LEUKEMIA, like any other type of cancer, may be caused by a number of factors, some of which are known and others which may still have to be discovered. These factors may vary quantitatively or qualitatively. There is no doubt that genetic factors play an important part in the origin of leukemia at least in some species, to mention only mice. In addition, estrogens, carcino- 
genic hydrocarbons and radiation are known to cause leukemia in mice, either acting separately or synergistically. Viral agents are also known to 
play a part in the origin at least of certain types of leukemia in animals of 
certain species. Viral agents may also be involved in leukemias in other 
species, but this remains to be proved experimentally. To argue whether viral 
agents or other factors play a primary role in the origin of leukemia may seem 
large a waste of time. It would be a mistake to attribute the causation of leu-
kemia to the sole action of a viral agent, which may be more or less promi-
nently involved in the origin of certain types of leukemia in certain species. 
While in some types of leukemia a viral agent may be involved, in other 
types of leukemia, even of the same histologic appearance, other factors may 
play a part, for example hormonal stimulation, either of internal or external 
origin, or radiation energy. This must be borne in mind until such time when 
experimental evidence is at hand that a viral factor is involved in other types 
of leukemias in mice and in other species. Even then, should this prove to 
be the case, the contributory part played by other factors should not be over-
looked, but thoroughly explored, as they may have an important bearing on 
the final outcome—the development of leukemia. No primary or secondary 
importance should be ascribed to one or other set of factors until such time, 
when as many factors as possible in the origin of a particular type of leukemia 
in a given species are thoroughly known.
The cell-free transmission of chicken leukemia has been known since the original discovery of Ellermann and Bang.\(^{24}\) In time, it was shown that similar transmission may occur not only under experimental but also under natural conditions. Some forms of chicken leukemia (visceral lymphomatosis) are transmitted in the embryo and by contact.\(^{6}\) A fact of great interest is the observation of a change in the affinity for specific tissues of a chicken leukemia virus following storage at \(-70^\circ\text{C}\). The virus, following such treatment, may induce other types of tumors such as lymphosarcoma, fibrosarcoma, osteochondrosarcoma, myxosarcoma and endothelioma.\(^{12,58}\) It is, however, well known that the response of various hosts at different times may be quite variable and may have accounted, at least in part, for the results.

For the last seven years evidence has been accumulating that viral agents play a part in the origin of lymphatic and myeloid leukemia in mice. Gross\(^{31,32}\) first demonstrated that lymphatic leukemia of mice can be transmitted by cell-free preparations of leukemic organs of these mice into susceptible, newborn mice of certain strains. The cell-free preparations induce also parotid gland carcinomas and fibrosarcomas.\(^{33-35,37}\) Like chicken leukemia,\(^{10}\) mouse leukemia appears to be transmitted in the embryo.\(^{36}\) While certain types of lymphatic leukemia are transmitted by cell-free material to newborn mice, others (myelogenous) can be transmitted to adult mice.\(^{27}\) These original observations have been amply confirmed, although in some cases with somewhat different interpretation.\(^{28,57,60,65,72}\) Induction of myeloid leukemia in mice with cell-free preparations from mouse transplantable tumors of epithelial and mesenchymal origin has also been reported.\(^{30,58}\) In some of these studies not only induction but also acceleration of both lymphatic and myeloid types of leukemia in mice have been recorded. Whether these observations are the result of stimulation of a latent virus still remains to be proved. It should be mentioned that recently Hays, Simmons and Beck\(^{39}\) have not only confirmed the original observations of Gross, but have also induced leukemia in certain strains of mice with preparations, from both leukemic and nonleukemic tissues of mice with a high incidence of spontaneous leukemia, containing deoxyribonucleic acid and ribonucleic acid. This is an observation of great interest, and it remains to be proved whether these cell-free preparations contained active virus nucleic acid. There appears to be no doubt that a viral agent or agents are implicated in the origin of certain types of leukemia in mice. Scarcely anything is known as yet about the relationship of viral agents to other factors known to be leukeinogenic such as, for example, \(x\)-irradiation, although some preliminary observations have already been recorded\(^{14}\) and will later be briefly mentioned.

