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TRANSMISSION OF JAAGSIEKTE (OVINE PULMONARY ADENOMATOSIS) BY MEANS OF A PERMANENT EPITHELIAL CELL LINE ESTABLISHED FROM AFFECTED LUNGS

SUZANNE COETZEE, H. J. ELS and D. W. VERWOERD, Veterinary Research Institute, Onderstepoort

ABSTRACT

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An epithelial cell line, designated JS–15.4, has been established in culture from jaagsiekte lesions and subcultured *in vitro* for almost 2 years. It exhibits morphological and other features of transformed cells and has been shown by electron microscopy to consist of type B ovine alveolar epithelial cells. Jaagsiekte was successfully transmitted to 3 new-born lambs by the intratracheal injection of cells following immunosuppressive treatment with either anti-thymocyte immunoglobulin alone or combined with anti-macrophage immunoglobulin. Incubation periods as short as 10 weeks were recorded. Evidence was also obtained that natural transmission may result from the inhalation of viable cells.

Résumé

LA TRANSMISSION DE LA JAAGSIEKTE (L'ADÉNOMATOSE PULMONAIRE OVINE) AVEC UNE LIGNÉE DE CELLULES ÉPITHÉLIALES ÉTABLIE EN CULTURE PERMANENTE À PARTIR DE POUMONS AFFECTÉS

Les auteurs ont pu établir en culture cellulaire une lignée de cellules épithéliales, la lignée JS–15.4, à partir de lésions de la jaagsiekte au niveau des poumons. Révélant un aspect de cellules métamorphosées, ces cellules en microscopie électronique paraissent appartenir au type B des cellules épithéliales alveolaires. La jaagsiekte a pu être transmise avec succès à 3 agneaux nouveau-nés en les injectant par voie trachéale avec ces cellules à la suite d'un traitement immuno-suppresseur comprenant soit l'immunoglobuline anti-thymocyte seule, soit cette dernière en combinaison avec l'immunoglobuline anti-macrophage. Des périodes d'incubation aussi courtes que 10 semaines ont étées enregistrées. Les auteurs ont obtenu de la preuve que la transmission naturelle peut arriver à la suite de l'inhalation de cellules viables.

INTRODUCTION

Jaagsiekte, or ovine pulmonary adenomatosis, recently reviewed by Tustin (1969) and Wandera (1971), has been transmitted artificially to experimental sheep by cohabitation, by droplet infection, by parenteral inoculations of homogenized diseased lung tissue and of filtrates thereof, and by inoculation of primary cell cultures derived from lesions and grown for 22 days *in vitro* (Tustin & Geyer, 1971). These transmissions proved the infectious nature of this neoplastic disease but did not elucidate its cause, although transmission by means of cell-free filtrates obviously implicates a virus. To date, the only virus particles observed by electron microscopy in tumour tissue had the typical morphology of type C RNA tumour viruses (Perk, Michalides, Spiegelman & Schlom, 1974), but these have not yet been isolated and their possible significance in the aetiology of the disease remains unknown. An ovine herpes virus was isolated independently from adenomatous lung material by Mackay (1969), Malmquist, Krauss, Moulton & Wandera (1972) and De Villiers, Els & Verwoerd (1975), but attempts to transmit the disease with this virus were unsuccessful, however. The failure to do this may indicate that either the virus is an innocuous "passenger" having no part in the aetiology of jaagsiekte, or that, for oncogenesis to occur, another as yet unknown secondary factor is required.

The fact that crude extracts or primary cultures of affected lungs are usually infected with a variety of bacteria, mycoplasms, chlamydia, viruses and fungi, prompted us to investigate the possibility of eliminating contaminants by culturing the epithelial tumour cells *in vitro*. A permanent cell line capable of transmitting the disease would obviously be invaluable in investigations on the aetiology of jaagsiekte.

Epithelial cell lines have been established from various tumours, such as mammary carcinomas (Bassin, Plata, Gerwin, Mattern, Haapala & Chu, 1972), human alveolar cell tumours (Coalson, Nordquist, Coalson, Mohr & Rhoades, 1973), hepatomas (Williams, Weisburger & Weisburger, 1971) and mouse lung adenomas (Stoner, Kikkawa, Kniazeff, Miyai & Wagner, 1975). The most common problem in the establishment of epithelial cell lines, viz. overgrowth of the cultures by fibroblasts, has been overcome in various ways by other workers, such as by cloning (Brown, 1973; Coon, 1968), collagenase treatment (Iype, 1971), mechanical destruction of undesired cell types and differential adsorption (Williams, Weisburger & Weisburger, 1971) and selective trypsinization (Owens, Smith & Hackett, 1974). The various methods were tested and a combination of those found to be most effective were used for establishing the JS–15.4 cell line from jaagsiekte lungs.

