

Filter-passing Viruses in Disease.

AT the Bath meeting of the British Medical Association a discussion on the filter-passing viruses occupied the first day of the meeting of the Section of Pathology and Bacteriology. The discussion was opened by Dr. W. E. Gye. Dr. Gye criticised the expression "filter-passing viruses," which is generally used to include certain viruses which have hitherto not been proved to be filterable. The use of the term "filterable viruses," though convenient, has at present no real significance. His own experience has been particularly with Rous's chicken sarcoma, and even with this virus, filtration can only be conducted if certain experimental conditions are observed. If the tumour emulsion be insufficiently diluted, and if distilled water be employed instead of saline, the filtration is much more difficult, and it is seldom that an infective filtrate is obtained. By using a small volume of saline, or by using distilled water as a diluent, one obtains a viscous solution of mucin and nucleo-protein which will not pass the filter readily. The hydrogen ion concentration of the liquid also affects the rate of filtration; within limits not yet exactly determined, acidity increases the difficulty of filtration and alkalinity makes the process easier. The properties of the liquid in which the virus is suspended thus play an important part in the results of filtration.

A further important factor in filtration is the adsorptive capacity of the filter. Under certain conditions the filtrates of the infective material in Rous's sarcoma failed owing to the adsorption of the virus on the surfaces of the filter. The only method readily available by which the particulate character of viruses can be tested is that provided by the centrifuge. In the case of Rous's sarcoma, long centrifugalisation, at 8000 revolutions a minute at least, is necessary to obtain a definite difference in the infective actions of the upper and the lower layers. There is an urgent need for a machine which will spin at a higher speed with safety and is so constructed that fluids can be spun without running the risk of contamination. It is very improbable, however, that we shall ever get a centrifuge with which it will be possible to spin out completely an organism of the size, roughly, of 0.1μ .

The identification of the filterable viruses by staining methods is beset with fallacies, and the results obtained up to now have proved in the main disappointing. The microscope proved of very little value except in revealing contaminating microbes. It is possible that, with increasing knowledge of the ultramicroscopic organisms, we shall find that the doctrine of fixity of form holds here as well as with ordinary microbes. With regard to the cultivation of the filterable viruses, each virus must be considered by itself. The virus of pleuro-pneumonia is apparently the only organism of this group that grows readily on artificial media. The production of cloudiness in Noguchi tubes, which has been put forward as a proof of culture, so far as chicken sarcoma is concerned, is entirely erroneous.

The animal test provides the final proof of the presence of the organism in an inoculum. The difficulty of microscopic investigation in this field makes us fall back in a very special way upon animal experimentation. It is because ordinary laboratory animals are not susceptible or only slightly so to human filterable viruses such as measles or influenza that such small progress has been made. We shall be compelled to work at animal diseases if we want to discover a method of handling and identifying the very small microbes. This will,

however, involve great expense and is open only to those who have ample funds behind them.

In addition to his opening address (which was circulated but not read) on the more general aspects of the filterable viruses, Dr. Gye also discussed some aspects of the recent work he has done in collaboration with Mr. J. E. Barnard on cancer. The mere conjunction of host and parasite does not necessarily produce disease. There are accessory factors that must be searched for. Some years ago Dr. Gye and Dr. Cramer investigated the accessory action of certain chemical substances in the production of gas gangrene, and Dr. Gye had formed the opinion that the conception of disease as being due to the conjunction of living organisms involving a non-living factor was capable of further development. In this way he was led to his experiments on cancer. Any theory of cancer had to provide an explanation of its particular specificity both as regards the animal species the tumour is able to attack, and the type of growth produced. Yet cancer can be looked at from some points of view as one homogeneous specific disease, which has probably some common cause underlying it. The difficulty of obtaining a theory of cancer capable of reconciling the "specificity" of new growths with the general properties they hold in common has been overcome, in Dr. Gye's opinion, by his experimental work on Rous's sarcomas.

