

AN INVESTIGATION ON THE NATURE OF ULTRA-  
MICROSCOPIC VIRUSES (1)

By F. W. TWORT, L.R.C.P. Lond., M.R.C.S.  
(From the Laboratories of the Brown Institution, London.)

During the past three years a considerable number of experiments have been carried out at the Brown Institution on filter-passing viruses. Many of these, previous to the outbreak of the war, were performed by Dr. C. C. Twort, and, unfortunately, circumstances during the present year have made it difficult to continue the work.

In the first instance attempts were made to demonstrate the presence of non-pathogenic filterpassing viruses. As is well known, in the case of ordinary bacteria for every pathogenic microorganism discovered many non-pathogenic varieties of the same type have been found in nature, and it seems highly probable that the same rule will be found to hold good in the case of ultra-microscopic viruses. It is difficult, however, to obtain proof of their existence, as pathogenicity is the only evidence we have at the present time of the presence of an ultra-microscopic virus. On the other hand, it seems probable that if non-pathogenic varieties exist in nature these should be more easily cultivated than the pathogenic varieties; accordingly, attempts to cultivate these from such materials as soil, dung, grass, hay, straw, and water from ponds were made on specially prepared media. Several hundred media were tested. It is impossible to describe all these in detail, but generally agar, egg, or serum was used as a basis, and to these varying quantities of certain chemicals or extracts of fungi, seeds, &c., were added. The material to be tested for viruses was covered with water and incubated at 30° C. or over for varying periods of time, then passed through a Berkefeld filter, and the filtrate inoculated on the different media. In these experiments a few ordinary bacteria, especially sporing types, were often found to pass through the filter; but in no case was it possible to obtain a growth of a true filter-passing virus.

Attempts were also made to infect such animals as rabbits and guinea-pigs by inoculating two doses of the filtered material, or by rubbing this into the shaved skin. In other cases inoculations were made directly from one animal to another in the hope of raising the virulence of any filter-passing virus that might be present. All the experiments, however, were negative.

Experiments were also conducted with vaccinia and with distemper of dogs, but in neither of these diseases was it found possible to isolate a bacterium that would reproduce the disease in animals. Some interesting results, however, were obtained with cultivations from glycerinated calf vaccinia. Inoculated agar tubes, after 24 hours at 37° C., often showed watery-looking areas, and in cultures that grew micrococci it was found that some of these colonies could not be subcultured, but if kept they became glassy and trans-

(1) Reproducido de "The Lancet", diciembre 4 de 1915, págs. 1241-1243.