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MATERIALS AND METHODS

Cell cultures

Adenomatous lungs were collected from freshly killed sheep suffering from jaagsiekte. The affected tissue was minced and trypsinized in a 0,25 % solution of trypsin(1) in phosphate buffered saline (PBS) for 48 h at 4 °C. Cells were seeded at 1×10^6/ml in BHK medium(2), and supplemented with the following antibiotics: penicillin(3), streptomycin(4), neomycin(5), Tylan-50(6), the fungicide nystatin(7) and the glucocorticosteroid betamethason(8). During the later stages of the work, this medium was replaced by the enriched

(1) Bacto-Trypsin, Difco, U.S.A., diluted 1:20
(2) Modified Eagle's medium prepared in our laboratory
(3) Penicillin: Hoechst Pharmaceuticals (Pty) Ltd, Johannesburg, 100 mg/l
(4) Streptomycin: Novo Industries (Pty) Ltd, Johannesburg, 200 mg/ml
(5) Neomycin: Fabrus Pharmaceuticals (Pty) Ltd, Bethlehem, 100 mg/ml
(6) Tylan-50: Elanco division of Lilly Laboratory S.A. (Pty) Ltd, Isando, 100 mg/ml
(7) Nystatin: Mycostatin Squibb, 25 000 u/l
(8) Betamethasone: Betsolan, Glaxo Laboratories Ltd, Greenford, England, 4 mg/l

F12 medium described by Weinstein, Orenstein, Gebert, Kaighn & Stadler (1975). Growth medium was renewed twice weekly and at confluency the culture was trypsinized and divided into 3 or 4 culture flasks.

Electron microscopy: Cells were scraped off the culture flask surface and centrifuged at $300 \times g$ for 5 min. The pellets were fixed in 5% glutaraldehyde, washed twice in phosphate buffer and post-fixed in 1% osmium tetroxide at pH 7,2–7,4 (Millonig, 1961). Portions of these pellets were embedded in Epon (Luft, 1961) and sectioned with glass knives. The sections were double-stained with 5% uranyl acetate for 30 min, and then with lead citrate for 3 min. A Siemens Elmiskop 1A electron microscope, employing a double condenser system, was used.

Test for Mycoplasma *infection:* Cultures were tested for nucleoside phosphorylase activity by the method described by Schneider, Stanbridge & Epstein (1974). Positive cultures were examined with the electron microscope and attempts made to culture the organism on standard *Mycoplasma* broth and solid media.

Colony formation in semi-solid media: Cells were suspended at a concentration of 5×10^3/ml in enriched F12 medium containing 10% foetal calf serum and either 0,4% agar or 1,0% methyl cellulose, and overlayed on 1,0% agar-containing F12 medium in 30 mm plastic dishes. Plates were incubated at 37 °C in a humidified CO_2 atmosphere and examined for growth of colonies for 14 days. Groups of cells containing more than 10 cells were regarded as colonies.

Karyotype analysis: The karyotype of JS–15.4 cells were analyzed after 19, 23, 27 and 30 *in vitro* passages, using a modification of the procedure described by Rothfels & Siminovitch (1958). Cells were grown on well cleaned microscope slides in a petri dish in enriched F12 medium containing 10% foetal calf serum. When the monolayers were almost confluent, colchicine was added to a final concentration of 0,001%, and the cultures incubated for a further 5 h at 37 °C. Distilled water was added to the medium in the ratio of 3:1 and the cells allowed to swell for 20 min. before being fixed in 50% acetic acid for 10 min. Slides were then air-dried at room temperature before being stained with freshly filtered 2% orcein in 50% acetic acid. After being washed in tap water and dried, the slides could be examined directly for metaphase spreads. Chromosome counts were done microscopically on at least 100 spreads for each passage level, and a few of the best preparations were photographed for a preliminary karyotype analysis.

Oncogenicity studies: For transplantation studies, confluent monolayers of JS–15.4 cells at different passage levels were scraped off, washed once by low speed centrifugation and suspended in culture medium without serum. Cell counts were estimated by counting trypsinized parallel flasks. Cells were injected as a concentrated suspension into various experimental animals by the routes indicated in Table 1. Immunosuppressive treatment was given where applicable, by the method described below.

Rabbits were observed for clinical signs of jaagsiekte for 3 months, hamsters and mice for 2 months and lambs for 1 year. After this period, or when showing clinical symptoms, animals were autopsied and examined both macroscopically and microscopically for evidence of jaagsiekte.

Immunosuppression: Antiserum against sheep thymocytes (ATS) was first prepared in a group of 5 rabbits and subsequently in a horse by repeated injections of 10^8 and 10^{10} freshly prepared foetal thymocytes, respectively, following the schedule recommended by Fahey (1973). The rabbit serum was pooled and used without any attempt at determining its activity *in vitro*. The activity of the horse serum was estimated by titrating its ability to agglutinate sheep thymocytes (Abaza & Woodruff, 1966). A titre of 1:2 560 was obtained. Immunoglobulin was prepared from this serum by repeated precipitation with $\frac{1}{2}$ volumes of saturated ammonium sulphate. The anti-thymocyte immunoglobulin (AT–IgG) was dissolved in $\frac{1}{8}$th of the original volume in PBS, sterilized by filtration and stored frozen until used. Anti-macrophage immunoglobulin (AM-IgG) was prepared in the same way, except that alveolar macrophages obtained from adult sheep lungs and purified by adsorption to glass were used instead of thymocytes. Immunosuppressive treatment of experimental lambs consisted of 10 ml ATS, AT-IgG, or AM-IgG+AT-IgG containing 200 mg of ampicillin, given intraperitoneally on Days −1, +1, +3, +5 and +7 (relative to the transplantation on Day 0), unless otherwise indicated.

Antisera against mouse spleen lymphocytes (ALS) and peritoneal macrophages (AMS) were prepared in rabbits and used in essentially the same way.

