An Electron Microscopic Study of the Leukemia Virus in AKR and Hybrid Mice Inoculated with Ascites Passage or Tissue-cultured Leukemic Cells

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SUMMARY

Various organs and tumors from AKR and hybrid mice and leukemic cells grown in tissue culture were studied by electron microscopy. Only spleen and thymus were examined in fetuses and newborn mice. In adults, solid tumors, lymph nodes, and ascites cells were also studied.

AKR mice dying of spontaneous leukemia showed typical virus particles (Types A and C) in their lymph nodes. No virus particles could be identified in organs from newborn or 7- to 10-week-old mice. Virus particles were not observed in the thymus or lymph nodes of mice injected with long-term ascites transfers of the leukemia, but characteristic particles were found in all solid mesenteric and ascites tumors examined. Organs from F1 hybrids (C57BL/6 × AKR) were uniformly negative. However, in one solid tumor, produced by inoculation with tissue-cultured cells, numerous virus particles were present. The virulence of these lines, for AKR or hybrid mice, did not correlate with either the chromosome number or the presence of virus particles.

INTRODUCTION

Various aspects of AKR mouse leukemia have interested many investigators since Gross first succeeded in transmitting it by a filterable agent (12). Some studies, to list only a few, have stressed the maintenance of ascites or tissue culture cell lines (1, 7, 8, 17), variations in chromosome patterns (1, 6, 23), or antigen specificity (18). One of us (R. C.) has been interested in factors involved in the transmission of AKR leukemia and the possible production of immunity. Several mouse ascites and in vitro tissue culture lines were established (6, 8) and periodic chromosome counts made. Virulence tests on AKR and hybrid mice were studied in an attempt to correlate loss of virulence with changes in metabolic activity, growth patterns, or chromosome configuration.

The evidence that a virus is involved in the transmission of AKR mouse leukemia has been well-documented through animal experimentation (9, 13, 14); therefore, we felt it unfruitful to repeat those studies. Recent electron microscope studies have shown that the virus-like particles associated with AKR leukemia may assume a variety of forms (3, 10, 15, 20). Thus, it was of interest to know whether: (a) typical virus-like particles found in AKR spontaneous leukemia still existed in our in vitro and ascites cell lines; (b) the presence or absence of these particles correlated with the ability of such cells to produce leukemia.

The following report stresses primarily the electron microscopic identification of virus-like particles in AKR and hybrid mice after inoculation with tissue culture or ascites lines of leukemic cells. These lines, isolated originally from leukemic tissue of AKR mice dying of spontaneous leukemia were also examined for the presence of virus. Pertinent studies on chromosome configuration and virulence are included only to emphasize the lack of correlation between them and the presence of virus-like particles. A more detailed report on the effect of long-term culture on chromosome configuration and changes in virulence is in preparation.

MATERIALS AND METHODS

AKR mice were obtained from Simonsen Laboratories of Gilroy, California (raised from stock originally supplied by the NIH), or bred in our own laboratories from the Simonsen stock. C57BL/6 mice were generously provided by Dr. Donald Bailey of this Institute, from his highly inbred strain. All hybrids were produced by matings of the above two strains.

\[ \text{AF}_1 = \text{C57BL/6 } \varphi \times \text{AKR } \sigma \]

\[ \text{BF}_1 = \text{AKR } \varphi \times \text{C57BL/6 } \sigma \]

All mice were killed by cervical dislocation and examined as rapidly thereafter as possible.

Spontaneous Leukemia and Solid Tumors

Adult mice 8-14 months old were killed when large palpable lymph nodes were noted. Histologic sections of the affected tissues (thymus, spleen, lymph nodes, solid mesenteric tumor, and occasionally kidney) revealed the characteristic picture of spontaneous leukemia [as described by Gross (13)].

Since these results remained constant, we discontinued histologic tests, except in special instances, and limited our observations to the gross changes noted in the autopsy of every animal sacrificed.

