gloves were then put on and each plate was held with both hands outside the opening; the cover was then raised and the medium exposed to the wind for a half to one minute. The cover was then replaced and the dish was wrapped in gauze and paper and stored in the container. Cultures were taken in this manner every 1,000 feet, and in two instances where there was a fault in the technique a second culture was taken.

The wind coming in contact with the plates and with my hands had a velocity of 150 miles an hour. About 7,000 cubic feet of air passed over the plate in one minute. The calculated temperature at 28,500 feet was 34° F. below zero.

My hands soon became so numb and stiff that I could not carry out the technique as I had planned it. There were faults in opening and closing the plates and it was found impossible to protrude the hands far enough outside or to hold the plate longer than half a minute. It must be acknowledged that due to these errors there may have occurred possible contaminations from the inside of the plane. Then, too, it was found that the medium in several of the plates was completely frozen when I got back to the laboratory.

The plates were put in incubators and were examined very carefully during the next ninety-six hours by a competent bacteriologist.

The report was as follows:

A plate exposed	at 19,000 feet	was negative	
" " "	" 20,000 "	" "	
" " "	" 21,000 "	" "	
" " "	" 22,000 "	" "	
" " "	" 23,000 "	" "	
" " "	" 24,000 "	showed one colony of *Staphylococcus albus* (surely a contamination)	
" second plate	" 24,400 "	was negative	
" plate exposed	" 25,000 "	" "	
" " "	" 26,000 "	showed one colony of *Staphylococcus albus* (surely a contamination)	
" second plate	" 26,300 "	was negative	
" plate exposed	" 27,000 "	" "	
" " "	" 28,000 "	" "	

In two plates, one at 24,000 feet and the other at 26,000 feet, the colonies of staphylococci found were most probably due to a contamination, because in each one of them definite faults had been observed and a second plate had been exposed.

The work was not done with sufficient accuracy to claim the establishment of any new facts, but it suggests that the atmosphere from 20,000 feet to 28,500 feet is sterile.

In this flight we attained the highest altitude at which cultures had ever been taken and in this sense it is a record.

The plates for fungi were not exposed.

The flight demonstrated the impossibility of carrying out the technique as planned and showed the necessity of having some kind of a mechanical device for opening, exposing and closing the plates. I am now trying to devise such an apparatus.

It may be of interest to say that I began the use of oxygen at 21,000 feet and did not experience any embarrassment of respiration, but my hands suffered quite severely from the cold. My right index finger was frost-bitten and my right hand did not regain its normal feeling for several hours after I came down. The finger remained numb for about one week.

The pilot and I used a container holding five liters of liquid oxygen. One tube went to his compartment and a second came to mine. We were entirely separated in the plane and I was unable to make any signal to him. This was a marked disadvantage and a matter of considerable mental discomfort.

I wish to express my sincere appreciation to General MacArthur for the use of the plane and to Captain Polk for his hearty cooperation and for his high degree of skill as an aviator.

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ON THE NATURE OF FILTERABLE VIRUSES[1]

THE defining characters of filterable viruses appear to be ultramicroscopic size and obligate parasitism. They are the smallest units showing the reproductive property considered typical of life. While it is recognized that obligate parasitism does not necessitate their extreme smallness, there is apparently something related to the ultramicroscopic size of filterable viruses that obligates them to a parasitic existence. From information at hand we must conclude that the ultraviruses demand direct association with living cells to display the most characteristic property of life, that of reproduction.

It is of great significance that modern intense studies of microbiology have failed to discover any evidence of the existence of free-living microbes of ultramicroscopic size. In any case, where changes occur in an organic or inorganic solution that indicate definitely the presence of living microbes, they can always be demonstrated by direct microscopic examination. Even more critical experience has been gained by the use of bacteria-proof filters. Whenever filtered solutions show biological change indicative of microbic growth, visible microbes appear always to be present. There are, of course, bacteria that will regularly pass the usual bacteria-proof filters. Cultures of certain sulfur bacteria which grow on inorganic

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sulfur in water are extremely minute and the smallest cells in such a culture are apparently just ultramicroscopic and will regularly pass a Berkefeld filter. However, the growth obtained from such a filtrate contains the usual visible bacterial cells. While it may be that a whole world of free-living ultramicrobes remains to be discovered, this now appears very improbable.