Results

Establishment of epithelial cell cultures

None of the methods described previously for the selective growth of epithelial cells from various carcinomas was found to be entirely satisfactory for jaagsiekte lung material. In the preliminary experiments, however, the procedure of Owens *et al.* (1974), using repeated selective trypsinization of cultures to remove contaminating fibroblasts, was the most successful. The greater susceptibility of fibroblasts to trypsin was therefore also exploited in the initial dispersion of lung material. Extended treatment in 0,25% trypsin for 48 h at 4 °C was found to eliminate most of the fibroblastic elements without unduly affecting epithelial cells. A combination of this long initial trypsinization and the use of a culture medium favouring the growth of epithelial cells, as well as repeated selective trypsinizations to eliminate any residual fibroblast growth, resulted in the establishment of a number of epitheloid cultures. One of these, designated JS–15.4, was derived from a ewe, and was further characterized in the present study.

Growth characteristics and morphology of JS–15.4 cells

Four days after new cultures had been seeded, small islands of closely cohering polygonal cells appeared (Fig. 1). The cells remained in contact after cell division, suggesting an epithelial origin (Owens *et al.*, 1974). Confluency was reached after 7 days when the culture displayed a cobblestone-like growth pattern. A few spindle-shaped fibroblasts were present, but the growth medium used selectively depressed their growth while promoting that of the epitheloid cells. Consequently, after a series of 3 selective trypsinizations followed by passaging at weekly intervals, a pure epitheloid culture consisting of various epithelial cell types was obtained. In confluent cultures the cells showed a lack of contact inhibition manifested by the formation of multilayered "clumps" or "chords" (Fig. 2). At the 8th passage the culture was tested for the presence of *Mycoplasma* and found to be free of contamination.

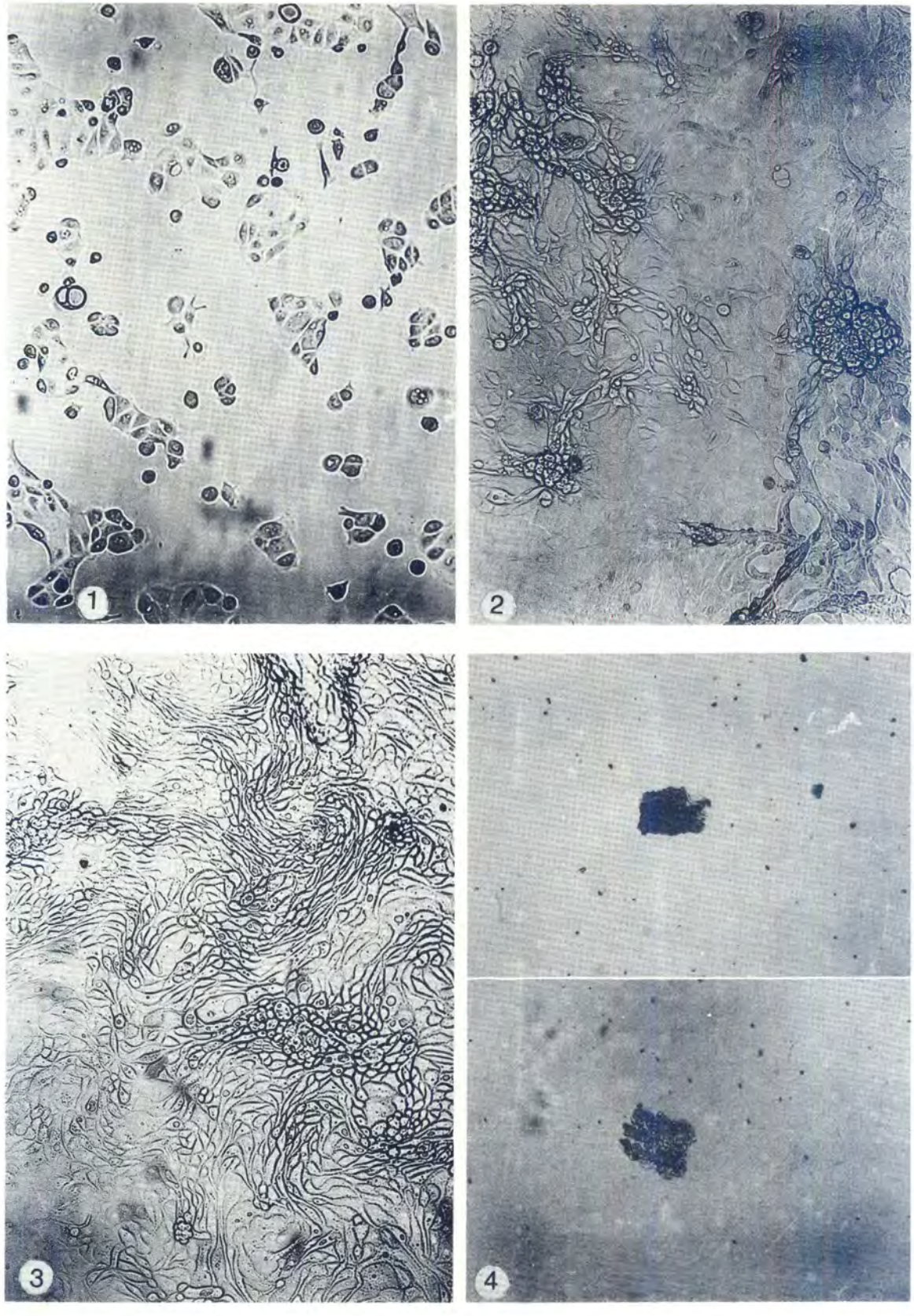

FIG. 1 Typical islands of epithelial cells in JS–15.4 cultures 24 h after the 24th subculture. Note the smooth edges of the cohesive islands of cells. Magnification ×75

