately before use.

Serum-free culture fluids were harvested from normal and virus-transformed cells as described [15]. These cultures had been grown on 35-mm plastic Petri dishes and were at or near confluence at the time of assay for PA activity. Culture fluids were centrifuged at low speed to eliminate debris and frozen until use in fibrinolysis assays. Test supernatant fluid (200 μl) was added to a mixture of 0.02% Triton X-100 and 10 μg of purified bovine plasminogen in 0.1 M Tris buffer, pH 8.1, to a total volume of 1 ml. This mixture was added to the [^{125}I]-coated Petri dishes for 2 hr at 37°C. In this assay, the PA which is present in culture fluids causes the conversion of its substrate plasminogen to plasmin, a proteolytic enzyme which digests the [^{125}I]-labelled fibrin. Tests were carried out using duplicate samples. The total number of counts per plate was determined by addition of 1 ml of a 0.1% versene–0.25% trypsin mixture. Following repeated scraping, fluids and washings were counted in a gamma counter. The percentage of specific isotope release was calculated as the ratio of counts/min released under test circumstances to that obtained with control plates using versene and trypsin.

Sugar transport studies

Confluent 35-mm Petri dishes were used for all cell types approximately 24 hr after addition of fresh medium. The details of the transport assay have been described elsewhere [16]. In all experiments, zero time controls were subtracted. At the time of assay for sugar transport, the cell monolayers were washed twice at 37°C with sugar-free BSS, pH 7.4, containing 20 mM *N*-2-hydroxyethylpiperazine *N'*-2-ethanesulphonic acid (HBSS–HEPES). Then 0.8 ml of HBSS–HEPES containing varying concentrations of [^{3}H] - deoxy - D - glucose (2-DG) and [^{3}H] - L - glucose were added to the cell monolayers and the latter were incubated for 1 or 2 min at 37°C. The specific activities of the 0.1–0.3 mM 2-DG and L-glucose solutions were 22.5 μCi/μmol, and those of the 0.5–3.0 mM sugar solutions were 4.5 μCi/μmol hexose. After incubation the radioactive medium was aspirated and the monolayers were washed four times with 2 ml (each time) of cold PBS (pH 7.4). The monolayers were dissolved in 1 N NaOH and aliquots taken for liquid scintillation counting and protein determination [12]. In data expressed on a cellular basis, triplicate plates were counted to determine the cell number per plate at confluence. The contribution of L-glucose uptake was subtracted from the total 2-DG uptake before determining the K_m and V_{max} for transport [9, 16].

RESULTS

Growth studies

Our initial experiments dealt with the relative growth abilities of normal CEF cells, PrA-transformed CEF cells and avian sarcoma cells (PrA-induced) which had been derived from each of progressively growing and regressing neoplasms. Progressive tumors were obtained from 3-week-old animals, whose tumors would almost invariably prove fatal, and from 11-week-old hosts at times prior to the onset of regression. Regressing tumors were taken from animals of the latter category but at later periods (e.g. four weeks after viral inoculation). Tumor growth was followed by means of a pair of calipers at least twice weekly. Virtually all of the tumor-derived cells studied could be specifically stained in indirect immunofluorescence assays by rabbit anti-viral antibody, thus indicating that they had been infected by ASV (data not shown).

The results (Fig. 1) show that each of normal CEF cells, virus-transformed CEF cells and avian sarcoma cells derived from progressing tumors had approximately equivalent growth rates in culture. Doubling times in the cases of both types of embryonic fibroblast were about 22 hr, while for the tumor cell populations the

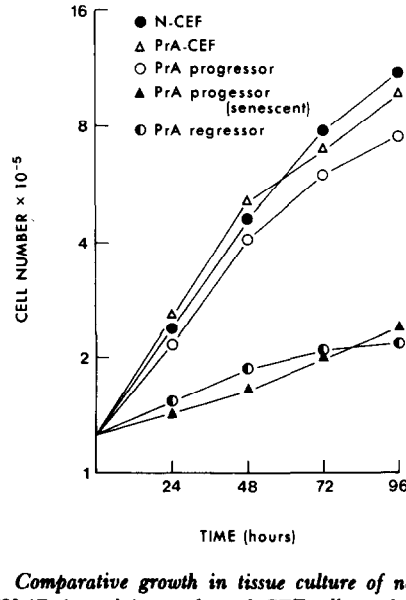

Fig. 1. Comparative growth in tissue culture of normal (N) and ASV (PrA strain)-transformed CEF cells, and of regressor and progressor cells derived from PrA-induced neoplasms.

