I have called the specific factor. Superficially, it might be thought that Bail's term "aggressin" would appear satisfactory, but a casual analysis of the facts relating to an aggressin shows that this is not so. As an example of an aggressin the toxin of *B. welchii* will be taken. The toxin is separated from the bacilli by filtration and the minimal lethal dose for mice determined. Bacilli, conveniently grown on solid medium are washed free of toxin, and are then found to be perfectly harmless to mice. If, now, mice are inoculated with a sublethal dose of toxin to which has been added washed *B. welchii*, an infection is set up, more toxin is produced in vivo, and the mouse eventually dies of a spreading gangrene with toxæmia. When the bacilli added to the toxin are *B. welchii* the infection produced is characteristic of *B. welchii* infections; when *Vibrion septique* is added the disease produced is characteristic of *Vibrion septique*;[7] and similarly, toxin-free harmless tetanus spores mixed with sublethal doses of *B. welchii* toxin produce tetanus.[8] Thus, the specificity in these cases depends upon the microbes; the aggressin, acting equally well for all three, produces a microscopic lesion which in some way, not yet fully understood, enables these anaerobic organisms, which alone are harmless saprophytes, to proliferate in the tissues and there to make their characteristic poisons. These facts have been established by experiments similar to, but much easier to perform than, those which have been employed in the work described in this paper. The differences between an aggressin and the specific factor of a tumour are evident.

These researches have led me to look upon cancer —using the term in its widest sense—as a specific disease caused by a virus (or group of viruses). Under experimental conditions the virus alone is ineffective; a second specific factor, obtained from tumour extracts, ruptures the cell defences and enables the virus to infect. Under natural conditions continued "irritation" of tissues sets up a state under which infection can occur. The connexion between the specific factor of a tumour and an irritant remains to be investigated. Some of the relatively unimportant "irritants" are known, such as coal-tar, paraffin oils, &c. The virus probably lives and multiplies in the cell and provokes the cell to continued multiplication.

The methods of experiment now described, and the obvious extensions of them, give the means of classifying the viruses, of investigating the nature of the specific factor, and of testing suspected "irritants," such as preservatives in foods, which may play a rôle in the genesis of a tumour.

I am indebted to the Executive Committee of the Imperial Cancer Research Fund for placing at my disposal their unrivalled series of animal tumours. My thanks are due to my former chief, Dr. J. A. Murray, F.R.S., for his continued critical interest in the work. To my colleague, Dr. W. J. Purdy, I am indebted for assistance in many experiments, for help in the routine inoculation of tumours, and for the preparation of charts; over and above this direct help, I desire to acknowledge the advantages I have derived from his criticisms both of experiments and the interpretations placed upon results. Finally, I wish to state that I owe much to my laboratory servant, Mr. W. Hall, for his willing service.

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THE MICROSCOPICAL EXAMINATION OF FILTERABLE VIRUSES

ASSOCIATED WITH MALIGNANT NEW GROWTHS.

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(From the National Institute for Medical Research, Hampstead, N.W.)

(With Illustrative Plates.)

Introduction.

THE search for filterable viruses by microscopical methods presents problems of great difficulty, due for the most part to the optical limitations of the microscope. The evidence that such organisms exist is based upon numerous experiments in which infective material has retained its infectivity after passing through a filter that will not pass the smallest known visible organism. There is at present no method in general use by which such filters can be standardised, and it is therefore not certain that filterable viruses can be regarded as organisms of any definite order of size. Other factors than size of the organism influence the result of filtration experiments, not the least being variability of the filters themselves and the viscosity of the filterable material. If filtration did give more exact information as to the size of any given organism it would then perhaps be advisable to adopt some other standard of reference than permeability. Such a standard might be the limits of microscopical resolution, by which all bodies of less than 0·25 μ in their greatest diameter should be regarded as filter-passers, although in that case some other more suitable name might well be found.

It is necessary to consider the main factors limiting both visibility and resolution in the microscope as these limits operate so definitely in any attempts to see or to photograph small bodies. It is also of interest to realise the probable size of filterable viruses in relation to the smallest micro-organism on one hand, and a large molecule on the other. The latter limits are shown diagrammatically in Figs. 1 and 2. The inner circle A in Fig. 1 may be regarded as a hæmoglobin molecule, molecular weight 16,600 approximately. The circles B, C, D, represent the orders of microscopical resolution obtainable if certain ultra-violet radiations are used of wave-lengths 232 μμ, 257 μμ, and 275 μμ respectively; they do not indicate the magnitude of relative wave-lengths. The circle E represents the relative position of the green line in mercury wave-length 546 μμ, with which the visual observations have been made. Between the positions A and B, therefore, no method at present exists by which microscopical resolution can be obtained. (The millimicron μμ = one-thousandth of a micron, 10^{-7} cm.) It is convenient to use the micron for microscopic dimensions and the millimicron for wave-length estimations. To enable a comparison to be made with an organism of known size, reference must be made to Fig. 2, which is on one-tenth the scale of Fig. 1. In this the relative wave-lengths are represented by the small inner circles B, C, D, and E, and the outer circle F represents a body of 1·0 μ in diameter, the average size of a small micrococcus. On reference again to Fig. 1 it will be seen that to obtain the necessary microscopical resolution, a term which will be explained later, and which is proportional to the wave frequency of the light used, it is necessary to use light of wave-length 275 μμ if a body of 0·1 μ in diameter is to be represented, whereas wave-length 257 μμ would resolve down to approximately 0·075 μ, a dimension that is of importance in the present investigation. Fig. 2 gives some idea of the possible sizes of filter-passers. It is certain that they are made up of a considerable number of complex molecules, but no exact limit is assignable in either direction. There are, however, two physical considerations that do suggest a limit of smallness. One is the difficulty there would be for a small body to overcome its

relatively great surface tension and to divide and reproduce itself by binary fission as larger organisms do; the other is the mean free path, or Brownian motion, which it would acquire if it exists as a separate particle. There are reasons to think that these conditions are provided against in the life processes of small organisms, and the evidence that this is so will be detailed later; at the same time these factors must and do operate at times, and they may therefore be regarded as limiting factors in any estimation of size. There are other circumstances that cannot be detailed here suggesting that filterable viruses are of sizes represented by the distance between B and D, and can therefore be photographed by ultra-violet light of the wave-lengths indicated. No objective has yet been constructed which works to or near the theoretical limit that the shortest of these wave-lengths should give, but experimental efforts indicate that such will be evolved in due course.

FIG. 1.