Solid mesenteric tumors usually occurred in mice inoculated...
with our ascites lines while solid tumors at the site of injection were generally found when tissue culture cells were used. Histologic sections of both types of tumor showed typical lymphosarcoma or leukemic infiltration.

Ascites Tumors

Ascites tumor lines were originally produced by intraperitoneal injections of minced fragments and cells from spleen, thymus, or lymph nodes obtained from AKR mice showing typical symptoms of advanced spontaneous leukemia. Once the ascites lines had been isolated, they were maintained by serial intraperitoneal transfer every 7–11 days. Each ascites line was injected at a level of $1 \times 10^6$ to $1 \times 10^7$ cells into several 6- to 9-week-old AKR mice.

Tissue Culture Lines

These lines were established in vitro from ascites lines which had been maintained for several months in AKR mice. Once established, the tissue culture lines were grown on various media and kept in 2 or more incubators to minimize the danger of loss or contamination and to observe the effects of these variables upon the relative virulence or chromosome pattern.

Virulence

Preliminary studies were carried out with our ascites lines to establish their potency for AKR mice. Doses ranging from $1 \times 10^5$ to $1 \times 10^6$ were used and all were found to be 100% lethal. Tests were made on 2 to 10 animals at each dose level and male and female mice of different ages were compared. A dose of $1 \times 10^5$ was chosen as the standard against which the relative virulence of the tissue-cultured cell lines could be evaluated. The median survival time of AKR mice inoculated with $1 \times 10^5$ ascites cells ranged from 15 to 19 days depending upon the ascites line used.

Chromosome Preparations

Mouse, with spontaneous leukemia or with ascites transfers, were inoculated 1 to 2 hr prior to sacrifice with colchicine (0.1 ml per 10 gm body weight of a concentration of $1 \times 10^{-4}$).

Tissue culture cells were transferred to a 15-ml centrifuge tube and incubated 24–48 hours before the addition of colchicine to give a final concentration of $1 \times 10^{-4}$. The method of Fox and Zeiss (11) was used for chromosome preparations, with minor modifications added to provide greater spreading in certain preparations.

Chromosomes were considered “normal” when over 70% of the countable metaphases contained 40 chromosomes and no apparent abnormalities (such as metacentric, minute, abnormally long, or short chromosomes) could be observed. (As it is practically impossible to karyotype correctly more than 3 or 4 pairs of mouse chromosomes by the present technics it is difficult to say with certainty that only “normal” chromosomes exist in certain of our long-term cultures, but it is equally difficult to prove that they are not “normal.”)

Complete details of our methods for establishing ascites and T.C. cell lines and for chromosome preparations have appeared elsewhere (6).

Preparations for Electron Microscopy

Tissue culture and ascites cells were washed in Tyrode’s or Hank’s solution, centrifuged at 500 rpm for 5 minutes, and the supernatant decanted. The cells were then fixed as a pellet directly in the centrifuge tube. After 15 minutes, the fixative was poured off and, in order to maintain these loose cells as a solid pellet during the subsequent procedures, they were infiltrated with 1% agar at 40°C. The agar was hardened by immersing the tubes in ice water, after which the jellied plugs were removed. The agar tips containing the pellets of cells were then cut off and placed in fresh fixative for an additional 1.5 hours. Organs and tissues were removed from mice killed by cervical dislocation and minced directly in fixative.

The fixatives used for preparing the above materials were either Veronal-acetate or s-collidine-buffered OsO$_4$ (2) at pH 7.5. Glutaraldehyde in sodium cacodylate buffer (22) followed by OsO$_4$ also proved to be a satisfactory fixative. It was found necessary to add 0.02% CaCl$_2$ to the OsO$_4$ fixatives to obtain satisfactory preservation of ultrastructural components.

