studies on a transplantable chicken tumor
(rpl-12 lymphoma)

iii. cytological changes during virus-induced oncolysis

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the histological changes following inoculation of the rpl-12 lymphoma into the pectoral muscle and brain of the chicken have been described (29, 30). superimposed infection of the host with the n.f.t. strain of st. louis encephalitis virus results in the development of prominent cytoplasmic "inclusions" in the tumor cells during the period when the virus concentration in the tumor is highest (30). apart from some displacement or indentation of the nucleus by the "inclusions" and occasional margination of the chromatin, there is no further morphological evidence of degeneration before the tumor cells in the pectoral muscle undergo phagocytosis. when the virus is inoculated into the host with tumor growing in the brain, phagocytosis is deficient, and an abnormally large number of metaphase mitotic figures are observed in the tumor cells containing "inclusions" (30). the present experiments were designed to analyze the formation and structure of the so-called "inclusions," and to determine the effect of virus infection on the mitotic process by comparing the cytological and cytochemical properties of the tumor cell before and after infection with virus.

materials and methods

complete details of the biological procedures and the plan of the experiments have already been published (29, 30).

tissues.—in addition to the routine histological examination of all organs and tissues, the following material was specially prepared for cytological and cytochemical study: (a) pectoral muscle and brain tissue containing tumor, daily from the time of inoculation of virus until tumor cells in the muscle had undergone phagocytosis, or until the birds with tumor in the brain had died; (b) uninfected tumor tissue on the same days as (a).

preparation of the tissues.—thin fragments were scraped with a sharp knife from the cut surface of the tissue and smeared on slides. when no fixative was used (table 1), the smears were allowed to dry for 5 minutes before staining; when fixation was desired, the slides were immersed in the fixative while still wet. small pieces were placed in 0.85 per cent nacl or mixed with supravital staining solutions on slides and examined directly by ordinary and phase microscopy. blocks of tissue were fixed as described below, and paraffin and frozen sections were prepared. except where otherwise stated (table 1), the final preparations were mounted in permount, and the paraffin-imbedded material which had been fixed in formal sublimate was treated with iodine and thioulate before staining.

fixation and staining procedures.—paraffin sections of material fixed in formal sublimate were stained with barrett's stain (4) to observe the general cytological detail. for the demonstration of golgi bodies and mitochondria, blocks were fixed and prepared by standard methods: (a) cajal's silver and the sopwall and kolatchew osmic acid methods for golgi bodies (35) and (b) altmann's and regaud's methods for mitochondria (35). in addition, the more recent method of harman (22) was applied to blocks fixed in regaud's fluid. supravital staining properties were investigated by placing small fragments of tissue in freshly prepared mixtures of stock solutions of 0.006 per cent janus green and 0.01 per cent neutral red in 0.85 per cent nacl in the proportions of 2:1, 1:1, and 1:2. a comparison of the sudanophilic with the supravital stainable material in the cells was made by replacing the supravital stain under the cover slip with baker's sudan black b (1) and differentiating with 50 per cent alcohol.

to minimize repetition and clarify the rationale, the cytochemical procedures are outlined in table 1.

analysis of mitosis.—owing to the rapid phagocytosis of infected tumor cells in the pectoral muscle, only brain material was available for sustained analysis of the mitotic process in sections stained by the feulgen method. beginning on the day when inclusions were first observed, 1,000 tumor cells in each section were analyzed, the number of cells in the various phases of mitosis was noted, together with the proportion of binucleate cells and the incidence of gross mitotic abnormalities as defined by glucksman (17). multipolar division, bridge formation, and lagging of an occasional chromosome in anaphase were not regarded as abnormal for the purposes of the count, since they do not necessarily lead to death of the daughter cells.

results

uninfected tumor cells

characterization of the structures of the cell.—supravital staining with neutral red and janus green revealed numerous rounded green mitochondria of regular size distributed throughout...
### TABLE 1