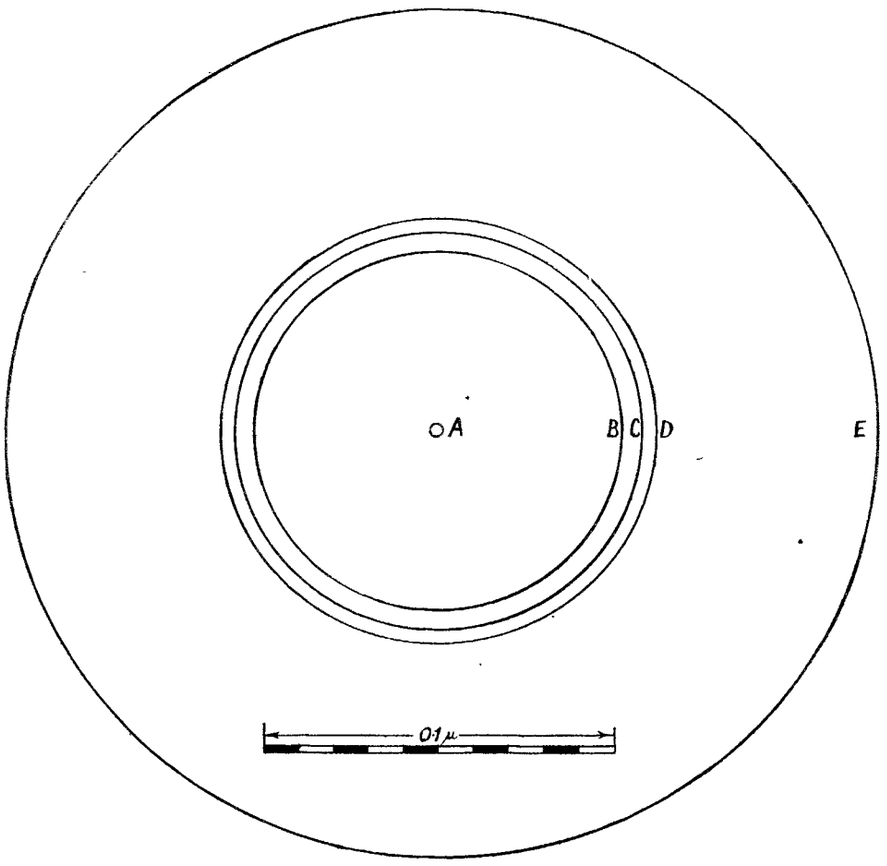
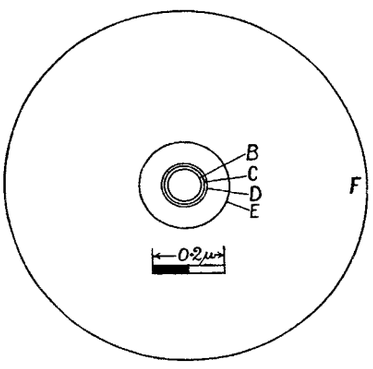


FIG. 2.



Diagrams to show relative magnitudes.

The Microscopical Limits of Observation.

Microscopic objects may be divided into two classes, those that are seen by transmitted light and those that can be made self-luminous. The former are seen as the result of partial or of selective light absorption, that is, they may be semi-opaque, the elements of structure absorbing more or less light, or they may be seen in colour as the result of selective absorption as in a stained preparation. For an object to be self-luminous, it must be illuminated in such a manner that no direct light reaches the micro-objective but only that which is refracted, diffracted, or scattered by the object itself. Under these conditions an object is seen as a bright image on a dark background and the method is known as dark-ground illumination. The main purpose in all microscopical observations is to obtain the greatest resolution, and the term, "limits of resolution," is applied to that state of affairs in which no further separation of elements of structure is obtainable. Only late in the last century was it definitely recognised that there are theoretical limits beyond which the microscope as ordinarily employed cannot go, and these theoretical limits are now all but reached under proper conditions of use. If two points in an object are within a certain limiting distance of one another they cannot be separated by any known means. Further, if an isolated element of structure is smaller than a certain limit the image seen does not represent its exact form or size. Magnification as such does not help, as, however much the image may be enlarged, there is no resulting increase of resolution. The main factors governing resolution are the numerical aperture of the objective used and the wave-length of the light employed. The best results are therefore to be expected when numerical aperture (N.A.) is large and the light used of the shortest wave-length practicable. While this power of delineating fine detail is of primary importance in all observation, another factor comes in with self-luminous objects which is hardly less important, and that is visibility. The term "ultra-microscope" is often inaccurately applied to all illuminating appliances that render an object self-luminous, but it should in fact only be used to describe apparatus which renders particles visible that are smaller in all dimensions than the resolution limits. In actual practice this limit is reached with isolated objects or elements of structure that are less than 0·25 micron in diameter. When objects larger in any dimension than this limit are being so observed the method may be described as "dark-ground" illumination, whereas, when the objects are smaller, "ultra-microscopy" is the correct term. Neither dark-ground illumination nor the ultra-microscope can be regarded as a means of increasing resolution, their main purpose being to obtain greater visibility, although the full resolving power of any given lens may be secured by this means. In the light of recent experiments it is, in fact, clear that full resolving power is obtained more certainly by an efficient dark-ground illumination method than by any other means. In theory there is no limit to the smallness of an object that can be made visible if a sufficiently powerful illuminant is used. In practice, however, the dark-ground illumination limits are governed by the following conditions:—

(1) That the particles under observation can themselves scatter enough light to enable them to be seen.

(2) That the particles are separated from one another by intervals that are within the limits of resolution of the microscope objective used.

(3) That there is sufficient difference of refractive index between the particles and the medium in which

they are immersed. Only under certain very limited conditions can the full resolving power of the best oil-immersion objectives available for visual work (those in which N.A. = 1·4) be secured by this means. The limits in biological work are less than this, and are imposed by the refractive index of the medium in which the objects to be examined are immersed. As these are rarely widely different from water (with a refractive index of 1·33) it follows that the N.A. of any objective used could not exceed about 1·27. In practice the use of even this N.A. introduces difficulties when there is but a small difference of refractive index between object and medium. The method that appears to be most satisfactory, therefore, is to use an illuminator with such an illuminating angle that it admits of the use of an objective of 1·20 N.A. for all preliminary and routine observations. The highest practicable N.A. 1·27 is reserved for use in cases where circumstances are favourable, a point that can only be determined by experience. The highest practicable resolution, therefore, by dark-ground methods is that which enables an object of the order of 0·35 μ to be seen and perfectly resolved, although much smaller bodies may be rendered visible and some of their physical properties determined. The limits of dark-ground methods in the search for filterable viruses are therefore obvious, but it has been found in practice that much preliminary work can be done. The only other method likely to be of service is, therefore, to use light of a short wave-length as indicated already. Approximately a body may be photographed of one-half the size of the wave-length used. The simple formula governing resolution is given by the relation, $a = \frac{1}{2}\lambda/\sin a$, where λ is the wave-length of the light employed when passing through the medium used, and a is the angle emitted by half the diameter of the back lens of the objective. Thus the value of d, which is the limiting distance between two particles, or the size of a single particle if a correct image is to be seen, can be reduced by increasing aperture or decreasing the wave-length of light used as an illuminant. The former factor is determined by the objective and for visual work cannot be increased. The latter can in theory be altered to any extent, and the usual expression for attainable resolution $\frac{\lambda n}{2\mu \sin a}$ can be diminished so that a filterable virus of any probable size should be resolvable. This is at least what theory indicates, and the direction of experimental work in the department of applied optics is to make such radiations available as are needed for any particular purpose. The present position is not discouraging, but it is admitted that many practical difficulties have yet to be overcome.

