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THE SURFACE FEATURES OF *DROSOPHILA* EMBRYONIC CELL LINES

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Schneider's cell lines 1, 2 and 3 derived from embryonic stages of *Drosophila melanogaster* were examined by scanning electron microscopy, histochemical staining procedures, and SDS polyacrylamide disc gel electrophoresis. Although the three lines showed some similarities when compared by each of these methods, differences between the lines were observed as well. The surface features of the cells in each line showed morphologic as well as age dependent distinctions when examined in the scanning electron microscope. The intracellular distribution and amount of periodic acid-Schiff positive (PAS+), α-amylase sensitive material was also distinct for each of the lines. Approximately one-third of line 1 cells contained PAS+ hyaluronidase-sensitive material localized within a cytoplasmic vesicle whereas less than 1% of the cells of lines 2 and 3 contained such material. SDS polyacrylamide disc gel electrophoresis revealed PAS+ material in a soluble fraction of line 2 cells and in the 100,000 × *g* pellet of line 3 cells, but no PAS+ bands in similar fractions of line 1 cells were detected.

Methods for establishing stable cell lines of *Drosophila melanogaster* have been developed within the past several years (10, 24), and it is anticipated that this material will prove particularly useful for studying the biology of cells *in vitro* as well as analyzing various developmental features in this species which is so widely used for genetic experimentation. Schneider's cell lines 1, 2 and 3 (24) have already been exploited to examine a number of problems in cell biology. Of particular interest are the nuclear transplantation studies of ILLMENSEE (14) using these *in vitro* cells. To test the ability of nuclei from these cultured cells to promote differentiation of *Drosophila* tissues, ILLMENSEE (14) injected the nuclei into cleavage embryos thus allowing the implanted nuclei the opportunity to become integrated into cells during cellular blastoderm formation. By the use of genetically marked eggs, the fate of the descendants of the implanted nuclei could be recognized as mosaic patches of cells among the cells of the host embryos. These implantation studies demonstrated that the potential for differentiation of the three cell lines differed. Nuclei of line 1 participated in the development of both larval and adult tissues, line 2 nuclei were restricted to larval tissues, and nuclei of line 3 cells had lost these embryonic potentialities.

The specific tissue from which Schneider's lines originated is not known because the lines were derived from trypsinized fragments of late embryos. Since embryonic cells from the primary cultures underwent metamorphosis to form adult structures when tested in host larvae, SCHNEIDER (24) suggested that the cell lines are derivatives of imaginal discs. She reported differences in the growth characteristics of the three lines, and more recently quantitative differences in the agglutination responses to wheat germ agglutinin and concanavalin A were found among the cell lines (3). The present study was undertaken to further characterize these three cell lines. Since heterogeneity of response to lectin-induced agglutination may be due in part to differences in carbohydrate-containing surface structures, the surface topography of

the cells in these lines was examined by scanning electron microscopy and the carbohydrate moieties of the macromolecules in these cell lines were compared by cytochemical methods and SDS polyacrylamide disc gel electrophoresis.

MATERIALS AND METHODS

Scanning electron microscopy Cells were grown in Schneider's medium (22, 23) supplemented with 15% fetal calf serum (Rehatuin Fs, Reheis Chemical Company) on 24 X 50 mm coverglasses in Leighton tubes. Some cultures were fixed for electron microscopy after 2 days of growth at 25°C while others were allowed to grow for 7 days prior to fixation. The cell density of 2 day cultures ranged between 5×10^3 to 5×10^4 cells/cm^2 as determined by counting a sample of cells in a hemacytometer. Doubling time for lines 1 and 3 was 19–20 hr, and for line 2, 16 hr. Cells on the coverglasses were rinsed with phosphate-buffered saline (25) and fixed in nascent formaldehyde in phosphate buffer at pH 7.1 for 1 hr. The coverglasses were then rinsed with phosphate buffer and transferred to oxmic fixative for 20 min. They were processed through a graded series of ethyl alcohol, amyl acetate, and finally dried by the critical point method recommended by ANDERSON (1). Portions of the coverglasses were mounted on aluminum stubs, and the cells were sputter-coated with gold. A JEOL (Model JSM-U3) scanning electron microscope was used to examine the cells.

Cytochemistry Cells growing on coverglasses were rinsed with phosphate-buffered saline prior to fixation for at least 1 hr in either 100% ethanol or Carnoy (three parts 85% ethanol: one part glacial acetic acid), or in 100% methanol for 5 min followed by air drying. Carbohydrates were stained by the periodic acid-Schiff reaction (PAS), and acid mucopolysaccharides in the cells were stained with Alcian Blue 8GN (18). The bromphenol blue method of BONHAG (5) was used to stain protein.

The presence of glycogen was confirmed by incubating the cells in α-amylase (Sigma Type II-A) at a concentration of 100 μg/ml in 0.02 M phosphate buffer containing 0.0067 M NaCl, pH 6.9, for 30 min at room temperature. Control coverglasses were incubated in buffer solution. Bovine testicular hyaluronidase (Sigma Type I) was used at a concentration of 100 μg/ml in 0.02 M sodium acetate buffer containing 0.4 M NaCl at pH 3.8 for 30 min. Controls were incubated in buffer only.

