CYTOLOGICAL AND CYTOCHEMICAL PROPERTIES OF TEN ASCITES TUMORS

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PLATES 1 TO 3

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A considerable amount of information about ascites tumors is now available. The origin, growth characteristics, and a number of cytological features of these tumors have been described (13–16, 19, 29, 30, 33, 35). Extensive studies of the chromosomes and the mechanisms of endomitosis and endoreduplication have been made by Levan, Hauschka, and Tjio (21, 37). Determinations of the modal chromosome ploidy by these workers have been confirmed by estimations of DNA by chemical methods and by ultraviolet spectrophotometry (15, 20). A number of enzymic and metabolic studies of these tumors have been reported (18, 22). A detailed study of the supravital staining of the Ehrlich ascites tumor was begun by Seeger (32). The nature of the lipochondria and large cytoplasmic lipide droplets, the presence of parachromatin bodies and some cytochemical properties of this tumor were demonstrated by Love, Koprowski, and Cox (24). The present investigation is an extension of this work to provide a comparative study of the cytological and cytochemical properties of 10 ascites neoplasms.

Degeneration and other cytological and biological changes which occur at different stages in the growth of tumors (2, 8) necessitated the selection of a fixed interval after transplantation as a means of studying comparable phases of development. The experiments were originally devised to establish the characteristics of uninfected ascites tumors at the time when oncolysis could be induced in some of them by infection with viruses. Since virus was usually introduced 4 days after transplantation, and oncolysis was commonly observed 3 days later (24), 7-day-old ascites were selected for intensive study.¹

Materials and Methods

Mice, Tumors, and Biological Procedures

Mice of both sexes weighing 20 to 25 gm. were used. A list of the tumors employed, the original histological classification, approximate modal chromosome ploidy, the number of

¹ The Krebs 2B ascites tumor was examined on the 5th day after transplantation because it frequently killed all mice in less than a week.

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cells in the inoculum, and the mouse strain into which they were transplanted are shown in Table I. For the cytochemical studies, the tumor inoculum consisted of undiluted ascitic fluid, except for the TA 3 diploid tumor where the fluid was diluted 1:20 with 0.85 per cent saline, and the MC1M sarcoma which was diluted 1:4. We are greatly indebted to Dr. Theodore Hauschka and Dr. George Klein for providing us with the tumors, the origins of which have been recently reviewed (21).

**TABLE I**

Characteristics of Tumors, Inocula, and Mouse Strains Employed in the Experiments

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Original histological classification</th>
<th>Approximate ploidy</th>
<th>Number (in million) of cells in inoculum</th>
<th>Mouse strain used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ehrlich tetraploid</td>
<td>Spontaneous Carcinoma</td>
<td>4n</td>
<td>10-12</td>
<td>Swiss (ICR)</td>
</tr>
<tr>
<td>Ehrlich hyperdiploid</td>
<td>Mammary adenocarcinoma*</td>
<td>2n+</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Sarcoma 37</td>
<td>Spontaneous Carcinoma</td>
<td>4n</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>TA3 tetraploid</td>
<td>Mammary adenocarcinoma</td>
<td>4n</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>TA3 diploid</td>
<td>Mammary adenocarcinoma</td>
<td>2n</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Krebs 2B‡</td>
<td>Spontaneous Carcinoma</td>
<td>4n</td>
<td>5</td>
<td>Swiss</td>
</tr>
<tr>
<td>DBA lymphoma</td>
<td>Lymphosarcoma</td>
<td>2n</td>
<td>12</td>
<td>DBA</td>
</tr>
<tr>
<td>MC1M</td>
<td>Induced Rhabdomyosarcoma</td>
<td>4n</td>
<td>§</td>
<td>C3H</td>
</tr>
<tr>
<td>6C3HED</td>
<td>Lymphosarcoma</td>
<td>2n</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Lymphoma §1</td>
<td>Lymphosarcoma</td>
<td>4n</td>
<td>58</td>
<td>A</td>
</tr>
</tbody>
</table>

* After the first 22 passages became a sarcoma (6).
‡ Derived from the original Krebs 2 ascites by transplantation of 4 cells (21).
§ Since the ascitic fluid was too viscous for hemocytometer pipettes, a 1:4 dilution in 0.85 per cent saline was used.