The possible viral origin of leukemia in man has been considered by a number of authors.\(^{50,58}\) Human leukemia, especially acute leukemia in children, because of the clinical picture—its onset, course, exacerbations and remissions—is considered by some authors to be very similar to an infectious disease.\(^{59}\) It may be considered doubtful if at the present state of our knowledge a comparison\(^{58}\) can be made between chicken leukosis and mouse leukemia on one hand and human leukemia on the other. There appear to be certain similarities in the behavior of cell-free preparations of tissues from
patients with leukemia and similar preparations from leukemic organs of
certain strains of mice. The former, like the latter, have been reported to
accelerate and to induce leukemia in suitable mice. Further studies are required
before any opinion can be expressed about the nature of these observations.
In spite of some claims, no experimental proof as yet has been produced of
a viral agent being implicated in the origin of any type of human leukemia.
Chemical and physical agents are believed to produce human leukemia, but
the relationship of a possible etiologic agent to an individual instance of hu-
man leukemia is usually uncertain.

In any study of tumors in general and of leukemia in particular, in which
an attempt is made to establish their possible viral origin, a number of criteria
have to be met before the viral etiology can be considered as established.
Not only the cell-free transmission of a particular type of leukemia has to be
carried out successfully, but also the viruses implicated in this transmission
have to be isolated and identified with the disease. Only then can the etiology
of the leukemia studied be clearly understood. Purification of animal viruses
and characterization of their properties require a number of methods which
are only useful if applied in a correlated and complementary sequence. Simi-
lar requirements apply to tumor-inducing viruses. These have been recently
discussed in an excellent review by Beard. Fractionation, quantitative and
analytic centrifugation, electrophoresis, electron microscopy of viral partic-
 spectra, each present difficulties of varying degrees which depend on
the physical and biologic properties of the examined viruses. Electron micro-
scopy alone cannot substitute for complete and convincing identification of
the viruses. It helps, however, in the characterization and assessment of the
centrifugal, electrophoretic and chemical procedures employed in identification
of viral agents. A good deal of literature on electron microscopy of viruses
has been reviewed by Bang. The following criteria for identification of viruses
seen in the electron microscope have been proposed in this review: (1) char-
acteristic appearance of individual and groups of particles; (2) association
of these particles with the infectivity of the preparation; (3) physical testing
(centrifugation, filtration) of the association of the observed particles with
infectivity; (4) association of the particles with other known activities of the
virus; (5) immune reactions between the particles and specific antisera; (6)
induction of specific lesions by a small number of particles. So far, only in a
few instances have all these criteria been fulfilled.

It is now known that many viruses, including some tumor-inducing viruses,
can be studied in cells grown in tissue culture. The cells and tissue culture
fluids can be used for the study of tumor-inducing viruses by the outlined
methods. Here again electron microscopy of these cells and tissue culture
fluids may be of real help.

A new approach to the study of viruses in general and tumor-inducing
viruses in particular has been provided by the comparatively recent method
of electron microscope study of ultrathin sections of the examined material.
In the past few years, electron microscopy of ultrathin sections has provided
a basis for what could be described as submicroscopic cytology of mammalian
cells.
The cytoplasmic membrane with details of its structure and composition, various characteristic cell surfaces, the structure of mitochondria, Golgi apparatus, the small particles or granules of the basophilic substance, the membrane system of the ergastoplasm, the structure of the nuclear membrane, nucleoplasm, nucleolus, mitotic figures and chromosomes have all been described in great detail. The various types of blood cells have also been examined and their characteristic structures visualized.

These studies made possible attempts at comparison between normal and malignant cells, which so far have failed to reveal qualitative differences between these two types of cells. Only quantitative differences were observed, and only one qualitative difference between a series of normal and malignant cells. In some mouse tumors, but not in all, extracellular (sarcoma 37) or intracytoplasmic (melanoma S-91) particles were observed within the size range of viruses. These particles found in the two transplantable mouse tumors were not observed in two other transplantable tumors, a lymphoma and a hepatoma. The only qualitative difference would therefore be related to an extrinsic factor present in these malignant tissues.