Cell fractionation and SDS polyacrylamide disc gel electrophoresis Cells were grown in Pyrex glass milk dilution bottles for 10 days. They were washed 3 times in phosphate-buffered saline followed by suspension in 0.0625 M Tris at pH 6.8. Disruption of the cells in this medium was accomplished by freeze-thawing 5 times in a dry ice-acetone bath. The suspension was centrifuged at 100,000 X *g* in a Beckman ultracentrifuge at 4°C for 60 min, and the supernatant was retained as the soluble fraction. The pellet was dispersed in buffer and recentrifuged to give the insoluble fraction.

Electrophoresis followed the method of LAEMMLI (16) with a final length of stacking and separating gels of 1 cm and 7.5 cm respectively. Protein concentration of each sample was determined by the method of LOWRY *et al.* (17) and sample volumes adjusted so that 100 μg of protein was applied to each gel for PAS and 15–20 μg for protein staining. Electrophoresis was carried out at room temperature with a current of 5 mA per gel.

Gels were placed in a solution of 0.05% Coomassie blue in 50% methanol and 9.0% glacial acetic acid for 2–3 hr to stain proteins. Destaining of the gels was completed in 5% methanol and 7.5% glacial acetic acid, and the gels were then stored in 7.5% glacial acetic acid. Glycoproteins and other carbohydrate-containing materials were detected by the PAS method of FAIRBANKS *et al.* (11) except that each gel was carried through the procedure in a 25 X 150 mm test tube. Selected samples were incubated in 0.01% pronase in 0.0625 M Tris, pH 6.8 for 1 hr at 37°C or in 0.1% α-amylase in the same buffer for 1 hr at room temperature prior to electrophoresis.

RESULTS

Surface morphology

Line 1 cells form colonies with dense multilayered central foci (Fig.1) whereas cells of line 2 are scattered at random over the coverglass (Fig.2). In line 3 aggregates of cells are often

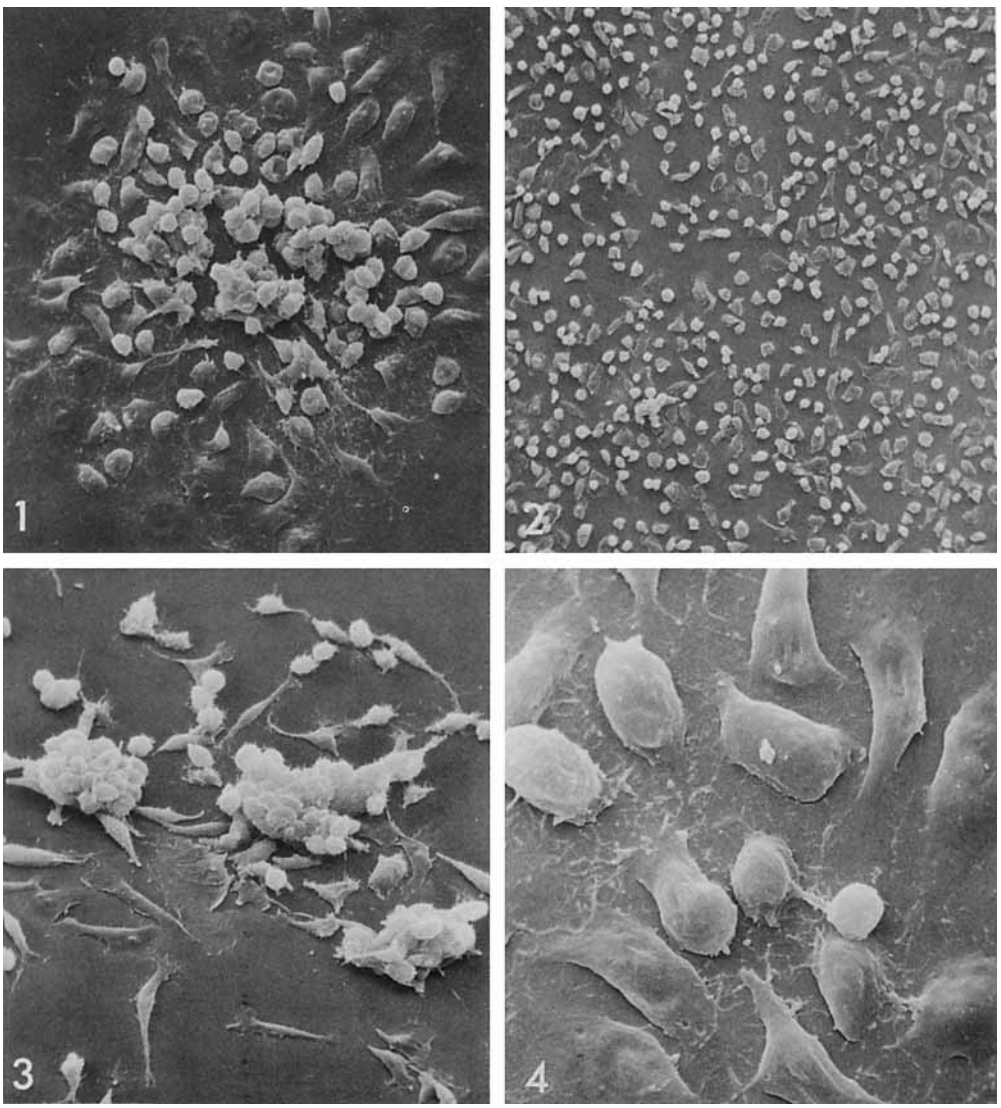

Fig.1. Line 1 cells. Squamate types of cells appear at the periphery of this intermediate sized colony in a 2 day culture while cells in the colony center are spheroid. X 670.
Fig.2. The mode of dispersion of cells in line 2. X 200.
Fig.3. Cells of a 7 day culture of line 3. Some cells grow in aggregates but other cells remain dispersed over the coverglass. Spindle shaped cells are common and highly flattened cells are also present. X 740.
Fig.4. Note the smoothness of cell surfaces in a small colony of line 1 cells in a 2 day culture. X 2,000.

encountered, but many cells remain dispersed over the coverglass as well (Fig.3). These distinctive growth characteristics of the cell lines were reported by SCHNEIDER (24) and the pattern for each line has thus been maintained. A variety of cell shapes occurs in all three lines, including spherical, spindle, and flattened epithelioid forms.