parent. On examination of these glassy areas nothing but minute granules, staining reddish with Giemsa, could be seen. Further experiments showed that if a colony of the white micrococcus that had started to become transparent was plated out instead of being subcultured as a streak then the micrococci grew, and a pure streak culture from certain of these colonies could be obtained. On the other hand, if the plate cultures (made by inoculating the condensation water of a series of tubes and floating this over the surface of the medium) were left, the colonies, especially in the first dilution, soon started to turn transparent, and the micrococci were replaced by fine granules. This action, unlike an ordinary degenerative process, started from the edge of the colonies, and further experiments showed that when a pure culture of the white or the yellow micrococcus isolated from vaccinia is touched with a small portion of one of the glassy colonies, the growth at the point touched soon starts to become transparent or glassy, and this gradually spreads over the whole growth, sometimes killing out all the micrococci and replacing these by fine granules. Experiments showed that the action is more rapid and complete with vigorous-growing young cultures than with old ones, and there is very little action on dead cultures or on young cultures that have been killed by heating to 60° C. Anaerobia does not favour the action. The transparent material when diluted (one in a million) with water or saline was found to pass the finest porcelain filters (Pasteur-Chamberland F. and B. and Doulton White) with ease, and one drop of the filtrate pipetted over an agar tube was sufficient to make that tube unsuitable for the growth of the micrococcus. That is, if the micrococcus was inoculated down the tube as a streak, this would start to grow, but would soon become dotted with transparent points which would rapidly extend over the whole growth. The number of points from which this starts depends upon the dilution of the transparent material, and in some cases it is so active that the growth is stopped and turned transparent almost directly it starts. This condition or disease of the micrococcus when transmitted to pure cultures of the micrococcus can be conveyed to fresh cultures for an indefinite number of generations; but the transparent material will not grow by itself on any medium. If in an infected tube small areas of micrococci are left, and this usually happens when the micrococcus has grown well before becoming infected, these areas will start to grow again and extend over the transparent portions, which shows that the action of the transparent material is stopped or hindered in an overgrown tube; but it is not dead, for if a minute portion is transferred to another young culture of the micrococcus it soon starts to dissolve up the micrococci again. Although the transparent material shows no evidence of growth when placed on a fresh agar tube without micrococci it will retain its powers of activity for over six months. It also retains its activity when made into an emulsion and heated to 52° C., but when heated to 60° C. for an hour it appears to be destroyed. It has some action, but very much less, on staphylococcus aureus and albus isolated from boils of man, and it appears to have no action on members of the coli group or on streptococci, tubercle bacilli, yeasts, &c. The transparent material was inoculated into various animals and was rubbed into the scratched skin of guinea-pigs, rabbits, a calf, a monkey, and a man; but all the results were negative.

From these results it is difficult to draw definite conclusions. In the first place, we do not know for certain the nature of an ultra-microscopic virus. It may be a minute bacterium that will only grow on living material, or it may be a tiny amoeba which, like ordinary amoebae, thrives on living microorganisms. On the other hand, it must be remembered that if the living organic world has been slowly built up in accordance with the theories of evolution, then an amoeba and a bacterium must be recognised as highly developed organisms in comparison with much more primitive forms which

once existed, and probably still exist at the present day. It is quite possible that an ultra-microscopic virus belongs somewhere in this vast field of life more lowly organised than the bacterium or amoeba. It may be living protoplasm that forms no definite individuals, or an enzyme with power of growth.

In the vaccinia experiments described above it is clear that transparent material contains an enzyme, and it is destroyed at 60° C. It also increases in quantity when placed on an agar tube containing micrococci obtained from vaccinia, and this can be carried on indefinitely from generation to generation. If it is part of the micrococcus it must be either a stage in its life-history which will not grow on ordinary media but stimulates fresh cultures of the micrococcus to pass into the same stage, or an enzyme secreted by the micrococcus which leads to its own destruction and the production of more enzyme. The fact that the transparent portion cannot be grown except on the micrococcus makes it impossible to obtain any definite evidence on these points. There is this, however, against the idea of a separate form of life: if the white micrococcus is repeatedly plated out and a pure culture obtained, this may give a good white growth for months when subcultured at intervals on fresh tubes; eventually, however, most pure strains show a transparent spot, and from this the transparent material can be obtained once again. Of course, it may be that the micrococcus was never quite free from the transparent portion, or this may have passed through the cotton-wool plug and contaminated the micrococcus, but it seems much more probable that the material was produced by the micrococcus. Incidentally, this apparent spontaneous production of a self-destroying material which when started increases in quantity might be of interest in connexion with cancers. In any case, whatever explanation is accepted, the possibility of its being an ultra-microscopic virus has not been *definitely* disproved, because we do not know for certain the nature of such a virus. If the transparent portion were a separate virus, it might be vaccinia or it might be some contaminating non-pathogenic ultra-microscopic virus, for it is conceivable that whereas a non-pathogenic variety might grow on micrococci or bacilli, a pathogenic variety might grow only on the animal it infects. As the animal experiments were negative there is no evidence that it is vaccinia, although such a virus might lose its virulence when grown outside the body. On the other hand, no evidence was obtained that it was a non-pathogenic contaminating ultra-microscopic virus. On the whole it seems probable though by no means certain, that the active transparent material is produced by the micrococcus, and since it leads to its own destruction and can be transmitted to fresh healthy cultures, it might almost be considered as an acute infectious disease of micrococci.

