time to avoid flattening the egg by pressure of the coverslip. In practice this presents little difficulty.

The fixative noted above is used simply because it appears to give the best fixation in our material. Others may be substituted if desired. Contrary to early reports the Feulgen method of staining appears to be applicable to material fixed in any of the ordinary fixatives. We have used it after Fleming, Gilson's mercuric nitric solution and Bouin, as well as Carnoy.

As an aid in transferring the eggs during dehydration, staining, etc., any of the standard methods of handling small objects may be used. We use the method of packing them in Drosophila pupa skins when they are in 70 per cent. alcohol after fixation[2] and then pushing them out of these cases either singly or in groups during the final process of mounting in balsam.

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RECONSTRUCTION WORK BY THE USE OF CELLOPHANE

IN 1930–31 the writer began investigations on the development of the jugular lymph sacs in turtles. In review of the literature on the vascular system, one observes that the only available extra-vitamin methods of studying the development of vascular elements, are by plastic reconstructions and by injections. Excellent as these methods are in studying vascular anatomy, they merely show the extent of the system at any stage and many questions as to the exact way in which growth takes place can not be determined by them. For that reason a new method for studying the development of the system was being sought. It seems hardly necessary to emphasize the advantage of using all possible methods in studying systems, if not always in one's own work, certainly through understanding the value of the observations of others and the necessity of comparing and testing the limitations of different methods.

Material was being sought which was thin and transparent, so depth could be observed when used in reconstructions. Cellophane answered the purpose, it being material of transparency, which is strong, durable and .0017″ in thickness.

Camera lucida drawings were made of serial sections on separate sheets of cellophane. A projection microscope was used for the drawings, so that a relatively large field with a high magnification could be obtained. This material made it possible to check each drawing carefully and quickly with the one preceding, before they were placed in reconstruction form. It was found to be the best method to get every vascular element in the reconstruction and to establish, thereby, a graded series of stages of embryos of different ages, complete in all details, in which the vascular elements could be studied and compared in their proper relations. In some cases it seemed highly desirable to use different colored inks to represent the arteries, veins, lymphatics and nerves.

It seems to the writer that where wax reconstructions are necessary, the use of cellophane marks a decided advancement in the preliminary steps for making the plastic reconstructions. The transparency of the material, making observation of depth possible, gives one an accurate course of the parts under observation in relation to other structures.

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SPECIAL ARTICLES

SIMULTANEITY IN THE ONSET OF POLIOMYELITIS

THE observation has often been made that when multiple cases of poliomyelitis occur in a family or a similar group of children, the onset of symptoms in all the individuals affected is simultaneous or nearly so. Sometimes the first symptoms are weakness or paralysis of muscles; at other times the symptoms are indefinite and mild, with weakness or paralysis of muscles following only after several days, or not at all.

These occurrences point to a common and coincidental exposure to the virus of the group of children affected. From these children, secondary cases of the disease may arise in other children, according to circumstances varying from group to group. The secondary cases will be separated from the primary ones by an interval of one, two or even more weeks, which interval is called the "incubation period."

A corresponding simultaneity is shown in a remarkable manner by groups of monkeys (*Macacus rhesus* and *cynomolgus*) inoculated experimentally with a potent virus by simple nasal instillation. The incubation period in these animals is regular and

[2] C. W. Metz, "A Simple Method of Handling Small Objects in Making Microscopic Preparations." *Anat. Rec.*, Vol. 21, No. 4, pp. 373–374, 1921.

accurate; it falls, in many of the tests, between the tenth and the fifteenth day following the first instillation, or between the seventh and the tenth day after the last instillation.

The striking precision of the phenomenon may have an important bearing on the still discussed question of the mode of infection in epidemic poliomyelitis. No other means of producing the infection in monkeys gives a corresponding, regular result; and no other experimental method of inoculation, through an external portal, gives reliable results at all. The digestive organs in monkeys are impervious to the virus, or the virus penetrating the tract is destroyed quickly. Not only is infection almost never produced by artificial feedings of virus, but monkeys which have resisted repeated artificial feedings are found to be as susceptible as normal or control animals to the nasal instillations.