When line 1 was inoculated at low cell densities and examined after 2 days of growth, numerous small colonies were found on the coverglasses. Most of the cells in the smaller colonies had relatively smooth surfaces whether cell shape was spherical or elongate (Fig.4). A few cells in slightly larger colonies showed surface projections, primarily membrane folds and small knobs, but this surface activity occurred generally where cells were in contact with one another. Seven-day aging of the cultures resulted in the growth of large colonies in which most of the cells had filamentous surfaces (Fig.5). Filaments of adjacent cells were in contact, but single cells located away from the colonial masses also displayed a highly filamentous topography. Bubbles, or blebs, were prominent in these older cell cultures as well. A few spherical cells with smooth surfaces were seen in the dense centers of large colonies, and some of the flattened, elongated cells at the periphery of large colonies also showed a smooth surface.

Cells of line 2 showed little change in surface topography as cell density on the coverglass increased with growth and aging of the culture. The cells were smooth, and surface morphology was limited to membrane folds and warts. Even a cell such as that in the lower right hand corner of Fig.6 which appears extremely rough textured at low magnification showed only membrane folds and knobs when its surface was examined at high magnification. The spindle-shaped cells in this line have the same surface characteristics as the spherical cells, but the flattened cells, which are particularly common in this line, tended to be especially smooth.

Most of the cells in both 2 and 7 day cultures of line 3 were ocvered with fine filamentous projections (Fig.7). This type of surface development occurred on spindle-shaped cells as well as spherical cells. Bubbling of the surfaces was also more pronounced in this line than in lines 1 and 2, and some cells had numerous large bubbles attached to their surfaces.

The cells on the coverglasses were asynchronous with respect to the stages of the cell cycle, so changes in surface morphology associated with cell division were an expected variable. Since examination in this study was restricted to cell exterior, recognition of cells in division was limited to the final event, or cytokinesis. In an early culture where a fair number of colonizing foci are formed, pairs of cells interconnected by a cytoplasmic stem, or twin cells located a distance away from other cells and colonies, represent cells in cytokinesis and the stage immediately after cell division respectively. Such isolated pairs of cells were therefore sought to compare topographic features of this specific period in the cell cycle among the three lines. The dividing cells in line 1 were characterized by a smooth texture except for a few folds and wartlike protrusions. Line 2 cells in division also showed membrane folds and warts (Fig.9), but dividing cells in line 3 had considerably more surface projections, and filaments were found on these cells as well (Fig.8). In all three lines where material was favorably positioned, one of the twin members showed a pad of cytoplasm adhering to the glass substratum. The other member of the cell pair apparently buds off into the culture medium; this phenomenon is clearly illustrated in Fig.8. The retention of microvilli or filaments on the surfaces of line 3 cells was particularly intriguing since these structures were clearly present over the area undergoing the greatest stretching, the stem body of the cleaving cells.

To compare the ultrastructural topography of cells in these three lines, the cells were scanned at a magnification of × 30,000. Fig.10 shows the filamentous surface of a cell from a 7-day culture of line 1 for comparison with the filaments on a line 3 cell (Fig.12) from a culture of similar age. The filaments were erect, nonuniform in thickness, and highly variable in length. Some filaments appeared to rise from a slight fold of the cell membrane. Fig.11 illustrates the surface smoothness of line 2 cells and shows the wartlike projections occurring

