The Golgi Complex and Endoplasmic Reticulum in Tissue-cultured Human Melanoma Cells with Phase Contrast Microscopy*

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SUMMARY

A human melanoma was cultivated in tissue culture chambers on coverslips, under strips of dialysis cellophane. It was found with phase contrast microscopy that cells emigrating from these cultures contained a Golgi complex and forms suggestive of the endoplasmic reticulum. Both of these observations are documented with photographs and discussed with respect to findings in melanomas by electron microscopists.

It was previously reported (20–22, 24, 25) that osteoblasts emigrating from embryo chick bone fragments on tissue culture coverslips under strips of dialysis cellophane (24) contained prominent phase gray juxtanuclear masses. These masses were considered to be living representatives of the Golgi complex, since they were juxtanuclear in most cases, approximately the size of the nucleus, generally conformed to cytochemical and vital staining reactions reported by others, and because time-lapse cinematographs (20–22) revealed an emanation of droplets from them. Further, cultures of human tissues (adenocarcinoma, melanoma, and fetus) established in the same manner had a variety of emigrating cells, some of which when viewed by phase contrast microscopy were observed to have cytoplasmic forms considered to be the endoplasmic reticulum (21, 23). 1

By use of the cellophane methods (24) a human malignant melanoma explant was cultivated. The emigrating elements had juxtanuclear masses which, unlike the Golgi complex of embryo chick osteoblasts, varied considerably in size and morphology. The melanoma cells also contained filamentous forms which were similar in size and phase display, but not in distribution, to the endoplasmic reticulum (ER) formerly described (7, 21, 23). Nevertheless, there was convincing evidence that these juxtanuclear masses were also representatives of the Golgi complex in melanoma cells and that a close association existed between this organoid and the ER.

The data of this report consist of morphological descriptions of this Golgi-ER complex in cellular emigrations from a tissue-cultured melanoma as observed through the phase contrast microscope. It is regrettable that the use of special cytochemical technics was not possible with these specimens, but the amount of material available and the variety of technics utilized resulted in a limited cellular yield of the malignant element for study. The preferred choice for a morphological and time-lapse cine analysis of these unusual organoidal displays, therefore, was considered as the fundamental goal and one upon which more advanced technics could be supported in the future.

The Golgi-ER complex in tissue-cultured melanoma cells will be discussed with respect to: (a) the recently reported electron microscope (EM) studies of melanoma cells (26–29), (b) cellular morphology of emigrating melanocytes from tissue-cultured melanomas in studies of others.

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1 Endoplasmic reticulum—the term is used in reference to the membrane system of the cytoplasm first described by Porter with the electron microscope, but the images may include the ribosomes as well and, therefore, be representative of the ergastoplasm.
(1, 3, 9, 12–15, 26), and (c) the potential usefulness of studies of the Golgi-ER complex for increasing basic knowledge of cellular function of melanomas.

MATERIALS AND METHODS

Specimen.—A metastatic melanoma was obtained from a node in the right axilla of a 45-year-old white male and immediately placed in a test tube containing a synthetic tissue culture fluid nutrient (V-614, Difco Laboratories). The specimen was rinsed several times in additional V-614 solvent (penicillin G, 2000 units/ml) and then cut with surgical scalpels into fragments approximately 1 × 1 mm. for tissue culture explantation.

Culturing chambers and technique.—Sixty pieces of the tumor specimen were placed on the coverslips of twenty standard multipurpose culture chambers (17–25) under strips or sheets of Visking dialysis cellophane. The strips provided an environment which was very flat and one which was directly continuous with the fluid nutrient vault of the chamber, whereas the sheets completely isolated the explants from the fluid nutrient except by dislaysis. A more complete description of this procedure may be found in another publication (24).

Nutrients.—The basic nutrient was composed of Fischer’s V-614, which contained whole egg ultrafiltrate2 (6), 5 per cent, and serum, 20 per cent. In half of sixteen cellophane strip cultures the nutrient was used in the nutrient formula, and in the other half heterologous (calf) serum was used. In four cellophane sheet cultures there was no serum in the nutrient.

Studies of living cells.—Observations of the emigrating cells were made with a phase contrast microscope (Bausch and Lomb). Still photographs were made on Agfa Isopan IFF 13, #120 roll film with a Hasselblad 1000F camera. The photographs in Figures 1, 2, and 10 were taken through a 21X phase objective, whereas all others were taken through a 97X phase objective. In both cases a 10X projection ocular (Bausch and Lomb) was used, and all the photographs were enlarged exactly three diameters. Time-lapse cine analyses were made on Ektachrome Commercial (Type 7255) 16-mm. film with a Cine Kodak Special attached to an EMDECO time-lapse unit.3 Other sequences were made through the AO-Baker interference microscope (19).