It is now known that in ultrathin sections, cells infected with a virus contain particles which usually but not always are spheroid or spherical in form and possess an electron dense core of varying shape and size, centrally or eccentrically placed, and a single or multiple peripheral membrane separated from the core by a less dense region. The particles may also have a core of low electron density. The size of viral particles varies from 400 Å to 5000 Å. From the size and the location (nuclear, cytoplasmic or extracellular) of the viral particles, an insight can be gained of the developmental stages of the viral agent examined as well as of its relationship to other nuclear or cytoplasmic constituents. The size of the particles of certain viruses may vary according to their location in the cell and stage of development. The particles of some viruses may also exhibit a different internal structure, according to their place of location.

Particles of certain size and characteristic structure have not only been observed in sections of cells infected with a number of viruses but also in sections of cells of a number of tumors of benign or malignant character. In addition, electron microscope studies have also revealed different host-virus relationships in the different viral infections, the reaction of cells and of their various constituents to the presence of the particular virus. It should be repeated at this point that the observation of virus-like particles in tumor tissues does not imply that these particles are agents specifically connected with the particular type of neoplasia. Additional criteria have to be met before a specific relationship can be established with any certainty.

There is no doubt that more valuable information about the size and structure of a number of viruses has been obtained by the study of sections of the infected cells than by an electron microscope study of suspensions of the particular viruses which had been shadowed by atoms of heavy metals. The study of viruses in thin sections of infected cells appears, therefore, to constitute a convenient first step in the study of viruses. Electron microscopy cannot
substitute for bio-assays, but is very useful if combined with the latter. It provides a background which should make possible the fulfillment of the majority of criteria for establishing the causal relationship between a viral particle and the specific infection. It certainly constitutes a novel approach to the study of viruses, their morphology, their life-cycle, and of the part played by different viruses in the life of the cell. The value of this approach is enhanced by the fact that it is possible to distinguish viral particles from other structures present in the uninfected control cells. This method provides also, perhaps, the only means at the moment available for a search of specific viral agents in tissues of the apparently healthy host. A stimulating application of the ultrathin section method in electron microscopy is the study of ultrathin sections of tumor cells of suspected or of unknown viral etiology in search for the presence of specific cellular structures resembling the structures of known viruses or for the presence of viral structures already observed in tumors of known viral origin.

In erythroblastosis, the only disease of the chicken leukosis complex so far studied in thin sections, characteristic virus particles have been observed. Virus-like particles have been described in preparations, coated with atoms of heavy metals, and obtained from the plasma of chickens with lymphomatosis, erythroblastosis and myeloblastosis. Their association with specific activity was established by differential centrifugation, electrophoresis and certain enzyme activity. Few or no particles were found in the plasma of control chickens. These studies, although of fundamental importance, do not permit an examination of the viral particles for their internal structure or of their relationship to various cell constituents in the tumor cells.

On the basis of all the available evidence, a series of electron microscope studies was initiated in an attempt to search for structures resembling viral agents in sections of tissues of animals with leukemia in which experimental proof was already available of the viral etiology.

Different forms of the chicken leukosis complex were, therefore, the initial object of this study. As a second step in this study, the examination of spontaneous and induced leukemia in mice was carried out. As an extension of these studies on leukemia, examination of some types of human leukemia was undertaken. At the outset it was realized that should these studies prove successful, they would constitute only the initial steps in attempts at characterization of the different viral agents encountered. At least some, and preferably all, criteria, already enumerated, should be met in any attempt at identification of virus-like particles seen in thin sections in the electron microscope.

It should be mentioned that these initial studies would not have been possible without the collaboration of a number of investigators. In collaboration with Dr. B. R. Burmester of the Regional Poultry Research Laboratory of the Agricultural Research Laboratory of the Agricultural Research Service, U. S. Department of Agriculture, East Lansing, Michigan, the study of chicken leukemia was undertaken. Leukemia in mice was studied in collaboration with Dr. L. Gross of the Cancer Research Unit, Veteran’s Administration Hospital, Bronx, New York, and Dr. L. W. Law of the National Cancer Institute,
Bethesda, Maryland. Studies on human leukemia have been initiated through
the collaboration of Drs. C. C. Shullenberger and C. D. Howe of the Depart-
ment of Medicine of the M. D. Anderson Hospital and Tumor Institute.