The infective filtrates of ground-up Rous's tumours contain not the virus only, but also an accessory substance or substances which are necessary to produce the disease. The accessory substances are responsible for the more specific peculiarities of the tumour, such as its histological structure and the susceptibility of certain animals to it.

The actual virus is probably the same in every case, and by means of prolonged spinning in a centrifuge, the virus may be obtained apparently free from the other factors. By suitable treatment with chloroform the virus can be destroyed without affecting the "specific factor." Neither the virus nor the accessory factor is separately able to produce sarcoma. The fact that "virus factor" from human carcinoma is able to produce sarcoma in the chicken if reinforced by the "non-virus" or "specific" factor from Rous's tumour brings the infectious sarcomas into line with the mammalian tumours, which up to now have been considered by most authorities to be non-infectious.

Mr. J. E. Barnard followed Dr. Gye with an account of the optical part of the work they have done on cancer. He dwelt on the limitations of microscopical research into filter-passing viruses, and stated his opinion that the present dark ground illumination apparatus such as is used at the National Institute for Medical Research is of such perfection that further improvement in direct microscopy upon present lines cannot be expected. The method which has proved so valuable in his and Dr. Gye's work depends upon the use of an apparatus by which the object can be focussed in visible light, and by a mechanical device can be refocussed in any desired wave-length, and thus a photomicrograph is obtained using light with a very short wave-length. What is believed to be the virus of cancer has thus been photographed, and what are considered to be phenomena of growth were seen in the preparations. The real difficulties in the work depend upon biological rather than physical considerations. The material is, in fact, more important than the apparatus, and much depends upon the absorptive capacity for light of the media, and

of the virus itself. The study of the absorption of the material will be the key of further optical improvement in microscopy.

Dr. M. H. Gordon dealt with the problem of the filter-passing viruses in the light of his experimental work on vaccinia and variola. These diseases are particularly suitable for the preliminary attack on the problem since a small animal is available that is susceptible to the virus, and the lesions produced by the virus are so characteristic that there is no likelihood that they will be mistaken for accidental lesions. In his work Dr. Gordon decided that since the cultivation of the virus and any attempts to make it visible by microscopic means are beset with difficulties, the most promising line of attack is by the methods of immunity. Vaccinia also satisfied the criterion of particulate matter, and centrifugalisation can separate infectious material into layers of varying infectiousness. Vaccinia is filterable, but only with special precautions, *e.g.* after previous treatment of the material with trypsin.

Active immunity to vaccinia can be produced not only by normal living virus but also by means of heated attenuated virus. In rabbits a passive immunity can also be transferred. Agglutination and complement fixation experiments have been done with the virus and its immune serum. Agglutination of the virus is easily visible with the naked eye or with a hand lens. Preparations of the agglutinated material show objects which bear a close resemblance to those figured by Dr. Gye and Mr. Barnard in their paper on cancer. The use of the agglutination method has given some interesting results. Material from confluent smallpox and from the so-called Alastrim absorbs the agglutinin in the same way as the homologous virus of vaccinia. From the point of view of agglutination, the close relationship, if not identity, of these three diseases was confirmed. Dr. Gordon finds a very striking susceptibility of vaccinia virus to potassium permanganate, which is more destructive than the customary disinfectants. Potassium permanganate in 1 in 100,000 dilution destroys

the virus in a short time. This curious susceptibility of the virus may, indeed, to some extent, explain the result credited to this drug in the treatment of smallpox. Dr. J. E. McCartney stated that in the case of herpes virus he has been unable to separate virus from its suspension fluid by means of spinning. This he ascribed to the activity of the virus at much greater dilutions than that of the sarcoma or vaccinia viruses.

