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March-April 1959
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Princeton N.J.

"Whether all viruses multiply in exactly the same manner as 'phage is not entirely clear but available evidence indicates that they behave in a similar or analogous manner. Like phage, they all behave like transmissible mutagenic agents; all (with the possible exception of some large viruses) have only a reproductive physiology; all multiply only inside of those living cells, producing death and disintegration or mutation, directing the synthetic mechanisms of the infected cell to the making of virus substance. Animal viruses, like 'phage, show a latent period, varying from minutes to hours, in various systems." --M. Frobisher, Fundamentals of Microbiology, 72, W.B. Saunders Co., Philadelphia, (1958).

It may appear strange that we should begin with a quotation. However, this particular one takes us to the heart of the matter: All viruses seem to multiply in a similar or analogous manner. With this in mind we propose the following statement: ALL VIRUSES REPRODUCE IN BACTERIA! Since we know that viruses ('phages) can and do reproduce in bacteria, let us say that viruses can reproduce only in bacteria. (What justification is there for this statement? Namely, that we may be led by it to account for the facts and make some verifiable predictions, or else led by its error to a better hypothesis.)

This hypothesis seems to be stymied when it comes to explaining how plant, animal and insect viruses reproduce. Electron microscope studies clearly show that viruses are found within the plant, animal or insect cells. We could get around this by suggesting that bacteria are present amongst the tissue cells, and that the viruses enter the cells after reproducing in bacteria. However, this idea falls down when we realize that viruses are cultivated (multiply) in tissue cultures in which bacteria have been inactivated by antibiotics or other means. For example, polio virus is cultured in bacteria-free monkey kidney tissue preparations. Thus it seems that the original hypothesis is defeated. Surely, all viruses cannot reproduce in bacteria. (But! We are not yet entirely defeated.) We have only eliminated the multiplication of viruses by EXTRACELLULAR bacteria. We have not eliminated this possibility for INTRACELLULAR bacteria. It is now apparent that we must make a big assumption in order to retain the original hypothesis. We must assume that there are intracellular bacteria.

In order to look for these bacteria, a definition is in order. "Probably the best positive definition of the bacteria that can be made at present would run as follows. The bacteria are a morphologically varied collection of small microorganisms with a primitive cellular organization, like that of the blue-green algae. Most of them are non-photosynthetic. The photosynthetic representatives differ from blue-green algae physiologically, for they carry out a special kind of photosynthesis in which oxygen is never evolved." --Douderoff, Stanier, and Adelberg, The Microbial World, 106, Prentice Hall Inc., Engelwood Cliffs, N.J., (1957).

Since we require the presence of these bacteria in the cells of a multitude of organisms (perhaps even one-celled organisms other than bacteria) it would be inconceivable to think that there are bacteria present which have escaped detection. These bacteria are then necessarily of a curious kind--they have been discovered and yet remain undiscovered. Scrutiny of cellular components 'universally' found in cells is limited by our definition of bacteria. Also, it would seem that we require a

component not restricted in location to either cytoplasm or nucleus, for viruses have been observed in both areas. Therefore, we need a specific cellular component which has some freedom of motion within the cell or several components found in both areas. Also, if we are looking for bacteria, we might be suspicious particularly of moving bodies anyway.

We might now venture a guess as to which cellular component might be accused. In animal cells, it is the MITOCHONDRIA THAT SEEM TO SATISFY THE CONDITIONS OUTLINED THUS FAR. They are present in a multitude of organisms; they have some freedom of motion within the cell; and they fit our definition of bacteria. This last point might be disputed on the grounds that mitochondria are not microorganisms with a primitive cellular organization, as the definition requires. However, such a dispute would find its basis in turn in the definitions of 'microorganism' and 'primitive cell', and in this regard it can be said that mitochondria are within the size range of some bacteria, and that electron microscopy has recently revealed the membranous structure of the general mitochondrion; one which could be labelled as a primitive cellular organization. (See Fig. 1). Another important criterion to be used in deciding whether a cellular component could be considered a 'microorganism' is that concerning the mode of reproduction of the cellular component. Strikingly, both animal and plant mitochondria, like bacteria, appear to be self-replicating bodies. Further, it has been established that this division process is not necessarily coordinated with the cell's nuclear division. This apparent independence in reduplicating argues for a division apparatus incorporated within the mitochondria.

So far we have pointed to the mitochondria as the bacteria of the animal cell. But what about plants? What component of normal plant cells will fit the criteria thus far laid down? The obvious component is again the mitochondria, as we mentioned earlier. However, there is a second component which also might be accused. Going back to our definition of bacteria, we find that "most of them are non-photosynthetic. The photosynthetic representatives differ from blue-green algae physiologically, for they carry out a special kind of photosynthesis in which oxygen is never evolved." Briefly, the similarity is seen in the following equations:

Green Plants: $CO_2 + 2H_2O \longrightarrow CH_2O + H_2O + O_2$

Sulfur Bacteria: $CO_2 + H_2O + 2H_2S \longrightarrow CH_2O + 2H_2O + 2S$

However, the remarkable thing is not just that these equations are similar, but that photosynthesis is common to both plants and bacteria. That such a process should occur in two places in nature is not so remarkable, however, when we realize that it is not the plant cells themselves that are doing the photosynthesizing, but rather a plant cell component. This is of course, the CHLOROPLAST, WHICH IS PRESENT WHEREVER PLANTS ARE FOUND. The chloroplast, like the mitochondrion suits our definition of bacteria in that it is within the size range of bacteria and has a 'primitive cellular organization' bearing a distinct relationship to the mitochondrion. Chloroplasts also seem to have a division apparatus comparable to mitochondria since chloroplasts often differentiate from ? ← ? plant mitochondria. (See Fig. 2).

Thus we maintain our original hypothesis, namely that "all viruses reproduce in bacteria", by including the mitochondria and chloroplasts under the heading 'bacteria'. We will show, in the following arguments, why we feel justified in doing so.

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We shall now attack the problem of virus-cell interaction in a different way, namely, by focusing on evolution. We will have to adopt one of two roads immediately. Either mitochondria evolved from materials inside the cell, or they evolved from materials outside the cell. To us, the second possibility seems the more probable because mitochondria are self-replicating units with characteristic genetic content and a distinct membranous structure which is not fixed within the cell but rather appears to move freely through the cytoplasm. On this basis we suggest that MITOCHONDRIA AND BACTERIA HAD DIRECT COMMON ANCESTRY FROM POPULATIONS OF 'PRIMITIVE CELLS'. Further, we also state that CHLOROPLASTS had common ancestry with these primitive populations.

Some of the smaller population members(primitive bacteria) found good living conditions within the larger members and a mutualistic relationship developed and was selected for in the evolution of the larger cell types. The primitive bacteria adapted to their new envirnment and as a result lost many functions which were no longer of use to them and which were of no evolutionary advantage to their host. Further, the interplay between two genetic systems PROVIDED A GREAT POTENTIAL FOR VARIATION. This interplay made possible the development of multicellular organisms from one-celled and colonial forms. Both systems are passed on from generation to generation in an integrated manner. Thus, the mitochondria and chloroplasts in cells today appear to function as vital organelles, and indeed they are vital in that many of their capacities were exploited in the course of evolution.