In order to increase contrast, the tissues were stained in block with KMnO$_4$ during dehydration in a graded acetone series (19), or before dehydration with 0.5% uranyl acetate in Michaels buffer (16). After embedding in Araldite, silver to silver-gold sections were cut with a diamond knife on a Porter-Blum ultramicrotome and mounted on Parlodion-coated, carbon-evaporated grids. The sections were stained with 2% uranyl acetate at 45°C for 1 hour, and for 20 minutes with lead citrate (21). An RCA EMU-3G electron microscope with a 35-µ objective aperture and 100-kv accelerating voltage was used for viewing and photography.

Where viruses were present, practically all sections contained virus profiles. In those cases where negative results were obtained, at least two blocks from each organ were sectioned and 4–5 grids (approximately 10–20 sections/grid) from each block examined.

RESULTS

Lymph nodes from AKR mice with spontaneous leukemia showed virus particles and served as our positive control for the electron microscope studies. In Figs. 1 and 2, immature or doughnut-shaped particles (90–100 µm$^2$), characterized by Bernhard and Granboulan (4) as Type A', can be seen in cells from lymph nodes of AKR mice. This tissue also contains the mature or Type C particle (Figs. 2, 3), which consists of a dense central nucleoid about 50–60 µm$^2$ in diameter surrounded by a double membrane. The total diameter of this particle is about 100 µm. In the two samples tested, chromosome studies of the tissues revealed no abnormalities in appearance and number (Table 1).

Newborn and young AKR mice, 6 to 8 weeks old, showed no signs of leukemia, had normal-appearing organs (thymus and spleen), and did not reveal any virus in these tissues. Similarly, no virus particles could be found in the thymus or lymph nodes of AKR mice carrying long-term transfers of the ascites form of the leukemia. Autopsy findings in these mice were generally as follows: scant to abundant ascitic fluid and cells (0.2 to 3.5 ml); variably enlarged spleen, thymus, and lymph nodes; and diffuse
TABLE 1

<table>
<thead>
<tr>
<th>Source</th>
<th>Sex</th>
<th>Age</th>
<th>Tissues examined</th>
<th>Virus*</th>
<th>Chromosome number</th>
<th>Remarks—autopsy data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Newborn</td>
<td>?</td>
<td>&lt;1 day</td>
<td>Thymus and spleen</td>
<td>-</td>
<td>N.D.</td>
<td>Organs from 2 mice pooled</td>
</tr>
<tr>
<td></td>
<td>?</td>
<td>&lt;1 day</td>
<td>Thymus and spleen</td>
<td>-</td>
<td>N.D.</td>
<td>Organs from 2 mice pooled</td>
</tr>
<tr>
<td>Normal, young</td>
<td>♂</td>
<td>7 wk.</td>
<td>Thymus and spleen</td>
<td>-</td>
<td>N.D.</td>
<td>All tissues normal</td>
</tr>
<tr>
<td>Adults, with spontaneous leukemia</td>
<td>♂</td>
<td>12.5 mo.</td>
<td>Lymph node</td>
<td>-</td>
<td>40</td>
<td>Moribund: Large palpable lymph nodes, enlarged thymus</td>
</tr>
<tr>
<td></td>
<td>♀</td>
<td>9.5 mo.</td>
<td>Lymph node</td>
<td>-</td>
<td>40</td>
<td>Same, with large solid mesenteric tumor</td>
</tr>
<tr>
<td></td>
<td>♀</td>
<td>9.5 mo.</td>
<td>Lymph node</td>
<td>+</td>
<td>N.D.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>S.M.T.</td>
<td>+</td>
<td>+</td>
<td>63</td>
<td>Enlarged liver, spleen, and S.M.T. plus solid tumor at site of injection</td>
</tr>
<tr>
<td>Young, injected i.p. with 1 × 10^6 T.C. cells (B1)</td>
<td>♂</td>
<td>3-4 mo.</td>
<td>S.M.T.</td>
<td>+</td>
<td>63</td>
<td></td>
</tr>
<tr>
<td></td>
<td>♂</td>
<td>3-4 mo.</td>
<td>S.T.S.</td>
<td>+</td>
<td>63</td>
<td></td>
</tr>
<tr>
<td>Young, injected i.p. with 1 × 10^6 cells (AI ascites, 138th transfer)</td>
<td>♂</td>
<td>2.5 mo.</td>
<td>Spleen</td>
<td>+</td>
<td>41</td>
<td>Typical ascites type of leukemia. Enlarged thymus, spleen, and lymph nodes plus solid mesenteric tumor</td>
</tr>
<tr>
<td>Young, injected i.p. with 1 × 10^6 cells (AI ascites, 143rd transfer)</td>
<td>♂</td>
<td>2.5 mo.</td>
<td>Ascites cells</td>
<td>+</td>
<td>41</td>
<td>Typical ascites type of leukemia, as above</td>
</tr>
</tbody>
</table>

