Some Cultural and Cytological Characteristics of Human Tumors in Vitro*†

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INTRODUCTION

The present study was undertaken to determine both the general growth patterns and some of the cytological details of frequently occurring human tumors. For this purpose the roller tube method, which provides good nutritional conditions, and the hanging drop method, which, less favorable nutritionally, provides optimal conditions for cytological investigation, were used.

The cultural behavior of human tumors grown in roller tube cultures has been observed by Gey and Gey (8) and Coman (4, 5). Other investigators, among them Kredel (11); Buckley (1); Russell and Bland (16); Zakrzewski and Kraszewski (17); Pinkus (15); Höfer (10); Grand, Chambers, and Cameron (9); and Murray and Bradley (14), have studied the cultural characteristics of human tumors in hanging drop slides or Carrel flasks and incidentally have included observations on the cytology of their cells. It therefore seemed worth while to study primarily the cytology of the stroma and parenchyma, including the shapes of the cells and their nuclei, the condition of the cytoplasm and nucleoplasm, the form and distribution of mitochondria and neutral red granules, the shapes and number of nucleoli, and the process of division whenever possible; and, secondarily, to note any individual peculiarities in the cultural behavior of these neoplasms.

METHOD

Twenty human tumors, obtained from the operating room, were cultured in a roller tube (Coman and Stabler, 3) and subcultured in hanging drop slides. Specimens were grown in the roller tubes in a chicken plasma and chick embryo extract clot and a fluid medium of 10 drops of physiological saline (8) and 15 drops of fetal cord serum according to the method described by Coman (4). The subcultures on slides were grown in a medium of 5 parts embryo extract, 3 parts plasma, 3 parts physiological saline (8) and 2 parts fetal cord serum. Most of the cytological data were obtained when the preparations were 1 to 4 days old. When cultures were studied beyond this point the medium was renewed.

Studies of mitochondria were made on hanging drop preparations placed in a 1:50,000 Janus Green B solution for 15 minutes, and studies of neutral red granules on hanging drop cultures stained in 1:2,500 neutral red solution for 15 minutes. Preparations were discarded 1 hour after they had been treated with one or both of these dyes. Some of the vitally stained and some unstained hanging drop cultures were fixed in Bounin's solution and subsequently stained with iron-alum hematoxylin and light green. These were later studied and compared with observations on living cells.

Nucleolar counts in epithelial cells and in fibroblasts were made on a total of 10 hanging drop preparations. Since there were few epithelial outgrowths in all explants of these preparations, cells in every epithelial projection of every explant of a culture were investigated; the number in the outgrowths was small enough so that there was no possibility of counting the same cells twice. However, in fibroblast outgrowths that were more prolific, fields were mapped on the upper, middle, and lower portions of only 1 explant of a culture, and the nucleoli of the cells at only 1 focus of the microscope were counted.

RESULTS

Of the 20 tumors cultured, 3 carcinomas of the breast and 1 adenoma of the thyroid have been selected for presentation. The malignant tumors are discussed in one group, the benign tumor of the thyroid separately.
ADENOMA OF THE THYROID

Cultural characteristics.—Within 24 hours after the roller tube cultures had been set up, tufts of fibroblasts projected from the explants and increased in number during the 55 days the cultures were grown until finally they formed solid sheets of cells.

Epithelial cords and tongues projected into the plasma clot by the second day. These cultures were notable in that their first epithelial outgrowths were cords of cells that bent and twisted around and, within 2 days after cultures had been set up, formed acinar structures. Such organized groups of epithelial cells have been previously reported by Cameron and Chambers (2) in a carcinoma of the breast and by Coman (4) in a benign tumor of the breast. Morphologically, the acini found in the cultures of the thyroid adenoma resembled those described by Coman more closely than those described by Cameron and Chambers. They differed, however, in that they appeared after 2 days of culture, whereas in Coman’s cultures they were found only after a month in vitro. Coman does not report their fate; Cameron and Chambers state that the acinar organizations in their cultures later resolved into epithelial sheets. Those here reported similarly resolved into sheets of epithelium, which grew luxuriantly during the life of the cultures.