Perhaps it is time to stop speculating about the occurrence of such events, and see just how feasible this plan is. There is no need to question the general concept of mutualism, as it is a wide spread natural phenomenon. In its extreme would be found a relation such as that between man and his crop plants, while in its more restricted use, a relation such as that found today in Hydra which contain, within certain cells, photosynthetic algae. In this latter case, the Hydra benefits by gaining carbohydrates, while the protist in turn receives water, nutrients, and 'shelter'. Now exactly what is the difference between this situation and that of the chloroplasts in plants? If we throw away for the moment the traditional concept of the chloroplasts as an endogenous part of the cell, we will see that there is no difference. But! Is there not a big difference in the way algae and chloroplasts are passed from generation to generation? The algae are passed on from 'parent' to 'child' by entering eggs as they are extruded, whereas the chloroplasts develop from the nucleus by differentiation from other cell constituents under control of the nucleus. We are back to the question of origin, here, the immediate origin of chloroplasts. On the basis of our hypothesis, we might expect that these pre-chloroplast constituents would maintain an independence even in the seed--and there is some evidence that they do! Chloroplasts develop from small cytoplasmic particles included in the seed, and their continuity from generation to generation depends on their ability to form these 'spore-like' particles. This ability of chloroplasts to form spores parallels the ability of several bacterial species to form spores as a part of their life cycle.

It is apparent that the hypothesis that chloroplasts, mitochondria, and bacteria have common ancestry must lead us to change somewhat our conception of the nature of living organisms. For with regard to plants, nature utilized the potential that lay ready in the form of photosynthesizing 'primitive bacteria' to build larger and more complex living structures which displayed these bacteria in great numbers and to great advantage. However, a problem may now arise. Mutualism means a mutually beneficial relationship between organisms. Here though, it is apparent that since chloroplasts and mitochondria function so intimately with the rest of the cell that they are considered to be cell parts, these structures are heavily responsible for the evolution of plants in the first place. Thus we must stretch the meaning of mutualism somewhat in that the participants of the system can no longer get along successfully without each other.

Thus far we have ignored the mitochondria somewhat. Mitochondria participate mutually also, but in a more subtle manner. They are best known as centers of enzymic activity within the cell. Further some investigators believe that the enzymes found within mitochondria initiate cytokinesis. Their biochemistry will be discussed in more detail later on.

One point left untouched is the mechanism whereby one organism should get into another, as we have suggested by having primitive bacteria within primitive larger cells. One reasonable way we could propose is that the larger cells ingested the smaller, but in this case did not digest them. Such a possibility is supported by observation. (See Fig.3) The bacteria in this case appear to help in digestion within the flagellate. Here then is a case of bacteria living within a living cell, a flagellate. A similar situation is proposed for the origin of chloroplasts and mitochondria.

Thus it is with this evolutionary argument that we justify the claim that chloroplasts and mitochondria may be included under the overall heading of bacteria.

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If mitochondria, chloroplasts, and bacteria are shown to have some properties in common, and we hypothesize that they have a direct ancestry, we might search with confidence for other properties held in common. Therefore we might logically look at the mitochondria and chloroplasts as the bodies in which viruses reproduce since one well-known property of bacteria is their ability to reproduce viruses.

Since we intend to deal particularly with the problem of virus reproduction, it would be appropriate to pose the problem: What is a virus? The most readily studied viruses are those which infect bacteria, namely the bacteriophages. So we shall start by trying to understand the nature of the bacteriophage-bacterium relationship. Probably the major characteristic of this relationship is its <u>specificity</u>. Certain viruses infect certain bacteria and cannot infect others unless a mutation of the virus or bacterium occurs. If we can explain this specificity, we probably shall have gone a long way toward discovering the nature of the relationship.

The pictures of phages 'attacking' a bacterium strikingly resemble pictures of sperm 'attacking' an egg, and an individual phage itself has a certain resemblance to a sperm. Resemblance alone however is certainly little basis on which to draw a connection; we can say only that these are

analogies. However, in a certain light these relations may be viewed as more than analogies. Let us call a bacteriophage a degenerate sperm. As a sperm, it should be incapable of self reproduction in isolation, which it is. Now let us call the bacterium an elaborate egg. As an egg it should also be incapable of isolated self reproduction, which it is not. Here, parthenogenesis seems to be the rule rather than the exception. While the 'analogy' fails somewhat at this point, it still may be carried further.

What we have suggested is that the 'phage-bacterium relationship is a type of sexual relationship in which the phage would demand a specific host just as a sperm demands a specific egg. When the phage contents (genetic message--DNA) enters a bacterium two things can happen:

1. New phages may be produced which escape the host through lysis. This may be likened to spermatogenesis, in which the explosion of phages is really only the phenomenon corresponding to liberation of sperm by meiosis.
2. The genetic content of the phage complements that of the bacterium giving prophage. This may be likened to fertilization, just as the genetic content of the sperm complements that of an egg. When the sperm contents attaches to the corresponding and recipient structures in the egg, the sperm constituents are replicated faithfully in all of the progeny of that egg cell. The same can be said of the prophage phenomenon. We need only substitute the word bacterium for egg, and 'phage for sperm. In the multicellular organism this replication continues until some of the progeny of the original fertilized egg separate out as the sperm or egg contribution for succeeding generations. Here recombination of genetic material can give rise to different eggs or sperms. This is also typical of the conversion of prophage to lysogenic phage in which some of the phages (spermlike) are like the original genetically, and some are not. Here is what M. Frobisher and W. Weidel have to say on the subject:

> "The essential feature of fertilization in all forms of life exhibiting sex is at the microscopic, single-cell level. It is the complete fusion of the nucleus of a haploid, male gamete with a haploid female gamete. A diploid cell results. This, in essence, is true for the simplest sexually reproducing protozoan or fungus as it is for violets, human beings, or whales. The essential event is the transferrence of genetic DNA from male to female gamete. The male DNA enters the genetic mechanism of the female cell, becomes self-replicating and contributes to the genetic character of the resulting diploid cells. Does this suggest infection with a virus? Transduction? Transformation with DNA? Kappa in Paramecium, etc.?
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> We may if we wish, for purposes of argument, regard sex in higher animals as an evolved, improved, very selective, very certain means of transmitting certain, particular, genetic material from one particular cell to another particular cell. On the unicellular level of protozoa, yeasts, etc., simpler and less perfected means exist: simple cell fusion or conjugations, without differentiation of male and female. At the still lower level of Bacteria and viruses, the mere carrying of a bit of genetic DNA by any hit-or-miss, catch-as-catch-can means to any 'competent' cell in the vicinity appears to suffice: by 'phage, by mere bathing of the cell in a solution of sterile DNA, by agents like Sigma, etc."
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> --M. Frobisher, Fundamentals of Microbiology, 220.

Further Weidel has the following to add to Frobisher's inferred sexuality.