RESULTS

Cellophane strip cultures with calf serum nutrient.—The immediate emigration was composed of cells which are generally termed as spindle cells, fibroblastoid cells, fibrocytes, and stromal cells. In about 2 weeks these elements were abundant, and isolated multinucleated giant cells were also located near the explants. Some of these giant cells contained a light melanin pigmentation in a cytoplasmic aggregation, whereas others had a coarse accumulation. Cultures were maintained for 3 months, and over this period of observation all the giant cells degenerated in about 2 months, leaving ghost cells on the coverslips. There was a continuing development of a dense and intertwining fibroblastoid stroma; however, malignant elements as described in the next section were not observed.

Cellophane strip cultures with autologous serum.—These cultures, unlike the heterologous serum group, were characterized by few outwandering fibroblastoid cells and a slow emigration of pleomorphic epithelioid cells (Figs. 1, 2) which had many features of malignancy. Although no mitoses were observed, these cells were widely different with respect to nuclear size, cellular mass, and contour. In most of these epithelioid elements the nucleus was associated with a spheroidal mass approximately its own size. These juxtanuclear masses were basically phase white, although they often contained dark streaks which made them appear as a whorl (Figs. 3, 5). There was a considerable amount of cellular debris (Figs. 1, 2), and this interfered with the microscopic observations. Other juxtanuclear masses appeared to be surrounded by delicate nets or parallel forms (Figs. 4–9, 11–19), which suggested a similarity to the endoplasmic reticulum; and phase-white droplets (Figs. 4–8) were often in and about them. Time-lapse cinematography showed that these droplets originated in the juxtanuclear masses (Golgi complex) and emanated from them in the centrifugal direction indicated by arrows in Figure 6. Cells in some cultures became detached from the main explant and floated into a very narrow area between the glass and the cellophane. Such isolated cells frequently were among debris (Fig. 10) but in this environment continued to contain an evident Golgi complex and endoplasmic reticulum (Fig. 11).

Cellophane sheet cultures with V-614 nutrient.—The explants cultivated under full sheets of cellophane and provided with only a dialysate of the basic nutrient without serum showed an emigration of cells which at first had the juxtanuclear body with the parallel forms, but later the Golgi complex as shown in Figures 12–14. There were fewer of these epithelioid and more fibroblastoid elements than in the autologous serum nutrient.
Cultures of an advanced age (2 months) had emigrating cells with a foamy cytoplasm (Figs. 12–14), but still the nucleus and Golgi complex appeared as in the nonfoamy cells, which also were present at this time. These cells ultimately became ghost cells, as the cytoplasmic walls opened and the nuclei and Golgi complexes disintegrated.

A closer inspection of the Golgi-ER complex.— After 2 weeks of cultivation, when the malignant epithelioid cells first became evident, the juxtanuclear zone was more like that of the cell in Figure 18, so that the first interpretation was the presence of an endoplasmic reticulum in close association with the nucleus. As other cells emigrated into observable fields, these juxtanuclear zones were more circumscribed, and some of them did not have an appearance of the endoplasmic reticulum at all. Further observations indicated that many of these circumscribed areas contained phase-white droplets, which, as already indicated, were proved by time-lapse cine analyses to have their origin at this site (Golgi complex). At one instance, then, the interpretation for these areas was that they represented the endoplasmic reticulum, whereas another interpretation was that they represented the Golgi complex. After repeated analyses and photographic studies had been made, it became apparent that both interpretations were correct and that in one case the endoplasmic reticulum was so overlying the Golgi complex that it appeared to be the most demonstrable, whereas in other situations the endoplasmic reticulum was to one side or only slightly covering the Golgi complex so that it did not conceal it. Further, the Golgi complex in the embryo chick bones had been shown by phase contrast to be gray. In sharp contrast, the Golgi complex in these melanomas. In these reports they stated that melanin granules appeared in the Golgi apparatus of the Cloudman S91 mouse melanoma. Continued examinations by Wellings and Siegel (28, 29) also revealed evidence for the Golgi complex as the site of melanin granule origin in human melanomas. In these reports they stated that melanin granules were never observed within spaces enclosed by the ER membranes whose outer surfaces were provided with ribonucleoprotein particles. They did not infer that melanin synthesis took place in any one area, since they felt this was beyond the range of the EM but that several organeloids, such as the ER, mitochondria, and Golgi complex, all probably participated in its production. In other words they considered the origin of the melanin pre-granule unknown but that of the melanin granule as probably occurring first in the Golgi complex.

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DISCUSSION

Four publications on the electron microscopy (EM) of human malignant melanoma cells have been presented recently by Wellings and Siegel (27–29) and Wellings et al. (26). In their initial report (1958) they indicated that preliminary observations showed melanin granules were first visible diffused throughout the endoplasmic reticulum (27). Dalton (4) then reported in 1959 that melanin granules appeared in the Golgi apparatus of the Cloudman S91 mouse melanoma. Continued examinations by Wellings and Siegel (28, 29) also revealed evidence for the Golgi complex as the site of melanin granule origin in human melanomas. In these reports they stated that melanin granules were never observed within spaces enclosed by the ER membranes whose outer surfaces were provided with ribonucleoprotein particles. They did not infer that melanin synthesis took place in any one area, since they felt this was beyond the range of the EM but that several organeloids, such as the ER, mitochondria, and Golgi complex, all probably participated in its production. In other words they considered the origin of the melanin pre-granule unknown but that of the melanin granule as probably occurring first in the Golgi complex.