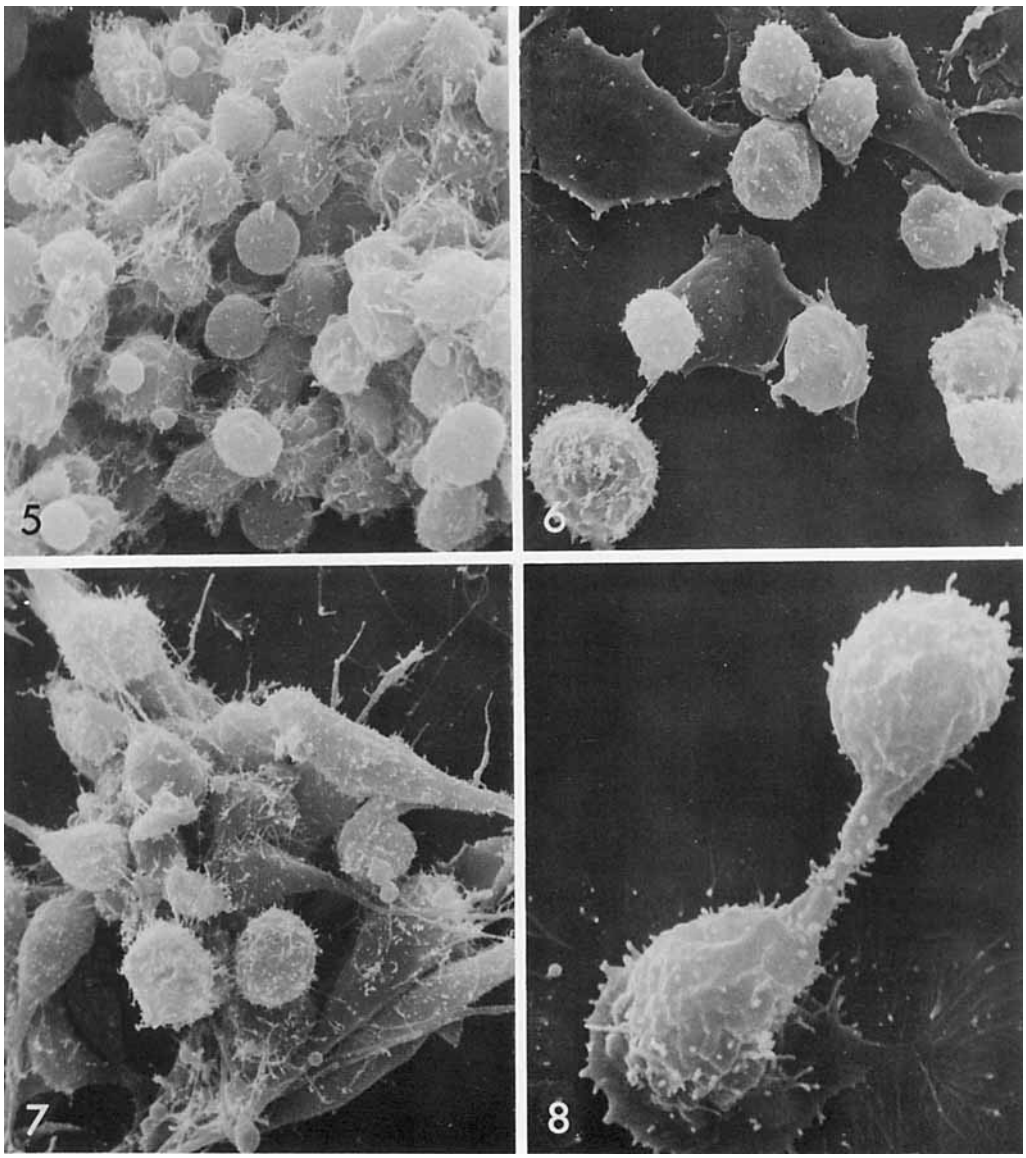

Fig.5. The crowded cells in the center of a line 1 colony (7 day culture). There is heterogeneity among the cell surfaces; some cells are smooth surfaced while other cells have filaments and blebs. The filaments on the cells tend to adhere to neighboring cells. X 2,000.

Fig.6. Note the smooth texture of the flat cells in line 2 and the presence of membranous fringes with knobs on the cell in the lower left. Knobs on the surfaces of other spheroids are also visible. X 2,000.

Fig.7. An aggregate of line 3 cells illustrating the highly filamentous surfaces. X 2,000.

Fig.8. Cells from line 3 (2 day culture) illustrating the attachment of the member in the lower left frame to the glass substrate. The cytoplasmic connection between the twins is covered by microvilli which can also be found on other regions of the cell surface. X 5,000.