**Cytological and Cytochemical Procedures**

Smear preparations of the ascitic fluid were made on the 7th day after implantation of tumor, except for Krebs 2B (5th day). When no fixative was used, the smears were allowed to dry in air; otherwise, they were immersed face downward in the fixative while still wet.

The procedure and rationale of most of the cytological and cytochemical investigations and the various controls employed have been published (24-26). Supravital staining with mixtures of neutral red and Janus green was performed as previously described (26); in addition, cells were stained separately and in succession with the two dyes. Barrett's stain (1) was employed for general cytological detail as before (24); the Carazzi hematoxylin (1) was diluted 1:2, and the best results were obtained after fixation at 37°C, for 4 hours in formal sublimate, by removal of most of the lipide by brief immersion in xylool, and increasing the ratio of red to blue mixtures in the stain to 2 parts red:1 part blue (1). Stage 4 of Barrett's method was omitted, and the cells were adequately stained after 4 hours in the staining mixture.
In addition to the staining methods already described, the following were used: (a) Dam’s method for fatty peroxides (11); (b) Moog’s method for cytochrome oxidase (27); (c) a modified Sakaguchi test for arginine (4); and (d) Cain’s Nile blue sulfate after incubation for 1 hour in buffered ribonuclease (28) or buffer alone.

Analysis of Tumor Cell Population

Ten smears of each tumor were prepared between 8 and 10 a.m. on a given day and stained by Leishman’s method. Five hundred tumor cells on each slide were analyzed. The number of cells in mitosis was noted, together with the proportion of multinucleated forms and the incidence of gross mitotic abnormalities as defined by Glücksmann (12). Bridge formation, lagging chromosomes, multiple spindles, d mitosis, and other minor abnormalities which do not lead to the death of the cell were not included in the counts. Cells with one large nucleus and one or more micronuclei were not included in the multinucleated group but were placed in a separate category (accessory micronuclei, Table III). All types of nuclear fragmentation associated with disintegration of the cytoplasm were grouped as karyorrhexis for the purpose of the count. Abortive organization of the tumors, as evidenced by cell clumping, was also noted.

The Leishman preparations were projected onto a graduated scale and the nuclear and cytoplasmic diameters of 100 cells on each smear were measured.

RESULTS

Cytology

Fresh preparations were examined by phase microscopy and compared with the results of supravital staining, Barrett’s method (1), silver and osmic acid Golgi technics, and various mitochondrial stains (25, 26). These observations revealed that the same structural components were present in the cells of all 10 ascites tumors.

The nuclei of intermitotic cells were enclosed by a delicate chromatin membrane (Figs. 1, 2, and 3). Within the nucleus, the chromatin formed membranes around a variable number of prominent nucleoli. In preparations stained with hematoxylin or by the Feulgen method, the nucleus also contained a few small granules and some fine threads which occasionally linked the nucleoli to the nuclear membrane or to one another (Figs. 1, 2, and 3). When cells were stained by Barrett’s method, in addition to the hematoxyphil chromatin, the nuclei contained acidophilic parachromatin material which seemed to consist of granules of variable size (Table II). The largest granules have been called parachromatin bodies (24), and were present in small numbers in occasional cells; their presence was always associated with some margination of chromatin. In the majority of cells the parachromatin was finely granular; less commonly, granules could not be resolved in the light microscope and the parachromatin appeared amorphous.

Three types of sudanophilic granules with distinctive morphological, tinctorial, and cytochemical properties were distinguished in the cytoplasm (Figs. 4 and 5). Small regular rounded particles, evenly scattered throughout the cytoplasm, with the

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8 Since this material is associated, but not identical, with chromatin and because it may be granular, the term “parachromatin” is used instead of “thin network,” which is an artifact, or “nuclear sap,” which implies a structureless fluid.
exception of the region of the nuclear hof, were identical in shape, size, and distribution with the mitochondria as stained supravitally with Janus green. Secondly, a variable number of larger refractile lipide bodies were observed at the periphery of the cell. Until their nature can be more clearly understood, these have been termed lipide globules (26). The remaining sudanophilic material consisted of granules of variable size, but smaller than the lipide globules, mainly located in the nuclear hof of the cytoplasm. After the mitochondria had been stained with Janus green, supravitally staining with neutral red revealed a smaller number of red granules in the nuclear hof and occasional semilunar forms adhering to the rim of a large lipide globule (cf. Fig. 5). The introduction of Sudan black stain beneath the coverslip of