This position was fortified by an additional report (26) involving malignant melanoma cells
cultured in vitro. The tissue-cultured melanocytes were said to have contained a Golgi complex in which the melanin granules could also be observed by EM, but the identification of the melanocytes in vitro was made by tyrosine-tyrosinase and silver impregnation methods (8). Regarding the appearance of Golgi bodies in the living or fixed cells with light microscopy, Wellings et al. did not mention having observed any; yet in their electron micrographs of these tissue-cultured cells such melanin-producing bodies were discernible.

As a matter of fact, none of the reported studies of tissue-cultured melanomas or of other melanin-producing tissues in recent years (1, 3, 9, 12-15) has indicated the presence of a demonstrable Golgi complex in emigrating melanocytes with light microscopy (LM), even though some of the cells of emigration have been regarded as differentiated elements. The most recent and comprehensive survey by Hu et al. (14) and the very inclusive study on melanomas from 38 patients by Cobb and Walker (3) do not indicate the appearance of a Golgi complex in the in vitro cells.

There seems to be, therefore, some discrepancy with respect to the manner in which cells are demonstrated by electron microscopy and the way cells are actually observed in their tissue culture environments. The EM studies have shown that melanoma cells contain a prominent Golgi complex and that from this Golgi complex melanin is, in all likelihood, aggregated and released. Yet, those engaged in tissue culture analyses of emigrating cells from melanomas have labeled certain elements as melanocytes even though they have not been associated with an obvious Golgi complex, a structure approximately the size of the nucleus. The concluding questions may be asked: (a) why have the results of the present study supported the EM studies in that the melanoma cells contained very prominent and seemingly functioning Golgi complexes; (b) why was melanin not observed in these Golgi complexes; (c) why have tissue culture studies of others failed to show melanoma cells containing the Golgi complex; and (d) why does melanoma, an unusually savage malignant condition, contain such a pronounced Golgi complex, and organoid generally associated with a purposeful function to the animal host? Malignant conditions are conventionally considered as disorderly cellular growths which have recognizable though diminishing Golgi complex forms (5, 11) and capacities as viewed with the EM, whereas cells from melanomas and perhaps a few other tumors, which show normal or hypertrophied Golgi bodies (10), cannot be thus categorized. Melanoma cells continue in a normal functioning of melanin production, albeit a superactivity, but at the same time produce the disorderly and metastasizing malignant growth.

In response to these questions it can be pointed out that malignant strains of cells will undergo more vigorous growth rates when they are not confined under sheets of dialysis cellophane, whereas normal embryonic tissues undergo a high degree of morphological and functional differentiation though still a slow growth rate when they are so confined. It was indicated in the present study that the outgrowth rate of the melanoma cells was slow, that mitosis was not evident, and that the Golgi complex was observed to be functioning with respect to droplet emanation as revealed by time-lapse cinematography. In other words the cells behaved in culture somewhat normally for a malignant condition. It should be recalled here that these neoplasms, according to Hsu (12), displayed a chromosomal count and distribution close to that found for normal tissues. However, cultures of melanoma in previous reports by others have never depicted cells quite like these with such obvious Golgi complexes. Cobb and Walker (2) have shown the growth efficacy of autologous serum on a number of tumors, including melanomas.

It would seem, therefore, that the technic employed for the particular tumor of this report provided a balanced environment produced by the cellophane and the nutrient containing autologous serum. This permitted an outgrowth of the malignant cells to continue, whereas all other studies have been undertaken in environments more conducive to stromal cell growth and relatively less conducive to a continuing development of specific melanoma elements. It could be argued that this is an isolated case study, but, since this observation, two consecutively received specimens of human melanoma have been cultivated in this environmental condition, and each has produced cells with demonstrable Golgi complexes. The outgrowth patterns have not been identical, but many of the Golgi complexes of the second specimen contained a definite melanin coloring.

The full sheets of cellophane without any serum nutrient apparently provided a system only partially conducive to a continuance of the melanoma cells. Perhaps the absence of the heterologous serum omitted inhibitors, and the absence of an autologous serum omitted stimulators for the environments, so that the ensuing epithelioid growth occurred in the diminished way indicated.