* - = no virus observed; + = virus present but not abundant; ++ = abundant virus in many fields.

b N.D., not done.
c S.M.T., solid mesenteric tumor; S.T.S., solid tumor at site of injection; T.C., tissue culture.
d Immature virus only.

TABLE 2

<table>
<thead>
<tr>
<th>Mouse</th>
<th>Sex</th>
<th>Age</th>
<th>Tissue</th>
<th>Virus</th>
</tr>
</thead>
<tbody>
<tr>
<td>AF_1 fetuses (2) (C57Bl/6♀ × AKR ♂)</td>
<td>?</td>
<td>20 days i.u.*</td>
<td>Thymus</td>
<td>-</td>
</tr>
<tr>
<td>AF_1 fetus</td>
<td>?</td>
<td>20 days i.u.</td>
<td>Thymus</td>
<td>-</td>
</tr>
<tr>
<td>AF_1 newborn</td>
<td>?</td>
<td>&lt;1 day</td>
<td>Spleen</td>
<td>-</td>
</tr>
<tr>
<td>BF_1 newborn (AKR ♀ × C57Bl/6♂)</td>
<td>?</td>
<td>&lt;1 day</td>
<td>Thymus and spleen</td>
<td>-</td>
</tr>
<tr>
<td>AF_1 adult given i.p. 3 doses ascites tumor cells (AI), 1 × 10^6</td>
<td>♀</td>
<td>1 yr.</td>
<td>S.M.T.</td>
<td>-</td>
</tr>
<tr>
<td>AF_1 adult given i.p. 1 dose T.C. cells (B1), 1 × 10^4</td>
<td>♂</td>
<td>4 mo.</td>
<td>S.T.S. at site</td>
<td>+</td>
</tr>
<tr>
<td>AF_1 adult given i.p. 4 doses T.C. and ascites tumor cells (B1)</td>
<td>♀</td>
<td>9 mo.</td>
<td>Spleen</td>
<td>-</td>
</tr>
<tr>
<td>BF_1 adult given i.p. ascites C tissue culture and AI ascites tumor cells</td>
<td>♂</td>
<td>1 yr.</td>
<td>Spleen</td>
<td>-</td>
</tr>
</tbody>
</table>

* i.u., in utero; S.M.T., solid mesenteric tumor; S.T.S., solid tumor at site; T.C., tissue culture.

b See Footnote a, Table 1.
Electron Microscope Study of Leukemia Virus in Mice

TABLE 3
Virus, Chromosome Complement, and Virulence of Several Tissue Culture Lines of AKR Mouse Leukemia