A conviction that there are no free-living ultramicroscopic microorganisms brings us at once face to face with the curious sharp limitations of the size of free-living forms at a dimension that also represents the limits of microscopic visibility. If we survey generally the magnitudes of living forms, we see that those of extremely large size are relatively few in number and kinds. With a decrease in magnitude there is an increase in both kinds and numbers. In the microscopic world kinds become difficult to distinguish and individual numbers become enormous. There is a sudden limitation in numbers just above the smallest visible magnitude. Beyond that size occur only those curious parasitic forms which we designate as filterable viruses. It appears of great significance that all life smaller than a certain size is obligately dependent upon life of greater magnitude than that size. We could express that limiting dimension in terms of any unit we please, but as long as it corresponds to the range of the visible spectrum, it may be of some significance to consider it related directly to the wavelength of light.

It is the apparent absence of free-living ultramicrobes on this earth that indicates a microbic origin of filterable viruses. Our knowledge of the relationship of visible parasites establishes that parasitic forms are derived from free-living forms. We must accept also that ultramicroscopic parasites have also originated from independent forms. The ultraviruses can be adaptives from only two possible sources. First, they may be surviving parasitic forms developed from free-living ultramicrobes formerly inhabiting the earth and now extinct. Secondly, they may be parasitic forms of life developing by retrograde evolution from visible microbes similar to the visible forms now existent. The assumption of a present or past world of free-living ultramicrobes is pure conjecture, with no single fact to substantiate the assumption. A theory of microbic origin of filterable viruses by retrograde evolution from visible microbes naturally follows our knowledge of evolution in the visible world.

The primary characteristic of retrograde evolution under parasitism is a loss of function and associated substance. A common example of this is the tapeworm which, adapting itself to life in the gastrointestinal tract, has lost all vestiges of a functional or anatomical digestive system of its own. As far as its adult form and physiology are concerned, it is mainly a collection of vestiges with an exaggerated reproductive ability. It is an obligate parasite because it has become an incomplete form of life.

The obligate features of parasitism by microorganisms likewise represent a loss of function for which the parasite becomes dependent upon the host. Many pathogenic microorganisms are obviously dependent for growth on substances existent in their hosts, but generally the nature of these compounds is unknown. *Pasteurella tularensis* of McCoy is a clear-cut example of a known loss of function, which in this case concerns the metabolism of cystine. This organism can lead a saprophytic existence, independent of a living host only when cystine is made available. It does not take any stretch of the imagination to visualize this retrograde process extending to a considerable loss of function and finally resulting in a loss of all mechanism formerly concerned with this function.

The development of parasitism depends primarily upon conformity of the parasite to the host. The parasite must propagate its kind before it destroys its host. This conformity becomes very great for parasitism within tissue and exceedingly so for intracellular parasitization. With parasitism established comes the opportunity for acceptance of host functions by the parasite. For an intestinal parasite this would be limited largely to digestive functions. While adaptation to a parasitic existence within tissue in the intercellular spaces would require great conformity, the opportunity for function acceptance would be limited. Once, however, a microorganism has developed the conformity and ability for intracellular parasitism, the opportunity for function acceptance becomes extremely great. The intracellular microbe is surrounded by protoplasm functioning basically in a manner similar to its own protoplasm. The opportunity is afforded to depend upon the host protoplasm for certain metabolic functions and these the parasite can accept. The specific location of intracellular microbes either within the protoplasm or within the nucleus as seen for members of the Rickettsia group is visible evidence of the same intracellular selection and adaptation indicated by physiological deficiencies.