Some of the examined material required, when in 70 per cent alcohol, trans-
portation to the laboratory extending over a period of two to three days,
after fixation in 1 per cent osmic acid according to the method of Palade,53
modified by Rhodin,52 and followed by dehydration in successively stronger
alcohols up to the stage of 70 per cent alcohol. Storage of the material in 70
per cent alcohol was found to have no deleterious effect. All steps, from
the stage of the immersion of tissue in a mixture of equal volumes of 100 per cent
alcohol and methacrylates through the final stage of polymerization at 48° C.,
following addition of Luperco to the methacrylates, were carried out in
vacuum obtained by 15 pounds of negative pressure. Final embeddings of the
same consistency were thus obtained. For imbedding, a mixture of methacry-
lates, consisting of 1 part methyl and 6 parts of n-butyl methacrylates was
used in size “00” Eli Lilly gelatin capsules. Porter and Blum54 microtome with
mechanical advance mechanism (Servall) and glass knives were used for thin
sectioning. Sections were collected in 30 per cent acetone on specimen screens
covered with a thin film of collodion and thinly coated with evaporated car-
bon particles. An RCA EMU 3A electron microscope was used for study of
the sections of the different types of the examined material.

Preliminary results of electron microscope studies on the submicroscopic
structure of several neoplastic conditions of the chicken leukosis complex,
known to be transmitted by cell-free preparations, have either already been
reported13,14,17,18 or are in press.15,19,20

The neoplastic conditions of the fowl leukosis complex, which have been
studied, were lymphomatosis,5 erythroblastosis and granuloblastosis (myelo-
blastosis).2

For study of visceral lymphomatosis, material was used from chickens with
naturally occurring and induced disease. The donor birds were either field
cases of spontaneous lymphomatosis or had the disease induced by inocula-
tions of cell-free preparations from RPL-12 tumor or from affected livers of
chickens which had been inoculated with cell-free preparations of the chicken
transplantable RPL-12 tumor. This transmissible tumor was originally obtained
from a field case of visceral lymphomatosis.51 Spleen and liver from chickens
with either naturally occurring or induced visceral lymphomatosis showing
tumorous changes were studied in thin sections.

Burmester,5 early recognized several pathologic entities in chickens inocu-
lated with cell-free preparations of the RPL-12 tumor. Two of these have
been examined in the present study: (1) visceral lymphomatosis, characterized
by extravascular accumulation of neoplastic lymphoid elements, also termed
extravascular visceral lymphomatosis; (2) the form characterized by intra-
vascular involvement with features resembling erythroblastoid leukemia,5 and
classified as intravascular visceral lymphomatosis.9 In the light of more de-
tailed studies, to be reported shortly, the intravascular form should be called
erthroblastosis.9 On the suggestion of Dr. Burmester, the former form will
be termed visceral lymphomatosis and the latter form described as erythroblasto-
stasis - strain RPL-12.

The two other forms of the chicken leukemia complex studied were erythro-
blastosis, started by Engelbreth-Holm,25 and used extensively by Beard,2 and
granuloblastosis (myeloblastosis) originated by Hall, Bean and Pollard,38 and
also studied by Beard.2 In the study of these forms, spleen and liver were used
from chickens inoculated with plasma from donors with either erythroblastosis
or granuloblastosis, both originally obtained by Dr. B. R. Burmester from Dr.
J. W. Beard.