doubling time was about 28 hr. This was true regardless of whether progressor tumor cells were derived from very young (i.e. 3-week-old) or more mature (11 to 12-week-old) chickens.

In contrast, tumor cells which derived from regressing neoplasms displayed much poorer growth in culture. Doubling times generally varied between 72 and 120 hr, although occasionally, faster-growing cultures were observed. In addition, we noted distinct variations between the different types of tumor cell cultures in terms of the length of time for which they could be maintained *in vitro*. Most sarcoma cultures which derived from progressively-growing sarcomas could be sustained for as long as 90–100 days before dying out. Parenthetically, these cells behaved much like regressing sarcoma cells with respect to growth and doubling time toward the end of their maintenance in culture (Fig. 1). In contrast, those cultures which were initiated from regressing tumors died after 3–4 weeks.

By the end of the 96-hr experimental period, each of the normal CEF cells, ASV-transformed CEF cells and tumor cells which were derived from progressively growing neoplasms were at confluence. Tumor cells from regressors appeared to be greatly enlarged and were only semi-confluent.

Cell size, protein and DNA

We further compared each of the above cell types with respect to overall diameter, as described in Materials and Methods. Dramatic differences were noted among the various cell types, as determined by flow cytophotometry (Fig. 2). Normal CEF cells were relatively homogeneous and possessed an average diameter of approximately 10 μm. A somewhat greater degree of heterogeneity was documented for PrA-transformed CEF cells. This was reflected in a somewhat greater average diameter (11.5 μm) than that observed for untransformed cells, as well as a slightly broader distribution curve. Tumor cells which derived from progressively-growing neoplasms were slightly larger than transformed CEF cells with which they otherwise shared a similar distribution profile. In contrast, cells which were obtained from regressing sarcomas were considerably enlarged on average, with diameters ranging between 10 and 25 μm. These cells were by far the most heterogeneous of all in terms of size distribution.

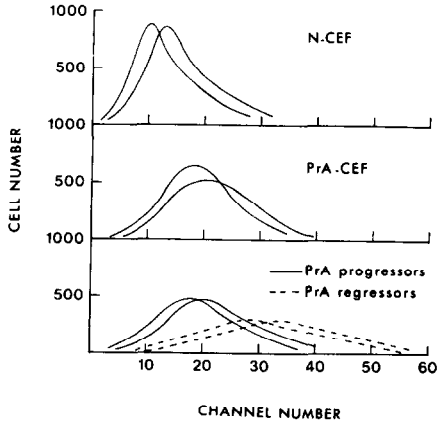

Fig. 2. Comparative cell sizing of normal (N) and ASV (PrA strain)-transformed CEF cells and of progressor and regressor cells derived from PrA-induced neoplasms.

Differences among these various cell types were also noted with respect to protein content and less marked with respect to DNA content. The results of Table 1 indicate that for each of normal and ASV-transformed CEF cells, average protein contents were in the neighborhood of 100 μg protein/10^6 cells. These assays were performed on cells which had either just reached confluence or which were almost confluent. In the case of tumor cells derived from progressively-growing sarcomas, protein content appeared elevated by approximately 50%. In contrast, those tumor cells which were obtained from regressors apparently contained as much as 10 times more protein than either normal or ASV-transformed CEF.