It follows, therefore, that there are at present only two methods of attack if a microscopical image of a filterable virus is to be obtained :—

1. The observation of the living material by dark-ground illumination methods in which visibility would be secured in terms of the size of the particular virus.

2. The use of light of short wave-length (that is by means of invisible radiations) resulting in higher resolution, proportionate to the wave frequency.

Both of these methods exclude any process such as staining, in which changes will certainly occur owing to the preparation or fixing of the material. Gye has already referred to the difficulties he experienced in endeavouring to demonstrate organisms in infective material. It is evident that in cases where the body to be observed is below the limits of microscopical resolution, any staining method would be useless. Even more, such processes would destroy the only recognisable characteristic that such bodies possess, the difference that there might be in physical, and particularly in optical qualities, between them and inert particulate matter. In the dark-ground arrangement this would be evident mainly in terms of visibility, but by the use of ultra-violet light it is possible to obtain a real image of a small body providing a short enough wave-length is used.

Dark-ground Illumination Methods.

The apparatus used for this method may now be briefly described. The source of light is a quartz mercury-vapour lamp of the non-vacuum type as designed for microscopy. Colour screens are used to give either monochromatic blue, green, or yellow. The green line in mercury is peculiarly suitable as it is a narrow one and so approaches closely to a true monochromatic light source. Further, as this line is in that part of the spectrum to which the eye is most sensitive it is possible to work with light of low intensity, a point of the utmost importance in dark-ground work.

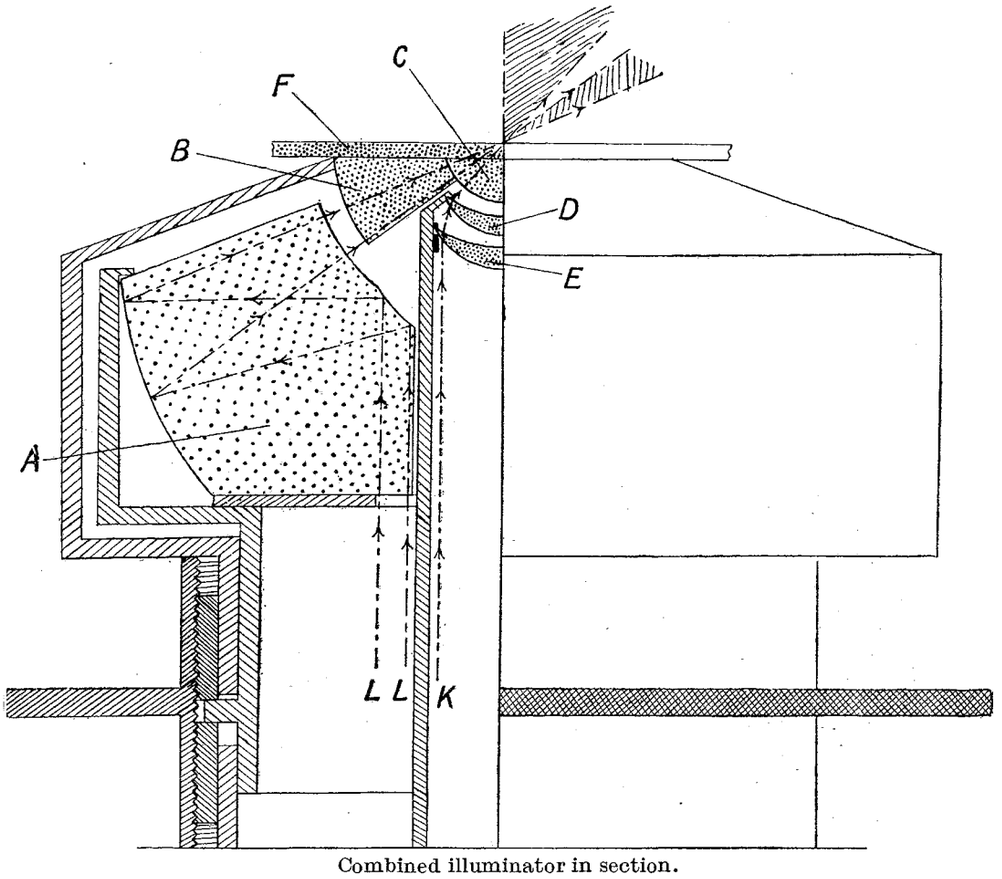
It is commonly stated, even in text-books, that a powerful light source is necessary, but this only results in such irradiation from larger particles that all fine detail is lost. A stable, well-made microscope is essential as all adjustments must be made with the utmost care. Thus, perfect centration of every part must be secured and the slide and cover-glass must be truly normal to the optical axis of the microscope. Full instructions for setting up a dark-ground illuminator will be found in the Special Report Series No. 19 issued by the Medical Research Council. The illuminators used have been designed to work with 2 mm. apochromatic objectives of 1·2 N.A. and 1·27 N.A., and the whole outfit is made by Messrs. R. and J. Beck, Ltd. Both these condensers work at their best with slides of the thickness of a No. 2 cover-glass. The material to be examined is therefore mounted between two cover-glasses and these are held in a chamber designed for the purpose. Provision is made to ensure that the layer under examination is as thin as possible, regard being paid to the necessity of preventing undue compression. A semi-darkened room is essential for this work, and the illuminant must be so shielded that no direct light reaches the eye at any time. Another essential is to provide means for reducing the light intensity without altering its quality. This is best secured by using semi-platinised glass screens prepared by the method described in the writer's book on photomicrography. A high-power compensating ocular × 18 to × 25 is necessary for final observations and the objectives mentioned will tolerate these perfectly.

The Microscope for Ultra-violet Photography.