In all the examined tumors of the leukosis complex, whether visceral
lymphomatosis, erythroblastosis-Strain RPL-12, erythroblastosis-Beard, and
granuloblastosis, the tumorous cells in the spleen and liver showed various
stages of destruction of submicroscopic elements. Characteristically, the nu-
cleus was seldom found altered. The cytoplasm showed vacuolization, de-
genative changes and destruction of mitochondria and of ergastoplasm, ap-
pearance of dense osmiophilic bodies and breakdown of cytoplasmic mem-
banes. Characteristic structures resembling virus particles were observed to
form within these osmiophilic bodies and were also seen scattered in the
cytoplasm. These particles were also present in the intercellular spaces.

The frequency with which these changes were observed in all the forms
of the chicken leukemia was found to be similar. In erythroblastosis-strain
RPL-12 and visceral lymphomatosis, both naturally occurring and induced,
virus-like particles were observed with equal frequency within the inclusion-
like bodies and in intercellular spaces (figs. 1, 2, 3). In erythroblastosis-Beard
strain and granuloblastosis, virus-like particles were found in greater num-
ber within the cytoplasm of cells than outside the cells. Inclusion-like bodies ap-
peared to be more numerous than in erythroblastosis-RPL-12 or in visceral
lymphomatosis (figs. 4, 5a, 6a).

The virus-like structures observed in all the diseases examined are spherical
and have a similar appearance comprising a dense central core and an outer
lighter zone surrounded by a sharply delineated outer membrane. The average
size of virus-like particles in the different diseases was found to differ. It
measured 880Å in erythroblastosis-RPL-12 strain; 840Å in visceral lympho-
matosis; 670Å in erythroblastosis-Beard strain and 670Å in granuloblastosis.

It should be emphasized that size and appearance of viral-like particles
cannot constitute the sole basis of their identification or of differentiation be-
tween the viral agents. These observations, only if combined with biologic
and other tests, now in progress, can establish the etiologic relationship be-
tween the various forms of the chicken leukemia complex. In sections of pellets
obtained by differential centrifugation of cell-free preparations from visceral
lymphomatosis tumors, particles have been observed similar in size and appear-
ance to those observed in sections of organs from chickens with visceral
lymphomatosis.

Characteristic changes and structures resembling virus particles have been
observed in different forms of chicken leukemia known to be transmissible by
cell-free preparations. These could not be found in similar organs of young,
Fig. 1.—Part of a cell of tumorous spleen from a chicken with naturally occurring visceral lymphomatosis (extra-vascular visceral lymphomatosis). In the cytoplasm (CY) of the cell a number of vacuoles (V) with virus-like particles (VP) and an altered mitochondrion (M) can be seen. X 42,000.

control chickens of the same breed (so-called line 15.) While this difference may be only quantitative, it nevertheless appears to be significant. It is believed that the present studies will help in additional characterization of the agents responsible for chicken leukemia which had already been extensively studied by different means by Beard and his collaborators.
Fic. 2.—Break-down cells in a tumorous spleen from a chicken with erythroblastosis strain RPL-12 (so-called intra-vascular visceral lymphomatosis). In the cytoplasm (CY) mitochondria (M) in different stages of break-down can be seen; inclusion-like bodies (IB) with virus-like particles (VP) are present; virus-like particles are also seen scattered in the cytoplasm. Nucleus (N) of an adjoining cell may be seen. X 12,000.

In connection with the present observations, it may be of interest to mention the presence of viral activity in the cytoplasmic portion of cells in erythroblastosis.71 The ribose nucleic acid was also found to follow viral activity.71 These studies led to the conclusion that only the cytoplasm is connected with viral activity and may prove to be in agreement with the results of the present electron microscope studies of thin sections of erythroblastosis tumors.
Thus, in several forms of chicken leukemia, known to be transmissible, particles have been observed similar in shape and appearance to particles encountered in tissues infected by several known viruses. It should not be forgotten that visceral lymphomatosis is the only, so far known, tumor caused by a virus which is also contagious.