> "Yet adsorption/penetration of a phage particle is just one example of how nature solves a ubiquitous problem: for biologic entities of all sorts, it is essential to establish specific and durable surface contacts to others as a prerequisite for uni-or bilateral exchanges of material or even for complete fusion. The infection mechanism of a phage particle is thus bearing the marks of what would be called a typically sexual process in more obvious cases."
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> "Chemically defined macromolecular complexes serve as mediators for 'sexual' attachment as well as for virus attachment. Whether these complexes are called receptor substances in the latter case or gamones in the first, functionally they must be considered as belonging to the same class."
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> "Establishment of specific surface contacts is always a matter of two, possibly complementary, structures participating in the binding reaction. In the case of bacterial viruses, one is of bacterial origin, and the other is part of the anatomy of the phage particle." --W.Weidel, Bacterial Viruses, Ann. Rev. Microbiol. (1958).

S.E. Luria also has commented on the resemblance of viral infection to sexual fertilization. --Protoplasmatologia, Band IV,52,(1958).

Let us adopt the sexual scheme temporarily, since it offers a rather simple description of what might otherwise appear to be a complicated and perplexing phenomenon. We see that from this point of view, a bacterium plays a part in the phage life cycle, and the phage plays a part in the bacterial life cycle. The two cycles are intimately related.

Now, since we have been talking mainly about phage, perhaps it is time to see how viruses in general fit into the picture. If Weidel's 'less obvious' sexual process and Frobisher's 'lower' sexual process are truly more than analogies with higher sexual processes, then they should hold for all viruses, not just bacteriophages. But what do we mean by 'lower'? Bacteria, as our definition admits, have a primitive constitution. We call it primitive because it seems so by comparison with other cells, like the cells of multicellular plants and animals, and thus we come to the conclusion that bacteria are a 'lower' form of life, maintaining this lower sexuality. Now, if a phage, as we have maintained, is truly a part of the sexual life cycle of a bacterium, or was a more legitimate part before degeneration to a parasitic nature, then viruses in general must somehow be a part of the sexual life cycles of plant, animal and insect cells. BUT WHAT IS SUCH A LOW FORM OF SEXUALITY DOING IN SUCH HIGH FORMS OF LIFE??

We intend to resolve this paradox by claiming that there is a certain part of plant, animal, and insect cells, which is 'lower', and with which viruses enjoy their sexual relations. But sexual relations implies that genetic materials are involved--DNA,RNA--and since these are in the nucleus of the plant and animal cells, shall we not have to claim that the sacred nucleus of these cells is 'lower'? No, there is another alternative and one which perhaps makes up feel better. We need only claim that the genetic materials required are in the cytoplasm. In plants the most obvious extranuclear vehicle of heredity is the CHLOROPLAST. MITOCHONDRIA are also found in plants although they appear to be more prominent in animals.

Both mitochondria and chloroplasts appear to contain RNA and possibly DNA. Further, recent studies by DuBuy and Wood have shown that plant mitochondria can mutate to become unusual chloroplasts. Furthermore, both mitochondria and chloroplasts exhibit what might be called a 'lower' structure. (See Fig.1,2)

Thus we contend that in plant and animal cells, all viruses maintain their sexual cycle with chloroplasts, mitochondria, or derivatives of these structures.

We might wonder why, if mitochondria and chloroplasts contain a self replicating material --RNA and/or DNA, and are able to mutate, they might not have changed in the course of evolution so as to become unsusceptible to viruses, thereby safeguarding the organisms in which they are found. Here are three possible answers to this question:

1. Mitochondria and chloroplasts have not given up this capacity for the same reason that extracellular bacteria have not given it up. It appears that this reason is found in the intimacy of the bacterium-virus relationship. Furthermore, the phenomenon known as prophage suggests that the bacterial division (we would use 'mitotic' but there is a current dispute over bacterial division and recombination) apparatus is used, just as a sperm uses that of an egg, so that we might be asking the bacterium, chloroplast or mitochondria to give up or completely modify its division mechanism. The accomplishment of such a feat would seem unlikely.
2. Capacities for viral synthesis might go hand in hand with capacities for synthesis in general. For example, in Streptomyces (bacteria-like fungi), antibiotic --streptomycin--production appears to be related to viral reproduction and release of the phage particles. These capacities in the case of chloroplasts and mitochondria are exploited and integrated into the organismal system; they cannot be given up.
3. Some mitochondria and chloroplasts may presently be evolving in a direction that will lead to loss of virus reproducing capacity, or perhaps some organelles have already lost this ability to promote viral reproduction.

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SUMMARY

The bacterium-bacteriophage relation is to be considered a bacterium-virus relation. While in the conventional literature, a phage is regarded as a virus, a virus is not a phage. We wish to state otherwise, by maintaining that all viruses operate in the same way that phages do, namely through specific action on and in a bacterium chloroplast, or mitochondria.

There are two prevalent hypotheses: One says that there are phages, animal viruses, plant viruses, and insect viruses. The other says that there is not enough difference in the mode of reproduction of these four types to classify them as such, but that they are all just plain viruses, operating on bacteria. The second hypothesis suggests that terminology alone might cloud the field of virology. For example, we may call a bacteriophage a parasite, but when a seed-bearing pod bursts open, we do not call the seed parasites of the pod. Nor do we state a burst size for the particular species of plant, which like the burst size of a bacterium, is probably fairly constant. Nor do we say that the pod is lysed.

The second hypothesis is the simpler of the two and should be adopted before the first for this reason, at least until it is thoroughly disproven by experiment.

Viruses reproduce in host bacteria, chloroplasts, or mitochondria. They are restricted by host specificity. This applies to bacteriophages and the so called plant, animal, and insect viruses(including the tumor viruses). That viruses should reproduce in mitochondria and chloroplasts is understandable in the light of the mutualistic theory of plastid origin.

We have found no reasonable basis or experimental evidence supporting the view that viruses reproduce in cell nuclei. However, we have found some evidence as well as several theoretical schemes to support our view that reproduction of viruses occurs in chloroplasts, mitochondria, or bacteria. This evidence follows.

I. In this section we intend to show several similarities between mitochondria, chloroplasts, and bacteria, as found in the literature.

A. Mitochondrial and chloroplast activity in isolation from the rest of the cell.

1. Leon, H.A., S.F.Cook Mitochondrial reduplication in vitro, Science 124, 123, (1956).

" Ephrussi and others advocate cytoplasmic genetic continuity (of mitochondria), thus requiring autoduplication of these cytoplasmic elements. Such a process was reported for mitochondria in 1910 by Faure-Fremiet. In the course of some studies we performed on the morphology of mitochondria, this phenomenon of self-duplication was also found to occur in vitro."

2. Kardos, J. and R. Kis Bagoly, Studies of the extracellular developmental possibilities of mitochondria in the cells of plants, Acta. Biol. Acad. Sci. Hungaricae 6 (½), 19-30, (1955)

"What were believed to be mitochondria broke into rods, coccus, etc. forms, and moved in various directions. The question as to whether the bodies are microorganisms or mitochondria is briefly discussed.

3. Allen, M.B., F.R.Whatley, and D.I.Arnon, Photosynthesis by isolated chloroplasts , Biochim. et Biophysica Acta. 27, 16 (1958)

B. The action of antibiotics on mitochondria and chloroplasts.

1. Loomis, W.F., On the mechanism of action of Aureomycin, Science 111 474, (1950).

It was observed that low concentrations of aureomycin depressed phosphorylation in normal mitochondria without inhibiting respiration.