**Cytocchemical Procedures**

<table>
<thead>
<tr>
<th>Purpose to investigate</th>
<th>Fixation</th>
<th>Preparation</th>
<th>Staining</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid</td>
<td>F. sub., f. calc. and none</td>
<td>Smear or inc jelly</td>
<td>Sudan black B (1)</td>
<td>All material for lipid investigation, and for comparison with any lipid stain, mounted in glycerine jelly</td>
</tr>
<tr>
<td>Neutral and acidic lipid</td>
<td>F. sub. and f. calc.</td>
<td>Smear or Inc jelly</td>
<td>Sudan black B after lipid extraction</td>
<td>Lipid extracted by 6 hours' immersion of slides in hot chloroform-methanol. To insure that sudanophilia was not due to chemical combination with Sudan black it was shown that the dye, but not the lipid, could be removed by acetone (20).</td>
</tr>
<tr>
<td>Fatty aldehydes and precursors</td>
<td>None</td>
<td>Smear or Inc jelly</td>
<td>Cain's Nile blue sulfit (6)</td>
<td>Compared with preparations stained with Sudan dyes to confirm the lipid nature of the material stained.</td>
</tr>
<tr>
<td>Unaturated fatty acids</td>
<td>F. sub., f. calc. and none</td>
<td>Smear</td>
<td>Perfuming acid Schiff (86)</td>
<td>M.R. stained simultaneously as a positive control.</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>F. sub. and f. calc</td>
<td>Smear</td>
<td>Schults (30)</td>
<td>Mouse adrenal stained simultaneously as a positive control.</td>
</tr>
<tr>
<td>P.A.S.-positive material</td>
<td>F. sub. and f. calc. and none</td>
<td>Par. and fros. or smears</td>
<td>P.A.S. (84)</td>
<td>In all cases where P.A.S. stains were used, control sections of the same material were treated identically except that oxidation with periodic acid was omitted.</td>
</tr>
</tbody>
</table>

**1,2-Glycol groups**

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<tr>
<td>F. sub. and f. calc.</td>
<td>Smear</td>
<td>P.A.S. after acetylation in pyridine (86)</td>
<td>Control section stained after treatment with pyridine alone to determine reduction in P.A.S. stain due to solution of cell constituents in pyridine.</td>
</tr>
<tr>
<td>F. sub. and f. calc.</td>
<td>Smear</td>
<td>P.A.S. after lipid extraction</td>
<td>The lipid extraction process did not affect the intensity of the P.A.S. reaction of control sections of clam. A difference between the unextracted and the extracted material must be due to P.A.S.-positive lipid.</td>
</tr>
<tr>
<td>F. sub. and f. calc.</td>
<td>Smear</td>
<td>P.A.S. followed by Sudan black B</td>
<td>To determine whether location of the lipid coincides with that of the P.A.S.-positive material.</td>
</tr>
<tr>
<td>F. sub. and f. calc.</td>
<td>Smear</td>
<td>P.A.S. after bromination</td>
<td>Control section of clam stained before and after bromination showed considerable reduction in the intensity of the P.A.S. reaction. Bromination by exposure to bromine vapor in bright sunlight and by Lillie's method (85).</td>
</tr>
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**Acid mucopolysaccharides**

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<tr>
<td>Par.</td>
<td>Sudan black B (1)</td>
<td>Lipid extraction, hyaluronidase (27), P.A.S.</td>
<td>Activity of enzyme shown by its ability to reduce metachromasia of clam acid mucopolysaccharides.</td>
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<tr>
<td>F. sub.</td>
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<td>Lipid extraction, diastase (27), P.A.S. after acetylation in pyridine</td>
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<td>Stained before and after incubation for 1 hour in buffered ribonuclease (97); staining of control sections unaffected by incubation in buffer alone. Association of stainable material with lipid constituents of the cell investigated by staining with toluidine blue after lipid extraction. Fixation of toluidine blue stain by the method of Mansley (5).</td>
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**Glycogen**

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**DNA**

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**Tyrosine, tryptophan, histidine, ? purines and pyrimidines**

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<td>Susa</td>
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**Ascorbic acid**

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*Section of clam in which presence of acid mucopolysaccharides and alkaline phosphatase and absence of lipids was demonstrated (97).*
the cytoplasm and a small number of slightly larger irregularly sized neutral red staining particles, mainly located in the nuclear hof.\footnote{The term “nuclear hof” indicates the zone of cytoplasm which is the site of the centrosome and the lipochondria or Golgi apparatus and is usually adjacent to a slight indentation of the nucleus.} A few large refractile globules in the cytoplasm did not stain. Instillation of Sudan black stain beneath the cover glass of supravital preparations clearly demonstrated the lipid nature of the refractile globules, which stained in a few seconds, and of the mitochondria, which were visibly stained after about 10 minutes. Unfortunately, the Sudan black solution rapidly destroyed the supravital staining properties of the cell, and there was a latent period when the cell was unstained and considerable shrinkage occurred. It was not possible, therefore, to visualize the exact fate of every neutral red particle. A comparison of drawings of the supravital stained and the Sudan black-stained cell revealed that some of the neutral red vacuoles were probably identical with sudanophilic granules which became visible at about the same time as the mitochondria, but were mostly confined to the nuclear hof.