Cytological characteristics.—Whether in sheets, cords, or tongues the epithelial cells were for the most part polygonal in shape, though variations of this form were observed on the edge of an outgrowth; such cells were often slightly fusiform. The epithelial nuclei were oval in living cells, and round in fixed and stained hanging drop preparations. Within the nuclei, 2 nucleoli of various sizes were seen; although their shape varied in different cells, they were most often round with an irregular periphery.

The cytoplasm was homogeneous in appearance with the exception of a few granular areas, which either bordered on or were localized at one of two ends of the oval nuclei. Refractile fat globules followed the same pattern of distribution as the granulations. Small clusters of fat surrounded the granular areas at the tips of the nuclei or formed a perinuclear ring between the periphery of the nuclei and cell membrane. Occasionally a row of 2 to 4 globules was found in the peripheral regions of the cells.

Similarly neutral red granules were clumped at either or both ends of the nuclei. Cells varied in the size and quantity of granules contained; in some they were scattered throughout, with the greatest concentration in the region of the nucleus. Perinuclear distribution of mitochondria was also evident in these epithelial cells. Rod-like, filamentous, and spherical mitochondria were seen, the latter most frequently.

No complete data were gathered on the division cycles of this tumor, although cells in mitosis were often found in fixed and stained preparations. Observations were made on an epithelial cell of the adenoma in telophase, at which time the cell was a long oval shape, with its spindle drawn into a long cylinder, at each end of which chromosome rods were visible. Granules and fat globules were aligned on the long axis of the cell between the margin of the spindle and the periphery. Twelve minutes after the observation was begun the central portion of the spindle had thinned, the chromosomes had clumped at either end, and the cytoplasm had started to constrict. As soon as the connection between the divided chromosomes had broken (8 minutes later), granules and fat, previously aligned along the margin of the cell, moved to the center, through which the line of cytoplasmic cleavage ran. The chromosomes lost their identity, and the outlines of the daughter nuclei became irregular and difficult to see. The granules and fat, divided between the two cells, were scattered. Four minutes later, the daughter nuclei started to reorganize and the fat became localized around the periphery of the cell. The time required from the beginning of telophase to the end of cytoplasmic division was 30 minutes. These cells were not observed again until 40 minutes later, when they had the appearance of typical epithelial cells with clearly defined nuclei and faint nucleoli.

The course of the final stages of division of this cell corresponded to that of similar stages in the division of normal animal cells (12) and the epithelial cells of a human squamous cell carcinoma (10).

The stroma cells of the adenoma were usually bipolar, spindle-shaped cells, although broad tripolar fibroblasts with 1 process at the proximal end and 2 at the distal end were also seen in the outgrowths of this tumor. The oval nuclei usually contained 2 nucleoli, which were bent, twisted, and dumbbell-shaped or triangular in surface view. The nucleoplasm was finely granular and appeared much denser than the smooth, homogeneous cytoplasm. The only granules in the cytoplasm were localized at the poles of the nuclei.

The granular areas contained small accumulations of fat globules. In tripolar cells, 2 small clusters of fat appeared peripherally and on opposite sides of the cell body in the region where the 2 cell processes extended. In all fibroblasts an occasional row of 2 to 6 fat globules, resembling a string of beads, appeared in the cell processes parallel to the long axis of the cells. Filamentous mitochondria, the predominant form in these cells, were located in the cell extensions, although filaments, rods, and spherules were also grouped around the nuclei. Unlike mitochondria, neutral red granules were seldom seen in the outer
portions of the cell processes. Most neutral red granules accumulated in the granular parts of the cytoplasm.