2. Van Meter, J.C., and J.J.Oleson, Effect of aureomycin on the respiration of normal rat liver homogenates, Science 113, 273, (1951)

Evidence is presented showing that aureomycin causes an inhibition of respiration of whole rat-liver homogenates-- possibly may block some part of the Krebs tricarboxylic acid cycle.

3. De Deken-Grenson, M., and S.Messin, The genetic continuity of Euglena chloroplasts. I. Mechanism of the appearance of white clones in streptomycin-treated cultures Biochim. et Biophys. Acta, 27 (1) 145, (1958).

Streptomycin selectively decreases the growth rate of the chloroplasts which, when lost cannot be formed de novo. (note the bacteriostatic action similarity)

4. Vavra, J., The action of streptomycin on chloroplasts of the flagellate Euglena gracilis Klebs., Folia Biol., 3 (2), 108 (1957)

The decrease in the size of the plastids under the influence of streptomycin is explained as beind due to the loss of the ability of the plastids, during division, to continue growing.

5. Robbins, W.J., A.Hervey, and M.E.Stebbins, Euglena and vitamin B 12 , Ann. N.Y.Acad. Sci., 56, 818, (1953).

Streptomycin in sufficient amounts produced permanently bleached strains of Euglena. Intermediate amounts of the drug produced pale green and yellow strains. Bleached strains were also produced by aureomycin and by growth in the dark at elevated temps.

C. Viruses associated with mitochondria and chloroplasts.

1. Sukhov, K.S., and G.S. Nikifarova, Crystalline inclusions of tobacco mosaic virus in the plastids of mosaic tobacco, Doklady Akad. Nauk. SSSR, 104 (5) 786, (1955).

Washed chloroplasts of mosaic tobacco were studied with the electron microscope. Crystalline aggregates of virus were observed, which suggests that they can reproduce in these organs.

2. Leyon, H., Virus formation in chloroplasts, Exptl. Cell Res. 4, 362 (1953)

"Examination with the electron microscope has revealed that the filamentous particles characteristic of leaves infected with beet yellows virus are frequently found in association with chloroplasts, and appear to have been extruded from them. Similar investigations have been carried out on tobacco mosaic virus. The assumption is made that at least some viruses are formed within chloroplasts."

3. Ackermann, W.W. and H.Kurtz, The relation of Herpes virus to host cell mitochondria, J. Exptl. Med., 96, 151, (1952)

"It has been demonstrated previously that certain of the oxidative reactions of the Krebs cycle which are localized in the mitochondria are essential to the propagation of influenza virus. Furthermore, changes in these activities have been observed in tissues infected with herpes virus (both by author--Ackermann). The data reported here shows existence not only of a biochemical but also a physical relationship of this virus to mitochondria. The interpretation is tentatively advanced that these organelles are a site of viral synthesis in the cell. The virus found in the cytoplasm may result from a deterioration of the mitochondria at the time of viral maturation. Data indicate that in livers infected with herpes virus, there is a selective destruction or deterioration of mitochondria.

4. Dempsey, E.W., Variations in the structure of mitochondria, J.Biophys. and Biochem. Cytol. 2 (4), Suppl. 305, (1956).

"A final instance should be noted in which mitochondria are altered in cells, the metabolism of which has been changed. Luse and Smith (unpublished data) have infected splenic cells in mice by injecting massive doses of salivary gland virus intraperitoneally. They have also studied human fibroblasts grown in tissue culture after infection with virus. In both instances, the cells enlarge and exhibit an increased number of mitochondria, with frequent "open" forms, at times just prior to the appearance of recognizable viral bodies within the cell."

D. Viruses, mitochondria, and chloroplasts associated with pathology.

1. Huxley, J., Biological Aspects of Cancer , Harcourt,Brace, & Co. New York, (1958).

---NOTE-- In the following quotations we would particularly like to point to Huxley's analogy of tumor-virus masking to alteration of prophage, as being more than an analogy. Viruses well may be capable of maintaining a "prophage" or "latent" state within the mitochondria and chloroplasts if one considers the bacterial nature of the mitochondria and chloroplasts, and this may be significant in connection with the differences between a viral infection such as polio, and the development of a cancer by virus. Possibly, in the former, viral replication takes place within the mitochondria in a similar fashion to phage replication, while in the latter, the virus assumes the latent "prophage" form which permanently mutates the mitochondria so as to produce the abnormal cellular functions distinguished as cancer.

p.71 "The fact that they (tumor viruses) must primarily affect the cytoplasm is important, and points the way to further research."
p.75 "The closeness of association between virus particles and host cytoplasm can vary greatly. The virus is more readily liberated from slow-growing benign tumors. Increased growth rate and malignancy increase its 'masking'--ie, the closeness of its associations with host tissues-- and diminish its freedom."
p.86 "The analogy between the masking and unmasking of tumor viruses and the alternation of prophage and active phage virus is striking."
"In all lysogenic strains of bacteria occasional individuals will be lysed; in them the prophage becomes 'unmasked' or detached, and proceeds to multiply disproportionately in the usual way.Environmental agencies can 'induce' active phage from prophage; eg., ultra-violet irradiation somehow detaches certain prophages from the bacterial genome and frees them for replication."

2. Frobisher, M., Fundamentals of Microbiology, W.B.Saunders Co. Philadelphia...London, (1957)

p.586 "It has been suggested, on reasonable grounds, that the true role of carcinogenic agents is to induce cancer viruses which may, like 'phage in lysogenic bacteria, be latent in the tissue cells of certain people."

3. Bernhard, W. Electron Microscopy of tumor cells and tumor viruses, a review, Cancer Res. 18, 491, (1958)

"The general impression is that cancers have fewer mitochondria than normal cells. This is only a general rule, and it is easy to find exceptions to it." "As mitochondria in tumor cells often appear swollen, the density decreases rapidly and the characteristic double membrane of their outer walls and cristae may disappear completely. The swelling of mitochondria, preceded by ultrastructural defects, is very frequently encountered." "It would be of great interest to know whether injured and swollen mitochondria in cancer cells are linked with the malignant process and represent a primary lesion, or if these defects are only the result of the rapid aging and degeneration of these cells, or of poor nutrition and respiration, very common in necrobiotic areas of tumor tissues." "The author has observed many tumor cells which were well preserved in all respects and probably very actively growing when being fixed; the only visible lesion was the swelling of some of their mitochondria." "An apparently opposite process is observed in many tumor cells: tumor mitochondria are not swollen, but, on the contrary, may be much denser and smaller than normal." "Among these small and dense mitochondria one finds all intermediate aspects between typical mitochondria and dense, spherical formations which have been called "microbodies"-- very dense particles without any visible inner structure and limited by a single membrane. They have been considered as precursors of mitochondria." "Ferritin-hemosiderin granules can be found within microbodies in pathological paraerythroblasts of fowl erythroblastosis. Even viruses may be observed within some mitochondria of this disease. Whether the agent as such has penetrated incidentally or whether it has been formed in a matrix, filling previously the mitochondrial body, is still unknown. Summarizing all the observations reported on tumor mitochondria, one is struck by the extraordinary variations in their number, size, form, and density, and by the frequent lesions they present."