The results of electron microscope studies of organs of mice from strains...
Fig. 4.—Break-down cell surrounded by more normal-appearing cells of a tumorous spleen from a chicken with erythroblastosis-Beard strain. In the break-down cell virus-like particles (VP) may be seen and mitochondria (M) in different stages of break-down. The surrounding cells show nuclei (N), cytoplasm (CY) with mitochondria (M) and cellular membranes (CM). X 26,000.

characterized by a high incidence of spontaneous lymphatic leukemia have already been described.\textsuperscript{14,16,21} The results of studies of thin sections of leu-

cemic organs of mice with leukemia induced by cell-free preparations of the 

affected organs of mice with spontaneous lymphatic leukemia have also been
Fig. 5.—Tumorous cell of a spleen from a chicken with erythroblastosis—Beard strain. Cytoplasm (CY) showing vacuolization, with virus-like particles (VP) in vacuoles and in what may be degenerating mitochondria. A mitochondrion (M) with internal structure may be seen. Nucleus (N) of the cell. X 25,000.

Fig. 5a.—Part of the cytoplasm in figure 5 shown at higher magnification. Virus-like particles (VP) are seen. X 42,000.
FIG. 6.—Part of a cell of a tumorous spleen from a chicken with granuloblastosis (myeloblastosis). Inclusion-like body (IB) with virus-like particles (VP), some of which are present in the cytoplasm and vacuoles. Mitochondria (M) in different stages of break-down. X 34,000.

FIG. 6a.—Part of figure 6 at higher magnification, showing an inclusion-like body (IB) with virus-like particles. X 60,000.
reported. These results will be briefly described in an attempt at comparison with the changes encountered in leukemic organs of chickens. Some new evidence will also be presented, suggestive of the specificity of some of the changes observed.

Leukemic lymph nodes, thymus gland and spleen from mice of AKR, AKn and C58 strains with spontaneous leukemia, and similar organs from C3Hf Bi strain mice with leukemia induced by cell-free preparations were studied by the same method as that used in the study of chicken leukosis.

A number of changes of a varying degree were observed in the cytoplasm of malignant cells, the nucleus as a rule remaining either unaltered or showing comparatively small changes. The changes found in the cytoplasm consisted of vacuolization either due to the formation of vacuoles within the cytoplasm itself or due to changes in the ergastoplasm (endoplasmic reticulum) which showed disruption of its membranes; swelling and vacuolization of mitochondria, fragmentation of their cristae and their breakdown; formation of finely granular osmiophilic material within the mitochondria; appearance of large densely osmiophilic bodies; formation of what appeared to be inclusion bodies with the appearance of virus-like particles within these bodies; breakdown of cytoplasmic membranes. The structures resembling virus particles were found in varying numbers both within the cytoplasm and in the intercellular spaces (figs. 7, 8, 9).

These changes were found in leukemic organs of mice with both spontaneous and induced leukemia. The size and appearance of virus-like particles in these two types of lymphatic leukemia appeared similar. The particle size in spontaneous mouse leukemia ranged from 570Å to 1650Å, and in leukemia induced by cell-free material from leukemic organs of mice with spontaneous leukemia it ranged from 900Å to 1650Å. The average diameter of particles in spontaneous leukemia varied in different blocks of tissue from 670Å, 870Å, 950Å to 110Å. The average size of the internal dense core varied from 210Å to 690Å.

None of the changes were observed in the organs of young, 6 to 8 week-old mice from the same strains. Again, it should be pointed out that these differences may only be of quantitative but not qualitative character, as it is quite easy to miss structures of characteristic appearance in ultrathin sections if they are present in only small numbers within the cells.

Mice are known to carry a number of latent viruses, some of which are frequently encountered, like the LCM virus, other viruses, or hepatitis virus. The contamination with LCM virus, which could have easily concentrated preferentially in the tumor tissue, does not appear likely in view of our own studies (unpublished) and those carried out by Stewart et al. These studies showed no evidence that the leukemia inducing agent in cell-free preparations is an LCM virus.

Preliminary studies on x-ray induced leukemia in mice of the same strains, in which characteristic virus-like particles were found in leukemia induced by cell-free preparations, failed to reveal any characteristic changes.

An interesting aspect of the studies on cell-free transmission of lymphatic leukemia is the induction of salivary gland carcinomas in some of the in-
oculated mice, originally observed by Gross, and confirmed by other workers. On the basis of these studies, a conclusion was reached that the parotid gland tumor-inducing virus is a separate and distinct agent from the leukemia-inducing virus.