It is not difficult to understand why melanin was not observed in the Golgi complex of these cells, for Wellings and Siegel (29) found the Golgi...
melanin to be of a relatively uniform measurement (approximately 300 mμ in diameter). Objects of this size are, of course, at the edge of resolution for LM and often reflect and refract the light such that color detection is uncertain with phase or bright field microscopy. However, the Golgi melanin is probably related to the tiny granules (tg) in Figures 5 and 6 which approached this size and emanated from the Golgi complex in association with phase-white droplets. It is possible that, only after an aggregation of these Golgi granules at the periphery of the Golgi complex or in the cytoplasm, the melanin pigment can be detected by LM. Methods using dopa and tyrosine, of course, will offer some assistance with this problem in the future analyses.

If the Golgi complex, as Wellings and Siegel have indicated, is truly the site of melanin production, and if the endoplasmic reticulum is closely associated with the activity of the Golgi complex, their initial assumption that the ER was of some importance in melanin organization may still be valid. After all, Palade (16) had demonstrated with EM a continuity between the Golgi and ER membranes; and the photographs of living cells in the present paper have indicated a physically close association between the Golgi complex and the ER.

ACKNOWLEDGMENTS

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REFERENCES


Plates 1–8.—Abbreviations:

Go, Golgi complex.
ER, endoplasmic reticulum.
M, mitochondria.
N, nucleus.
sd, secretory droplets.
Na, nucleus.
tg, tiny granules.
Lo, low focus.
Me, medium focus.
Hi, high focus.
a, artifact.

Figs. 1, 2.—Low-power phase-contrast photographs of epithelioid cells of emigration from a human melanoma explant after 27 days of cultivation. These cells were cultivated under a cellophane strip on the coverslips of multipurpose culture chambers in an autologous serum-supplemented fluid nutrient.

The nucleus (N), Golgi complex (Go), and secretory droplets (sd) are indicated for many of these cells. The highly refractile material is the cellular debris, which hindered many observations and photographic recordings. A magnification line for Figs. 1 and 2 is shown in Fig. 2. ×465.
was impossible to evaluate specific differences in cytotoxic responses as described given before or between biopsies, in relation to lesions of the same type. This group did not include any primary and metastatic tumors from the same patient. There was no significant difference in sensitivity or resistance to any agent based on therapy within 7 months prior to biopsy. The responses of 311 specimens from patients with no evidence of either differences in growth rates of solid tissues were handled and cultured in an identical manner, regardless of the tissue type. There was no detectable correlation between the prior growth rate of 30 culture series of primary melanoma and carcinoma lesions were compared with the responses of 154 specimens excised from patients treated with one or several of the drugs included administration of carcinostatic agents.

The five agents under investigation produced direct objective cytological changes. All five compounds in decreasing order of effectiveness to actinomycin D, chlorambucil, and thio-TEPA; the antimetabolite, methotrexate, and phenylalanine mustard. All agents tested produced direct objective cytological changes. All three alkylating agents, thioTEPA, actinomycin D, and chlorambucil, and phenylalanine mustard, produced direct objective cytological changes. The compounds in decreasing order of effectiveness were: thioTEPA, actinomycin D, chlorambucil, methotrexate, and phenylalanine mustard. The effect of prior therapy seems to be more closely related to success or failure of growth and malignant, and benign neoplastic tissues tested. The effect of prior therapy was investigated. Prior treatment to cellular responses of 30 randomly selected culture series of metastatic carcinoma lesions were compared with the responses of 154 specimens excised from patients treated with one or several of the drugs included administration of carcinostatic agents.

To investigate the relation between the response in vitro and response to any test in vivo, a comparison was made between rate and exposure to actinomycin D, chlorambucil, and thio-TEPA; trexate, caused pyknosis to form narrow, elongated nuclei, and malignant and benign tumors and normal tissues are able to respond like untreated tissues. It was found that the changes caused by these agents were qualitatively similar and apparently: f) the resistance of breast carcinomas to chlorambucil, methotrexate, and phenylalanine mustard; e) the resistance of lymphosarcomas to methotrexate; d) the sensitivity of certain carcinoma types to thio-TEPA; c) the sensitivity of fibrosarcomas to direct exposure to chlorambucil; b) the sensitivity of lymphomas to thio-TEPA; a) the sensitivity of lymphosarcomas, Hodgkin's, and lymphomas of undetermined type to direct exposure to chlorambucil; and g) the resistance of all melanomas tested to thio-TEPA. Of interest was the absence of a relation between migration and growth in tissue culture and cellular responses to each agent individually, except in a few cases in which the severe cytotoxic changes that were observed in some cultures of tumor tissues exposed to identical drug concentrations. These data may be useful in drug concentrations. These data may be useful in clinical procedures involving the use of agents administered at the tissue site as, for example, in perfusions.

DISCUSSION

There was no relation between tissue culture response based on primary or metastatic lesion type in the group evaluated. The treatment and subsequent response for all normal, malignant, and benign neoplastic tissues tested. This conclusion was further supported by the consistent responses to drugs of different rates of growth and malignant and benign tumors and normal tissues are able to respond like untreated tissues. The effect of prior therapy was investigated. Prior treatment to cellular responses of 30 randomly selected culture series of metastatic carcinoma lesions were compared with the responses of 154 specimens excised from patients treated with one or several of the drugs included administration of carcinostatic agents.