4. Dounce, A.L., M.P. O'Connell, and K.J.Monty, Action of mitochondrial DNAase I in destroying the capacity of isolated cell nuclei to form gels. J. of Biophys. and Biochem. Cytol. 3 649,(1957)

"The work reported in this paper shows what mitochondrial DNAase I can do to the nucleoprotein of cell mitochondria upon breakdown of compartmentalization of this enzyme within the mitochondria. The firm binding of DNA to chromosomal protein is broken by the DNAase and some depolymerization of the DNA occurs. In view of the probable genetic role of DNA such effects could be expected to cause irreversible damage to the cell. Anything that tended to cause mitochondrial disruption within the living cell therefore could be expected to cause the eventual death of the cell or to produce genetic effects. We thus become aware of a possible basis of cytopathology that seems not to have been emphasized previously."

5. Clerici, E., and G. Cudkowicz, Certain effects of p-dimethyl amino azobenzene, deficient diet, and hypotonic media on mitochondrial enzymes in experimental rat-liver carcinogenesis, J. Nat. Cancer Instit. 16, 1459, (1956).

"Recent investigations have emphasized a close correlation between the morphology of isolated mitochondria and their enzymic activities. It is therefore possible that some enzymes may be influenced, during carcinogenesis, through modifications of the mitochondrial structure." "The decreased activation of ATPase in the precancerous liver mitochondria could be interpreted as an example of an enzymic alteration related secondarily to a modification of the mitochondrial structure brought about by experimental carcinogenesis."

6. Warburg, O., On the origin of cancer cells, Science, 123, 309, (1956)
--NOTE- in the following quotation Warburg refers to the mitochondria as grana.

"The connection of respiration with the grana also explains a carcinogenesis that I have not mentioned previously, the carcinogenesis by X-rays. Rajewsky and Pauly have recently shown that the respiration linked with the grana can be destroyed with strong doses of X-rays, while the small part of the respiration that takes place in the fluid protoplasm can be inhibited very little by X-rays. Carcinogenesis by X-rays is obviously nothing else than a destruction of respiration by elimination of the respiring grana." "It should be mentioned here that grana, as Graffi has shown, fluoresce brightly if carcinogenic hydrocarbons are brought into their surroundings, because the grana accumulate the carcinogenic substances. Probably this accumulation is the explanation for the fact that carcinogenic hydrocarbons, although almost insoluble in water, can inhibit respiration and therefore have a carcinogenic effect."

7. Woods, M.W. and H.G. DuBuy, Hereditary and pathogenic nature of mutant mitochondria in Nepeta, J.Natl.Cancer Inst., 11, 1105, (1951)

1951b "Present results strongly support the view of the autonomous hereditary (extranuclear) nature of plastids. They demonstrate that many distinct types of mutant mitochondria can be recognized by their plastid forms in the cells, and all possible combinations observed without transition forms between normal and mutant types. Such transition forms might be expected if diffusable extramitochondrial cytoplasmic factors were responsible for the plastid abnormalities (plasmagenes). Some of the abnormal mitochondrial derivatives cause pronounced derangements in cell growth and function. They constitute the continuing cause of neoplasia."
"Virus multiplication and the multiplication of strongly aberrant derivatives could be expected to compete for certain loci on normal mitochondrial elements. Data support suggestions that certain plant viruses may have evolved and are evolving through chondriogene mutations and natural selection of progressively

more virus-like forms thus produced. Through influence of nuclear genes mutation rate of chondriogenes can be enormous."
--NOTE* Here is an example of the direct interaction of the two genetic systems (as mentioned previously).
"There appear to be no reasonable grounds for doubting that plant and animal mitochondria are basically homologous cellular organelles, eg. as homologous as are plant and animal nuclei. It is believed, therefore, that the results of the present study suggest that a considerable range of pathologic conditions may exist in animal tissues as a result of chondriome mutations. Such a view has already been expressed."

8. Woods, M.W., and H.G.DuBuy, The action of mutant chondriogenes and viruses on plant cells with special reference to the plastids, Amer. J. Bot., 38 (6), 419, (1951).

"Normal mitochondria of Nepeta cartaria and Nicotiana differentiate into chloroplasts containing grana. Mitochondria possessing mutant chondriogenes however, develop into abnormal plastid forms characteristic of the given chondriogene mutant. These modifications include changes in plastid size, pigment content, size and number of grana, vacuolation, enzyme activities, etc. Cells infected with certain strains of tobacco mosaic virus undergo modifications in plastid development that parallel those produced by mutant chondriogenes in Nepeta."
"Mutation of tobacco green mosaic virus to yellow mosaic types results in characteristic changes in action of virus on chloroplast development. Cytologic evidence of multiple infection of single cells with both parent and mutant viruses was obtained. In cells containing both normal and mutant mitochondrial systems each plastid system is independently modified by the virus. This supports the previous theory that certain plant viruses have had a mitochondrial origin."
--NOTE* underlined sentence appears to support our view of the specificity of viruses for mitochondria as being similar to phage specificity for bacteria-- specificity is altered by mutation of the phage, the bacterium, or both.

E. Similarities in the biochemistry of mitochondria, chloroplasts, and bacteria.

1. Hogeboom, G.H., W.S.Schneider, M.J.Striebich, Localization and integration of cellular function - mitochondria, Cancer Res. 13, 617, (1953)

"The mitochondrial content of enzyme systems related to the respiratory activity of the cell is their most striking biochemical property. The utilization of reduced cytochrome c appears to be localized EXCLUSIVELY in the mitochondria."
"Systems closely related to cytochrome oxidase are also found in mitochondria. Mitochondria play an important role in the Krebs series of reactions as indicated by presence of the fraction succinic dehydrogenases and oxidation of a-keto glutarate, oxalacetate, and octanoate. Glutamic and b-hydroxy butyric acid dehydrogenase are also present. A reaction that has an important

bearing on the role played by mitochondria in integrated activities of the cell is the synthesis of ATP through oxidative phosphorylation. In the presence of an oxidizable substrate ADP is converted to ATP at a rapid rate by isolated mitochondria." Ribonuclease and desoxyribonuclease are found in the mitochondrial fraction . Finally, a number of vitamins and coenzymes are found in mitochondria as well as citric acid and phospholipid.

2. Aerobic Bacteria

Aerobic respiration serves as the principle source of energy for growth of many bacteria- the coupling of oxidation of substrate to reduction of molecular oxygen. Respiratory enzymes catalyze the oxidation to water and hydrogen peroxide. Peroxidases then decompose the peroxide produced. The cytochromes are the principle catalyst that mediate between initial dehydrogenation of substrates and reduction of oxygen to water. Energy derived from respiration is transferred to energy consuming reactions thru ATP. Complete oxidations of the citric acid cycle occurs via coenzyme a. Thus there is a considerable similarity between respiration in aerobic bacteria and in mitochondria.

3. Chloroplasts- photosynthesis

a. Pigments- chlorophylls, carotenoids, phycobilins, floridorubin
b. Fluorescence of chlorophylls
c. Reaction: $CO_2 + 2H_2O \rightarrow CH_2O + O_2 + H_2O$
d. Photosynthetic phosphorylation

4. Anaerobic bacteria- photosynthesis

a. Pigments- chlorophylls, carotenoids, etc.
b. Fluorescence of chlorophylls
c. Reaction: $CO_2 + H_2O + 2H_2S \rightarrow CH_2O + 2H_2O + 2S$
d. Photosynthetic phosphorylation-- obtaining ATP from inorganic phosphate and ADP under anaerobic conditions.