In an attempt to obtain a comparison between the submicroscopic appearance of leukemic organs of mice and of salivary gland carcinomas of mice,
Fig. 8.—Inclusion-like bodies (IB) with virus-like particles in a cell of an axillary lymph node from a mouse with spontaneous leukemia. Degenerating mitochondria (M) may be seen. X 86,000.

studies on ultrathin sections of carcinomas of the parotid gland were carried out. These studies will be reported in detail elsewhere. It should be mentioned, however, that while in organs of mice with spontaneous and induced leukemia, cells in various stages of breakdown were observed, comparatively few signs of cellular breakdown have been found in the parotid gland tumors. Alterations in the structure of mitochondria and occasional vacuoles in the cyto-
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Fig. 9.—Thymus tumor from a mouse with leukemia induced by a cell-free preparation from leukemic organs of a mouse with spontaneous leukemia. Break-down cell with virus-like particles (VP) surrounded by more normal-appearing cells showing nucleus (N), cytoplasm (CY) with degenerating mitochondria (M) and a large osmiophilic body (UB). X 24,000.

Plasm which were filled with homogeneous material and surrounded by dense osmiophilic material have been observed. Comparatively few changes have been observed in the ergastoplasm. Structures resembling virus particles were found mostly in the vacuoles of the cytoplasm and in the intercellular spaces.
These particles were similar in appearance to those observed in leukemic tissues; their size, however, was considerably smaller, between 650A-700A (fig. 10a).

The separation of the leukemia-inducing agent from the parotid gland

Fig. 10.—Parotid gland tumor induced in a mouse by a cell-free preparation from mouse leukemic organs. Cells showing nuclei (N), nucleolus (NU), mitochondria (M), endoplasmic reticulum (ER) and an osmiophilic body (OB) may be seen. X 11,000.

Fig. 10a.—Virus-like particles in a parotid gland tumor. X 42,000.
tumor-inducing agent by means of ultracentrifugation and ultrafiltration, as well as by other means, has been reported by Gross.\textsuperscript{33,37} Preliminary studies of ultrathin sections of centrifugal pellets from cell-free extracts of leukemic organs were therefore carried out. In such pellets, obtained after centrifugation of leukemic tissue extracts at 125,000 x g for 30 minutes, preceded by centrifugation at 1,400 x g and 7,000 x g (for 15 minutes), particles were observed of the size of particles found in parotid gland tumors (fig. 11). Although these pellets still contain a considerable amount of tissue debris, it appears encouraging that the supernate from which the pellets were obtained has, so far, induced only parotid gland tumors in suitable test mice. The size of the virus-like particles observed in the pellets obtained by ultracentrifugation is in fairly close agreement with that of the biologically active particles calculated on the basis of ultrafiltration studies.\textsuperscript{37} Further studies will have to be carried out to correlate the parotid gland tumor-inducing activity with the structures observed in the centrifugal pellets and in sections of parotid gland tumors.

These studies, although preliminary in nature, indicate that electron microscopy of thin sections, if combined with biologic and biophysical tests, may help to solve the question of whether in the cell-free transmission of mouse

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig11.png}
\caption{Section of a pellet obtained by differential high-speed centrifugation of a cell-free preparation from mouse leukemic organs. Virus-like particles (VP) are present surrounded by a still considerable amount of cell debris. X 90,000.}
\end{figure}
leukemia an exogenous viral factor or a viral-like endogenous factor plays a part. They may also help in characterization of the different agents which may be involved in the whole range of biologic activity of cell-free extracts of leukemic tissues. The observation of viral-like structures in the affected tissues must be followed by isolation, purification of these particles and correlation of tumor-inducing activity with characteristic particles observed in the different types of leukemia.