Phase contrast microscopy confirmed the existence of spherical particles of variable size in the cytoplasm but did not distinguish different types. In sections stained by Altmann’s, Regaud’s, and Harman’s methods for mitochondria, and the Cajal silver, Sjövall, and Kolatchew osmic acid methods for Golgi bodies, the results were variable; cytoplasmic particles corresponding to the Janus green and the neutral red staining material were present in parts of sections stained by all methods except the first, in which only a few coarse granules could be seen. In the less intensely stained preparations only the smaller granules were stained, while, as the density of staining increased, the larger granules stained around the periphery and finally appeared as solid bodies. Even in the most intensely stained preparations scanty unstained vacuoles were present in the peripheral cytoplasm. No vacuoles, however, were present in Sudan black preparations, indicating that the vacuoles were probably produced by the largest cytoplasmic globules whose sudanophilia was observed by direct staining of cells under the microscope.

Since the standard Golgi stains failed to show any structures other than those which were revealed by supravital staining and by phase microscopy, it seems justifiable to consider the larger argentophilic and osmiophilic granules which resemble the sudanophilic, neutral red staining bodies as Golgi bodies or lipochondria (38).

In smear preparations of tumor cells (but not paraffin sections) fixed in formol sublimate and stained with dilute toluidine blue, there were numerous dark blue cytoplasmic granules, identical in shape, size, and distribution with the supravitaly stained mitochondria (Fig. 1).

In summary, therefore, at least three types of sudanophilic material were demonstrated in the cytoplasm of the tumor cells. The smallest and most numerous particles were mitochondria; a few larger granules, mainly in the nuclear hof, were lipochondria or Golgi bodies; and, until they can be more clearly defined, the largest will be called lipid globules.

**Cytochemistry.**—The tinctorial and cytochemical properties of the cytoplasmic and nuclear structures of the tumor cells are summarized in Table 2.

**Nucleus and nucleolus.**—The nuclear chromatin, including the nucleolar membrane, was the only material in the cell which gave a positive Feulgen reaction. In acid solutions of aniline blue and orange G the nucleolus and parachromatin granules had a stronger affinity for orange G than any other cell component, and therefore contained histone (42). The nucleolus was poorly stained by toluidine blue in smear preparations, and the intensity of staining was not decreased by ribonuclease digestion (Figs. 1 and 2). The nucleolus had the same staining range as the cytoplasm in the methylene blue extinction test, and was similarly affected by previous ribonuclease digestion (Table 2). The nucleolus, like the rest of the cell, stained dark red by the coupled tetrazonium reaction. Benzoylation eliminates the reaction of tyrosine, tryptophan, and histidine with this reagent but, according to Danielli (10), has little effect on the reactivity of compounds containing purine and pyrimidine, which may not react at all under the conditions of this test (37). After benzoylation the nuclear desoxyribonucleic acid (DNA) was not stained by the tetrazonium method, but the cytoplasm was still faintly positive. Tyrosine, tryptophan, and/or histidine were therefore present in the nucleus, nucleolus, and cytoplasm. Extraction of the nucleic acids by treatment with sulfuric acid completely abolished the reactivity of all the cell constituents (Table 2). Ribonucleic acid (RNA) may have been responsible for the residual staining after benzoylation. The amount of RNA in the nucleolus and of DNA in the nucleus was not sufficient to react with the tetrazo compound after benzoylation. Confirmatory evidence of the paucity of RNA in the nucleolus was provided by the failure of ribonuclease to decrease the int-
| Method       | Barret's stain | Feulgen | Lipid extraction | Acetylation in pyridine | Lipid extraction hydrazine | Sudan | Nile blue sulfate | Acid hematin | Toluidine blue | M.B.E. | Ribonuclease | M.B.E. | pH of extinction | Tetrasomium | Strong acid benzoylethon | Tetrasomium | Aniline blue | Acid phosphatase | Supravital staining | Probable constituents |
|--------------|----------------|---------|------------------|-------------------------|----------------------------|-------|-----------------|--------------|----------------|---------|--------------|--------|----------------|--------|-------------------|-------------|-------------------|----------------|----------------|----------------------|---------------------|---------------------|
| Chromatin    |                |         |                  |                         |                            |       |                 |              |                |         |              |        |                  |        |                   |              |                  |                    |                   |                     |
|              |                |         |                  |                         |                            |       |                 |              |                |         |              |        |                  |        |                   |              |                  |                    |                   |                     |
| Nucleolus    | Pink           | 0       | 0                | 0                       | 0                           | 0     | Blue            | 0            | 0               | 3       | 7            | 0      | 0               | 0      | Orange            | 0            | 0                | Histone           | R.N.A. protein     | Triglyceride       |
| Cytoplasm    | Blue           | 0       | 0                | 0                       | 0                           | 0     | Blue            | 0            | +               | 3       | 7            | +      | +               | +      | Blue              | 0            | 0                | R.N.A. protein     | Triglyceride       | Glycolipid glycoprotein |
| Large lipid globules | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | Pink | Grey | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | Janus green | Neutral lipid | R.N.A. acid phosphate |
| Lipochondria | 0             | 0       | 0                | 0                       | 0                           | 0     | Pink            | ++           | 0               | 0       | 0            | 0      | 0               | 0      | 0                | 0            | 0                | Janus green | Neutral lipid | R.N.A. acid phosphate |
| Mitochondria | 0             | 0       | 0                | 0                       | 0                           | 0     | Pink            | ++           | 0               | 0       | 0            | 0      | 0               | 0      | 0                | 0            | 0                | Janus green | Neutral lipid | R.N.A. acid phosphate |