FIG. 2 Confluent cultures of epithelial cells established from jaagsiekte lungs, 16 days after subdivision. Note "chords" or "clumps" of multilayered cells, ×75

FIG. 3 The same JS–15.4 culture as in Fig. 1, 8 days later. Confluence has been attained but multilayered "clumps" have not yet formed

FIG. 4 Sheet-like cell colonies formed in JS–15.4 suspension cultures in semi-solid medium containing 0,4% agar

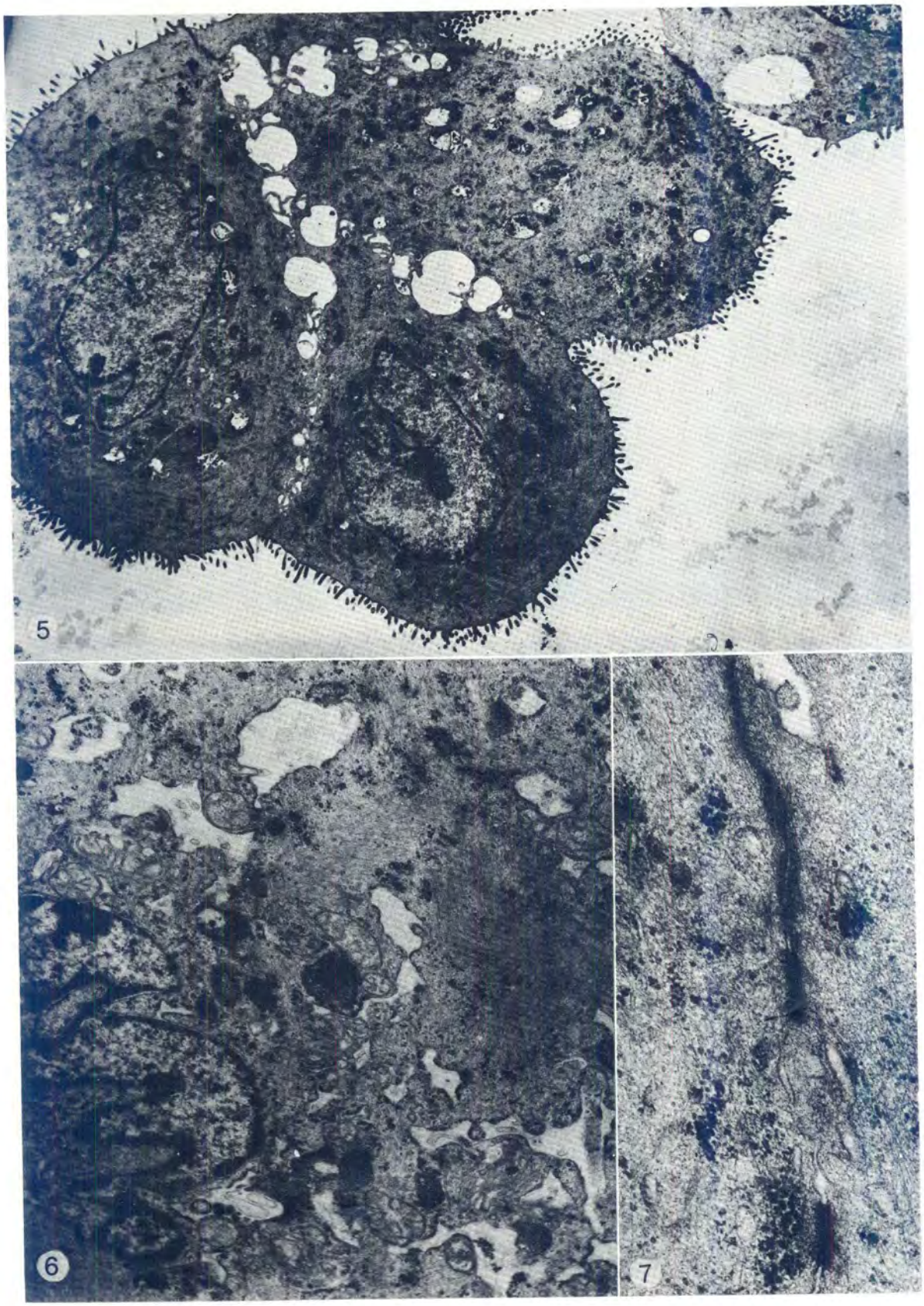

FIG. 5 Electron micrograph of a few JS–15.4 epithelial cells at low magnification, demonstrating numerous microvilli and connection by means of desmosomes. Note also cytosomelike bodies in cytoplasm. Magnification ×6 000

FIG. 6 Part of a cell demonstrating the invagination of the nucleus, various mitochondria, dilated cisternae, Golgi complex and bundles of fine filaments, ×15 000