<table>
<thead>
<tr>
<th>Method</th>
<th>Barrett's stain</th>
<th>Feulgen</th>
<th>PAS</th>
<th>Lipide extraction PAS</th>
<th>Bromination PAS</th>
<th>Acetylation PAS</th>
<th>Pyridine PAS</th>
<th>Lipide extraction diastase PAS</th>
<th>Lipide extraction diastase PAS</th>
<th>PAS</th>
<th>Sudan black</th>
<th>Nile blue</th>
<th>Toluidine blue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromatin</td>
<td>Hematoxyphil</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>Blue</td>
<td>+</td>
</tr>
<tr>
<td>Nucleolus and parachromatin</td>
<td>Pink</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Blue</td>
<td>+§ or 0</td>
<td></td>
</tr>
<tr>
<td>Cytoplasm</td>
<td>Blue</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Blue</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Large lipide globules</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Pink</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Lipochondria</td>
<td>Diffuse pink</td>
<td>++¶</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>Blue</td>
<td>++ or 0</td>
<td></td>
</tr>
<tr>
<td>Mitochondria</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>Blue</td>
<td>++ or 0</td>
<td></td>
</tr>
</tbody>
</table>

* Lipide extraction before staining.
† Mitotic chromosomes orange.
§ When treated with iodine and thiosulfate before staining.
|| No iodine and thiosulfate treatment.
¶ Indicates relative intensity of staining reaction. Number and size of lipochondria vary in different tu
0 indicates not recognizably stained.

No detectable preformed aldehyde, acetal phosphatide, or aldehydes secondary to oxidation of unsaturated phosphatase. Baker’s acid hematin test negative.

The term “nuclear hof” indicates the zone of cytoplasm which is the site of the centrosome and is usually adjacent to a slight indentation of the nucleus.
supravital preparations immediately decolorized the neutral red granules. Some of the sudanophilic granules which appeared later in the nuclear hof seemed to correspond to the neutral red staining particles. The standard Golgi stains gave erratic results, but demonstrated argentophilic and osmiophilic granules in this area of the cytoplasm. Since phase microscopy did not reveal any other structures in the cytoplasm, the argentophilic and osmiophilic granules which resembled the sudanophilic and neutral red staining bodies in the nuclear hof must be lipochondria (31) and may represent all or part of the Golgi complex of the cell.

**Cytochemistry**

The cytochemical properties of the 10 tumors did not differ qualitatively. The results of the cytochemical tests on all tumors and of some relevant staining methods are summarized in Table II.

### Plastic Cells of 10 Ascites Tumors

<table>
<thead>
<tr>
<th>Test</th>
<th>Lipide extraction</th>
<th>Tetrazonium</th>
<th>Benzoaldehyde tetrazonium</th>
<th>Saka- gachi for arginine</th>
<th>Azline blue, orange G</th>
<th>Acid phosphatase</th>
<th>Succinic dehydrogenase</th>
<th>Supravital staining</th>
<th>Probable constituents</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>Blue</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>DNA; arginine, tyrosine, tryptophane, or histidine</td>
<td></td>
</tr>
<tr>
<td>++ or ++ or ++ or ++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Blue</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Basic protein; tyrosine, tryptophane, or histidine</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>Blue</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>RNA; arginine, tyrosine, tryptophane, or histidine</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>Diffuse</td>
<td>Diffuse</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>Red</td>
<td>0</td>
<td>Saturated neutral triglyceride</td>
<td></td>
</tr>
<tr>
<td>0§§</td>
<td>0§§</td>
<td>0§§</td>
<td>+</td>
<td>0§§</td>
<td>0§§</td>
<td>Neutral red green</td>
<td>0§§</td>
<td>Acid phosphatase; cytochrome oxidase; succinic dehydrogenase; RNA; acidic lipid (± phospholipide)</td>
<td></td>
</tr>
</tbody>
</table>

* acids, no fatty peroxides, no birefringence, colored material, cholesterol, ascorbic acid or alkaline phosphatase.
Nucleus.—The Feulgen reaction demonstrated that desoxyribonucleic acid was confined to the nuclear chromatin. In acid solutions of orange G and aniline blue, the nucleolus, the nuclear parachromatin, and the mitotic chromosomes had a stronger affinity for orange G than any other cell component; according to White (39), this implies the presence of histone. Arginine could not be demonstrated in the nucleolus but was present in the chromatin.