The mechanical part of the microscope used is of special construction, as no ordinary one would fulfil the required conditions. Its essential features are great stability and high accuracy of all movements, and its general design is seen in Plate I., Fig. 1. Both the coarse and fine adjustment A_1 and A_2 are actuated through a large accurately-cut screw of the type usually employed on an interferometer, the bed being a heavy casting of girder type supporting the whole. The objective and ocular are carried on separate carriers O_1 and O_2, with no body-tube between them as in an ordinary microscope. This ensures that the adjustment of either optical part does not disturb the other. The mechanical stage is strongly supported so that it is truly at right angles to the optical axis. The two milled heads A_4 control the movements of the object on the stage within certain limits, but the quartz slide is held in a novel manner to give some freedom of movement by hand, while ensuring that the slide is always kept in close contact with the stage, a matter of moment in ultra-violet work. The combined dark-ground and ultra-violet light illuminator O_3, described later, is supported on a strong centreing sub-stage, which is moved by an accurate screw controlled by the large graduated head A_3. This is, in fact, a divided circle enabling the dark-ground or the ultra-violet light illuminating apparatus to be accurately focussed in any wave-length. The mercury-vapour lamp M_1 is mounted on a subsidiary optical bench which is arranged at right angles to the axis of the microscope. At M_3 a reflecting prism is mounted which can be swung into line, thus enabling the beam of light from the mercury-vapour lamp to be projected into the

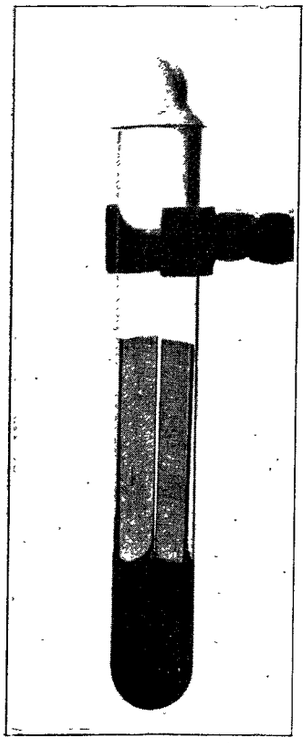
microscope. When the prism is swung out of line ultra-violet light from the spark-gap S_1 is projected by the quartz lens S_2 through the quartz prism S_3, and so into the central part of the microscope condenser. The arrangement therefore admits of either illuminant being brought into use by simply deflecting the prism M_3. This description is of necessity superficial, and assumes some knowledge of the orthodox methods of ultra-violet light microscopy, a full description of which by the writer can be found in the "Dictionary of Applied Physics." One of the main differences in principle here introduced is that focussing in any wave-length desired is performed in reference to the dark-ground image seen in the green line in mercury wave-length 546 $\mu\mu$. Focussing by means of the fluorescent image is both difficult and dangerous owing to the action of ultra-violet light on living organism. Changes can be set up which might be regarded as normal appearances in the organism. The actual exposure, therefore, of any material to ultra-violet must be the shortest possible. There is no reason to regard the action on living organisms as a trigger action, a certain time factor is involved, but precautions must be observed to ensure that no change due to the light itself is set up. A long series of investigations on the action of ultra-violet on bacteria has been carried out so that this part of the subject is understood; an account of these experiments will be published in due course. There is no reason to regard the filterable viruses that have been under observation as more sensitive to ultra-violet light than ordinary bacteria; there is, in fact, some evidence that they are less sensitive, but this point again requires further investigation. The actual microscopical method employed has therefore been the composite one indicated, and consists of the use of a high aperture dark-ground illuminator already described, mounted concentrically with and encircling a quartz condenser. The former secures visibility and is used for purposes of search and location; the latter is available for use with any suitable ultra-violet radiation and enables a transmitted light image to be secured on the photographic plate. The problem of the best quartz objective to use is still only partly solved; much remains to be done to ensure that the best result should be obtained in any given wave-length. In collaboration with my colleague, Mr. John Smiles, this part of the work is being attacked with every evidence that success will follow. One photograph is shown in which light of 257 $\mu\mu$ wave-length is used, and this is certainly the shortest wave-length recorded as having been so applied to microscopy.

FIG. 5.



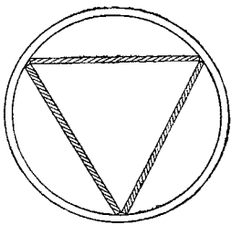
Combined illuminator in section.

FIG. 3.



Culture tube showing the disposition of glass slips.

FIG. 4.



Methods of Cultivation.

Cultural methods have been devised and carried out by Gye and are described by him in the foregoing paper. It was, however, found necessary to design some arrangement by which it could be determined whether colonies were developing, and to examine these colonies in situ. From the nature of the growth obtained in fluid there was some reason to doubt whether it would be possible to obtain growth on solid media. Further, as the quantity of growth in fluid, in relation to the volume of medium, is not so prolific as with ordinary organisms, it was regarded as probable that any colonies obtained would be few in number and small in size. A means of examining the surface of serum-agar was therefore desirable in which a 2 mm. oil-immersion objective could be used. Thus it was really necessary to apply to the medium for the detection of colonies a means that would ordinarily be used for the detection of organisms. Briefly, the arrangement that has been found successful is seen in Figs. 3 and 4. An ordinary test-tube is taken, preferably of rather large size, and into it three thin glass slips are introduced in the configuration seen in Fig. 3.

These three slips form a triangle in plan, Fig. 4, and are of such a width that they are free to move in the direction of the length of the tube but cannot

MR. J. E. BARNARD: THE MICROSCOPICAL EXAMINATION OF FILTERABLE VIRUSES.

PLATE I.