<table>
<thead>
<tr>
<th>Tissue culture cell line</th>
<th>Subculture</th>
<th>Virus</th>
<th>Chromosome No.</th>
<th>M.S.T.</th>
<th>Virulence in AKR mice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Age</td>
<td>No.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S-L4946</td>
<td>5 yr.</td>
<td>200+</td>
<td>++</td>
<td>40</td>
<td>N.L.E.</td>
</tr>
<tr>
<td>R-L4946</td>
<td>5.5 yr.</td>
<td>293</td>
<td>++</td>
<td>38</td>
<td>26 days</td>
</tr>
<tr>
<td></td>
<td>6 yr.</td>
<td>343</td>
<td>++</td>
<td>38</td>
<td>18 days</td>
</tr>
<tr>
<td>S-spleen</td>
<td>2 yr.</td>
<td>50</td>
<td>+</td>
<td>60-70</td>
<td>N.L.E.</td>
</tr>
<tr>
<td>St. H.</td>
<td>2+ yr.</td>
<td>140</td>
<td>+</td>
<td>65-73</td>
<td>27 days</td>
</tr>
<tr>
<td>CM1</td>
<td>2 yr.</td>
<td>166</td>
<td>+</td>
<td>63</td>
<td>59 days</td>
</tr>
<tr>
<td></td>
<td>2.5 yr.</td>
<td>188</td>
<td>+</td>
<td>63</td>
<td>N.L.E.</td>
</tr>
<tr>
<td>Bl</td>
<td>8 mo.</td>
<td>43</td>
<td>+</td>
<td>61-66</td>
<td>42 days</td>
</tr>
<tr>
<td>Ascites C</td>
<td>3.5 yr.</td>
<td>300+</td>
<td>++</td>
<td>40</td>
<td>45 days</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>59 days</td>
</tr>
</tbody>
</table>

* M.S.T., median survival time; N.L.E., normal life expectancy.
* See Footnote a, Table 1.

solid mesenteric tumors. In certain ascites transfers, the liver or kidney might also be involved. The chromosome number varied with the ascites lines, but once stabilized, remained constant throughout numerous transfers. The AI line tested in the 138th and 143rd transfer had retained the same chromosome number for months. Virus was found in the ascitic fluid cells (Figs. 6, 7) of these infected animals but not in the thymus or lymph nodes. The virus particles observed were of the immature variety, some of which were found in vesicles at different stages of development. With the 138th transfer, virus was found in small numbers in the spleen of the mouse examined (Fig. 8); however, virus was not found in this tissue on the 143rd transfer of the AI line.

All attempts to find virus in organs of our normal F1 hybrid mice were negative (Table 2). Virus could not be detected in the spleen, thymus, or lymph node of 20-day-old fetuses or newborn mice. In adult mice inoculated with cells from tissue cultures or ascites lines, these organs were also virus-free. Similar negative findings were noted in a solid mesenteric tumor produced in an adult F1 mouse by repeated doses of ascites cells. Abundant virus-like particles were found in only one F1 animal tested. This was in a solid tumor which developed at the site of injection of 1 x 10^6 virulent BI tissue-culture cells. Chromosome analysis of this tumor showed it to have the same karyotype as the line which was inoculated. Electron-microscope studies of this tumor showed numerous virus-like particles (Figs. 14, 15) which appeared to bud from thick membranes.

Our tissue culture lines, derived from different ascites tumors induced in AKR mice, presented a different picture (Table 3). All lines tested showed characteristic virus particles, both intracellular and extracellularly. Lines propagated in vitro longer than 5 years, and with more than 300 subcultures, always contained the virus. Chromosome numbers varied from hypodiploid [38] through diploid [40] to triploid or higher [63-70], but no differences in the amount or type of virus were apparent.

Virulence of either the ascites or the tissue-culture cell lines did not appear related to the presence or absence of virus. As shown in Table 3, Line S-L4946 was practically avirulent and had been for several years, yet its chromosome number had remained normal for over three years and the characteristic virus particles were present (Fig. 9). Virus found in cells of this line displayed a dense nucleoid and a surrounding double membrane typical of the type C particles characterized by Bernhard (3).

The R-L4946 line, established as resistant to amethopterin (5), has maintained its virulence throughout six years of testing. While its chromosome number has recently dropped from 40 to 38, virus particles are still demonstrable (Figs. 10, 11).

Another line (S-spleen), isolated in 1961 and tested repeatedly for 2 years at doses of 1 x 10^6 or greater, has never produced any reaction in the young (6-8 weeks) AKR mice tested. These cells, originally cultured from the enlarged spleen of an AKR mouse with spontaneous leukemia, contain Type C virus particles in abundance (Figs. 12, 13). The established cell line is composed of large fibroblast-like cells which grow into confluent sheets at a relatively slow rate. Their growth patterns differ considerably.