After fixation in formol sublimate and subsequent treatment with iodine and thiosulfate, the chromatin, nucleoli, parachromatin, and cytoplasm were stained by toluidine blue (Fig. 6). Ribonuclease digestion which abolished the staining reaction of the cytoplasm had no effect on that of the nucleolus (Figs. 6 and 7). The intensity of ribonuclease-sensitive staining of the cytoplasm was much reduced in cells undergoing mitosis; since this was associated with strong staining of the chromosomes, a redistribution of RNA from cytoplasm to chromatin may be inferred. The intense reaction of all nuclear structures with the coupled tetrazonium method (5) was completely abolished by benzoylation. Tyrosine, tryptophane, or histidine were, therefore, present in the chromatin, parachromatin, and nucleolus. Ribonucleic acid was absent or did not occur in sufficient amounts in these structures to be stained by toluidine blue or by the tetrazonium method.

Sudanophilic Cytoplasmic Particulates.—The cytochemical properties of the sudanophilic cytoplasmic components (Fig. 4) may be conveniently considered together. Their lipide nature was confirmed by failure to stain with Sudan dyes after 6 hours' extraction with hot chloroform–methanol. No cholesterol, preformed aldehyde, acetal phosphatide, aldehyde secondary to oxidation of unsaturated lipide, no fatty peroxides, no birefringent or colored material could be demonstrated (Table II).

The large lipide globules could only be stained by methods involving the simple solution of fat-soluble dyes, e.g., Sudan dyes, the oxazone of Nile blue sulfate (Fig. 5) and the red formazan formed in the succinic dehydrogenase stain (34) (Table II). The globules were, therefore, composed mainly of neutral saturated triglyceride.

The lipochondria were stained by the periodic acid–Schiff (PAS) method (Figs. 8 and 9). After extraction of lipides with hot chloroform–methanol, slightly smaller, less intensely stained PAS-positive granules were observed in the nuclear hof (Figs. 9 and 10). Thus two PAS-positive components, one of which was a lipide, were associated with the lipochondria. The presence of 1,2-glycol groups was shown by the abolition of PAS staining by previous acetylation in dry pyridine. Some of the PAS-positive material was removed by the pyridine (Table II). Bromination reduced the intensity of the PAS reaction, but no more than that of a similarly treated control section of clam mucopolysaccharide in which no lipide was present (23). The acidic reaction of the lipide fraction of the lipochondria was shown by blue staining with Cain's Nile blue sulfate method (Fig. 5), even after ribonuclease digestion. Since Baker's acid hematin reaction for phospholipides was negative, the acidity may have been due to the presence of free fatty acids. In the absence of lipide aldehydes and demonstrable ethylenic groups, the positive PAS reaction of the lipide component of the lipochondria must be attributed to glycolipide.

Since the non-lipide PAS-positive fraction of the lipochondria was unaffected by hyaluronidase or diastase and was not metachromatic (Table II), it probably contained neutral mucopolysaccharide or glycoprotein. The presence of protein was
confirmed by the intense diffuse staining of the nuclear hof area by the tetrazonium method, and the reduction of this coloration by previous benzoylation (Table II).