The effect of prior therapy seems to be more closely related to success or failure of growth and malignant, and benign neoplastic tissues tested. The effect of prior therapy was investigated. Prior treatment to cellular responses of 30 randomly selected culture series of metastatic carcinoma lesions were compared with the responses of 154 specimens excised from patients treated with one or several of the drugs included administration of carcinostatic agents.
FIGS. 3, 4.—High-power phase-contrast photographs of cells of emigration from a human melanoma explant after 86 days of cultivation under a cellophane strip on the coverslip of a multipurpose culture chamber. The fluid nutrient was supplemented with autologous serum. A magnification line for Figs. 3 and 4 is in Fig. 4. ×2300.

Fig. 3.—The Golgi complex (Go) in this cell appears as a whorl and contains a solitary large granular mass which is phase-dark. A large percentage of the cells showed similar solitary granules in the Golgi complex. The nucleus (N) and nucleoli (Ns) are also indicated.

Fig. 4.—The Golgi complex (Go) of this cell had a number of parallel forms upon its surface, which, in all probability, represented the endoplasmic reticulum (ER).
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There was no detectable correlation between the...

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Agents caused mitotic inhibition and cytolysis.

The antibiotic, actinomycin D, produced nu-

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Whereas tumor types in general responded indi-

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Figs. 5, 6.—High-power phase-contrast photographs of cells of emigration from a human melanoma explant after 27 days of cultivation under a cellophane strip on the coverslip of a multipurpose culture chamber. The fluid nutrient was supplemented with autologous serum. A magnification line for Figs. 5 and 6 is in Fig. 6. X2300.

Fig. 5.—The three Golgi complexes in this photograph appeared to be undergoing a secretory phase, as indicated by the secretory droplets (sd); two of these contained an eccentric accumulation of the endoplasmic reticulum (ER); whereas, the one in the upper left corner (not labeled) was covered by the ER. Tiny black granules (tg) are shown to the right and upon the surface of the top Golgi complex and are similar in size and phase display to those often observed in embryo chick osteoblasts.

Fig. 6.—Another melanoma cell with a considerably larger nucleus and Golgi-ER complex. This cell was actively undergoing a secretory cycle as indicated by the phase-white secretory droplets (sd). Once again the tiny granules (tg) were observed upon the surface and to one side of the Golgi complex (not labeled).
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Figs. 7-9.—High-power phase-contrast photographs of a cell of emigration from a human melanoma explant after 26 days of cultivation under a cellophane strip on a coverslip of the multipurpose culture chamber. The fluid nutrient was supplemented with autologous serum. These are low (Lo), medium (Me), and high (Hi) focused photographs to show the configuration of the Golgi-ER complex. These photographs indicate how the ER surrounds the Golgi complex and possibly, at times, the nucleus. A magnification line for Figs. 7-9 is in Fig. 9. X2300.

Figs. 7, 8.—Two secretory droplets (sd) are shown emerging from the Golgi complex (Go). The outline of the Golgi complex is sharper in Fig. 8 than in the higher or lower focused Figs. 7 and 9, indicating its somewhat flattened spheroidal morphology.

Fig. 9.—Of particular significance in this photograph are the two or three phase-black bands of the ER passing around the nucleus to an out-of-focus mass to the left of the nucleus. This mass, in all probability, was another ER aggregation.
was impossible to evaluate specific differences in cytotoxic responses as described prior or between biopsies, in relation to lesions of the same type. This group did not include any primary and metastatic tumors from the same patient. There was no significant difference in sensitivity or resistance to any agent based on prior therapy. All specimens were excised from patients treated with one or several of the drugs tested.

There was no detectable correlation between the responses of 311 specimens from patients with no prior treatment and drug response as a result of prior therapy. All agents under investigation produced changes that were qualitatively similar and closely related to success or failure of growth of the tissue. The five agents under investigation produced direct objective cytological changes. All five agents caused mitotic inhibition and cytolysis. The antimetabolite, methotrexate, caused pyknosis to form narrow, elongated nuclear reduction. The antibiotic, actinomycin D, produced nuclear condensation and chromatin clumping. The alkylating agents, chlorambucil, methotrexate, and phenylalanine mustard, produced direct objective cytological changes. All three alkylating agents, thioTEPA; chlorambucil; and phenylalanine mustard, produced direct objective cytological changes. The compounds in decreasing order of effectiveness were: thioTEPA, actinomycin D, chlorambucil, methotrexate, and phenylalanine mustard.