As was to be expected from the great interest that has recently been taken in the action of light and other physical agents in medicine, this subject was also prominent at the Bath meeting. Three discussions dealing with different aspects of this subject were included in the programme. Prof. S. Russ opened a discussion on the pathological basis of treatment by radiation with a general statement of the physical problems involved. Dr. E. T. Strangeways gave an account of experiments on the effect of X-rays on the division and development of tissue cells grown *in vitro*. He has been able to kill chick embryos while in the shell with X-rays, though after death of the embryo as a whole, successful tissue cultivation could still be made.

Miss M. E. Hume discussed the action of ultraviolet light on rickets, and the relation of light effects due to vitamin A. Dr. Canti described the action of radium on the mitosis of cells in human carcinoma.

In the Section of Therapeutics, Prof. W. E. Dixon opened a discussion on the therapeutic action of light; and in the Section of Public Medicine, Prof. Leonard Hill dealt with the influence of sunlight and artificial light on health. In all these communications there was the healthy sign that this branch of medicine, which a short time ago was in an empirical state, is now rapidly being co-ordinated on a scientific and experimental basis. Prof. Russ's concluding remarks seem to sum up the present position with regard to this branch of experimental medicine. "There is nothing so depressing as being told that we are only at the beginning of the subject. I do not think that this is the case, but rather that we are well in the middle of it."

Strong Electrolytes.

A SERIES of papers on the activities of strong electrolytes, by G. Scatchard, appears in the Journal of the American Chemical Society for March. The activity of hydrochloric acid was obtained from measurements of the E.M.F. of the cell $\text{Pt}(\text{H}_2)/\text{HCl}, \text{AgCl}/\text{Ag}$. The hydrogen electrode was of a rocking pattern suitable for use in a thermostat and simplified by the absence of a liquid junction. The activity coefficients obtained agree with those calculated from Randall and Vanselow's freezing-point measurements; the values at low concentrations are consistent with Debye and Hückel's limiting equation, the theoretical value of the constant in that equation being retained. The activities of potassium, sodium, and lithium chlorides are also considered; the existing data are compared and discussed in the light of Debye and Hückel's theory.

Individual ion activities have always been calculated through one of three assumptions, namely, (1) that the activities of the ions in a uni-univalent electrolyte are equal; (2) that the above is true in the case of potassium chloride, and that the activity of an ion depends only upon the total ionic concentration and not on the ions with which it is associated; (3) that the saturated potassium chloride bridge eliminates liquid-junction potentials. Assumptions (1) and (2) are in general incompatible; assumption (3), however, is quite distinct and is compared with the first two by G. Scatchard by measuring the E.M.F.'s of the cells,

$\text{Pt}(\text{H}_2)/\text{HCl}, \text{AgCl}/\text{Ag}$ and $\text{Hg}/\text{HgCl}, \text{KCl}(\text{sat.})/\text{HCl}/\text{AgCl}/\text{Ag}$, the latter containing a flowing junction.

The results indicate that assumption (2) above is correct and that the saturated potassium chloride bridge gives a liquid-junction which does not vary with the acid concentration. The liquid-junction potential alters slightly on stopping the flow. In more concentrated solutions the chloride ion appears to attain a maximum activity, whilst that of the hydrogen ion appears to drop to a minimum value. The results obtained enable certain single electrode potentials to be accurately calculated.

From a series of papers by H. S. Harned on the subject in the same issue of the Journal of the American Chemical Society, the activity coefficient and the vapour pressures of the solutions are calculated from the E.M.F. of the cell $\text{H}_2/\text{NaOH}(c_1)/\text{NaHg}/\text{NaOH}(c_2)/\text{H}_2$. The activity coefficient of potassium hydroxide is greater than that of sodium hydroxide at concentrations greater than 0.05 molar. In the presence of sodium chloride the logarithm of the activity of the hydroxide (concentration less than 0.5 molar) is a linear function of its concentration at constant total molality. For potassium hydroxide in potassium chloride solution the activity coefficient is greater than that of sodium hydroxide in a sodium chloride solution when the hydroxides and salts are at the same strength. W. Lucasse records the activity coefficients and transport numbers of the alkaline-earth chlorides in the same journal.