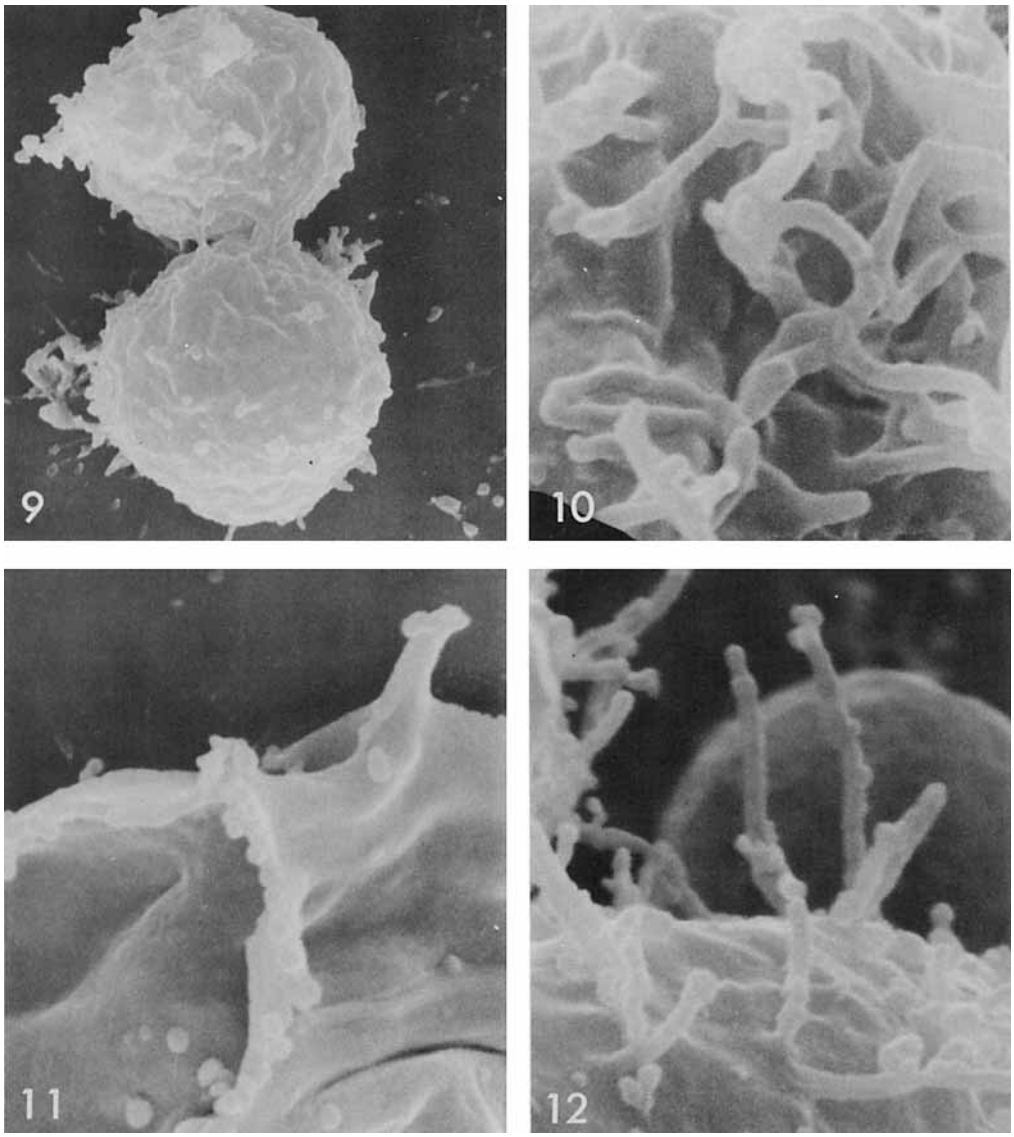

Fig. 9. A pair of line 2 cells completing cytokinesis. The lower member is attached to the glass substratum by radiating cytoplasmic extensions. X 6,700.

Fig.10. Detailed topography of a line 1 cell. Some of the microvilli are branched and nodular. X 20,000 (15 kv).

Fig.11. The surface of a line 2 cell showing knobs or microwarts over a smooth background and on surface fringes. X 22,100 (25 kv).

Fig.12. Topography of a line 3 cell surface. This surface is from a cell located in the central clump of the cells in Fig.3 and can be seen in back of three small cells closest to the viewer. The microvilli are clearly nodular. Many of them tend to show knobs along their body and particularly at the tips. The background is a bubble from the surface of another cell. X 22,100 (25 kv).

on an otherwise smooth terrain, or the development of membrane folds and thickened extrusions which contain a similar type of warts.

Cytochemical analysis

The nature of PAS-positive (PAS+) materials in the cells of the three lines differed as did the sensitivity of these materials to enzyme treatments. Furthermore, the percentage of cells in each line that contained PAS+ material also was different among the lines. Two types of PAS+ material occurred in the cytoplasm of line 1 cells. Small granules stained with PAS were found in a few of the cells. Many more of the cells, approximately one-third, contained pale pink, spheroidal or oblong inclusions localized within large vesicles (Fig.13–1). The PAS+ granular material was removable by α-amylase, but the staining of the inclusions within the vesicles was unaffected by this enzyme; it was removable by hyaluronidase. The PAS+ material in the vesicles did not react with bromphenol blue stain for proteins or Alcian blue staining for acid mucopolysaccharides.

Nearly all cells of line 2 reacted with PAS. A few cells (<1%) contained material in vesicles as described for line 1 cells, but most showed PAS+ inclusions which varied in shape and size, and were distributed in more than one region of the cytoplasm (Fig.13–2). Treatment with α-amylase removed almost all PAS+ material leaving only the PAS+ material within the vesicles. The latter material, as in the case of line 1 cells, was sensitive to hyaluronidase while all other PAS+ material was resistant. PAS+ materials did not correspond in position or shape to bromphenol blue-positive granules in the cells.

Approximately 75% of the cells of line 3 contained a single, PAS+ cytoplasmic inclusion

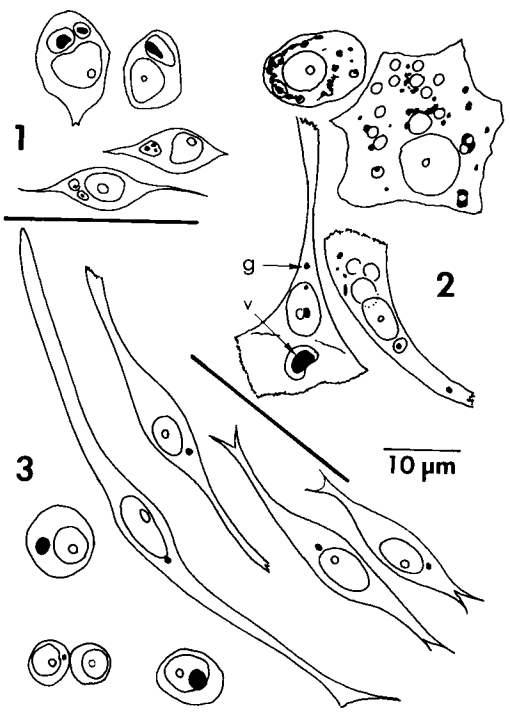

Fig.13. Camera lucida drawings of the cells to illustrate the distribution of PAS+ cytoplasmic materials (solid) in the form of granules (g) and in vesicles (v): line 1 cells-1, line 2 cells-2, line 3 cells-3. These outline drawings also indicate size and shape variabilities of the cells in the three lines. The line 1 cells correspond to the SEM photograph of a small colony center (Fig.1); line 2 cells represent spherical and flattened cells similar to those in Fig.6; line 3 cells are the isolated spindle-shaped or spherical cells as seen in Fig.3. The cell pair in the lower left of the drawing are daughter cells following cytokinesis.

with a smooth outline (Fig.13–3). A few cells showed more than one inclusion body. In spindle-shaped cells this material was near the nucleus along the long axis of the cell. It was α-amylase sensitive and hyaluronidase resistant. A few line 3 cells had PAS+ vesicular inclusions susceptible to hyaluronidase as described for the other lines.