FIG. 7 Junction between two cells showing desmosomes, ×30 000

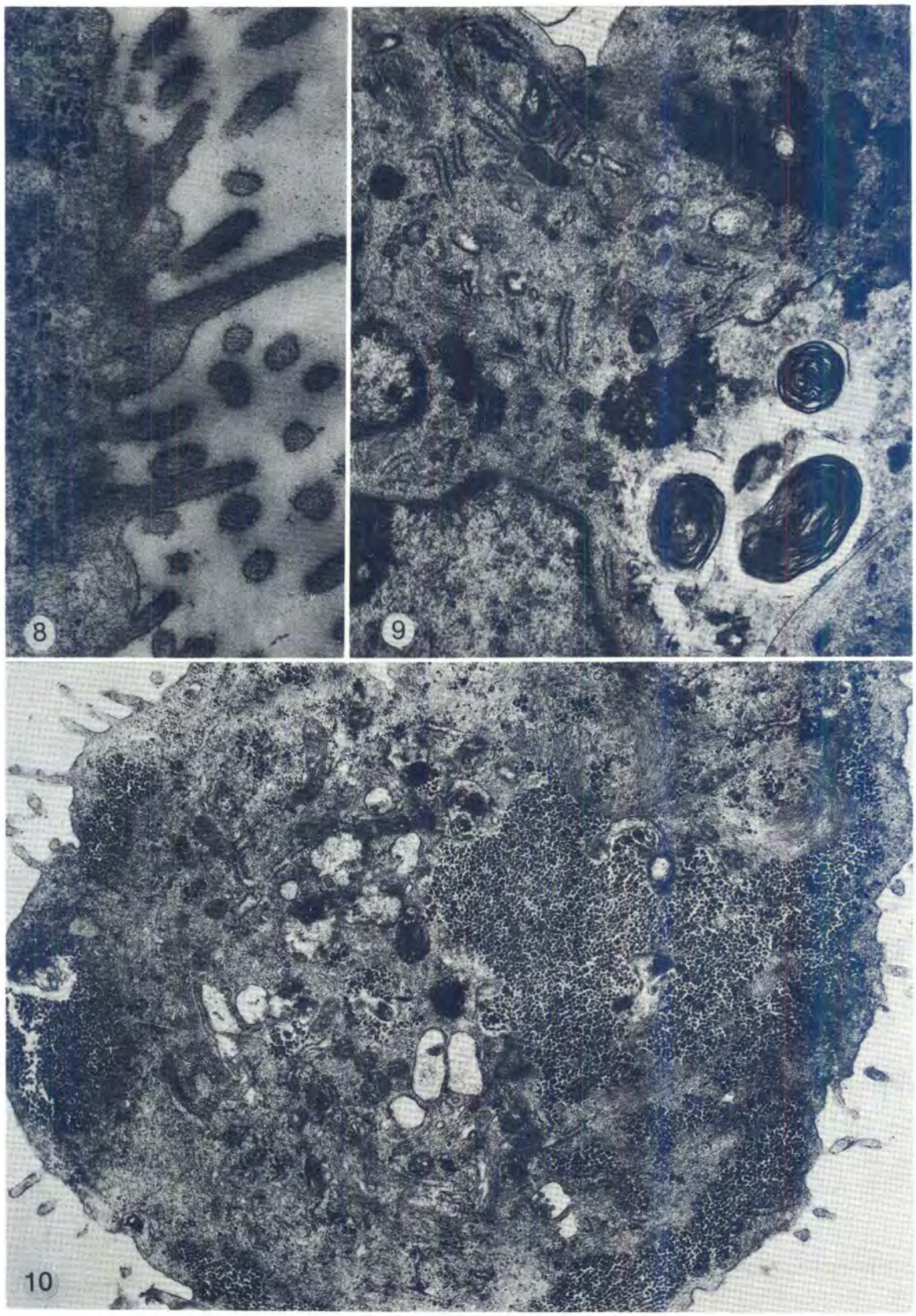

FIG. 8 Part of a cell wall illustrating the presence of fine filaments running from the cytoplasm into the microvilli, ×30 000
FIG. 9 Lamellar inclusion bodies, or myelin figures, in the cytoplasm of a typical cell also containing glycogen granules, cytosomes and polysomes. Magnification ×25 000
FIG. 10 Cytoplasmic region of a JS–15.4 epithelial cell possessing masses of glycogen granules as well as some mitochondria, Golgi complexes and fine filaments, ×20 000