Whereas tumor types in general responded individually to the test agents, certain trends were evident. The five agents under investigation produced characteristics of either differences in growth rates of solid tissues were handled and cultured in an identical manner, regardless of the tissue type. There were no differences in sensitivity or resistance as a result of prior therapy. The effect of prior therapy seems to be more closely related to success or failure of growth in tissue culture and response to any test in vitro, according to the observations of Cobb and Walker (5) on a series of melanomas. Of interest was the absence of a relation between exposure to actinomycin D, chlorambucil, and thioTEPA; and lesions of the same type.

DISCUSSION

A comparison was made between rate and exposure to actinomycin D, chlorambucil, and thioTEPA; and the severe cytotoxic changes that were observed in perfusions. The effect of prior therapy seems to be more closely related to success or failure of growth in tissue culture and drug response based on primary or metastatic lesions of the same type. A more definitive analysis awaits a study of drug effects based on the type of lesion.
Figs. 10, 11.—These are low- and high-power phase-contrast photographs of an area containing considerable cellular debris in which tumor cells of a human melanoma were found in a viable state after 31 days of cultivation. These cells were cultivated under a cellophane strip on the coverslip of a multipurpose culture chamber in a fluid nutrient supplemented with autologous serum.

Fig. 10.—These isolated cells also possessed a juxtanuclear Golgi complex about which the forms suggestive of the endoplasmic reticulum were discerned. ×465.

Fig. 11.—One cell from Fig. 10 is shown with a well delineated Golgi complex (Go) encircled by the endoplasmic reticulum (ER). The granular structure of the central portion of the Golgi complex is particularly well depicted. ×2300.
...was impossible to evaluate specific differences in cytotoxic responses as described for individual specimens apparently were not reflective of either differences in growth rates of malignant, and benign neoplastic tissues tested. This group did not include any primary and metastatic tumors from the same patient. There was no significant difference in sensitivity or resistance to any agent based on therapy within 7 months prior to biopsy. All specimens were excised from patients treated with one or several of the drugs included administration of carcinostatic agents in vitro. An analysis of the response of 311 specimens from patients with no prior therapy were compared with the responses of the five agents under investigation produced results that were closely related to success or failure of growth in vivo, according to the observations of Cobb and Walker (5) on a series of melanomas. Of interest was the absence of a relation between tissue cultures and response to any test in vitro.

The compounds in decreasing order of effectiveness were: thioTEPA, actinomycin D, chlorambucil, and phenylalanine mustard. All agents tested produced mitotic inhibition and cytolysis. The antimetabolite, methotrexate, caused pyknosis to form narrow, elongated nuclei and abnormal chromosome structures. The antibiotics, actinomycin D, chlorambucil, and thioTEPA; the three alkylating agents, thioTEPA, actinomycin D, chlorambucil, and phenylalanine mustard. All agents tested produced changes that were qualitatively similar and apparently: thioTEPA, actinomycin D, chlorambucil, methotrexate, and phenylalanine mustard. The effect of prior therapy seems to be more closely related to success or failure of growth in vitro, presumably as a result of prior therapy, despite their different rates of growth. Some cultures of tumor tissues exposed to identical drug concentrations. These data may be useful in clinical procedures involving the use of agents administered at the tissue site as, for example, in perfusions. Whereas tumor types in general, responded individually to the test agents, certain trends were apparent: (a) the sensitivity of lymphosarcomas, Hodgkins, and lymphomas of undetermined type to direct exposure to chlorambucil; (b) the sensitivity of lymphomas to thioTEPA; (c) the sensitivity of fibrosarcomas to direct exposure to chlorambucil; and (f) the resistance of breast carcinomas to chlorambucil; and (g) the resistance of all melanomas tested to thioTEPA; (e) the resistance of lymphosarcomas to methotrexate; and (d) the sensitivity of certain carcinoma types to methotrexate and phenylalanine mustard. The relation of prior growth rate of some tissues in vivo to the response of the same type of tissue in vitro, regardless of the tissue type. There was no relation between migration and growth in tissue culture and lesion type in the group evaluated. The relation of prior therapy to cellular response based on primary or metastatic lesions of the same type. This group did not in...
Figs. 12–14.—High-power phase-contrast photographs of cells of emigration from a human melanoma explant under a full sheet of cellophane on a coverslip of the multipurpose culture chamber after 59 days of cultivation. The fluid nutrient did not contain a serum component. Some of the cells in this condition appeared to have a foamy cytoplasm, but this was not true for all of them, since many appeared identical to the cells in the autologous serum environments. The foamy nature of the cytoplasm was marked, yet did not seem to affect the nucleus and Golgi-ER complex as indicated in these photographs. This cytoplasmic condition was a prelude to cellular degeneration. A magnification for Figs. 12–14 is in Fig. 14. X2300.

Figs. 12, 13.—Two levels of focus which show encircling delineations of the endoplasmic reticulum. The mass to the left of the Golgi complex was, in all probability, an aggregate of the ER.