Electrophoretic analysis

Electrophoresis of supernatant and pellet fractions in polyacrylamide gels containing SDS yielded many Coomassie blue-staining bands. Some bands were common to the three lines, but the pattern for each line was distinctive (Fig.14). The figure also shows the position of PAS+ bands in gels from some of these fractions. No PAS+ bands were detected in the soluble or insoluble fractions of line 1 cells, indicating that the PAS+ material observed *in situ* was either insufficient in concentration for detection in the gels, or was lost during the processing of the fractions or electrophoresis. The line 2 soluble fraction showed 7 bands of PAS+ material but the insoluble fraction was devoid of any such materials (Fig.14). When the soluble fraction was treated with α-amylase or pronase and then electrophoresed, the mobility of bands II-VII was increased but band I remained unchanged. These observations indicate that the

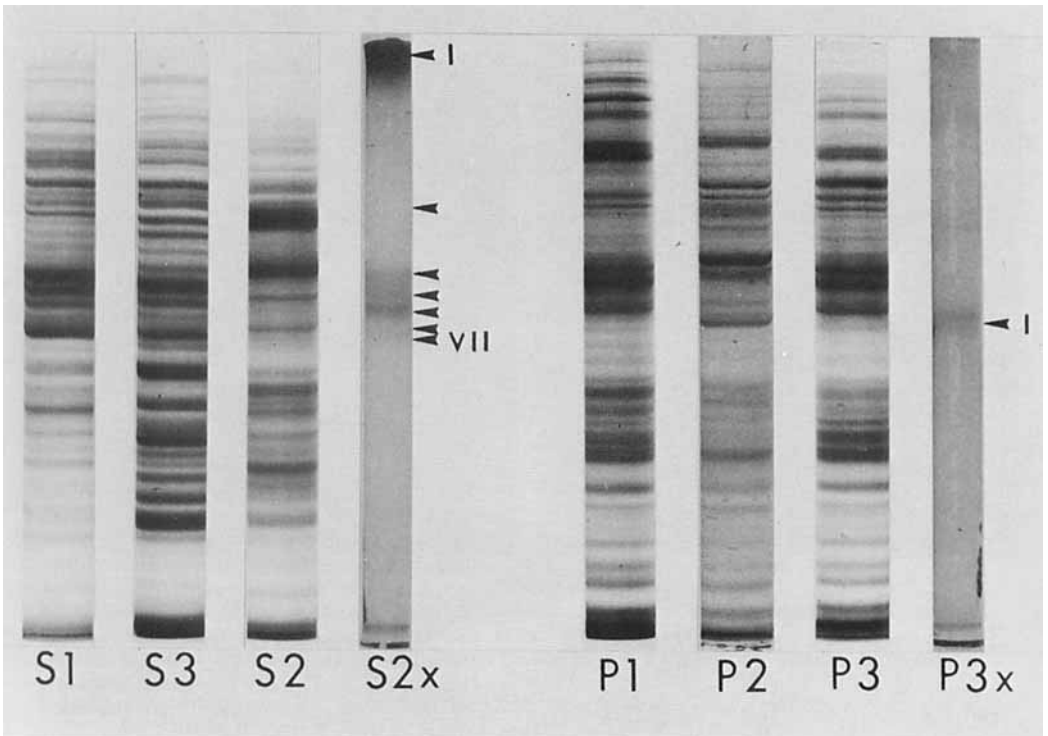

Fig.14. SDS polyacrylamide gels of supernatant (S1, S2, S3) and pellet (P1, P2, P3) fractions from the three lines stained with Coomassie blue. Gel S2x shows PAS+ bands (I-VII) in the supernatant from line 2 cells. The arrow near gel P3x indicates the PAS+ material in the pellet fraction from line 3 cells.

former PAS+ bands are soluble glycoproteins in which some of the carbohydrate moieties are attached by α-glycosidic links.

When PAS-treated gels of line 2 soluble fractions were stained with Coomassie blue after the positions of PAS+ bands were marked with India ink, no Coomassie blue bands were found to correlate with the positions of PAS+ bands. A similar observation was made by FAIRBANKS *et al.* (11) in an electrophoretic analysis of glycoprotein components in human erythrocyte membrane. These authors point out that Coomassie blue was a poor stain for some of the membrane glycoproteins as well as for the highly acidic protein marker (pepsin) used in their studies. It seems that the glycoproteins from the *Drosophila* cells may have atypical compositions of a type that are poorly stained by Coomassie blue.