Carcinomas of the Breast

Cultural characteristics.—In 2 of the 3 carcinomas, fibroblastic preceded epithelial outgrowth, and appeared on the first and second days; the epithelial cells were seen on the third day. In the third tumor, both components grew out on the fourth day. The patterns of growth were typical of those previously reported. Fibroblasts grew out radially and increased in number during the period of cultivation, so that the explants appeared on the first and second days; the epithelial cells grew out on the fourth day. The patterns of growth were similar to those previously reported. Fibroblasts grew out radially, increased in number, and eventually surrounded by a halo of densely packed cells. Mingled with these were cords and tongues of epithelium, which grew as sheets of flat polygonal cells.

Two of the breast tumors formed whorls of epithelial cells (Fig. 1) at the peripheries of the zones of outgrowth that often separated from the main outgrowth (Fig. 2), and subsequently became loci of epithelial colonies from which wide polygonal cells proliferated. In appearance the whorls resembled the pearls described by Cameron and Chambers (2) and by Coman (4) in cultures of squamous cell carcinomas; in behavior they resembled the groups of epithelial cells in a mouse carcinoma described by Fischer, Fischer, and Hollmann (7), in that both migrated from the solid areas of outgrowth to points on the margins of the clots.

It seems probable in the light of the work of Coman (6) that the whorls are a result of decreased cohesiveness in malignant compared with normal cells. Coman found that it required significantly less force to pull apart two malignant cells than to separate two normal cells from the corresponding normal tissue. The detachment of these clusters is interesting because metastasis is one of the distinguishing characteristics of most malignant tumors in vitro. The separation of the whorls in vitro, and the subsequent formation of loci of further growth from them, suggest that the behavior of carcinoma cells in vitro parallels their behavior in vivo.

Cytological characteristics.—Epithelial cells in cultures of the 3 carcinomas of the breast were polygonal, with one centrally placed, broad oval nucleus; possibly as a result of liquefaction of the clot or of the contact of neighboring cells, some were fusiform in shape. The cell membrane, with the exception of cells at the margins of the sheets, was not always visible, although the general limits of each cell were discernible by differences in opacity at the center and along the periphery.

Almost all carcinoma cells contained finely granular, dense cytoplasm, which extended to the periphery exclusive of the cytoplasmic layer and gave them a stippled appearance. Very infrequently carcinoma cells had localized granular areas in otherwise homogeneous cytoplasm. The nuclei of all epithelial cells of these tumors were filled with an even denser, more coarsely granular, heterogeneous nucleoplasm separated from the cytoplasm by distinctly visible nuclear membranes. The condition of the cytoplasm and nucleoplasm of these malignant tumors is in contrast to the condition of the cytoplasm and nucleoplasm of the adenoma of the thyroid, described above, in which granulations were localized in the cytoplasm and fine and evenly dispersed in the nucleoplasm. These observations suggest that diffusely granular, dense cytoplasm and coarsely granular, dense nucleoplasm may be a condition of malignancy in human tumors, as Lewis (13) has suggested in animal tumor cultures.

Counts of the number of nucleoli in malignant epithelial cells showed a range of 1 to 4 per nucleus, with 1 and 2 the most frequent number, 3 and 4 rare. There were obvious size differences in nucleoli in the same nucleus and in nuclei in different cells. Nucleoli were usually round, with smooth contours and occasionally with small indentations. Very few fat droplets were seen in these cells; when present, they were scattered irregularly throughout the cytoplasm or concentrated perinuclearily. Spherical mitochondria also accumulated around the nuclei. The rod-like and filamentous forms, which appeared less frequently in epithelial cells, were situated at the periphery rather than in the central region. The peripheral distribution of neutral red granules predominated when epithelial cells were unusually broad, but more often they banded the nucleus and extended into the region where there was the greatest amount of cytoplasm (Fig. 3).

The stroma in these cultures was composed of long, thin, bipolar spindle-shaped cells, both ends of which terminated in a long cell process. Their long, oval nuclei, frequently found in the broadest part of the fibroblasts whether this region was in the central area or near either end, was composed of dense but uniformly granular nucleoplasm.