The regularity and simultaneity with which experimental infection can be induced by dropping the virus of poliomyelitis into the nares of monkeys, afford additional support for the view that the portal of entry of the virus in human beings is the upper respiratory mucous membrane.

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PRIMITIVE OR FILTERABLE FORMS OF BACTERIA

IN a previous note[1] we reported the general occurrence of filterable forms of bacteria in such substances as soils, hay infusions, decomposing manure, human feces and milk. More recently, Sherman, Safford and Brueckner[2] have recorded a quantitative approach to this subject, which indicates that these primitive forms of bacteria are usually present in milk and milk products in greater numbers than are bacteria of familiar type.

The quantitative method in use in this laboratory was based on the assumption, later shown to be correct, that these primitive forms should occur in many substances in greater number than ordinary bacteria which are detected and enumerated by conventional methods. Serial dilutions of the substance under examination are made into glucose-beef infusion broth. These dilution cultures, representing from 10^{-1} to 10^{-13} gram of the original material, are incubated one or two days at 37° C. and then from two to three weeks at 30° C. After incubation, the higher dilutions, which contain no growth of ordinary bacteria, are seeded on the surface of glucose-infusion agar plates. The plates are incubated two days at 37° C. and, if necessary, longer at 30° C., after which they are examined for the delicate growth which Hadley has termed the "G" type colony. Growth has been recorded as positive when on microscopic study, after further culturing if necessary, definite minute cells which would be recognized as "bacteria" were found.

Aside from the quantitative approach, the most significant modification in this technique as compared with those previously used by Hauduroy,[3] Hadley, Delves and Klimek,[4] and others, is the longer incubation of the broth cultures. This prolonged incubation in broth appears to be desirable in order to get definite growth on the first agar plates seeded therefrom.

The numbers of primitive bacteria found by the application of this method are somewhat surprising. Nine samples of soil representing a wide range of productivity have yielded estimates ranging from 10^7 to 10^{12} per gram. Other materials have shown: Fresh human feces, two samples, 10^{12}; sour milk, two samples, 10^{11}; and raw market milk, six samples, 10^8 to 10^{12} per gram.

While the occurrence of these primitive microorganisms in such large numbers may be difficult to believe in the light of previous knowledge of "bacterial counts," they are perhaps not unreasonable. Ordinary bacteria are not infrequently found in numbers approaching and exceeding one billion per gram in certain types of decomposing and fermenting materials. When probable relative sizes are considered, the presence of bacteria in the filterable stage in numbers approximating one trillion per gram appears plausible. In this connection it is of interest to note that Clifton, Schultz and Gebhardt[5] have recently determined the size of the virus of poliomyelitis as probably less than "50 μμ in diameter."

With reference to the microscopically definite cells which are found in "G" type cultures, it should be remembered that workers in this field appear to be unanimous in the belief that these organisms represent a partially transformed state between the filterable (perhaps ultramicroscopic) and the non-filterable stages of the bacteria. Hadley, Delves and Klimek[4] have furnished rather definite evidence that this is in fact the case.

One of the most interesting points about the primitive forms of bacteria is their inertness on ordinary bacteriological media. When these forms are cultivated in the laboratory until they appear microscopically as true bacteria, they still make only very meager growth on agar and do not cause the familiar

[1] SCIENCE, 73, 448, 1931.
[2] *Proc. Inter. Dairy Congress*, Copenhagen, July, 1931.
[3] "Les ultravirus et les formes filtrantes des microbes," Paris, 1929.
[4] *Jour. Infect. Diseases*, 48, 1, 1931.
[5] *Jour. Bact.*, 22, 7, 1931.