Thus there is much similarity between photosynthesis of chloroplasts and anaerobic bacteria.

F. Similarities in the genetic chemistry of mitochondria, chloroplasts, and bacteria (Note- DNA and/or RNA in viruses , associated with host DNA and/or RNA)

1. Chiba, Y.,and K. Sugahara, The nucleic acid content of chloroplasts isolated from spinach and tobacco leaves, Arch.Biochem. and Biophys., 71, (2), 367, (1957).

RNA and DNA were extracted from chloroplasts isolated from spinach and tobacco leaves. It was inferred that not only RNA but also DNA may be real constituents of chloroplasts.

2. Laird, A.K., N.Y.Gaardo, H.Ris, Cancer Res. 12, 276, (1953)

RNA was found in mitochondria not contaminated to any appreciable extent by the microsomes.

Further there is some evidence for the presence of DNA in both animal and plant mitochondria. (Stafford, 1951, Physiol.Plantarum - 4 696; McClendon, 1952, Amer.J.Bot. 39, 275; Schneider et al, 1950, J. Natl.Cancer Inst. 10, 977.)

THEORIES (related to our work)

1. Altenburg, E., The 'viroid' theory, The symbiont theory, Amer. Nat. 80, 559, 561, (1946). He suggests that 'ultra-microscopic organisms akin to viruses' are universally present as useful symbionts in the cells of cellular organisms. These 'viroids' are precellular representatives that survived through adopting a symbiotic habit. Pathogenic viruses are derived from viroids during evolution.
2. Darlington, CD., The plasmagene theory of the origin of cancer, Brit. J. Cancer, 2, 118, (1948). He regards viruses as autogenous--originating from normal cytoplasmic proteins which have become abnormal; and also autonomous in being able to infect fresh cells.
3. Wallin, I.E., Symbionticism and the Origin of Species, The Williams & Wilkins Co., Baltimore, (1927).

Wallin presents the history of mitochondrial research, then a chapter on the bacterial nature of mitochondria (Chap. 3). This includes much of his own research on staining, chemical reactions, reactions to physical agents, thermal responses, and chemical constitution. It seems that Wallin's conclusion (p.31) is still justified today. He says "These facts, apparently, admit of no other interpretation than that mitochondria are living organisms, symbiotically combined with the cells of plants and animals." "The evidence for calling mitochondria bacteria, rests upon the following attributes: Their general behavior in the cell is similar to that of known microorganisms which live symbiotically in the cells of higher organisms; for example, the root-nodule bacteria of legumes. When grown independently in artificial media, they behave in all observed particulars like bacteria. They divide like bacteria. They are similar to bacteria in structure and shape. They exhibit no cultural characteristics foreign to bacteria." (p.41). Wallin then presents a chapter on the behavior of mitochondria in which he includes the following statement; "the possible relation of mitochondria to the etiology of disease is immediately indicated by their nature. The researches that have been made in pathology in this connection certainly give promise of ultimate fundamental discoveries. The earlier researches on mitochondria in this field were abandoned when Altmann's 'bioplasts' were rejected. The reestablishment of the fundamental result of Altmann's conception, namely, the bacterial nature of mitochondria, should again serve to stimulate investigations on the relationship of these ever-present microorganisms to disease." (53 Following this chapter is one on symbionticism as a fundamental significant biologic principle, then microsymbiosis, an analysis of symbiont reactions and symbionticism and the origin of species. Here (p. 111) Wallin includes the speculation that the chloroplasts are also symbionts, referring to the hypothesis of Merejkovsky (1920) "that the chloroplast is a microsymbiont, genetically related to the blue-green algae, and that all the higher green plants are symbiotic complexes." Wallin finishes with symbionticism in relation to heredity and development, and symbionticism and organic evolution.

Fin

Finally we would like to show how we may resolve one problem currently of interest to cancer research and virology. This problem concerns the nature of the KAPPA particles in Paramecium. From M. Sonneborn, Advances in Virus Research VI, 347, (1959); we have taken the following quotations:

"Hamilton and Gettner (1958) have made a suggestion which, if correct, would make kappa partly endogenous and partly exogenous in origin. They the possibility that kappa is a mitochondrion infected with an 'alien particle'. If the latter were rich in DNA, its distribution throughout the kappa particle could account for the observed staining reaction. In support of their suggestion, Hamilton & Gettner note that the membrane of kappa and the structure of its fine granules are like the membrane and microvilli or tubules of mitochondria, and that infected mitochondria might be expected to have reduced cytochrome oxidase such as Simonsen and Van Wagtendonk (1952,1956) reported for killers. In this connection the failure of others to confirm the existence of respiratory differences between killers and sensitives is pertinent. Furthermore, although Preer and Stark (1953) agree that kappa resembles mitochondria in size and shape more than does other structure in Paramecium, they also noted differences."

"Preer and Stark (1953) and Preer (1957) hold that the origin and phylogenetic relations of kappa are to be sought on the basis of as many traits as possible and through affinities to related chains of organisms. They have apparently eliminated relationship to normal components of Paramecium. They conclude that kappa is more like a bacterium than any other organism. This conclusion is based largely on size, shape, and mode of reproduction. Structure and composition, as currently reported, appear to suggest, in addition a relationship to viruses."

In terms of the theory we have presented involving the bacterial nature of mitochondria, the above controversy is obviously resolved.

Howatson, A.F. and A.W.Ham, Electron Microscope Study of Sections of Two Rat Liver Tumors, Cancer Res. 15, (1955) --FIG.6 (picture follows)

p.65 "The degenerating mitochondria were characterized by several features. They tended to be larger that normal; the largest one seen was 1.9 - microns wide (fig.6, right middle). Breaks in their limiting membrane were apparent. The double nature of their limiting membranes however could be clearly seen at some sites around their circumference, this being a good indication that these structures were indeed mitochondria. The degenerating forms revealed only remnants of transverse membranes, and such remnants as could be seen were detached from the limiting membrane (fig.6). The interiors of the degenerating mitochondria contained a great deal of granular material which was arranged in irregular clumps. The extent of this material together with the large size of the degenerating mitochondria, suggested that there had been a considerable increase in the amount of substance within them. The mitochondrion illustrated in the insert in fig.6 show irregular groups of poorly defined granules that range from 100A to 200A in diameter. Some of these could be seen to have light centers. The peripheries of these granules are irregular, relatively pale, and poorly defined."

p.68 "The apparent increase in substance within the degenerating forms described here is interesting and suggests the possibility of something, such as a virus, growing inside the mitochondria."