No detectable preformed aldehyde, aortal phosphatides or aldehydes secondary to oxidation of unsaturated fatty acids, no birefringence, colored material, cholesterol, ascorbic acid, alkaline phosphatase or succinic dehydrogenase.

Par = Paraffin section F. sub. = Formo1 sublimate M.B.E. = Methylene blue extinction test * Sudan black B or Sudan III and IV 0 = Negative or unstained F. calc. = Formo1 calcium P.F.A.S. = Performic acid Schiff + Trace after prolonged incubation
tensity of staining with toluidine blue. Only the methylene blue extinction test provided any evidence for the presence of RNA in the nucleolus, and this may have been caused by the presence of this material in the cytoplasm, which almost invariably covered the nucleolus in sections of the thickness used here.

**Lipid globules, lipochondria and mitochondria.—**

The cytochemical reactions of the sudanophilic cytoplasmic components (Fig. 4) may be conveniently considered together. All three components gave a pink color in all steps of Cain's Nile blue sulfate method and therefore contained neutral lipid (8). In material fixed in formol calcium they all stained with Sudan III and IV, which suggests, but certainly does not prove, that they contained triglycerides (37). With or without fixation, the cytoplasmic particulates were colorless, monorefringent, and gave a negative Schultz reaction for cholesterol. No preformed aldehyde or acetal phosphatide could be detected in unfixed smears. The performic acid Schiff test for unsaturated lipids was negative, and after 8 days' exposure to air no oxidation aldehydes could be demonstrated. Phospholipid could not be demonstrated by Baker's acid hematin test.

As already noted, the large cytoplasmic globules stained most readily with Sudan black B; they did not stain by any methods other than those involving simple solution of fat soluble dyes (Table 2) and probably consisted mainly of triglyceride.

In paraffin sections no P.A.S. positive material could be demonstrated in the tumor cells. In smears stained by the P.A.S. method and mounted in glycerine jelly, a few irregularly sized P.A.S.-positive granules were observed in the nuclear hof. When such a preparation was counterstained with Sudan black, all the red stain was obliterated by the black. After extraction of lipids, slightly smaller, less intensely colored P.A.S.-positive granules could be demonstrated in the nuclear hof. There were, therefore, two P.A.S.-positive components associated with the sudanophilic granules of the nuclear hof which, as already shown, were the lipochondria. One component, which could be removed by treatment with hot chloroform and methanol, was a lipid; the other was resistant to treatment with lipid solvents.