In view of the observations on chicken leukosis and mouse leukemia, preliminary studies have been carried out on acute lymphatic leukemia in man (Dmochowski, Grey, Shullenberger and Howe). In the cells of the affected lymph nodes from two untreated cases of lymphatic leukemia, a number of submicroscopic changes were observed. They appear to be similar to those already observed in chicken leukosis and mouse leukemia. Vacuolization of the cytoplasm itself, vacuoles due to the swelling of ergastoplasm, various stages of breakdown of mitochondria, appearance of large unidentified densely osmiophilic bodies and of smaller inclusion-like bodies were observed in the cytoplasm of the cells of the affected lymph nodes. Within these inclusion-like bodies and in the intercellular spaces virus-like particles have been found of approximately 800Å-1000Å diameter (figs. 12a, 13). Additional studies of the sections of biopsy material from other cases are now in progress, combined with other procedures, in an attempt to elucidate the nature of the observed changes. As in the case of mouse leukemia, the observed virus-like particles, if indeed they are a virus, may represent a latent or passenger virus not connected with leukemia.

While the studies on the nature of virus-like particles in the different types of leukemia constitute the main problem, the question of the nature of degenerative changes observed in the leukemic cells poses an additional question. Changes in the cytoplasm, similar to those observed in the cells of the leukemias studied, have been described in HeLa cells grown in vitro and classified as nonspecific. Furthermore, changes in the nucleus of HeLa cells grown in tissue culture, such as shrinkage of the nucleus, karyolysis, fragmentation, have also been observed as signs of nonspecific cell degeneration and not characteristic of a viral infection. Similar changes have also been observed in cells subjected to starvation. Nevertheless, the frequency and order of certain changes observed in ultrathin sections of cells may be suggestive of a viral infection.

In the present study, none of the changes observed in the leukemic cells have been observed in cells of organs from young, healthy chickens and mice. Similar studies on control human material will have to be carried out. On the basis of the available evidence, it appears that changes in cells infected with a number of contagious viruses are similar to those in cells subjected to various types of unfavorable environmental factors. Similar changes have been found in cells of several types of chicken and mouse leukemia and also human leukemia. The nature of these changes will have to be investigated. Some of these changes in the cells appear to be similar to changes described as developmental stages of viruses in certain known viral infections.
Fig. 12.—Cervical lymph node from a patient with acute lymphatic leukemia. Breakdown cells with cytoplasm (CY) showing mitochondria (M) in different stages of degeneration and virus-like particles (VP) in inclusion-like bodies (IB). Nucleus (N) of a cell may be seen. X 18,000.

Fig. 12a.—Part of figure 12 at higher magnification, showing an inclusion-like body (IB) with virus-like particles and a degenerating mitochondrion (M). X 42,000.
Fig. 13.—Cervical lymph node from a case of acute lymphatic leukemia. Break-down cell with degenerating mitochondrion (M) and virus-like particles (VP). On the left a normal appearing cell with nucleus (N) and cytoplasm (CY). X 43,000.

It appears that sufficient ground is now available for further study of leukemias of known viral origin and those of unknown etiology. Investigations of morphologic properties of virus-like particles must be combined with studies of their biologic as well as of other properties. Evidence, so far obtained, supports the hope that electron microscopy of sectioned virus-like
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particles may be of help not only as an initial step in the search for specific agents in neoplasia but also in all other methods required to establish the relationship between these virus-like structures and specific tumor-inducing activity.

SUMMARIO IN INTERLINGUA

Es presentate un revista del litteratura relative al transmission non-cellular de leucemias in animales experimental. Es reportate studios utiuisante le relativemente nove methodo del microscopia electronic de sectiones ultratenu, i.e. del cytologia submicroscopic de cellulas mammalian. Nulle del resultatos usque nunc obtenite es conclusive. Es formulate le opinion que le methodo, combine con tests biologic e biophysic, promitte resolver le question de si le transmission non-cellular de leucemia depende de un exogene factor viral o de un endogene factor virusoide. Es signalate le facto que—a parte le question del natura del particulas viral o virusoide in le varie typos de leucemia—etiam le problema del alterationes degeneratori observate in cellulas leucemic debe esser investigate.

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Studies on Submicroscopic Structure of Leukemias of Known or Suspected Viral Origin: a Review

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