The mitochondria were readily stained by the toluidine blue molybdate method, which is dependent upon the presence of lipide in the mitochondrion and of RNA in the cell (25) (Fig. 11, Table II). Since the blue staining of mitochondria with Nile blue sulfate was unimpaired after incubation with ribonuclease, the staining reaction must have been produced by acidic lipide and not by RNA. In unfixed and formalin-fixed preparations, the Gomori technic for acid phosphatase revealed minute positive granules throughout the cytoplasm of the tumor cells, reaching a maximum intensity after 2 to 4 hours incubation. After this period some diffusion occurred, and the nuclear chromatin was also stained. The reaction was more intense in the unfixed than the fixed material, but the number and distribution of the particles were identical and suggested that the enzyme was present in the mitochondria. Small blue granules resembling the mitochondria were stained by the blue tetrazolium method for succinic dehydrogenase (34); the other lipide components, and especially the large lipide globules, were stained red, probably as a result of diffusion of the diformazan which, in lipide solution, is red by transmitted light. The results of the Nadi reaction for cytochrome oxidase (27) were unsatisfactory. The positive reactions which occurred in the presence of azide and staining of the large lipide globules could not be attributed to fatty peroxides, since these were not demonstrable in the cells (Table II). Presumptive evidence of cytochrome oxidase activity in the mitochondria was provided by supravital staining with Janus green (36).

Cytoplasm.—The abolition of cytoplasmic staining with toluidine blue by previous ribonuclease digestion demonstrated RNA in this site (Figs. 6 and 7). The presence of tyrosine, tryptophane, or histidine was shown by the effects of benzoylation on the tetrazonium reaction (Table II). Residual staining after benzoylation might be attributed to the reaction of purines and pyrimidines of the RNA or the resistance of some aromatic amino acids to benzoylation. The modified Sakaguchi stain for arginine gave a strongly positive diffuse reaction in the cytoplasm.

Comparative Cytology of 10 Ascites Tumors

Despite the similarity in the structural components and the cytochemical properties of the 10 ascites tumors, certain morphological and biological differences were noted (Figs. 1–3). Some indication of the differences in the size of cells of the 10 tumors may be gained from Text-fig. 1. The extreme range of the nuclear and cell dimensions within each tumor is indicated by twice the average standard deviation (s.d.) of all the cells measured (Text-fig. 1). The differences in size were such that the dimensions of the largest cells of lymphoma 1 and the smallest cells of the MC1M tumor may be identical. The greatest variability in cell size was observed in the two sarcomas (Text-fig. 1, and cf. Figs. 1 and 3).

The variation in the mean diameter of the cell and nucleus in 10 samples of each tumor may be deduced from twice the s.d. of the means (Text-fig. 1). Despite the

4 In Text-figs. 1 and 2 the value of twice the standard deviation (s.d.) was used as an index of the variability of the observations.
variability of the mean in different samples, the mean diameter of the cell of the
MC1M tumor was significantly higher than that of all the other tumors except sarcoma
37. The sarcomas had more cytoplasm than the carcinomas and lymphomas, but the
difference was much less striking when the nuclear diameters were compared (Text-
fig. 2). The smallest tumor cells were found in the lymphomas, but the nuclei of the
near diploid carcinomas (TA3 and Ehrlich) were smaller than those of any other tumor.
A rough correlation between the modal number of nucleoli and the modal chromosome ploidy is also illustrated in Text-fig. 1. Fusion of nucleoli may have occurred in the MC1M and the TA3 diploid tumors where the modal chromosome ploidy was twice the nucleolar mode. Although the diploid nuclei tended to be smaller than the tetraploid, the size of the nucleus was also influenced by the type of tumor. Thus, the nucleus of the tetraploid lymphoma $\#1$ was larger than that of the Ehrlich hyperdiploid or the TA3 diploid tumors, but approximately the same size as that of the two diploid lymphomas (Text-fig. 1).

Considerable variation was observed in the mitotic rate in different samples of most tumors (Text-fig. 2). A fairly constant level of mitotic activity was noted, however, in the MC1M and DBA tumors. Although the mean mitotic index differed in the 10 tumors, there was greater variability in the mitotic rate in different samples of the same tumor. From Text-fig. 2 it may be seen that, while no particular mitotic index was characteristic of a given tumor, it is highly improbable that the extreme values would be found in the MC1M, DBA, and TA3 tetraploid tumors.

A number of other features of the 10 tumors are summarized in Table III. Clumping of chromosomes in metaphase was the commonest type of gross abnormality of mitosis.\(^4\) Chromosome clumping in prophase and anaphase and scattering of chromosomes in metaphase and anaphase were also observed. Abnormal mitosis was most common in lymphoma $\#1$, Krebs 2B, and sarcoma 37, and was less frequent in the Ehrlich, TA3, and DBA tumors.