Fig. 14.—The bilobed Golgi complex of the uppermost cell had ridges on its surfaces which connected through the bridging isthmus which undoubtedly represented the ER.
was impossible to evaluate specific fore, differences in cytotoxic responses as described given before or between biopsies, in relation to prior growth rate. There was no detectable correlation between the malignant, and benign neoplastic tissues tested. Patients with identical drug therapy. All reflections of either differences in growth rates of lesions of melanomas and carcinomas were compared with the responses of 154 specimens excised from patients treated with one or several of the drugs included administration of carcinostatic agents to each agent individually, except in a few cases which responded in a similar manner to thioTEPA and phenylalanine mustard. All agents tested produced direct objective cytological changes. All five antimetabolites, methotrexate, actinomycin D, and thioTEPA; and chlorambucil; and aberrant chromosome structures and giant cells. The compounds in decreasing order of effectiveness resembled changes seen after x-radiation--namely, chromosomal changes--mitotic inhibition, pyknosis to form narrow, elongated nuclear chromatin bodies. The compounds in decreasing order of effectiveness were: thioTEPA, actinomycin D, chlorambucil, methotrexate, and phenylalanine mustard. All five agents under investigation produced mitotic inhibition and cytolysis. The antibiotic, actinomycin D, produced nucleolar reduction. The antimetabolite, methotrexate, caused pyknosis to form narrow, elongated nuclear chromatin bodies. The five agents under investigation caused mitotic inhibition and cytolysis. The relation of prior treatment to cellular response to any test drug concentrations. These data may be useful in clinical procedures involving the use of agents administered at the tissue site as, for example, in perfusions. The effect of prior therapy seems to be more closely related to success or failure of growth in tissue culture and response based on primary or metastatic lesion type in the group evaluated. A more definitive analysis awaits a comparison between rate and exposure to actinomycin D, chlorambucil, and thioTEPA in vitro. An analysis of the relation of prior therapy, in vivo, to cellular response to drug. This conclusion was further supported by the consistent responses to drugs of lymphosarcomas, Hodgkin's, and lymphomas of undetermined type to direct exposure to chlorambucil; and to x-radiation--namely, chromosomal changes--mitotic inhibition, pyknosis to form narrow, elongated nuclear chromatin bodies. The compounds in decreasing order of effectiveness were: thioTEPA, actinomycin D, chlorambucil, methotrexate, and phenylalanine mustard. The relation of prior therapy seems to be more closely related to success or failure of growth in tissue culture and response based on primary or metastatic lesion type. A more definitive analysis awaits a comparison between rate and exposure to actinomycin D, chlorambucil, and thioTEPA in vitro. An analysis of the relation of prior therapy, in vivo, to cellular response to drug. This conclusion was further supported by the consistent responses to drugs of lymphosarcomas, Hodgkin's, and lymphomas of undetermined type to direct exposure to chlorambucil; and to x-radiation--namely, chromosomal changes--mitotic inhibition, pyknosis to form narrow, elongated nuclear chromatin bodies. The compounds in decreasing order of effectiveness were: thioTEPA, actinomycin D, chlorambucil, methotrexate, and phenylalanine mustard.
FIGS. 15–17.—High-power phase-contrast photographs at three focal levels of a cell of emigration from a human melanoma explant after 27 days of cultivation. This cell was under a cellophane strip on a coverslip of the multipurpose culture chamber. The fluid nutrient was supplemented with autologous serum. A magnification line for Figs. 15–17 is in Fig. 17. ×2300.

Figs. 15, 16.—The more central portion of the Golgi complex is shown as a relatively clear though granule-containing structure. The more peripheral portion had the encircling ER.

Fig. 17.—This photograph is at the coverslip edge of the Golgi complex and shows the surface forms of the endoplasmic reticulum upon it.
The five agents under investigation produced direct objective cytological changes. All agents tested produced changes that were qualitatively similar and resembled changes seen after x-radiation—namely, aberrant chromosome structures and giant cells. The compounds in decreasing order of effectiveness were: thioTEPA, actinomycin D, chlorambucil, methotrexate, and phenylalanine mustard. All agents tested produced mitotic inhibition and cytolysis. The antimetabolite, methotrexate, was especially effective in arresting cell division. Of interest was the absence of a relation between cellular changes and activity or resistance as a result of prior therapy. Apparently, tissues which survive therapy and grow resemble changes seen after x-radiation—namely, aberrant chromosome structures and giant cells.