These differences in protein content were not reflected in the amounts of DNA contained in these various cell types. Generally speaking, normal CEF cells, transformed CEF cells and tumor cells from progressors each contained between 20 and 25 μg DNA per 10^6 cells. The

Table 1. Protein and DNA content of various cell types

Sample No.	Cell type	μg protein/ 10^6 cells	μg DNA/ 10^6 cells
1	N-CEF*	110.4 ± 20.0†	22.4 ± 0.7
2	PrA-CEF	98.3 ± 3.1	24.8 ± 0.4
3	B_{77}-CEF	102.6 ± 3.5	19.7 ± 0.5
4	N-CEF	97.6 ± 2.7	18.6 ± 0.6
5	PrA-CEF	103.8 ± 4.9	25.2 ± 0.3
6	B_{77}-CEF	88.1 ± 1.7	20.4 ± 0.6
7	PrA progressor	136.9 ± 3.7	26.8 ± 1.1
8	PrA progressor	152.6 ± 5.9	24.3 ± 0.8
9	B_{77} progressor	163.5 ± 4.6	24.9 ± 0.8
10	PrA regressor	437.6 ± 5.0	31.6 ± 1.4
11	PrA regressor	728.0 ± 3.4	28.0 ± 0.7
12	B_{77} regressor	1049.1 ± 5.8	35.9 ± 1.0

*CEF cells employed were either normal (N) or transformed by either the PrA or B_{77} strain of ASV. Samples 1–3 and 4–6 were derived from two distinct embryos respectively.

†± Standard error of the mean.

corresponding figures for each of three cell samples derived from regressing tumors were 31.6, 28.0 and 35.9 μg DNA/10^6 cells respectively.

Plasminogen activator activity in culture fluids

We next compared levels of plasminogen activator production by each of normal and transformed CEF cells and ASV-induced tumor cells. In the ASV system such studies have hitherto been conducted only on virus-transformed CEF. The results (Table 2) show that very high levels of PA activity were associated with tumor cells which were derived from each of progressively growing and regressing neoplasms. In most cases, these levels were 2–3 times higher than those associated with ASV-transformed CEF cells. Normal CEF cells produced little or no PA activity. As indicated, we were unable to distinguish between progressor and regressor avian sarcoma cells in this regard.

Sugar transport

To further characterize the comparative cell biology of these cell types, we investigated their sugar transport kinetics. The data in Table 3 indicate that transformation of CEF led to an elevated V_{max} for 2-DG transport. Additionally, the V_{max} of the tumor cells (B_{77} or PrA) was higher than that found in B_{77}-transformed CEF. At all concentrations, L-glucose (non-saturable) uptake was less than 10% of the 2-DG (saturable) uptake at an equivalent concentration (data not shown). The data in Table 4 represent the transport of 2-DG per cell at a 1.0 mM 2-DG concentration. Of considerable interest is the observed elevation in 2-DG transport by the regressor tumor cells in culture. The rate of 2-DG transport in the regressor cells was two-fold higher than that in progressor cells which, in turn, was higher than the rate of transport in B_{77}-transformed CEF which was higher than that found in untransformed CEF. Although V_{max} determinations were not obtained, the data indicate that the V_{max} of regressor cells would be elevated above that of any other cell type. For example, in the V_{max} pattern observed in Table 3, progressors > B_{77} CEF > N-CEF and in Table 4 the observed uptake pattern was regressor > progressor > B_{77} CEF > N-CEF.

Table 2. Plasminogen activator (PA) activity in culture fluids of ASV-transformed cells and ASV-induced tumor cells

1	N-CEF*	0.8 ± 0.2†
2	PrA-CEF	6.8 ± 1.3
3	B_{77}-CEF	18.3 ± 5.4
4	N-CEF	1.6 ± 0.6
5	PrA-CEF	14.7 ± 2.7
6	B_{77}-CEF	18.0 ± 5.3
7	PrA progressor	58.6 ± 2.6
8	PrA progressor	59.3 ± 5.7
9	PrA progressor	35.9 ± 4.9
10	PrA progressor	54.0 ± 6.7
11	PrA regressor	53.1 ± 6.6
12	PrA regressor	37.2 ± 0.3

*CEF cells employed were either normal (N) or transformed by either the PrA or B_{77} strain of ASV. Samples 1–3 and 4–6 were derived from two different embryos respectively.

†± Standard error of the mean.