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from those of our leukemic cell lines; the latter are more sensitive
to pH changes and are composed of typical small lymphocytic
cells which usually do not attach themselves to the culture flask.

The chromosome complement of our S-spleen line is hyper-
triploid [60—70] and 1 or 2 minute marker chromosomes are
present. This abnormal chromosome number does not account
for the complete lack of virulence, as many other cell lines with
similar chromosome numbers have retained some degree of viru-
ulence ranging from high virulence at doses of 1 × 10⁶ to loss of
virulence with doses of 1 × 10⁴ cells (Table 3).

DISCUSSION

Information dealing with the presence of virus particles in
AKR mice at various stages in their life cycle has been ambigu-
ous. Until recently, most investigators have identified the virus
by the ability of cell-free extracts to produce characteristic leu-
kemia in susceptible animals which had to be prenat al or less than
21 days old. Awano et al. (1) have recently published experiments
on AKR leukemia using cell-free filtrates and ascites transfers.
Electron microscopic studies and chromosome counts were made
on the tissues of the infected animals. Chromosome numbers were
normal [40] or showed little change [41] in both the ascites and
spontaneous forms of the leukemia. Once chromosomal variations
occurred, they tended to stabilize for long periods. In most re-
spects our results support their findings. Awano et al. (1) found
that, after 30 or more transfers of ascites cells, no virus could be
shown in the spleen or lymph nodes of the AKR mice used, and
that cell-free extracts from these mice no longer transmitted the
leukemia. Our results (Table 1) showed no demonstrable virus in
thymus or lymph nodes of AKR mice inoculated with ascites
cells in their 138th or 143rd transfer passage. In one instance the
spleen appeared to contain virus and in another, virus was not
detected. However, ascites cells used for the transfers abounded
with virus as did the solid mesenteric tumor which was exam-
ined. In our experiments the leukemic ascites cells were grown in
vitro as long as five years and showed many variations in their
chromosome configuration and number. Upon electron micro-
scopic examination Type A and C particles were found in these
tissue culture cells. In most cases, however, the virulence of these
cells was greatly diminished. While an intraperitoneal inoculum
of 10⁶ or 10⁷ ascites cells invariably killed all AKR mice, the same
dose of tissue culture cells often killed less than 20% and then
only after a much longer period (7).

Opinions differ as to the infectivity of Type A or C particles,
but our results showed that the presence of either A or C particles,
or the two combined, had no relation to the virulence of the cell
line or to the chromosome number.

Thus, whereas Awano et al. (1) reported disappearance of virus
but retention of virulence by ascites or solid tumor cells, we found
the continued presence of virus-like particles but attenuation or
complete loss of virulence in our in vitro lines.

Our experiments with F₁ hybrids showed these mice to be
highly resistant to infection with leukemic cells. No virus was
found in the tissues from fetuses and newborn mice or in organs
from adult mice inoculated with multiple doses of tissue culture
or ascites cells. The cells in the inoculum contained virus and
usually proved lethal for AKR mice inoculated in parallel. Even
in a mesenteric tumor produced in one F₁ mouse after repeated
doses of line AI ascites cells, no virus could be seen. In only one
experiment, a solid tumor, occurring at the site of inoculation of a
large dose of virulent BI tissue culture cells, showed numerous
virus-like particles (Figs. 14, 15), which appeared to bud from
thick membranes not previously seen in our preparations.

Whether these unusual membrane formations, which appear to
be continuous with the virus-like particles, are a new cytoplasmic
component or part of the endoplasmic reticulum, cannot be
determined. All other preparations were fixed in OsO₄, buffered in
either Veronal-acetate or s-collidine. This preparation,
however, was first fixed in glutaraldehyde and then post-
fixed in OsO₄. The superior quality of preservation obtained after
the glutaraldehyde fixation seems to have maintained the material
of these membranes. It would be important to ascertain the com-
position of these thickened membranes. Such information might
be obtained by electron microscopic studies on ferritin-conju-
gated antibodies to the virus.