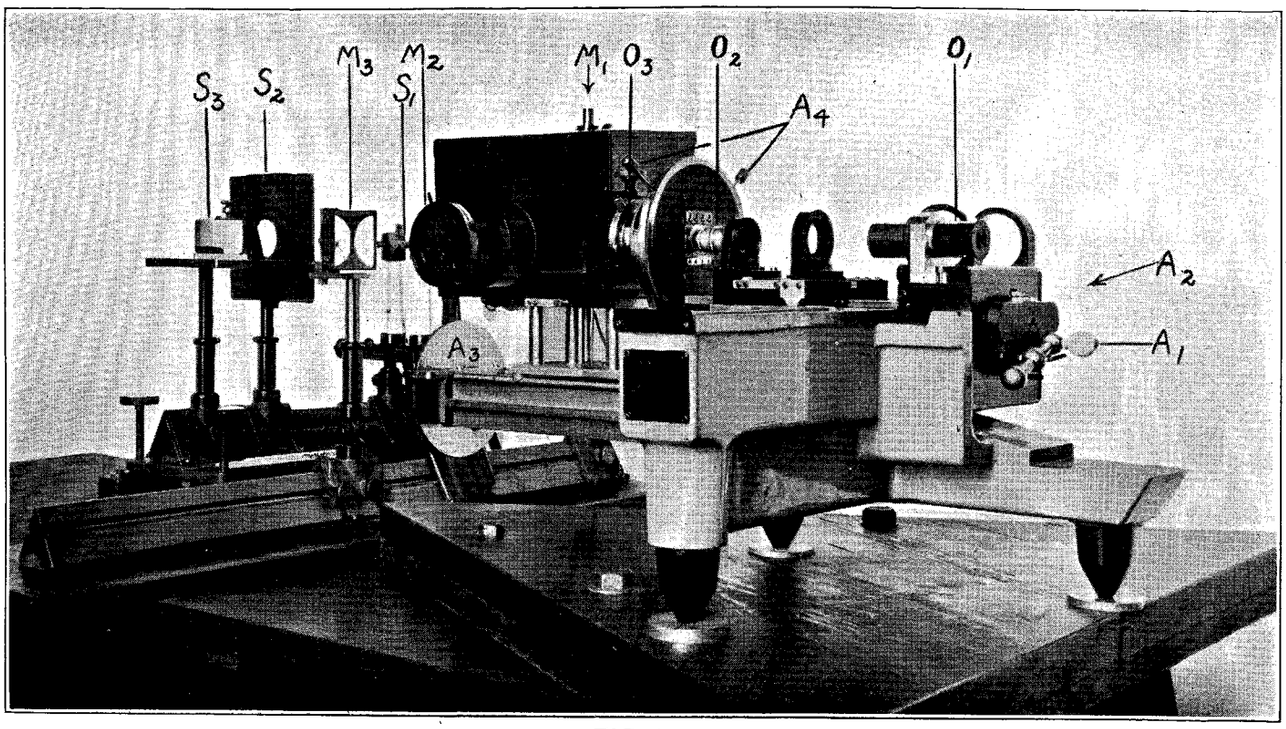


FIG. 1.

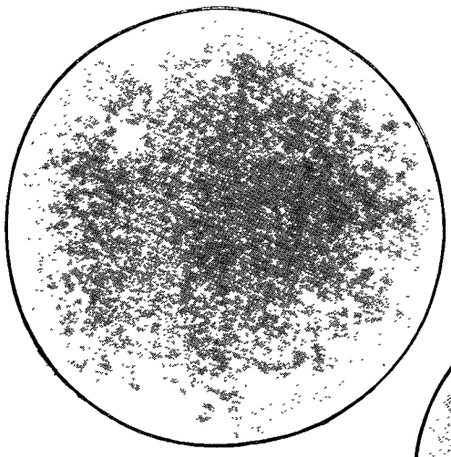


FIG. 2.

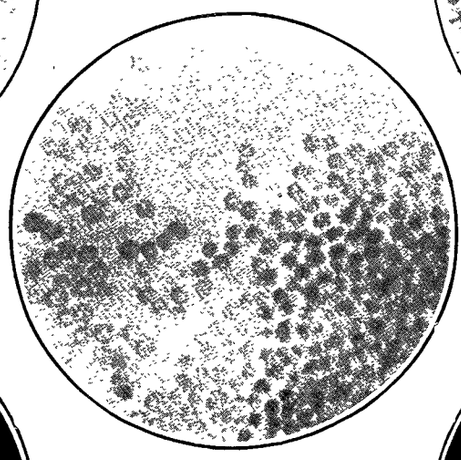


FIG. 6.

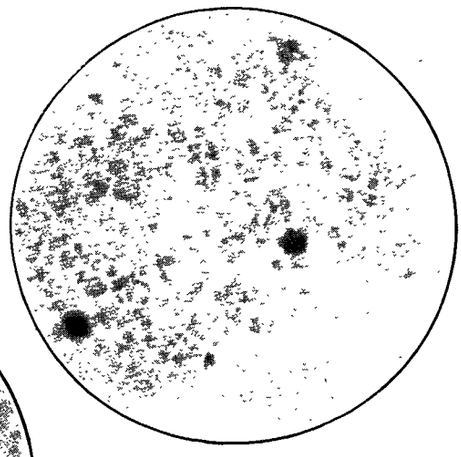


FIG. 3.

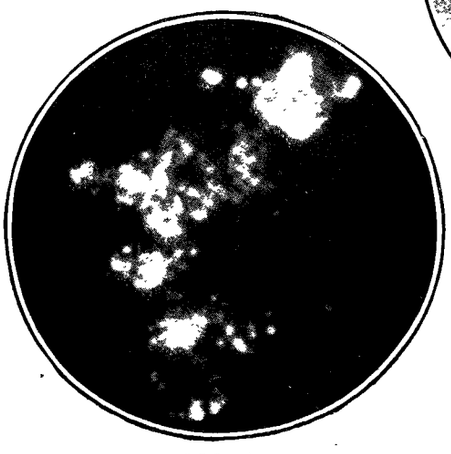


FIG. 4.

FIG. 5.

PLATE II.

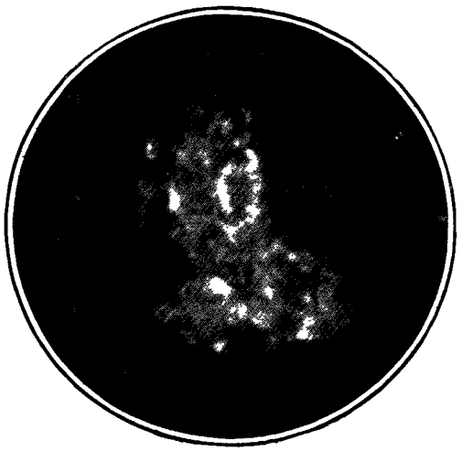


FIG. 1.

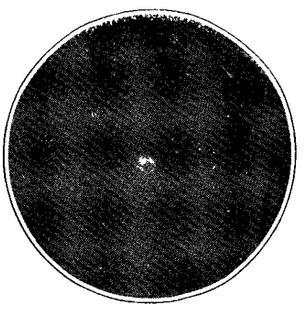


FIG. 2.

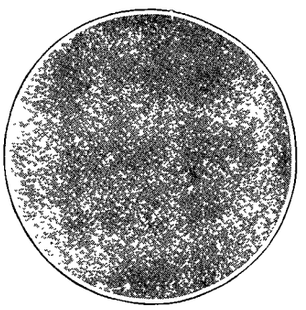


FIG. 3.

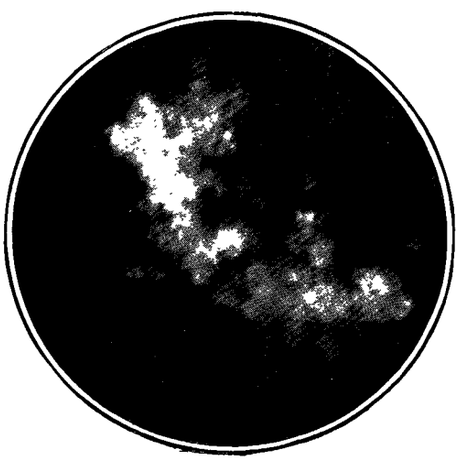


FIG. 4.

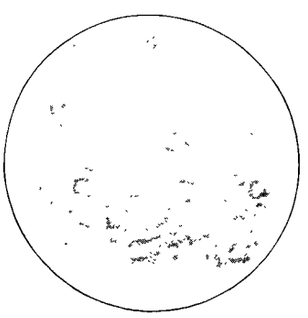


FIG. 5.