Glycolipids, some phospholipids, and some unsaturated lipids are the only colorless lipids reported to give a positive P.A.S. reaction (48). The presence of phospholipids in any quantity was excluded by the negative acid hematin reaction and the failure to demonstrate acidic lipids by Cain's Nile blue sulfate method (Table 2). Unsaturated lipid could not be detected by the performic acid Schiff reaction, and the reaction for aldehydes secondary to the oxidation of unsaturated fatty acids was negative (Table 2). Some reduction in the intensity of the P.A.S. reaction was produced by bromination, but no more than occurred in a lipid-free control section containing acid mucopolysaccharide. The reacting lipid was probably glycolipid.

The presence of acid mucopolysaccharide and glycogen in the lipochondria was excluded because the P.A.S.-positive reaction was unimpaired after hyaluronidase and diastase digestion and because metachromasia was absent. Acetylation in dry pyridine completely blocked the P.A.S. reaction of the cell, but, since pyridine is a lipid solvent, this did not provide any information about the reacting groups in the lipid component. It did, however, confirm that 1,2 glycol groups were probably responsible for the reaction of the other component which, by a process of exclusion, was either a glycoprotein or a neutral mucopolysaccharide.

In smears fixed in formol sublimate and stained with toluidine blue, the mitochondria were clearly stained dark blue (Fig. 1). The staining reaction must have been due to the presence of ribonucleic acid, since it was completely abolished by previous ribonuclease digestion (Fig. 2). The close association of RNA with the lipid particles was shown by the absence of mitochondrial staining with toluidine blue after extraction of lipid.

In unfixed and formalin-fixed preparations, the Gomori technic for acid phosphatase revealed minute positive granules throughout the cytoplasm of the tumor cells (Fig. 3), reaching a maximum intensity after 1 and 2 hours' incubation, respectively. After this period some diffusion occurred, and the nuclear chromatin was also stained. The reaction was more intense in the unfixed than in the fixed material, but the number and distribution of the particles were identical and suggested that the enzyme was present in the mitochondria. In preparations fixed in acetone, staining appeared after 3 or 4 hours' incubation and was more pronounced in the nucleus than in the cytoplasm.

**Cytoplasm.—** When paraffin sections were used, the mitochondria were no longer recognizable in toluidine blue preparations and the cytoplasm stained diffusely blue or faint purple. The staining reaction was removed by ribonuclease digestion, showing the presence of RNA. The presence of RNA in the cytoplasm was further confirmed by the alteration in the pH of extinction in the methylene blue extinction test after ribonuclease.
digestion (Table 2) and by the results of the tetra-
zonium reaction already described.

**Virus-infected Tumor Cells**

**Lipochondria.**—No perceptible changes oc-
curred in the tumor cells until the 2d or 3d day
after virus inoculation. At this time an increase
in the number and size of the lipochondria (Fig. 5)
could be demonstrated by all the methods de-
scribed except supravital staining, which did not
show any increase in the neutral red-staining par-
technic. In paraffin sections, however, the early stages
of the development of cytoplasmic aggregations
were not seen, and the first abnormality in hema-
toxylin and eosin sections was the appearance of
one or two large homogeneous eosinophil inclu-
sion bodies in the nuclear hof. (The term “inclusion
body” can be applied with some justification,
since no such structure can be seen in similarly
prepared sections of uninfected tumor cells.) These
bodies stained uniformly red by the P.A.S. method,
even after extraction of all stainable lipid. Like
the Golgi bodies, their ability to stain with the
P.A.S. technic was unaffected by diastase or hy-
auronidase digestion, was reduced by bromina-
tion, and abolished by acetylation. They stained
faintly with Sudan black B, and on high magnifi-
cation sometimes appeared to consist of a number
of smaller granules (Figs. 6 and 7). The “inclusions”
differed from the smaller lipochondria of the
uninfected tumor cell in that they were visible
in paraffin sections, where they acquired proper
properties which could not be shown in smear prepara-
tions of infected or uninfected tumor cells. In sec-
tions they stained more intensely than the cyto-
plasm, but exhibited the same staining reactions
with the tetrazonium method, toluidine blue stain
and the M.B.E. test, and were similarly altered by
previous destruction of RNA by ribonuclease or
treatment with strong acid. They contained,
therefore, protein (amino acids) and RNA. Ex-
cept for the presence of RNA and allowing for
some loss of lipid in the imbedding process, the
cytoplasmic “inclusions” observed in paraffin sec-
tions had the same cytochemical properties as
the lipochondria of infected and uninfected cells
in other preparations.