Further experimentation might indicate whether the visible
virus is essential for the transfer of leukemia, or whether the vi-
rus present in the cells grown in vitro has been transformed so
that it is less capable of inducing leukemia, while its ascites-
grown counterpart remains highly lethal.

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Figs. 1-3. Lymph node from an AKR mouse with spontaneous leukemia. Several doughnut-shaped particles (Type A') can be seen in Fig. 1 (arrow) both inside and outside a cell. Fig. 2 shows two types of intercellular particles: a Type C particle (arrow) with a central nucleoid and a Type A' virus particle (crossed arrow). Type C particles are present outside some cells in Fig. 3. Specimens fixed in 1.33% OsO4 in s-collidine buffer (pH 7.5), dehydrated in acetone, and stained in block with KMnO4. Fig. 1, X 95,900; Fig. 2, X 89,800.

Figs. 4-5. Cells from ascites fluid of a mouse injected with ascites leukemia AI, in its 138th transfer. In Fig. 4, several Type C particles (arrow) are present outside the cell, while inside the cell a vesicle (V) is seen to contain a budding doughnut-shaped particle. Many examples of Type C particles (arrow) can be seen next to the cell membrane (Fig. 5). Specimen preparation as for Figs. 1-3. Fig. 4, X 132,000; Fig. 5, X 99,780.

Figs. 6-7. Sections through solid mesenteric tumor from a mouse injected with the 138th transfer from AI-line ascites cells. In Fig. 6 there are many doughnut-shaped particles in the cytoplasm which are membrane-bound, and which show evidence of budding (arrow). Fig. 7 is a higher magnification of an area similar to that in Fig. 6. Notice the single doughnut-shaped particle, and the pair of half-particles (arrows). Specimens prepared as for Figs. 1-3. Fig. 6, X 24,300; Fig. 7, X 88,900.

Fig. 8. Portion of a cell from the spleen of a mouse injected with the 138th transfer of AI-line ascites cells. Some virus particles (Type C) are present (arrow). Specimen fixed in 2% OsO4 in acetate-Veronal buffer (pH 7.5), dehydrated in acetone, and stained in block with KMnO4, X 132,200.

Fig. 9. Tissue culture cell line S-L-4946. Many virus particles (Type C) are present in this space between cells. Specimen preparation as for Figs. 1-3, X 110,210.

Figs. 10-11. Tissue culture cell line R-L-4946. In Fig. 10, formation of a virus particle from the endoplasmic reticulum (ER), can be seen (arrow). Two membrane-bound Type A' virus particles are to be found at its left. Fig. 11 shows extracellular Type C virus particles, one of which is still connected to the cell membrane (arrow). Tissue preparation as for Fig. 8. Fig. 10, X 114,330; Fig. 11, X 89,800.

Figs. 12-13. Tissue culture cell line S-spleen. Many virus particles, surrounded by double-membraned vesicles, are present in Fig. 12. Fig. 13 shows a large quantity of Type C particles outside a cell. Specimen preparation as for Fig. 8. Fig. 12, X 99,800; Fig. 13, X 61,200.

Figs. 14-15. Solid mesenteric tumor of an F1 hybrid mouse injected with BJ tissue culture cells. The cytoplasm of the cell in Fig. 14 has several virus particles. Electron-dense membranous material is connected with some of these particles (arrow). Fig. 15 is a higher magnification of an area similar to that in Fig. 14. Notice the well-defined doughnut-shaped virus particles. The electron-dense membrane systems (M) seem to be connected to virus particles. Specimens fixed in glutaraldehyde in cacodylate buffer (pH 7.5), postfixed in OsO4, and stained in block with uranyl acetate. Fig. 14, X 28,600; Fig. 15, X 120,200.

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