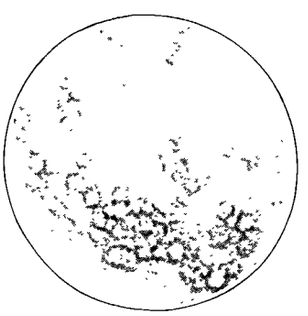


FIG. 6.

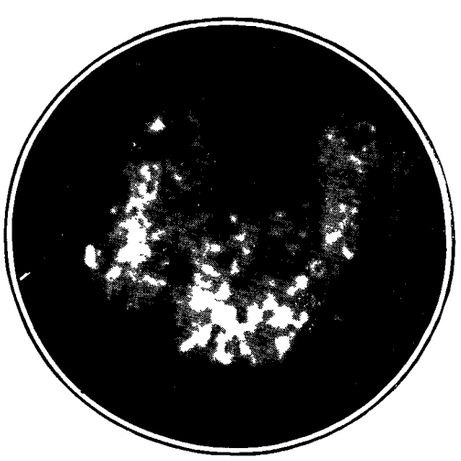


FIG. 7.

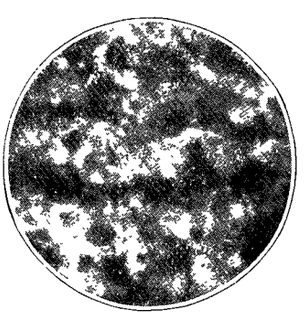


FIG. 8.

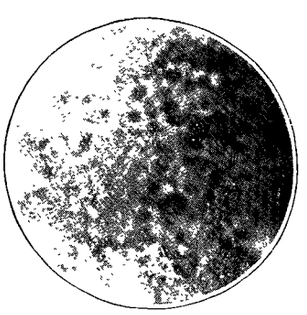


FIG. 9.

move in any other way. They dip into the agar medium seen at the bottom of the tube, and when the agar solidifies are quite stable. The agar is melted, the serum added in the ordinary way and inoculated with the infective material. After inoculation and before solidification the tube is tilted so that the agar runs along the tube and touches the external face of each slip of glass in turn. The amount of agar left on the glass is determined by the temperature, but it is always possible to repeat the tilting process, several times if necessary, until enough medium remains. The tube containing the three slips is incubated and at any suitable period one slip is removed for examination, the remainder going on for a longer period if desired. Either glass or quartz slips may be used so that examination is possible by the dark-ground method or photographs can be taken in ultra-violet light.

In either case a cover-slip of glass or of quartz is placed on the slide so that cedar-wood oil or glycerine may be used as an immersion medium. The serum-agar usually has sufficient surface moisture to secure optical contact between cover-glass and agar layer. It is doubtful whether colonies would have been detected without this method, and a fuller description will be published later by Mr. F. Welch, who has taken so large a part in working out the details.

Description of Combined Illuminator.

The design of this double purpose illuminator is seen in Fig. 5. The front lens of the central quartz condenser is seen at C, the other two lenses of this combination are D and E. The front lens is in glycerine immersion contact with the quartz slide F, on which the object is supported. The outer quartz ring B is part of the dark-ground illumination appliance and is mounted as shown to transmit the dark-ground beam without alteration of direction on striking the quartz lens C. It will be appreciated that this lens C is an essential part of both systems (in the drawing the closely dotted parts are of quartz, while the wider dotting indicates glass). The ultra-violet light therefore passes through the quartz system in the direction K, as shown, and is focused at the point of observation. The dark-ground illumination, portion A, is an annular glass reflector substantially as shown, which transmits its illuminating beam of visible light also on to the point of observation. By sliding a central stop into the apparatus the quartz system is obscured, while the outer dark-ground portion is covered at will by an annular stop. It follows, therefore, that either part may be used by simply inserting the suitable stop. The light energy required for ultra-violet work is supplied by a high-tension alternating spark between suitable metallic electrodes. The electrodes are selected to give any required wave-length, and a fuller description of this will be found in the author's book on Photo-micrography, now in the press. The method of focussing the image in ultra-violet work has hitherto been by means of a fluorescent screen. The image formed by the quartz ocular is thrown on to such a screen, and is then observed by a short-focus telescope. Focussing by this means is always difficult and sometimes impossible, if the object is a translucent one; further, it entails considerable exposure of the object to ultra-violet light. To minimise the difficulty small mercury droplets may be deposited by sublimation on the quartz slide before the material under observation is mounted. Such droplets can be deposited in almost any order of size by variation of temperature of the mercury and time taken for its deposition. Some droplets are seen as dark discs in Plate I., Fig. 3, and this method has proved of value. The combined illuminator has in most cases, however, rendered their use unnecessary, as visibility can usually be secured sufficiently to focus an image in monochromatic green light, and with the accurate microscope described the focussing in ultra-violet becomes a purely mechanical process.

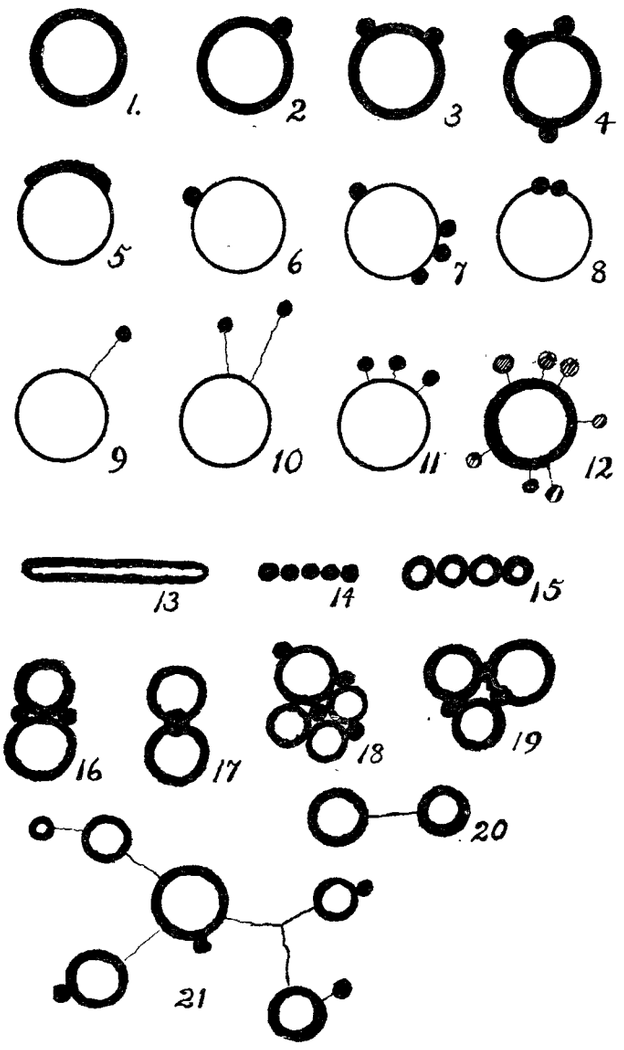
Observations.