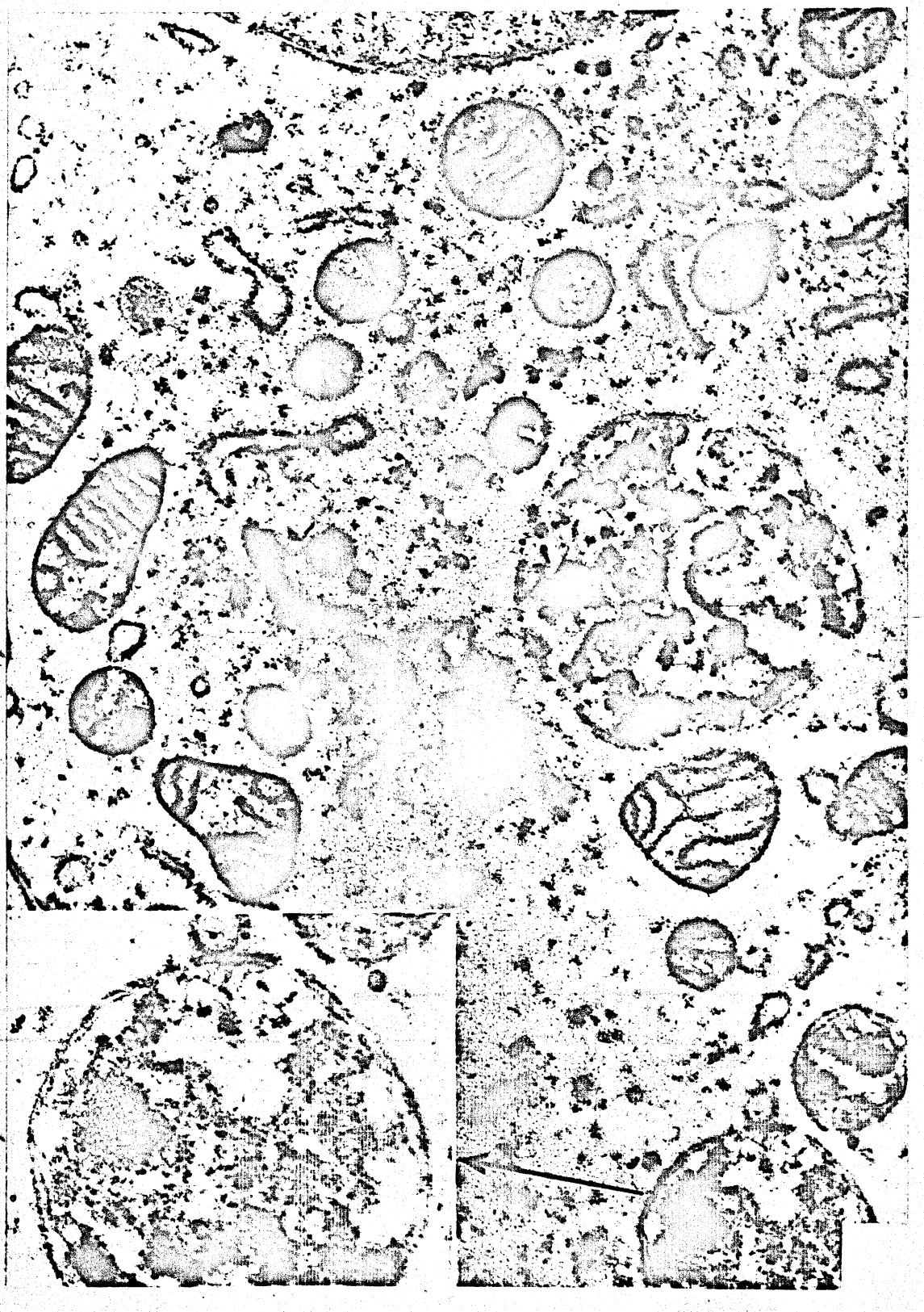
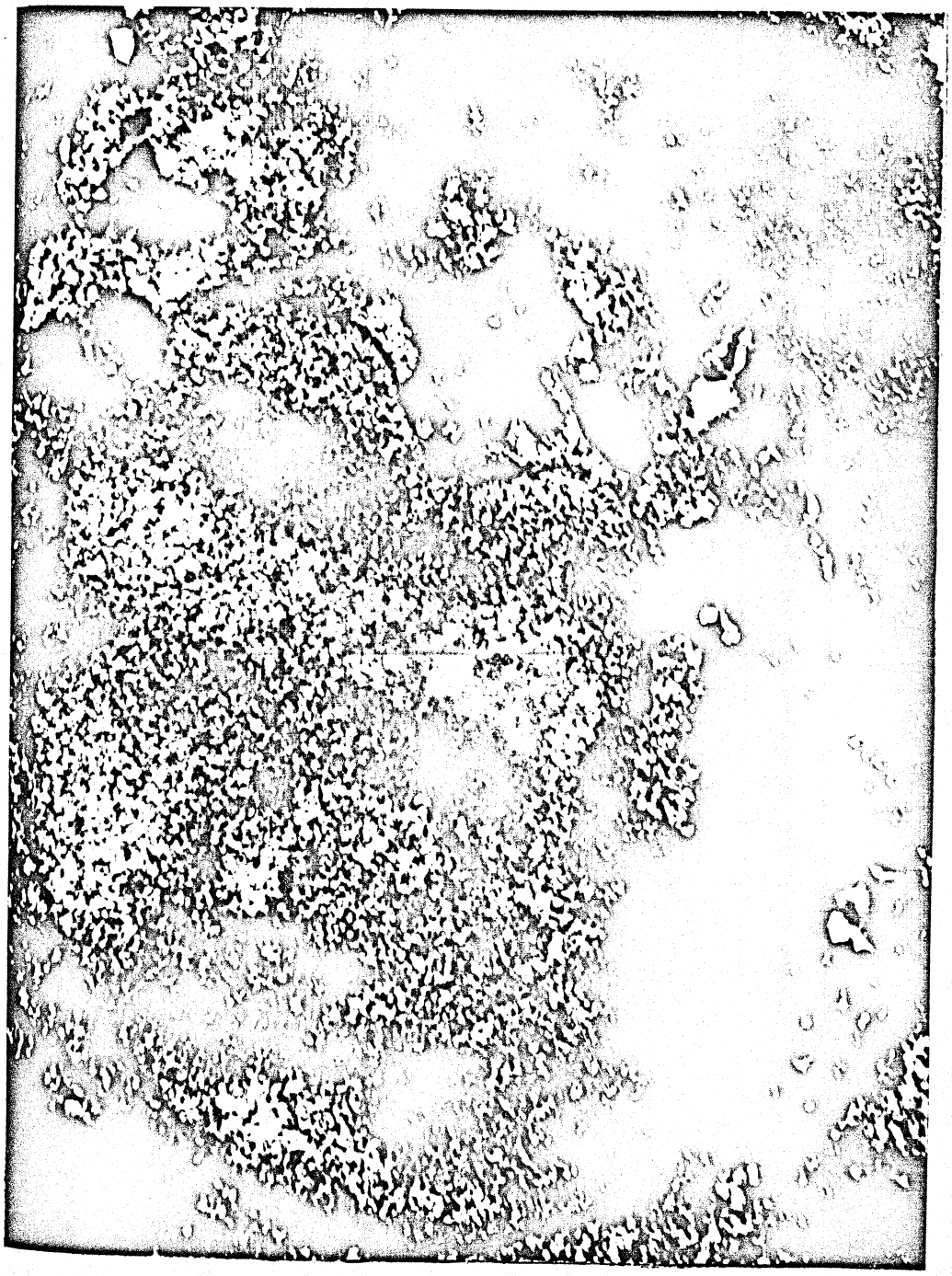


Fig. 6

Electron micrograph of a section of Novikoff tumor, ×30,000. A little of the nucleus of a cell shows above; the remainder of the field is cytoplasm. This figure illustrates the prominent and branching cristae of the mitochondria to advantage. It also illustrates degenerating mitochondria. A large one is to be seen at the right, middle. The insert represents a magnification of 51,000 and shows the granular material present in a degenerating mitochondrion, the double limiting membrane of which can still be seen.



