

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 functional 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 devel-

oped 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 a 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: $W_S = DT \int_a^b \int_c^d dy dx$, where

area of S is $\int_a^b \int_c^d dy dx$. Only its weight, W_S , need be determined. The ratio $\frac{W_F}{W_S} = \frac{DT \pi r^2}{DT \int_a^b \int_c^d dy dx} = \frac{A_F}{A_S}$ 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

can be determined by cutting the area of the outcrop from a map, and comparing the weight of the paper containing this area with the weight of the paper containing a known area, cut from the same map.

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A METHOD OF PREPARING SKELETONS OF SMALL VERTEBRATES

ALONG the entire Pacific Coast of the United States there occurs a marine isopod, *Cirolana harfordi*, which is an excellent agent for cleaning small vertebrate skeletons. I have used it a great deal in making osteological preparations, principally of fishes, and several colleagues to whom I have demonstrated the method have displayed great interest, while some have used it with considerable success.

The advantages of the method are that the finished skeleton is not disassociated, that it is cleaned within a day or two, and that no special malodorous place is required in the laboratory for storage during the process.

A fish to be skeletonized should first be skinned and eviscerated. The isopods will not attack the skin and it is practically impossible to remove it after skeletonization without tearing apart some of the smaller bones. In the case of large specimens, a little rough fleshing should be done. The specimen is then placed in a glass jar with a metal cap, such as the ones in which mayonnaise is commonly sold. The metal cap should be pierced by several 1/8 or 3/16th inch holes. These holes serve for entrance of the isopods which are small enough to leave even minute epipleurals intact and attached, while they exclude crabs which would tear the skeleton apart. The jar is placed securely under some large rock between tide marks and other stones heaped around it to keep it in place and to break up the force of waves which might damage the specimen. Twenty-four or 48 hours later the isopods have usually completed their task. The skeleton should then be gently washed in fresh water and hung up in the sun to dry. After drying, a drop of water placed on the proximal end of any bone which happens to be in an unnatural position softens the ligament and allows the bone to be rearranged.

A certain number of delicate specimens are bound to come apart in one or two places, but these may readily be patched or may serve very well as study material. A good percentage of skeletons turns out to be perfect museum material. A little practice enables any one to judge quite well the abilities of the isopods, and to balance accurately the size of the holes in the top of the jar, the amount of manual fleshing to be done, if any, and the length of time required in order to get the best results.

It should be mentioned that *Cirolana harfordi* will not attack animals which have been preserved in alcohol or formalin. Fresh material is required. The isopods also display a distaste for organisms in which putrefying bacteria have gained a good foothold. As a result 48 hours is usually their limit of activity on one specimen. In case their task is too great to be completed in this time the specimen must be thoroughly washed in order to remove all putrefying flesh, whereupon they can be induced to continue their work.

Keeping a supply of isopods in an aquarium in order to perform the work has been found to be unsatisfactory. While they eat the flesh very rapidly, they soon become satiated, and the number of individuals required to complete even a small skeleton is enormous. With the specimen placed in the ocean itself there is evidently a continuous parade of hungry workers into the jar and well-fed individuals out, resulting in the acme of efficiency.

In addition to fishes, I have made skeletons of toads, birds and small mammals. In these animals surplus pieces of ligaments should be clipped away while the skeleton is still wet. Drying the specimens in the sun for a few days generally gives them a satisfactory whiteness, but chemical bleaching agents may be used if desired.

While *Cirolana harfordi* is available to relatively few zoologists, it may well be that related forms along the Atlantic and Gulf coasts will prove equally efficient, while the fresh-water amphipods may possibly serve inland workers in the same manner.

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