The microscopical search for any filterable virus had been prepared for in part by a continual effort to improve the mechanical and optical parts of the microscope, and some definite advances had been made before any material was available. During the war some experimental work on trench fever suggested that something might be done by studying the optical characteristics of infective material. Further, a considerable amount of work had been done with bovine pleuro-pneumonia, as this appears to be the smallest organism known to grow under ordinary bacteriological methods of cultivation. There was, however, no cultural material available of an undoubted filter-passer until the successful cultivation of the Rous fowl sarcoma by Gye. The earliest efforts to observe this organism were of the simplest character, and consisted of the careful observation of uninoculated culture-media side by side with observation of inoculated tubes of subsequently proved infectivity. Dark-ground illumination as described already was employed, and to ensure that the comparison should be exact two microscopes of similar design and with the same optical equipment were set up. Thus infective and non-infective material could be examined and compared under identical conditions, an arrangement that has proved of great value. All the conditions described herein as being essential for accurate observation were carefully observed. Further, as a control, cultures of pleuro-pneumonia were kept going similarly, and observations were made on this organism in the same way. Any tube of infective material that contained bodies not to be seen in a similar uninoculated tube was then examined under the combined microscope, and if practicable an ultra-violet photograph obtained. Thus a dark-ground visual image was observed and ultra-violet photograph obtained that under favourable conditions would give an image of an isolated element of structure down to an order of size of 0·075 μ. The observations on pleuro-pneumonia were all carried out on living material, and not in reference to stained preparations. These are still being carried on by my colleague Mr. John Smiles at this institute, and will be described fully by him in due course. Briefly, however, these observations lead to some tentative conclusions. Thus the pleuro-pneumonia organism is a small particulate body about 0·2 μ diameter which can only grow and reproduce itself under certain conditions. The particulate form is accompanied by and is dependent for its reproduction on a larger spheroidal body which develops from the particulate form and which then forms the host on which the particulate form can develop further.

Fig. 6 shows diagrammatically the sequence of events as we understand them at present. After about 20 hours a tube that has been inoculated from an agar surface colony will show a number of spheroids that may be represented as No. 1. A few hours later a small refractile dot is seen on one part of the spheroid as No. 2, subsequently developing further bodies as in 3–4. Some spheroids appear to have a thickened wall, while others are thin and of low visibility, but recent observations suggest that this apparent thickening is due to a number of particulate bodies below the limits of size that can be visually resolved. The attached particle then tends to move away from the spheroid, but is still attached to it. This attachment is rarely observed visually, but ultra-violet photographs show it to be a fine filament which is derived from the spheroid and gives to the latter an amœboid appearance. Some particles soon develop into small spheroids and form groups, as in 16, 17, 18, 19, each of them developing attached particles substantially as shown. In most cases, however, the spheroid tends to disintegrate, or at least become less visible, and finally gets into an unstable physical state, somewhat as shown in 12 and 21, where the central spheroid is still evident but is surrounded by a number of smaller spheroidal bodies. This formation is quite

characteristic, but can only be appreciated when actually seen under the microscope. The grouping and movement in fluid is an interesting physical phenomenon in which a rotating spheroidal body unevenly loaded with particles appears to assume a form suggesting a spiral disposition. That the particles can and do develop into spheroids has been proved by careful filtration experiments. Thus a fluid culture has been filtered by us through a Bechhold collodion membrane of such porosity that it passes the particulate forms but keeps back all visible spheroids. The filtrate was incubated for 12 hours at 37° C. without any evident change, but a fresh tube of serum broth inoculated from it and incubated during the same period show that all the particulate forms had developed into spheroids, and the full life-cycle ensued. The explanation of the life-cycle may, therefore, be that the spheroids take the place of the living cell in providing conditions suitable for reproduction. In very young colonies of pleuro-pneumonia (see Plate I., Fig. 2) the particulate forms do apparently divide for the first few hours only, and are then immersed in some excretory material which reduces their surface-tension. Soon, however, the spheroids develop on the outer edge of the colony, and further reproduction takes place on these in the manner described. The argument is too long for full extension here, but it does appear to be the means by which the difficulty of subdivision by a small body is overcome. It is certain that this organism does not divide in the

FIG. 6.



Diagrammatic representation of bodies seen in cultures of bovine pleuro-pneumonia.

same way as do ordinary bacteria. The difficulty of growing them artificially, particularly on solid media, suggests that some special conditions are necessary, but speculation is futile until the exact physical conditions are better understood. A long series of comparative observations on cultures, all of which have ultimately been proved by animal experiments to be infective, has led to the conclusion that the same type of phenomenon, but on a smaller scale, is to be observed in the Rous fowl sarcoma, mouse sarcoma 37/S and latterly in human carcinoma, as can be seen in cultures of pleuro-pneumonia. In no essential point is there any difference, except that the cultures from malignant growths are slower in developing and do not produce such rich cultures. Probably no single factor has hindered the cultivation of filterable viruses so much as the assumption that a culture in broth, for instance, must become opalescent, or that a growth on solid media must develop colonies easily visible to the naked eye. If as we think probable the spheroids are an essential part of the life-history of these organisms then, owing to their low refractive index, they would require to be present in impossibly great numbers to produce evident cloudiness. The fluid cultures from malignant growths always remain clear; on solid media the colonies are so small that they require the use of a high-power objective to see them. The incubation period has not usually exceeded seven days, and examination of the cultures and similarly incubated uninoculated tubes has been made from three days onwards. During that period the same life-cycle has been observed in all cases as that seen in pleuro-pneumonia. Observation is more difficult than with pleuro-pneumonia owing to the small size and lower visibility of the malignant growth viruses. The microscopical technique has, therefore, to be carefully adapted to these more exacting conditions. Unless considerable care is exercised the spheroids may easily be confused with other spherical bodies occurring in most organic fluids. This can only be obviated by a careful preliminary study of uninoculated culture-media.

Description of Plates.

PLATE I.

FIG. 1.—Microscope described in text.

FIG. 2.—Young colony of bovine pleuro-pneumonia photographed in ultra-violet light. × 1200.

FIG 3.—Edge of an older colony showing spheroids. × 1800.

FIG. 4.—Young colony showing particulate form. Dark-ground illumination. × 1850.

FIG. 5.—Older colony showing development of spheroids. × 1850.

FIG. 6.—The edge of a young colony of *M. pyogenes aureus*, photographed in ultra-violet light to show normal development of an organism. In this case the line of subdivision is seen before the organism changes its external form.

Figs. 2 to 6 are colonies grown on slips by the method described.

PLATE II.

FIG. 1.—Rous fowl sarcoma colony on agar slip. Dark-ground illumination. × 1850. The particulate bodies are seen centrally in small bright masses, the spheroids developing on or near the edge.

FIG. 2.—Rous fowl sarcoma. An isolated spheroid with attached particle. Dark-ground illumination. × 1850.