**Mitochondria.**—In supravital and smear prepa-
rations of virus-infected cells the mitochondria
were unaltered in morphology and cytochemical
properties. Since mitochondria were not demon-
strable in paraffin sections of material fixed in
formol sublimate, they may also have been in-
volved in the fusion of lipid particles which gave
rise to the “inclusions,” and in doing so imparted
their RNA content to the final mass.

**Lipid globules.**—Coincident with the changes
in the lipochondria, the cytoplasmic lipid globules
were sometimes, but not always, increased in size
and number.

**Nucleus.**—As the Golgi bodies increased in
number and size the nucleus of the cell was pushed
to one side and even indented by the larger aggre-
gations (Fig. 7). Some margination of the nu-
clear chromatin was seen in an occasional tumor
cell with hypertrophied Golgi bodies, but for the
most part no further evidence of degeneration was
detected until the cell had undergone phago-
cytosis. This latter process was extremely rapid
when the tumor was growing in muscle, and tumor
cells were no longer present within 2 days of the
appearance of the first abnormality. In the brain,
however, phagocytosis was delayed, and “inclus-
ions” persisted in most of the tumor cells for 3
or 4 days, during which analysis of the mitotic
process was possible.

**Mitotic process.**—From the results presented in
Table 3 it will be seen that there was a significant
increase in the percentage of tumor cells in mitosis
after virus infection. Analysis of the data reveals
that this was almost entirely the result of an in-
crease of cells in metaphase. The percentage of
infected tumor cells in the other stages of mitosis
was decreased, though only the telophase results
are significant. There was, therefore, a delay or
partial arrest in metaphase. Further evidence of
interference with the process of cell division was
shown by a significant increase in the proportion
of binucleate tumor cells and in the percentage
of abnormal mitotic figures in the virus-infected
series (Table 3). Clumping of the chromosomes
in metaphase was the commonest abnormality
of mitosis, but scattering of chromosomes and
clumping in prophase, anaphase, and telophase
were fairly frequent. The same types of abnor-
mality were present in the uninfected and infected
groups, and the difference between the two was
entirely quantitative.

**DISCUSSION**

The results of this study lend support to the
more recent views of Baker on the nature of
Golgi bodies or lipochondria (3). The lipochondria
of the tumor cell consisted of spherical bodies
containing glycolipid, and some comprised an
outer lipid layer and an inner zone of glycoprotein
or neutral mucopolysaccharide. Cytochemical
differentiation between glycoprotein and neutral
mucopolysaccharide is impossible, but the co-
<table>
<thead>
<tr>
<th>Day after inoculation of virus</th>
<th>Second</th>
<th>Third</th>
<th>Fourth</th>
<th>Fifth</th>
<th>Means of observations</th>
<th>Difference between means</th>
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<tr>
<td>Percentage of tumor cells in:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prophase</td>
<td>2.2</td>
<td>2.2</td>
<td>2.2</td>
<td>2.1</td>
<td>1.864</td>
<td>-0.214</td>
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<tr>
<td>Metaphase</td>
<td>2.4</td>
<td>2.4</td>
<td>2.4</td>
<td>2.5</td>
<td>2.5</td>
<td>+2.504</td>
</tr>
<tr>
<td>Anaphase</td>
<td>0.4</td>
<td>0.4</td>
<td>0.4</td>
<td>0.7</td>
<td>0.042</td>
<td>+0.531</td>
</tr>
<tr>
<td>Telophase</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>0.7</td>
<td>0.042</td>
<td>+0.531</td>
</tr>
<tr>
<td>Mitosis (total)</td>
<td>6.0</td>
<td>6.0</td>
<td>6.0</td>
<td>5.5</td>
<td>5.665</td>
<td>+1.872</td>
</tr>
<tr>
<td>Abnormal mitosis</td>
<td>5.3</td>
<td>6.2</td>
<td>8.8</td>
<td>2.0</td>
<td>5.355</td>
<td>+5.158</td>
</tr>
<tr>
<td>(per cent of total mitosis)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Binucleate cells (per cent of tumor cells)</td>
<td>0.2</td>
<td>0.1</td>
<td>0.3</td>
<td>0.1</td>
<td>0.2</td>
<td>+1.056</td>
</tr>
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</table>
existent presence of protein suggested that the
former was more probable. A similar conclusion
reached by Gersh (16), who demonstrated
glycoprotein in the Golgi apparatus of the duo
denal cells in the rabbit and guinea pig.