As seen for gross parasites, loss of function is followed by loss of structure and substance. This is apparently true also for parasites composed of a single cell. That the protoplasm of microbes has sufficient plasticity for specialization is shown by the organelles of protozoa. It follows that the capacity is also inherent for retrograde simplification. There can be little doubt that loss of function by an intracellular microbe is followed by loss of structure and substance. This simplification and decrease in size is in itself a tremendous advantage to intracellular parasitism. The opportunity for such simplification is so

great and the result so much to the advantage of the intracellular microbe that the process must proceed to the development of a highly incomplete form of parasitic life obligately dependent upon the host cell for many of its vital functions.

Such partial forms of life could develop to various degrees of simplification. It is conceivable that the retrograde process could proceed until only those molecules concerned with reproduction remained as the parasitic unit. Such a residuum could be as small as a single colloidal molecule and would correspond to the smallest of the viruses. Such a virus would be a functionally complete unit of life only when immersed in living protoplasm. There it would complement its own limited vital processes with those of the surrounding protoplasm. While the host cell continued its metabolic activities, the virus would be a living functionary individual. This concept corresponds with the general observations that filterable viruses reproduce only when in association with living cells.

The great diversity observed for the group of filterable viruses is readily understood from this viewpoint. We should expect as great a diversity as among the microorganisms from which the viruses have been derived. The extensive intracellular adaptations seen for the protozoa, especially among the Sporozoa, indicate that many of the filterable viruses may have developed from these forms. The true bacteria with their many pathogenic races offer points of origin for many viruses of quite different properties.

While this concept of filterable viruses defines them as primarily simplified fragments of living protoplasm, it does not preclude the concomitant development of certain vital characteristics in the nature of increased complexity. It is entirely conceivable and probable that such occur. Many viruses definitely appear to stimulate an increased metabolism and proliferation in the host cell early in the parasitization, and this may well represent a specialization of the parasite's metabolism to increase its opportunity for reproduction.

From the very intensive investigations that have now been carried out on certain filterable viruses, their obligately parasitic nature, their ultramicroscopic size and their intracellular specialization appear established. No characters have yet been discovered for filterable viruses that require an unique explanation. Their origin from visible microbes and their known characteristic properties are to be expected from our knowledge of the evolution of life under the conditions of parasitism.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A SIMPLE METHOD OF DETERMINING AREAS IN MICROPHOTOGRAPHS

DETERMINATION of areas in microphotographs by the usual methods of mechanical integration is a lengthy process, requiring a skilled operator and often consuming more time than the job is worth.

By taking advantage of the fact that in a microphotograph the weight of the paper having as its surface the image of a given object varies directly as the area of the image, determination of areas can be greatly facilitated.

To apply this in the determination of the area of the image of a given object in a microphotograph, cut out of the print the image of the entire field, and weigh it. The weight of the image of the entire field on its paper is:

$$W_F = \pi r^2 D T$$

where D is the density of the paper, T its thickness and r the radius of the field as determined for the particular case. Only r and W_F need to be determined.

From the image of the entire field, cut out the image, S, whose area is desired, and weigh the paper having this image as its surface. The weight of this image on its paper is: $W_S = DT \int_a^b \int_c^d dydx$, where area of S is $\int_a^b \int_c^d dydx$. Only its weight, W_S, need be determined. The ratio $\frac{W_F}{W_S} = \frac{DT\,\pi r^2}{DT \int_a^b \int_c^d dydx} = \frac{A_F}{A_S}$ *i.e.*, the areas vary directly as the weights of the paper whose surfaces they are. From this it is obvious that the desired area, $A_S = \frac{W_S A_F}{W_F}$, where W_S and W_F were determined by weighing the paper having as its surface the respective area, and A_F was found by calculation. A more convenient form for some types of work is: Per cent. A_S is of $A_F = \frac{W_S}{W_F} \times 100$. When only relative results are desired, it is not necessary to find an arithmetical value for the area of the field.

The accuracy of this method, except for small, unpredictable errors, depends on the skill of the operator. If the cutting is done with a sharp scalpel or razor blade, held vertical, it is possible to keep the error down to about 1 per cent., this being due to the weight of silver in the emulsion, variations in the thickness of the paper, and varying moisture content in the paper.

Applications of this method outside of the field of microscopy are numerous. For example, the area of an irregularly shaped outcrop of a certain formation