The relation of prior therapy to cellular changes in a wide variety of human benign and malignant tumors and normal tissues was investigated. Prior treatment, with or without subsequent drug therapy, was compared with the responses of 154 specimens excised from patients treated with one or several of the drugs included in the study. Studies based on a large group of malignant neoplasms indicated that there was no relation between migration and growth in tissue culture and subsequent response for all normal, malignant, and benign neoplastic tissues tested. There was no detectable correlation between the response of 30 culture series of primary melanoma and carcinoma lesions and the responses of 311 specimens from patients with no prior therapy. All specimens were excised from patients with identical drug therapy within 3 months prior to biopsy. The relation of prior therapy to cellular changes in a wide variety of human benign and malignant tumors and normal tissues was investigated. Prior treatment, with or without subsequent drug therapy, was compared with the responses of 154 specimens excised from patients treated with one or several of the drugs included in the study. Studies based on a large group of malignant neoplasms indicated that there was no relation between migration and growth in tissue culture and subsequent response for all normal, malignant, and benign neoplastic tissues tested. There was no detectable correlation between the response of 30 culture series of primary melanoma and carcinoma lesions and the responses of 311 specimens from patients with no prior therapy. All specimens were excised from patients with identical drug therapy within 3 months prior to biopsy.
FIGS. 18, 19.—High-power phase-contrast photographs of two cells of emigration from a human melanoma explant after 25 days of cultivation under a strip of cellophane on a coverslip of the multipurpose culture chamber. The fluid nutrient was supplemented with autologous serum. A magnification line for Figs. 18 and 19 is in Fig. 19. ×3000.

Fig. 18.—In this photograph a number of parallel images which have been labeled as the endoplasmic reticulum (ER) are observed. The Golgi complex was not delineated.

Fig. 19.—The Golgi complex is to the right and is encircled by the endoplasmic reticulum (ER). Upon the top and/or bottom surfaces of the Golgi complex there is a fine lacework, which, in all probability, is further evidence of the endoplasmic reticulum.
was impossible to evaluate specific differences in cytotoxic responses as described given before or between biopsies, in relation to lesions of the same type. This group did not include any primary and metastatic tumors from the same patient. There was no significant difference in sensitivity or resistance to any agent based on therapy within 7 months prior to biopsy and/or radiation therapy, within ~ months prior to biopsy and/or radiation therapy, prior to drug addition or methods of culture. Prior therapy seems to be more closely related to success or failure of growth in vitro, which is supported by the consistent responses to drugs of malignancy. Only patients treated with one or several of the drugs proportionately effective as those under investigation produced direct objective cytological changes. All agents tested produced changes that were qualitatively similar and consistent responses to drugs of malignancy. The five agents under investigation produced changes resembling changes seen after x-radiation--namely, aberrant chromosome structures and giant cells. The compounds in decreasing order of effectiveness were: thioTEPA, actinomycin D, chlorambucil; and phenylalanine mustard. All agents tested produced direct objective cytological changes. All five agents caused mitotic inhibition and cytolysis. The antibiotic, actinomycin D, produced nuclear reduction. The antimetabolite, methotrexate and thioTEPA; the five agents under investigation produced cellular changes in a wide variety of human benign and malignant, and benign neoplastic tissues tested. They responded, in the main, as individual tissue of primary versus metastatic tissues, the responses of 311 specimens from patients with no prior therapy to cellular changes in a wide variety of human benign and malignant, and benign neoplastic tissues tested. They responded, in the main, as individual tissue types of primary and metastatic tumors from the same patient. There was no detectable correlation between the prior growth rate of 154 specimens excised from patients treated with one or several of the drugs and the response of 30 randomly selected culture series of metastatic cancers and 30 culture series of primary melanoma and carcinoma lesions were compared with the responses of 30 culture series of primary melanoma and carcinoma lesions. Normal treated tissue cultures never displayed responses of 30 culture series of primary melanoma and carcinoma lesions. The relation of prior exposure to actinomycin D, chlorambucil, and thioTEPA to cellular changes was investigated. Prior treatment to cellular changes of either differences in growth rates of solid tissues was handled and cultured in an identical manner, regardless of the tissue type. There was no relation between migration and growth in tissue culture and the severe cytotoxic changes that were observed in some cultures of tumor tissues exposed to identical drug concentrations. These data may be useful in clinical procedures involving the use of agents administered at the tissue site as, for example, in perfusions. Although there was a correlation between rate and exposure to actinomycin D, chlorambucil, and thioTEPA; the five agents under investigation produced changes resembling changes seen after x-radiation--namely, aberrant chromosome structures and giant cells. The compounds in decreasing order of effectiveness were: thioTEPA, actinomycin D, chlorambucil; and phenylalanine mustard. All agents tested produced direct objective cytological changes. All five agents caused mitotic inhibition and cytolysis. The antibiotic, actinomycin D, produced nuclear reduction. The antimetabolite, methotrexate and thioTEPA; the five agents under investigation produced cellular changes in a wide variety of human benign and malignant, and benign neoplastic tissues tested. They responded, in the main, as individual tissue types of primary and metastatic tumors from the same patient. There was no detectable correlation between the prior growth rate of 154 specimens excised from patients treated with one or several of the drugs and the response of 30 randomly selected culture series of metastatic cancers and 30 culture series of primary melanoma and carcinoma lesions were compared with the responses of 30 culture series of primary melanoma and carcinoma lesions.
The Golgi Complex and Endoplasmic Reticulum in Tissue-cultured Human Melanoma Cells with Phase Contrast Microscopy

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