Supravital staining clearly demonstrated that
in the infected tumor cells some of the lipochondr
did not color with neutral red. Jackson (23)
has recently reported a similar loss of affinity for
neutral red affecting the granules of degenerating
leukocytes.

The occasional presence of phospholipids in
Golgi bodies has been described by Baker (8) and
by Cain (9). Evidence for the existence of vitamin
C and alkaline phosphatase in the Golgi bodies
of many different cells has been reviewed by
Bourne (7). These substances could not be demonstra
ted in the tumor cells (Table 2).

Hyptertrophy of the Golgi bodies of virus-infect
cells has been reported in canine distemper
(11), Rift Valley fever (14), vaccinia (31), loping ill (21), myxomatosis (13), and fowlpox (32). The
“inclusions” in the virus-infected tumor cells
showed a remarkable resemblance to the Bollinger
bodies of fowlpox. In a detailed study of the evolu
on the Combined Effects of Fowl Pox Virus and
Acad. Sc., 54:977—99, 1952). The similarity of the
activation of latent fowl pox might have oc
curred. To investigate this possibility, a 10 per

ADDENDUM

Duran-Reynals has observed that fowl pox was activates by painting the skin of chicks with
methylcholanthrene (F. Duran-Reynals, Studies
on the Combined Effects of Fowl Pox Virus and
Acad. Sc., 54:977—99, 1952). The similarity of the
“inclusions” in the infected tumor cells infected
with St. Louis virus to Bollinger bodies suggested
that activation of latent fowl pox might have oc

curred. To investigate this possibility, a 10 per

SUM.MARY

1. A cytological and cytochemical study of the
RPL-12 tumor cell is presented. Three types of
lipid particles were identified in the cytoplasm.
The significance of the largest is unknown. The
smallest were mitochondria and probably con
ained neutral lipid, RNA, and acid phosphatase. The
remaining particles appeared to be lipochondr
d and contained glycolipid and glycoprotein.

2. Infection of the tumor with the N.F.T.
strain of St. Louis encephalitis virus induced an
crease in the number and size of the lipochondr,
partial arrest of mitosis in metaphase, and
creased numbers of mitotic abnormalities and
binucleate cells.

3. The mitochondria of the tumor cell were
affected by virus infection, but appeared to fuse
with the hypertrophied lipochondria in the pro
cess of paraffin imbedding and contributed to the
formation of large cytoplasmic “inclusion bodies”
seen in paraffin sections.
cent suspension of tumor, which had been infected 3 days previously with NFT virus and was known to contain "inclusion bodies," was inoculated in 0.2-ml. amounts onto the chorio-allantoic membranes of ten 7-day-old chick embryos. Two embryos were harvested daily, and no gross or histological evidence of fowl pox was observed.

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Magnification of all figures × 2,000.

FIG. 1.—Mitochondria in tumor cells. Note faintly stained nucleoli. Smear stained with toluidine blue.

FIG. 2.—Tumor cell stained with toluidine blue after ribonuclease digestion. Chromatin and nucleolus stain more intensely than in Figure 1. Mitochondria are unstained. Smear.

FIG. 3.—Multiple granular deposits of lead sulfide in the cytoplasm and some diffuse staining of the nucleus of tumor cell. Smear fixed in formalin: 3 hours' incubation; acid phosphatase stain.

FIG. 4.—Numerous lipid particles of varying size in the cytoplasm of uninfected tumor cells. Frozen section. Sudan black B stain.

FIG. 5.—Increased size of cytoplasmic lipid particles 2 days after inoculation of virus. The increase in number of particles cannot be seen from the photograph because of poor depth of focus at this magnification. Frozen section. Sudan black B stain.

FIG. 6.—Partial fusion of cytoplasmic lipid granules in two tumor cells 3 days after inoculation of virus. A macrophage (below) contains a fragment of lipid, probably from an ingested tumor cell. Paraffin section. Sudan black B counterstained with neutral red.

FIG. 7.—More complete fusion of lipid material to form a large "inclusion body." Paraffin section. Sudan black B counterstained with neutral red.

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Robert Love and George R. Sharpless

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