The line 3 soluble fraction lacked PAS+ bands. However, electrophoresis of the insoluble fraction of these cells showed the presence of PAS+ material either as one diffuse band or two adjacent bands. This material migrated just ahead of the 7th band of the line 2 soluble fraction, and had a molecular weight of approximately 50,000 daltons as based on the migration of protein standards. As in the case of line 2 PAS+ bands, the position of the PAS+ material in line 3 did not correspond to Coomassie blue-staining bands. Pretreatment of the insoluble fraction with α-amylase or pronase followed by SDS gel electrophoresis caused no significant change in the mobility of the PAS+ material but the band(s) no longer appeared diffuse and was sharply defined. Even when the incubation time with pronase was tripled and the enzyme concentration was increased fivefold resulting in loss of all Coomassie blue staining bands in the gels, the PAS+ band was still resolvable.

DISCUSSION

When the three *Drosophila* cell lines are judged by a number of parameters including the mode of growth, topography of the cell surface, and the surface features of dividing cells, the lines are distinct. Line 2 differs from lines 1 and 3 by its absence of colony formation or cell aggregation and the tendency of the cells to grow free in the medium after reaching confluency rather than attaching to the glass. Although line 3 does not form discrete colonies as does line 1, some of the cells in this line aggregate in compact clumps while others remain dispersed over the coverglass. The behavior of the cell surfaces in each line also follows its specific character as a function of culture age and conditions. These surface characteristics are consistent for the methods used in this study and have remained invariant in experiments performed 8 to 9 months apart on cultures maintained by serial transfer in Schneider's medium. Cells in line 2 show an overall smoothness of surface texture when compared with cells in lines 1 and 3. On the other hand, the surface activity of line 3 reflected in the form of filamentous projections and blebs is generally more pronounced than that of line 1, and this distinction between the two cell lines is especially apparent in cultures examined within 2 days after seeding at low density.

Dividing cells in lines 1 and 2 had a generally smooth texture with surface protrusions limited to membrane folds and small knobs, but the cleaving cell surface of line 3 had filaments as well. While the cortical layer of cells at cleavage is undergoing drastic changes associated with the elongation of the spindle, it is interesting to note that topographic characteristics such as knobs and filaments are not consistent features of this dynamic process in these *Drosophila* cell lines derived from late embryos. PORTER *et al.* (19) used synchronous populations of

Chinese hamster ovary cells to study changes in surface morphology during the cell cycle. In confluent cultures, progression through the G_1 and S periods correlated with changes in cell shape from spherical to flattened, and a round form was later restored prior to cell division. The surface of cells in G_1 was characterized by microvilli which decreased in the S phase as the cells assumed the flattened form. With the approach of mitosis and the return to a spherical shape, the cell surface showed extensive development of microvilli. These morphological alterations did not appear in Chinese hamster ovary cells plated at low density (21); the cells in this case remained spherical and highly blebbed through the G_1, S, G_2 sequence.

Modifications in surface features during stages of the cell cycle have also been reported for synchronized Chang's human liver cells which assume a flat form during interphase and a rounded form in preparation for mitosis (8). At the latter stage, long filaments extend from the edges of the retracted spherical cell body to cover an area equal to the size of a fully spread cell. Filaments or filopodia of a similar nature, which apparently function as holdfasts, have been noted on other cell types when these rounded up in the early mitotic period (19). The adhesion of spherical cells to the substrate at this specific period in the cell cycle thus appears to utilize a mechanism involving limited contact points, and it is interesting that this phenomenon is mimicked by treatments which induce rapid rounding up of cells in culture thereby yielding spherical cell bodies with long cytoplasmic filaments attached to the culture dish (20). The attachment of cells to the culture dish may be associated with extensive areas of intimate contact as well as limited contact points. In *Drosophila* cells at cytokinesis, cytoplasmic material in the form of a sheath against the glass substrate was often noted, but only one cell of the pair usually seemed to be anchored to the glass surface by this material. These cytoplasmic pads showed fine filamentous extensions from their borders which presumably strengthened cell attachment to the substrate during cytokinesis.

The role of filamentous surface projections in cell adhesion has been adequately documented (20, 6, 7), but other functions have also been proposed for these surface projections. Perhaps one of the most obvious functions for digitations of the cell membrane is to increase cell surface area; for example, microvillar borders on developing oocytes enlarge total exposure area by a factor greater than ten (9, 15). A physiological role for the microvilli on cells in culture has been suggested by FOLLETT and O'NEILL (12). The present study indicates that the more extensive surface changes in *Drosophila* cells appear with increase in cell density and aging of the cultures. On the other hand, surface fine structure as well as general cell shape may depend upon other factors as well. Line 1 cells in crowded areas overgrowing other cells remain compact and develop filaments whereas some of the cells in contact with the glass surface at the colony periphery assume a flattened or spindle form and have relatively smoother surfaces. Even within the multilayered aggregation at the colony center, however, a few cells with very smooth surfaces can always be seen. Since this *Drosophila* cell line exhibits an even texture at cytokinesis, it is possible that the spherical cells with the barren topography in the colony center are cells engaged in division. If so, then events associated with the cell cycle play the overriding role in determining surface features of the *Drosophila* cells, and cells at mitosis in this particular line will be smooth regardless of their immediate environment or those factors which modify cell surface features at other times in the cell cycle. The comparative study of cells of these lines at cytokinesis does not indicate, however, that generalities of surface texture are associated with this specific stage.