Table 3. K_m and V_{max} values for 2-DG transport in the various cell types

Cell type	K_m (mM)	V_{max} (nmol 2-DG/10^6 cells/min)
N-CEF	5.5	8.0
B_{77}-CEF	3.7	11.0
B_{77} progressor	1.4	18.0
PrA progressor	1.5	17.0

Corrected for L-glucose uptake. The data represent the average of triplicate determinations at each of 7 different sugar concentrations (0.1–3.0 mM).

Table 4. 2-DG Transport of the various cell types

Cell type	Deoxy-D-glucose transport (nmol/10^6 cells/min)
N-CEF	2.0 ± 0.29
B_{77}-CEF	6.1 ± 0.60
B_{77} progressor	15.9 ± 0.23
PrA progressor	15.1 ± 0.60
PrA regressor	31.9 ± 1.75

The concentration of 2-DG employed was 1.0 mM. The data represent the average of triplicate determinations ± S.E.M.

DISCUSSION

Avian sarcoma cells which are found in regressing neoplasms are apparently able to escape immune attack on the basis of their inability to express relevant viral antigens at their surface [6]. As discussed, such cells when cultured cannot synthesize transforming progeny virus but instead make glycoprotein-deficient, non-infectious particles which contain detectable levels of the enzyme RNA-dependent DNA polymerase. In contrast, sarcoma cells which are cultured from progressing

tumors produce wild-type virus and, accordingly, are susceptible to immune attack.

One problem which then remains is to explain the inability of those tumor cells which are cultured from regressors to grow *in vivo*. Since this deficiency cannot be easily understood on the basis of anti-viral or anti-tumor immune responsiveness, it is necessary to employ other approaches in answering this question. The present research was designed to explore certain phenotypic characteristics of progressing and regressing avian sarcoma cells, in the hope of providing some clues to this problem.

The results show that tumor cells which are cultured from progressors behave similarly to ASV-transformed CEF cells in a number of respects. In addition to the production of wild-type virus [6], both cell types have similar growth rates in culture and possess near-identical rates of glucose uptake. Several differences are also apparent. Namely, progressing tumor cells are slightly larger than transformed CEF cells and they secrete considerably higher levels of PA into the medium.

These differences are minimal, however, when contrasted to the behavior of regressing sarcoma cells in the same assays. First, the latter cells divide extremely slowly and possess a doubling time in culture which may be as high as 96–120 hr. In this respect there is a resemblance to progressively growing tumor cells which have been maintained in culture for over three months and become senescent. Other parallels to senescent cells emerge in the largeness of regressing tumor cells and their increased uptake of 2-DG. This increase in size is reflected in a protein/cell ratio up to ten times higher than that associated with transformed CEF cells. DNA levels in such cells are not, however, correspondingly elevated. This finding thus explains the increased uptake of glucose per cell associated with tumor regressors. Increased glucose uptake [17] and transport [9] on a per cell basis is also a characteristic of senescent human fibroblasts. Thus, in terms of *in vitro* growth, size, protein content and hexose transport, avian sarcoma cells derived from regressors behave like senescent cells. In contrast, tumor cells from progressors display characteristics associated with young growing cultures in most of these respects.

One area in which progressor tumor cells and regressors do not apparently differ is in regard to secretion of the enzyme plasminogen activator. This serine protease, which catalyzes the conversion of the zymogen plasminogen to its active form of plasmin, is secreted in large quantities by certain types of tumor cells but not normal cells [18, 19]. We have observed that both progressor and regressor tumor cells elaborate high levels of PA activity and that these levels may exceed those associated with transformed CEF cells by 2 to 3-fold. In actuality, however, the amount of PA produced per cell in the case of regressors probably greatly exceeds that elaborated by progressing tumor cells. This is due to the fact that a confluent culture of progressor cells contains many more cells than does an equivalent culture of regressors (Fig. 1).