In view of the results obtained with vaccinia similar experiments were carried out with other material. It will not be necessary to describe all these in detail; it will suffice to note that similar, though not such definite, results were obtained with a micrococcus and a member of the colityphoid group of bacilli which were obtained from the intestinal mucous membrane of a dog suffering from acute distemper, and there is some evidence that the difficulty often experienced in isolating certain known pathogenic micro-organisms may be due to the same cause. Experiments carried out with tuberculous pleural fluids and tubercle bacilli gave negative results.

More recently, that is when the investigation of infantile diarrhoea and vomiting was continued during the summer and autumn of this year (1915), similar experiments were carried out with material obtained from the intestinal tract. The general results of this investigation will be published later, and it will be sufficient here to note that after certain difficulties had been overcome it was found that in the upper third of the intestine, which con-

tained numerous bacilli of the typhoid-coli group, some larger bacilli were also present. In some cases they grew in far larger numbers than the coli types of bacteria; but this was only so when precautions were taken to eliminate the action of a dissolving substance which infected the colonies so rapidly that they were dissolved before attaining a size visible to the eye. Here, then, is a similar condition to that found in vaccinia, and the greatest difficulty was experienced in obtaining the bacilli free from the transparent dissolving material, so rapidly was the infection increased and carried from one colony to another. Finally, cultures were obtained by growing the bacilli with certain members of the typhoid-coli group for a few generations and then plating out. From the colonies cultures were obtained on ordinary agar. Some of these cultures being slightly infected with the dissolving material rapidly became transparent and were lost, while a few grew well. The bacillus has several curious characters, and these are now being investigated. It is in no way related to the typhoid-coli group. The relation of this bacillus and the dissolving material to infantile diarrhoea has not yet been determined, but probably it will be found also in cases of dysentery and allied conditions; and I greatly regret that I have not been afforded an opportunity of investigating the dysenteric conditions in the Dardenelles to determine this and other points.

When possible, experiments should be conducted to determine the relative toxicity of cocci and bacilli when free from and when associated with the dissolving material, and vaccines prepared with the transparent material should be tested.

I regret that financial considerations have prevented my carrying these researches to a definite conclusion, but I have indicated the lines along which others more fortunately situated can proceed.

*Y dos años más tarde se publica:*

## SUR UN MICROBE INVISIBLE ANTAGONISTE DES BACILLES DYSENTERIQUES LOS INSECTOS\*

Note (1) de M. F. D'HERELLE, présentée par M. ROUX

Des selles de divers sujets convalescents de dysenterie bacillaire, et dans un cas de l'urine, j'ai isolé un microbe invisible doué de propriétés antagonistes vis-à-vis du bacille de Shiga. Sa recherche est particulièrement aisée dans les cas d'entérite banale consécutive à une dysenterie; chez les convalescents ne présentant pas cette complication la disparition du microbe anti suit de très près celle du bacille pathogène. Malgré de nombreux examens, je n'ai jamais trouvé de microbes antagonistes, ni dans les selles de dysentériques à la période d'état, ni dans les selles de sujets normaux.

L'isolement du microbe anti-Shiga est simple: on ensemence un tube de bouillon avec quatre à cinq gouttes de selles, on place à l'étuve à 37° pendant 18 heures puis on filtre à la bougie Chamberland L<sub>2</sub>. Une petite quantité d'un filtrat actif ajoutée, soit à une culture en bouillon de bacilles de Shiga, soit à une émulsion de ces bacilles dans du bouillon ou même dans de l'eau

---

(1) "Comptes Rendus de l'Académie de Sciences", Paris, 10 de setiembre de 1917, págs. 373-375.