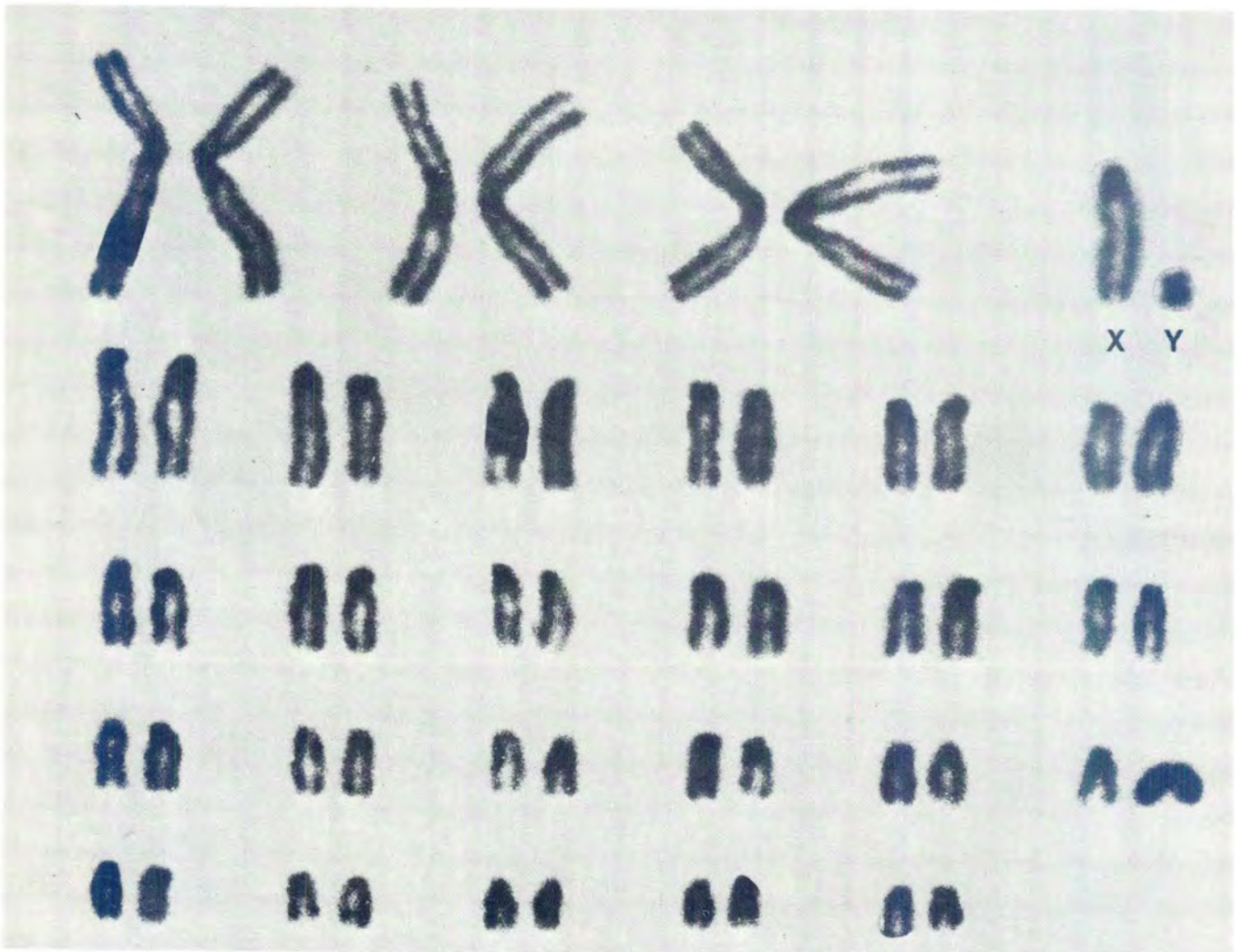

FIG. 11 Karyotype of a JS–15.4 epithelial cell. This particular cell had the normal diploid complement of male ovine chromosomes (2 n=54), consisting of 6 large metacentric, 47 smaller acrocentric and 1 small submetacentric (Y) chromosomes

Two main types of cuboidal cells were present in these cultures, viz. small rapidly dividing cells lacking contact inhibition, and larger flat cells the growth rate of which was slower and seemed to be inhibited by contact. It was observed that these large cells did not readsorb to the glass surface after trypsinization as readily as the small cells.

After the 11th passage, a period of slower growth was overcome by trypsinization and reseeding into the same flask. This procedure eliminated the large cells but retained the small, rapidly growing ones (Das, Hosick & Nandi, 1974). After 3 cycles of trypsinization and reseeding without subdivision, a homogeneous epithelial cell line (JS–15.4, Fig. 3) was obtained which was subsequently subcultured at weekly intervals for 50 passages. Cultures subdivided into 3 or 4 flasks were confluent after a week, attaining an average density of 1×10^5 cells/cm^2.

Ultrastructure

Electron microscopy showed cells in close apposition, with extensive interdigitation of plasma membranes and junctional complexes or desmosomes (Fig. 5 & 7). Cell surfaces not in contact with other cells had numerous microvilli (Fig. 5). The cells possessed many mitochondria, had well-developed Golgi complexes and smooth endoplasmic reticulum which often formed dilated cisternae (Fig. 6). Polyribosomes and microfibrils were abundant in the cytoplasm, the latter often extending into the microvilli (Fig. 8).

The cells also contained cytosomes (Fig. 9), which, according to Perk, Hod & Nobel (1971), are typical of ovine type B alveolar cells characteristic of jaagsiekte lesions. Large nuclei, often showing large indentations, multilaminated myelin figures and large masses of glycogen were also common features of the cells (Fig. 9, 10).

At no stage were any viral particles or recognizable virus-specific structures seen in any of a large number of preparations examined in the electron microscope.

Growth in semi-solid medium

At passage 22, low concentrations of JS–15.4 cells were seeded into 2 types of semi-solid media. In both agar and methyl cellulose containing media more than 1% of the cells formed colonies, which compared favourably with the 4%–7% obtained with the virus-transformed, oncogenic M(52)B mouse cell line included as a control.