FIG. 3.—Same as Fig. 2, but photographed in ultra-violet light. The attached particle is seen to be a small spheroid. × 2000.

FIG. 4.—Mouse sarcoma. 37/S. Colony on agar slip. Dark-ground illumination. × 1850. Considerable development of spheroids.

FIG. 5.—A small portion of the colony photographed in ultra-violet light. Wave-length 275 $\mu\mu$ showing that the spheroids may depart rather considerably from a spherical form at some stages of growth. × 2200.

FIG 6.—Same as Fig. 5, but photographed in ultra-violet light. Wave-length 257 $\mu\mu$. × 2200. Showing the ultimate particulate character of the spheroids. From this and other photographs in the same wave-length it is estimated that the particulate form of the virus is of the order of size of 0·075 μ. It is certainly appreciably less than 0·1 μ, as resolution cannot be obtained with any greater wave-length.

FIG 7.—Human carcinoma. Colony on agar slip. Dark-ground illumination. × 1850. The same general characteristics are to be seen as in Figs. 1 and 4.

FIG 8.—A small portion of the edge of a larger colony showing spheroids interspersed with particulate bodies. × 1850.

FIG. 9.—A portion of the edge of the same colony as seen in Fig. 8 photographed in ultra-violet, light wave-length 275 $\mu\mu$.

The general conclusions to be drawn from the photo-micrographs are that the developmental stages in each of the above are similar. It is feared that the reproduction will not show so well as the original negatives, particularly in those done in ultra-violet light. The difficulties are greatly increased owing to the relative opacity of serum-agar to ultra-violet light, the possibility of obtaining photographs at all being due to the thinness of the layer of culture medium. In addition, the negatives are very thin and lacking in contrast, although they do in the originals show much delicate detail.

NOTE ON THE PRECEDING PAPERS.

BY J. E. BARNARD AND W. E. GYE.

The separate parts of this investigation have now been described, and it remains to indicate how far the results obtained fit together. Our belief that the small bodies seen and photographed are the actual virus depends partly upon the fact that control uninoculated tubes of medium have been invariably blank, and partly upon the correspondence between the microscopical findings and the results of experiments upon animals. This correspondence—allowing for the real difficulties in both parts of the common task—has been so close that, although final proof has not been attained, we are convinced that our conclusions are sound. By final proof we mean the cultivation of the virus from a single colony, or if possible from a single spheroid, and the production of a tumour with the culture thus obtained. This work has been under consideration for a long time, and will be attempted when circumstances permit.

THE

Bowman Lecture

[ABRIDGED]

ON

THE FOUNDATIONS OF VISION.

Delivered before the Convention of English-Speaking Ophthalmological Societies on July 16th, 1925,

BY SIR JOHN HERBERT PARSONS, C.B.E.,
HON. D.SC. (BRISTOL), F.R.C.S. ENG., F.R.S.

[AFTER making introductory reference to Sir William Bowman and his work, the lecturer went on:—]

I propose to delve into the very foundations of the physiology of vision in the hope that a survey of the facts brought to light may elicit some general principles which will clarify our knowledge and point the way to further research.

In so far as the quest is successful its utility will not be limited to vision and to ophthalmology; its repercussions must inevitably be felt in the whole domain of the physiology of the senses and in the psychology which is the conscious expression of that physiology. For our waking life is a continuous cinematographic exhibition, in which varying perceptual patterns succeed each other upon the screen of each individual's experience. Each pattern is, in psychological language, a "presentation," a living picture, in which one feature occupies the focus of attention, surrounded by other more faintly apprehended features which form a relatively indifferent field. It must not be supposed that any such picture is a purely visual presentation, such as we see in a picture gallery. It is a composite picture made up of elements derived from all the so-called senses, and perhaps the object of consciousness is simply to combine and synthesise these diverse factors, in a way which is incomprehensible when viewed simply from the standpoint of the summation of physical quantities. Yet in man the prepotent factor is generally the visual factor, just as in many lower animals the prepotent factor is that derived from the sense of smell.

INTEGRATION OF PHYSIOLOGICAL IMPULSES.

The integration of these diverse physiological impulses is an active—kinetic—process, and it manifests itself in kinetic consciousness. But there are hierarchies of integration, and when a lower level integration has proved sufficiently serviceable to any given species it becomes part and parcel of the make-up of the individuals of the species. It then generally assumes the characters of potential consciousness, or in common terms is relegated to the "unconscious."

Further, there are many such integrations at every level—and there are many levels, only great groups of levels being collected within the confines of such terms as reflexes, instincts, and so on. Physiologically, the lower levels are, in vertebrates, associated with the activities of the spinal cord and medulla; higher levels with the mid-brain and thalamus; higher still with the palæo- and neo-pallium.

There are comparatively simple integrations and there are integrations of simpler integrations, forming different "patterns" *in* each level and *at* each level. And the most important point is that *all these patterns are of biological significance*—they are adaptations in the service of the individual or the species. Hence the most significant features of any pattern are themselves extremely complex. What I mean can be best shown by an example from ontogenesis. In very early days of life a child's attention is attracted by a bright light, and the eyes are moved so that the image of the light is formed on the macula of each eye. A child is soon most attracted towards its mother, and its behaviour towards her differs according to the tone of the mother's voice, her facial expression, and so on. Now, facial expression is a very complex visual presentation, and it is scarcely conceivable that at this very early age the child has succeeded in accurately correlating the innumerable visual impulses into which a facial expression can be analysed. We must rather regard the child's impression as a psychological pattern made up of the integration of a number of relatively undifferentiated auditory, visual, and other complexes, of which the still relatively undifferentiated visual complex forms the focus. It responds to this complex by appropriate behaviour simply because through countless preceding generations its ancestors have responded to similar and even less differentiated complexes.[1] The lower down the animal scale the less differentiated are the complexes to which the individual responds—and his responses are biologically determined—i.e., they are strictly utilitarian. Hence we conclude that the behaviour response is dominated by the psychological pattern, and that higher patterns dominate lower patterns. But, since the lower patterns are more primitive they are the least vulnerable—teleologically because they are concerned with vital processes upon which the survival of individual and species depends. The higher patterns are more highly differentiated and therefore more vulnerable. So that even these complex psychological patterns can be divided into two great groups—one, protopathic and of vital import, and another epicritic and of gnostic import.

PROTOPATHIC AND EPICRITIC SENSIBILITY.

I have adopted the terms protopathic and epicritic from the work of Head and Rivers on the tactile sensibility, feeling assured that the fundamental conception is justified, though I do not agree with all the detailed conclusions arrived at by Head.

Using the terms in the wider sense, we may say that the cognitive element of the crude protopathic response is diffuse, vague, and ill-defined. There is reason to think that the adequate stimulus produces the maximum response at the threshold—the reaction is of the all-or-none variety. The higher differentiation of the cognitive element introduces differentiation of