In conclusion, tumor cells which derive from regressing neoplasms are much larger, grow more slowly, have increased glucose transport activity and contain more protein than do sarcoma cells which are obtained from progressing neoplasms. In each of these respects, regressing tumor cells bear a resemblance to normal senescent cultures which have neared the end of their finite ability to be passaged. It is tempting to speculate that this fact may underlie the inability of tumor cells from regressors to sustain *in vivo* neoplastic growth.

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REFERENCES

1. RUBIN H. The immunological basis for non-infective Rous sarcomas. *Cold Spring Harbor Symp Quant Biol* 1962, **27**, 441–452.
2. RADZICHOVSKAJA R. Effect of thymectomy on Rous virus tumor growth induced in chickens. *Proc Soc Exp Biol Med* 1967, **126**, 13–15.
3. ISRAEL E, WAINBERG MA. Development of cellular antitumor immunity in chickens bearing tumors induced by Rous sarcoma virus. *J Immunol* 1977, **118**, 2237–2242.
4. WAINBERG MA, BEISS B, WAHI R, ISRAEL E. Thymic dependence of cell-mediated immunity to avian sarcomas in chickens. Immunological characterization of a nonvirion antigen in virus-infected cells. *Cell Immunol* 1979, **45**, 344–355.
5. MCARTHUR WP, CARSWELL EA, THORBECKE GJ. Growth of Rous sarcomas in bursectomized chickens. *J Natl Cancer Inst* 1972, **49**, 907–909.

6. WAINBERG MA, YU M, ISRAEL E. Decreased production of transforming virus and altered antigenic behaviour in cultured avian sarcoma cells. *J Gen Virol* 1979, **42**, 255–264.
7. PONTEN J. The *in vivo* growth mechanism of avian Rous sarcoma. *Natl Cancer Inst Monogr* 1964, **17**, 131–139.
8. CRISTOFALO VJ. Animal cell cultures as a model system for the study of ageing. *Adv Gerontol Res* 1972, **4**, 45–79.
9. GERMINARIO RJ, OLIVEIRA M, TAYLOR M. Studies on the effects of *in vitro* ageing on saturable and non-saturable sugar uptake in cultured human skin fibroblasts. *Gerontology* 1980, **26**, 181–187.
10. TEMIN HM, RUBIN H. Characteristics of an assay for Rous sarcoma cells in tissue culture. *Virology* 1959, **6**, 669–688.
11. SARMA PS, TURNER HC, HUEBNER RJ. An avian leukosis group-specific complement fixation reaction. Application for the detection and assay of non-cytopathogenic leukosis viruses. *Virology* 1964, **23**, 313–320.
12. LOWRY OH, ROSEBROUGH NJ, FARR AL, RANDALL RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951, **193**, 265–275.
13. BURTON K. A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of DNA. *Biochem J* 1956, **62**, 315–323.
14. UNKELESS JC, TOBIA A, OSSOWSKI L, QUIGLEY JP, RIFKIN DB, REICH E. An enzymatic function associated with transformation of fibroblasts by oncogenic viruses. I. Chick embryo fibroblast cultures transformed by avian RNA tumor viruses. *J Exp Med* 1973, **137**, 85–111.
15. COHEN SD, ISRAEL E, SPIESS-MEIER B, WAINBERG MA. Plasminogen activator is an apparent lymphocyte mitogen. *J Immunol* 1981, **126**, 1415–1420.
16. GERMINARIO RJ, OLIVEIRA M, LEUNG H. Saturable and non-saturable hexose uptake in cultured human skin fibroblasts. *Can J Biochem* 1978, **56**, 80–88.
17. GOLDSTEIN S, TRIEMAN G. Glucose consumption by early and late passage diploid human fibroblasts during growth and stationary phase. *Experientia* 1975, **31**, 177–180.
18. GOLDFARB RH, QUIGLEY JP. Synergistic effect of tumor virus transformation and tumor promotor treatment on the production of plasminogen activator by chick embryo fibroblasts. *Cancer Res* 1978, **38**, 4601–4609.
19. WOLF BA, GOLDBERG AR. Lack of correlation between tumorigenicity and level of plasminogen activator in fibroblasts transformed by Rous sarcoma virus. *Proc Natl Acad Sci USA* 1978, **75**, 4967–4971.