The morphology of the JS–15.4 colonies, however, was rather unusual as they tended to form a sheet-like cell aggregate, often at the lower surface of the semi-solid layer (Fig. 4). Similar colonies have been observed by Weinstein *et al.* (1975) in epithelial cultures from hepatomas.

Karyotype

Chromosome counts performed at various passage levels of JS–15.4 cells indicated progressive aneuploidy. At passage 19, between 70% and 80% of cells had the normal diploid complement of 54 chromosomes, whereas at passage 30 this had decreased to about 50%–60%. The number of chromosomes in aneuploids varied from 30 to 108.

A preliminary analysis of the karyotype of diploid cells proved the identity of JS–15.4 as a sheep cell line (Fig. 11). Attempts to determine the sex of the cells did not yield unequivocal results, however. The characteristic small submetacentric Y chromosome was present in some cells (Fig. 11), but absent in others.

Oncogenicity

As morphological and growth characteristics suggested that the JS–15.4 cell line probably consists of tumour cells, oncogenicity studies were carried out in sheep and various laboratory animals. The results are summarized in Table 1.

TABLE 1 *Oncogenicity of JS-15.4 cells for various experimental animals*

Experimental animal	Age when injected	Passage level cells	Number of cells transplanted	Route	Immunosuppression	Tumour development
Lamb 394	12 days	0	$3{,}0 \times 10^8$	I.T.	AT–IgG[1]	Advanced lesions 8 months p.i.
Lamb 660	New-born	5	1×10^7	I.T.	ATS (Rabbit)	Negative
Lamb 783	9 weeks	15	2×10^7	I.T.	None	Negative
Lamb 784	9 weeks	15	2×10^7	I.T.	ATS (Rabbit)	Negative
Lamb 1015	6 weeks	20	2×10^7	I.T.	ATS (Rabbit)	Negative
Lamb 1016	6 weeks	20	2×10^7	I.T.	None	Negative
Lamb 1462	New-born	25	2×10^7	I.T.	AT–IgG	Disseminated early lesions 4 months p.i.
Lamb 2442	New-born	23	$3{,}8 \times 10^7$	I.T.	AT–IgG+AM–IgG	Advanced lesions 4 months p.i.
Rabbits	Adult	36	1×10^7	I.P.	None	0/10
Hamsters	New-born	26	2×10^6	s.c.	None	31/40 (transient tumours)
Mice	New-born	26	2×10^6	s.c.	None	0/25
Mice	New-born	26	2×10^6	s.c.	ALS	0/25
Mice	New-born	26	2×10^6	s.c.	AMS	0/25

[1] Abbreviations: I.T.=intratracheally; I.P.=intrapulmonary; s.c.=subcutaneously; ATS=antithymocyte serum; AT–IgG=antithymocyte immunoglobulin; AMS=antimacrophage serum; AM–IgG=antimacrophage immunoglobulin; ALS=antilymphocyte serum; p.i.=post-injection

Transmission of jaagsiekte was successful in 3 out of 8 lambs given intratracheal injection of cells in conjunction with various immunosuppressive treatments (see Table 1). The 1st lamb (394) received 3×10^8 cells of the uncultured trypsinized lung material which was used to establish the JS–15.4 cell line, and thus served as a positive control. This lamb was 12 days old when injected, received immunosuppressive treatment with ATS and developed symptoms of dyspnoea after 8 months. Autopsy revealed extensive adenomatous lesions.

The 2nd case was a new-born lamb (1462) treated with ATS–IgG and injected with 2×10^7 JS–15.4 cells after 25 subcultures. This animal showed symptoms of respiratory distress after $2\frac{1}{2}$ months and at autopsy 6 weeks later widely disseminated small nodules in both lungs were histologically identified as typical early jaagsiekte lesions. The 3rd positive case was a new-born lamb (2442) which received $3,8 \times 10^7$ cells of the 23rd passage of JS–15.4 cells combined with strong immunosuppression by AT–IgG and AM–IgG. Autopsy 4 months later showed more advanced typical jaagsiekte lesions involving the greater part of the ventral lobes of both lungs.

Two pairs of lambs, injected when 6 and 9 weeks old, respectively, had not developed any jaagsiekte lesions when examined post mortem 1 year after injection. A new-born lamb (660) which was treated with immunosuppressants and injected with low passage cells, developed clinical signs characteristic of jaagsiekte 6 months later, but these dissappeared after some months. At autopsy a year after injection it was negative, suggesting a possible case of rejection.

Intrapulmonary injection of 2×10^7 cells into each of 10 full-grown rabbits did not produce any signs of tumours within 3 months. Subcutaneous injection of new-born hamsters with 2×10^6 cells produced transient growths after 4–6 days, but these had disappeared by the 10th day, probably as a result of an immune rejection reaction.

New-born mice were refractory to subcutaneous transplantation of JS–15.4 cells, even though they had received ALS or AMS.

Discussion

A new procedure for the selection and cultivation of epithelial cells from adenomatous lung lesions was developed. Based on previously described methods, it consists of extremely long initial trypsinization of tumour tissue, eliminating most of the fibroblasts, followed by repeated selective trypsinization of cultures to remove any remaining fibroblastoid cells. The gluco-corticosteroid beta-methasone, used as an additive to our growth medium, was found to promote the growth of epithelial cells while being detrimental to fibroblasts. This effect may be dependent on the epithelial cells being transformed while the fibroblasts are normal, as in the case of chicken embryo cells (Fodge & Rubin, 1975). Alternatively, the close coherence of the epithelial cells may account for their resistance to the corticoid (Behrens, Mashburn, Stevens, Hollander & Lampen, 1974). Amphotericin B was found to impair the growth of epithelial cells, and was therefore replaced by nystatin in our growth medium.