SECTION OF CHICK CHORIO-ALLANTOIC MEMBRANE INFECTED WITH A VIRUS OBTAINED FROM CASE OF DISSEMINATED VARICELLIFORM ERUPTION ×24,000

FIG. 13

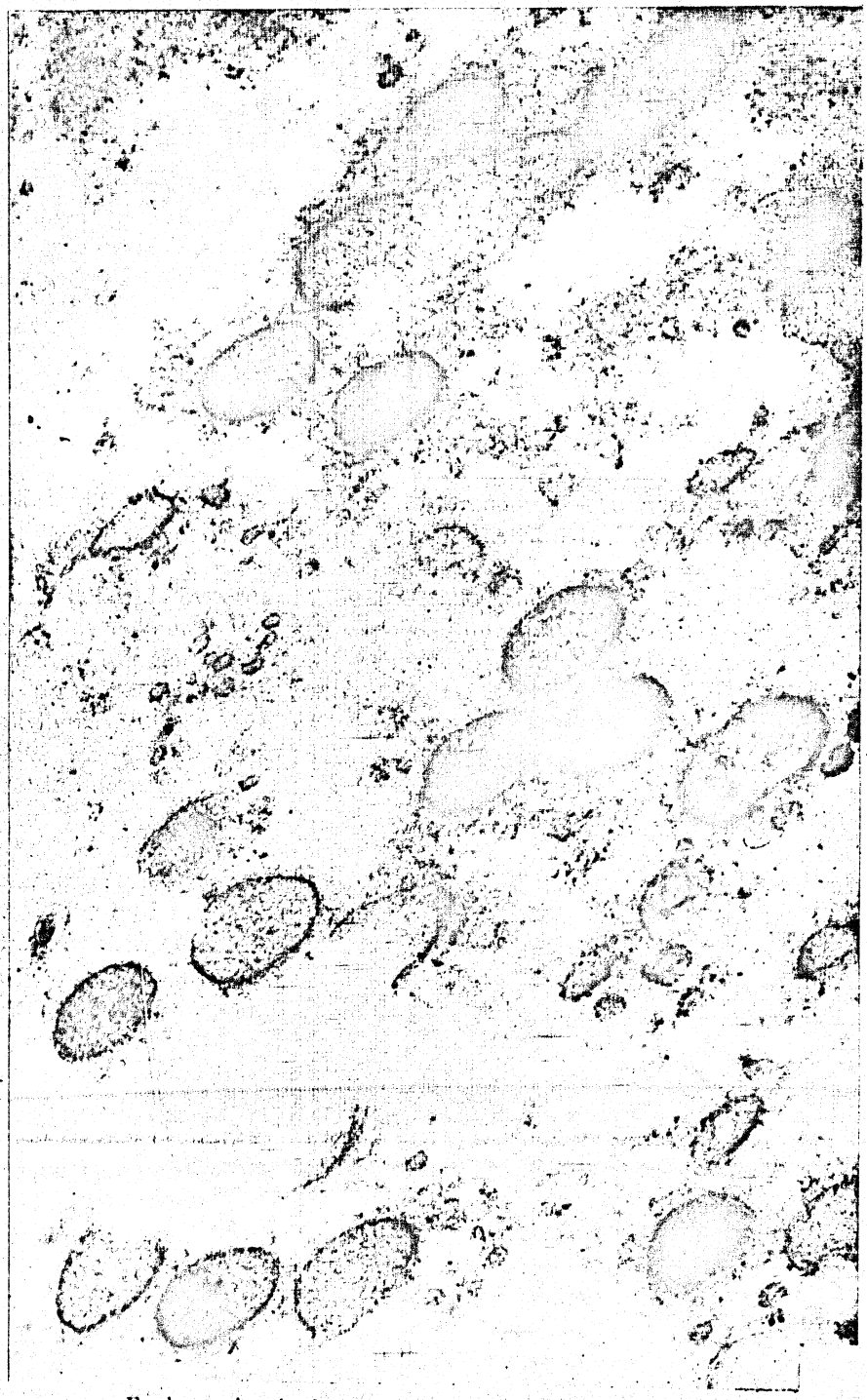
Section of intact nucleus and nuclear membrane. Masses of virus particles seen pushing in on nuclear membrane.

1. Fig. 36--C. Morgan et al., Structure and development of virus observed in the electron microscope, II Vaccinia and Fowl Pox Viruses, J. Exptl. Med., 100, 301, (1954).

2. Fig 32-- Dmochowski, L., and C. E. Grey, Electron microscopy of tumors of known and suspected viral etiology, Texas Reports on Biol. & Med 15, 753, (1957).

3. Fig. 15-9--Stanier et al., The Microbial World, Prentice Hall, (1957), 311.

Here is an electronmicrograph of a bacteria, Caryophanon which resembles the gross structure of the general mitochondrion.



Fowl pox virus in the cytoplasm of a host cell. Its structure resembles that of vaccinia virus. Incomplete membranes border aggregates of granular material which have replaced the characteristic components of cellular cytoplasm. Near the right border of the figure lies a mitochondrium which is nearly the same size as the virus but can be identified by the internal structure. × 56,500.

Fig 36

Q - ? Does "fowl pox virus" refer to the incomplete membranes plus granular material, or to the grains of the granular material? If to the former, then the virus is here approx the same size as the mitochondrium at right center. Is this not unusually large?

Part of cytoplasm of a cell of the cervical lymph node in human acute leukemia. Large osmiophilic unknown body (UB); mitochondria (M) undergoing degeneration. Inclusion-like bodies with virus-like particles (VP) present. x35,000.

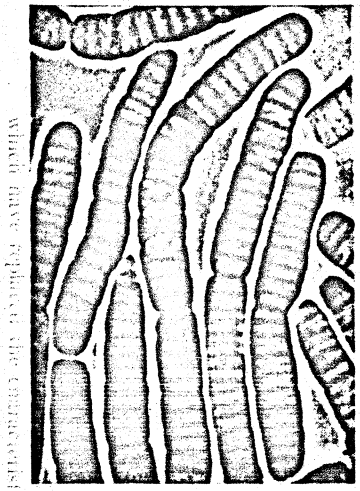


Fig. 15-9

EXPERIMENT

On the basis of the hypothesis, we would like to propose that viruses may be cultivated in mitochondrial or chloroplast cultures IN VITRO. This raises the immediate problem of finding media and conditions suitable for maintaining such cultures (if this is at all possible). We have pointed to recent experiments which illustrate the possibility of at least partially maintaining the metabolic processes of the mitochondria while in isolation. However, it is now generally known that bacteria can only reproduce 'phage if they are in a state of active growth. Thus the problem of finding a suitable medium so that mitochondria are still capable of growth in isolation may be a real and necessary one to solve before attempting to reproduce viruses in the mitochondria. For example, if we used rat liver mitochondria, then a suitable medium might consist of disrupted liver tissue. In this case the mitochondria would be in a medium most closely approximating its normal environment within the cytoplasm.