Some nucleoli also seemed granular, as if formed by the accumulation of many small nucleolar fragments. These often contained vacuoles. Others were smooth and nonvacuolated. All were irregularly shaped. Of a total of 476 cells counted, 174 contained 1 nucleolus, 239 contained 2, and 63 contained 3 or more.

If the nuclei were peripheral instead of central, granules accumulated at their sides; otherwise they clustered at one or both poles of the nuclei. The rest of the cell, except for areas in which fat, mitochondria, and neutral red granules were suspended, was non-granular and smooth.
Fig. 1.—Whorls of epithelial cells on margin of zone of outgrowth. Fifteen-day culture of carcinoma of breast. Mag. × 100.

Fig. 2.—Detached whorl showing initial stage of new proliferation. Fifteen-day culture of carcinoma of breast. Mag. × 100.

Fig. 3.—Epithelial tongue showing varying amounts of neutral red granules in perinuclear distribution and scattered in the cytoplasm. Four-day hanging drop preparation of carcinoma of breast. 1:2,500 neutral red. Mag. × 200.

Fig. 4.—Bipolar fibroblast with neutral red granules localized at end of oval nucleus and dispersed in cytoplasm at opposite pole. Highly refractile fat globules. Two-day hanging drop culture of carcinoma of breast. 1:2,500 neutral red. Mag. × 920.

Fig. 5.—Binucleate fibroblast with 2 irregular vacuolated nucleoli in each nucleus. Nucleoplasm homogeneous and nuclear membrane visible. Six-day hanging drop preparation of carcinoma of breast. Mag. × 920.

Fig. 6.—Trinucleate fibroblast. Nuclear components of varying sizes; vacuolated nucleoli with homogeneous contours. Three-day hanging drop preparation of carcinoma of breast. Mag. × 920.
Fat globules were most often scattered in the granular localizations or lined up in rows of several at the ends of the cell processes. Mitochondria were similarly situated, the rods and filaments the most common forms. When the cells were stained with neutral red the various sized granules in which the dye accumulated capped or flanked the nuclei and extended into the processes, except for the outermost tips of the cells (Fig. 4).

Multinucleate fibroblasts and epithelial cells varied in the shape and number of their nuclear components. In an epithelial cell there were 2 round nuclei, separated by a granular area in which mitochondria and neutral red granules accumulated. Other cells had a kidney-shaped and an oval nucleus, two oval nuclei (Fig. 5), or 3 ovoid nuclei of different sizes (Fig. 6). With the exception of one part of this trinucleate cell, which had no nucleoli, nuclear components in multinucleate cells had from 1 to 4 nucleoli each. Nucleoli were round or irregularly shaped, as shown in Figs. 5 and 6 respectively. In both of these, typical α- all, nucleoli were heterogeneous in appearance and contained small vacuoles.

A dividing carcinoma cell from one of the cultures containing multinucleate cells was studied for 23 minutes. It was first noticed in anaphase, during which time clear blebs of cytoplasm started to protrude from the cell surface. When it was observed 2 hours after telophase, the cytoplasm had still failed to divide completely. These observations suggest that the formation of binucleate cells resulted from a failure of the cytoplasm to cleave during division.

SUMMARY AND CONCLUSIONS
A study of 3 carcinomas of the breast and 1 adenoma of the thyroid, grown in roller tubes and studied cytologically in hanging drop cultures, is reported.

Cells in outgrowths of the cultures of 2 carcinomas of the breast formed whorls of epithelial cells that subsequently separated from the zones of outgrowth and formed loci of new colonies. It is suggested that the behavior of these whorls in vitro parallels the metastatic behavior of malignant epithelial cells in vivo.

Spherical mitochondria predominated in epithelial cells, rods and filaments in fibroblasts.

The neutral red granules in both parenchyma and stroma cells were usually in groups about the nucleus, but a few clusters were scattered at the periphery.

Diffuse granularity characterized most malignant epithelial cells, while a homogeneous cytoplasm with localized granular areas was more often present in the epithelial cells of the benign tumor and in the fibroblasts of both benign and malignant tumors.

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REFERENCES
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