It is not clear whether the topology of the cells in these lines is correlated with carbo-

hydrate metabolism and glycoprotein synthesis. Evaluation of the latter aspects at present is limited to a first approximation of differences between the cell lines. The cytochemical analysis of PAS+ material showed that the nature and packaging of these intracellular components differed among the lines as did the frequencies of the cells within each line containing the various types of inclusions. Presumably, the PAS+ granules or bodies that were removable by α-amylase contain glycogen. Line 2 cells were particularly rich in such glycogen inclusions. On the other hand, the bulk of the PAS+ material in line 1 cells was resistant to α-amylase and susceptible to hyaluronidase digestion. Since hyaluronic acid residues are not stained by the PAS reaction, this material may contain PAS+ carbohydrate moieties intercalated by hyaluronic acid residues.

Comparison of the cytochemical findings and SDS gel electrophoresis of the soluble and insoluble fractions of these lines brings out additional distinguishing features. The line 1 gels gave negative results with respect to the PAS reaction; hence, we may conclude that the cytochemically demonstrated intracellular inclusions in this line are not recovered in SDS gel electrophoresis. In line 2 gels, only the soluble fraction showed the presence of glycoprotein which was inferred from the fact that pretreatment of this fraction with both α-amylase and pronase changed the mobility of six out of seven PAS+ bands. The membrane bound fraction of cells should serve as the source material for a search for polysaccharide components of the cell surface. In this respect, only line 3 yielded a positive finding.

Adsorption of serum proteins to the surfaces of vertebrate cells in culture has been reported (4, 13), so the possibility that glycoproteins from the culture medium are loosely bound to the cell surfaces or taken up by the *Drosophila* cells must be considered. Yeastolate and fetal calf serum in the culture medium can serve as sources of PAS+ bands in SDS gels. ANDREWS (2) compared the mobility of the PAS+ bands from these medium components, which are by definition soluble, with the PAS+ bands from lines 2 and 3 cells. Yeastolate gels had a single band in the same region as band I of line 2, and serum showed bands in the regions where line 3 and line 2 bands II-VII are located. Since the soluble fraction of line 2 shows this material, it is possible that cells in this line selectively accumulate these materials from the medium. On the other hand, the PAS+ material in the line 3 cells was associated with insoluble fractions, again suggesting distinctions between the cell lines.

Inherent metabolic differences among the cell lines are indicated by differential growth responses of these cell lines to amino-sugars (2). Some of the metabolic differences as well as the characteristic surface properties of the cell lines must be correlated with the determined state of the cells in these lines, and must reside in the pattern of sequential activation of gene loci which regulate cellular properties as well as those gene loci which remain inactive in each cell line. The nuclear transplantation experiments of ILLMENSEE (14) provide evidence that certain gene loci are permanently turned off or have been lost among some of these cultured cells. It is not clear, however, whether the observed patterns are the result of different embryonic origins of the three cell lines or are due to selection and stabilization of specific determined states of cell types during *in vitro* culture.

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REFERENCES

1. ANDERSON, T. F., 1953. C. R. 1° Congr. Int. Microsc. Electron., **1**, 567–576.
2. ANDREWS, C. A., 1975. Ph. D. Thesis, The University of Michigan, Ann Arbor, Michigan, U.S.A.
3. ——— and T. M. RIZKI, J. Insect Physiol., (in press).
4. BISCHOFF, R. and M. LOWE, 1973. J. Cell Biol., **59**, 25a.
5. BONHAG, P. F., 1955. J. Morph., **96**, 381–411.
6. CORNELL, R., 1969. Exptl. Cell Res., **57**, 86–94.
7. CURTIS, A. S. G., 1962. Biol. Rev., **37**, 82–129.
8. DALEN, H. and P. SCHEIE, 1969. Exptl. Cell Res., **57**, 351–358.
9. DICK, E. G. and D. A. T. DICK, 1970. J. Cell Sci., **6**, 451–476.
10. ECHALIER, G. and P. SCHEIE, 1969. Exptl. Cell Res., **55**, 136–138.
11. FAIRBANKS, G., T. L. STECH and D. F. H. WALLACH, 1971. Biochemistry, **10**, 2607–2617.
12. FOLLETT, E. A. C. and C. H. O'NEILL, 1969. Exptl. Cell Res., **55**, 136–138.
13. HAMBURGER, R. N., D. A. PIOUS and S. E. MILLS, 1963. Immunology, **6**, 439–449.
14. ILLMENSEE, K., 1976. *In* Insect Development (P. A. Lawrence, Ed.), Blackwell Scientific Publications, Oxford.
15. KEMP, N. E., 1956. J. Biophy,. Biochem. Cytol., **2**, 281–292.
16. LAEMMLI, U. K., 1970. Nature, **227**, 680–685.
17. LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, 1951. J. Biol. Chem., **193**, 265–275.
18. PEARSE, A. G. E., 1960. Histochemistry, Theoretical and Applied. 2d edition, Little and Brown, Boston.
19. PORTER, K., D. PRESCOTT and J. FRYE, 1973. J. Cell Biol., **57**, 815–836.
20. REVEL, J. P., P. HOCH and D. HO, 1974. Exptl. Cell Res., **84**, 207–218.
21. RUBIN, R. W. and L. P. EVERHART, 1973. J. Cell Biol., **57**, 837–844.
22. SCHNEIDER, I., 1964. J. Exp. Zool., **156**, 91–104.
23. ———, 1966. J. Embryol. Exp. Morph., **15**, 271–279.
24. ———, 1972. J. Embryol. Exp. Morph., **27**, 353–365.
25. SEECOF, R. L., 1971. *Drosophila* Infor. Serv., **46**, 113.

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