The above procedure was used successfully to establish a number of epithelial cell lines from jaagsiekte lung material, but seems to be less satisfactory for normal lung tissue.

One of the cell lines obtained (JS–15.4) has now been in cultivation for about 2 years, and certain of its characteristics have been studied in some detail. Morphologically and ultrastructurally the culture complied with most of the accepted criteria for epithelial cells (Owens *et al.*, 1974; Weinstein *et al.*, 1975). These criteria include growth in the form of closely coherent islands of polygonal cells, the extensive interdigitation of plasma membranes with the formation of junctional complexes or desmosomes, the possession of numerous microvilli on free cell surfaces and the incidence of microfibrils in the cytoplasm. Lamellar inclusion bodies, which have been described by Stoner *et al.* (1975) as a typical component of type II mouse alveolar epithelium cells and the site of alveolar surfactant synthesis, were commonly seen. The cells also possessed some of the ultrastructural features described by Perk *et al.* (1971) for type B ovine alveolar cells in adenomatous lungs, e.g. multilayered myelin figures, probably identical with the lamellar bodies of Stoner *et al.* (1975), masses of glycogen, cytoplasmic clefts or dilated cisternae and large indented nuclei, in addition to cytoplasmic filaments and microvilli on the cell surface. These observations, in conjunction with the karyotypic evidence that it consists of ovine cells, suggest that the culture consists of the alveolar epithelial tumour cells of jaagsiekte.

When confluent, cultures had the appearance of being transformed, the cells piling up into multilayered clumps or chords. Similar structures were observed in epithelial cultures derived from normal livers by Weinstein *et al.* (1975), who considered that only growth in a semi-solid medium and not morphological appearance was a reliable criterion of transformation. The sheetlike colonies obtained in semi-solid media in the present study are morphologically similar to those observed by Weinstein *et al.* (1975) in semi-solid cultures of cells derived from hepatomas, substantiating a transformed character.

Transient growths obtained when JS–15.4 cells were transplanted in new-born hamsters indicated possible oncogenicity, and when tested in lambs, 3 of the 8 animals succumbed with typical jaagsiekte lesions. One was a control animal which received the parental cell suspension derived from diseased lungs; the other 2 new-born lambs treated with immunosuppressants received cells after 23 and 25 *in vitro* subcultures, respectively. Of the 5 negative lambs, 4 were older when injected. Two of these received no immunosuppressive treatment and the activity of the ATS used for the other 2 as well as for the 5th negative lamb is unknown. The immunosuppression attained in these lambs may consequently have been insufficient. The immune status of the experimental animal, as reflected by both its age and immunological competence, appears to be of prime importance in transmitting jaagsiekte by means of tumour cells, and probably accounts for the low percentage of positive transmissions previously obtained with lung material (Tustin, 1969; Wandera, 1971).

Another factor that may have contributed to the successful transmission of the condition, is the higher number of *in vitro* subcultures of the cells used in both positive cases. The change in growth pattern encountered at passage 12 suggests some kind of *in vitro* transformation. Stoner *et al.* (1975) found that cultures from mouse lung adenomas which were not oncogenic initially, acquired neoplastic potential after

prolonged culture in a medium containing hydrocortisone similar to the corticosteroid-containing medium used in the present investigation.

It is improbable that the positive lambs were infected by contact with other sheep, as the experimental animals were housed separately. Moreover, the incubation period (2–4 months) is much shorter than that previously observed in either natural or experimental infections. Extraneous infection can therefore be excluded.

The significance of our results is two-fold. Firstly, the reduction of the incubation period to 4 months or less will greatly facilitate future investigations on jaagsiekte, especially if a higher efficiency of transmission can be obtained by more efficient immunosuppression. Secondly, the cell cultures, which had been cultivated *in vitro* for an extended period, were shown to be free from all overt infectious agents, including *Mycoplasmas* and viruses. Therefore, the tumorigenic factor must be present in the cell itself.

One possible interpretation could be that jaagsiekte is not transmitted by an infectious agent, as hitherto suspected, but by transplantation of viable tumour cells, possibly in airborne droplets. This is supported by the evidence obtained that at least some of the cells in the JS–15.4 cell line have a male karyotype. As the cell line was established from a tumour obtained from a normal ewe, this result can only be explained in terms of natural transmission by means of viable cells from a male to a female animal. Further evidence for the feasibility of such a transmission is the unpublished observation from this laboratory that the lung exudate from an advanced case of jaagsiekte can contain 10^6 or more viable cells per ml.

On the other hand, various workers reported experimental transmission of the disease by means of cell-free filtrates (Markson & Terlecki, 1964; Wandera, 1971). An alternative hypothesis is therefore the presence of a latent virus, or integrated viral genome in the tumour cell. Such a system would be in close agreement with modern concepts of tumour virology and would allow for both transplantation of the tumour by viable cells not producing a virus and transmission of the tumour by cell-free filtrates after activation of the latent viral genome. The possibility of demonstrating such an integrated viral genome is being further explored.

Acknowledgements

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