Karyorrhexis was rare in all except the 6C3HED and lymphoma $\#1$ tumors. Multinucleated cells were most frequent in the MC1M sarcoma, but were not uncommon.

\(^4\)The less complete forms of scattering and of clumping in metaphase may indicate the development of a mitosis.
in all the tumors. The tendency of the MC1M tumor to form multinucleated cells was also reflected by a high incidence of accessory micronuclei in these cells (Table III). Cell clumping was observed only with the TA3 and the MC1M tumors.

Some quantitative differences in the number and size of the lipochondria as demonstrated by supravital and fixed preparations are indicated in Table III. The lipochondria were distinctly smaller and less numerous in the lymphomas, and were more conspicuous in the near diploid strains than in the tetraploid forms of the Ehrlich and TA3 tumors. The lipochondria and the large lipide globules were most striking in the two sarcomas (Table III, cf. Figs. 8 and 9) where the cell diameter was relatively greater in proportion to that of the nucleus (Text-fig. 1).

**DISCUSSION**

The results of this investigation confirm the existence of three types of cytoplasmic lipide particulates in the tumor cells of all 10 ascites neoplasms. The nature and significance of similar particles in the RPL-12 chicken lymphoma have been discussed in a previous communication (26). The large...
Lipide globules correspond to the L bodies described by Friedlaender et al. (9, 10) in electron micrographs of the Ehrlich tumor cell. These workers have also found that the Golgi complex is largely confined to the cytoplasm which occupies the nuclear hof (9), where lipochondria can be visualized by light microscopy in supravital and fixed preparations. The complex of membranous, vesicular, and other structures which are revealed by the electron microscope cannot be resolved in the light microscope; nor is it possible to be certain which part of the Golgi complex is responsible for concentrating neutral red under supravital conditions. The work of Weiss (38) has indicated that, under certain conditions, neutral red may stain mitochondria. We have been unable to achieve supravital staining of mitochondria with Janus green if the cells have previously been stained with neutral red. When the mitochondria were first stained with Janus green, the granules which were subsequently stained by neutral red sometimes adhered to the outer surface of the large lipide globules. The identity of the neutral red granules with some of the structures which were stained by Nile blue sulfate and by the PAS method in fixed preparations was suggested by the phenomenon illustrated in Figs. 5 and 9.

A relative scarcity of the cytoplasmic granules believed to be responsible for cytoplasmic basophilia in the nuclear hof has been observed in electron micrographs of the Ehrlich cell (10). The existence of RNA in these particles is confirmed by the paucity of ribonuclease-sensitive staining in this area (Figs. 6 and 7).

Analysis of the cytochemical properties of the nucleolus and the parachromatin of ascites tumor cells has given contradictory results which deserve further study. White's aniline blue-orange G method suggests that histone, or at least basic protein, is present in the nucleolus and that this material is concentrated in the chromosomes during mitosis (Table II). Arginine, which is one of the chief amino acids of histone, could not be demonstrated in the nucleolus. The apparent absence of RNA in the nucleolus might be attributed to its existence in a form which is unaffected by the ribonuclease preparations, or to the failure of the enzyme to penetrate to the center of the nucleus. On the other hand, as suggested by Dounce (7), the amount of RNA in mammalian nucleoli may be extremely small.

The acid hematin test of Baker for phospholipides is dependent upon controlled chromation and differentiation, and was initially devised for frozen sections of tissues. In using this empirical method on smears, the specificity of the test may be lost. Indeed, we have never been able to demonstrate phospholipide in smear preparations (24, 26). There is evidence, however, that mitochondria contain small amounts of phospholipide (3). Despite the negative acid hematin test, the acidic reaction of mitochondria of ascites cells (Nile blue sulfate, Table II) may still be produced by phospholipide, but it is more probably due to the presence of free fatty acids (3).
The comparative cytological studies reported in this paper have demonstrated significant differences in the tumor cell populations. The variability in one sample, as well as in several samples of a given tumor, was so great that single cells from different tumors could not always be distinguished by the methods employed here. In the course of investigations on the spectrum of oncolytic activity of a number of viruses against these 10 ascites tumors, it was found that the growth of Bunyamwera, West Nile, and Mengo viruses in 7 of them was accompanied by destruction of all the tumor cells in the peritoneal fluid (17, 24). The same viruses did not influence the growth of three tumors—DBA and 6C3HED lymphomata and the TA3 diploid tumor. Attempts have been made to correlate the response to virus infection with the chromosome ploidy of the cells (17) but no consistent cytological or cytochemical property can be definitely implicated.

SUMMARY

1. The cytological and cytochemical properties of the Ehrlich tetraploid and hyperdiploid, TA3 tetraploid and diploid, Krebs 2B, sarcoma 37, MC1M, 6C3HED and DBA lymphoma, and lymphoma #1 ascites tumors at a fixed interval after transplantation are described.

2. No qualitative differences were observed in the cytochemical properties of the nucleus, nucleolus, parachromatin, parachromatin bodies, large lipide globules, lipochondria, mitochondria, and cytoplasm of the tumor cells of the 10 neoplasms.

3. Quantitative differences were noted in the cells of the 10 neoplasms. These were reflected by variation in nuclear and cytoplasmic diameter, nuclear mode, mitotic index, incidence of gross abnormalities of mitosis, karyorrhexis, multinucleated cells, accessory micronuclei, cell clumping, and in the number and size of the large lipide globules and lipochondria of the cells.

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BIBLIOGRAPHY

EXPLANATION OF PLATES

PLATE 1

Fig. 1. Ehrlich ascites tumor. Barrett's stain. X 1333. Prominent nucleoli in the tumor cells.

Fig. 2. Krebs 2B ascites tumor. Barrett's stain. X 1333. In this smear the nuclei are more regular than those of the Ehrlich cells.

Fig. 3. MC1M sarcoma ascites tumor. Barrett's stain. X 1333. The tumor cells are distinguished by their deeply stained cytoplasm. Note the relatively greater amount of cytoplasm and extreme variability of cell size as compared with the Ehrlich and Krebs 2B tumors. The cytoplasmic vacuoles are the sites of lipide globules which have been removed during staining.

Fig. 4. Ehrlich ascites tumor. Sudan black. X 1333. Note the large number and the extreme range in the size of the sudanophilic granules.
(Love et al.: Cytochemistry of ascites tumors)
Fig. 5. Ehrlich ascites tumor. Nile blue sulfate. × 1333. In this photograph the large cytoplasmic neutral lipide globules, which stain red, are faint in comparison with the blue-staining acidic lipide particles which appear black. The arrows indicate points where some of the acidic lipide granules adhere to the periphery of the neutral lipide globules (cf. Fig. 9).

Fig. 6. MC1M sarcoma ascites. Fixed in formol sublimate, incubated at 37°C. for 1 hour in \( \frac{M}{50} \) phosphate buffer pH 7.7, treated with iodine and thiosulfate, and stained with toluidine blue. × 1714. Except for the nuclear hof, the cytoplasm is intensely stained.

Fig. 7. MC1M sarcoma. Fixed and stained like the material shown in Fig. 6 except that 0.1 per cent ribonuclease was added to the buffer. The cytoplasm is practically unstained but there is no decrease in the staining of chromatin or nucleoli. × 1714.

Fig. 8. Sarcoma 37 ascites. PAS. × 1333. Prominent granular PAS positive lipochondria in the nuclear hof.
(Love et al.: Cytochemistry of ascites tumors)
Plate 3

Fig. 9. Ehrlich ascites tumor. PAS. × 1744. Compare the accumulation of PAS positive granules in the nuclear hof with that of the small and medium sized sudanophilic granules in Fig. 4. Arrows indicate sites where the granules adhere to the neutral lipide globules, which appear as unstained vacuoles (cf. Fig. 5).

Fig. 10. Material similar to that shown in Fig. 9, but extracted for 6 hours with hot chloroform-methanol before staining. The PAS-positive granules are smaller and less intensely stained × 1744.

Fig. 11. Ehrlich ascites tumor. Toluidine blue mitochondria stain. × 1356. Compare the regular size and distribution of the mitochondria with the variable size and the eccentric concentration of the lipochondria as revealed by the PAS method (Fig. 9).
(Love et al.: Cytochemistry of ascites tumors)