THE APPLICATION OF PHOTO-ELECTRICITY TO THE DETERMINATION OF BACTERIAL GROWTH RATE.

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(With 3 Figures and 2 Graphs in the Text.)

In this paper a short account is given of the application of photo-electric methods to bacteriology. Although such methods are now well established, and have been used in colorimetry and nephelometry for some years, they have not received the attention they deserve. The apparatus required, apart from the cell, will be found in any well-equipped laboratory; and their simplicity, as well as the elimination of personal errors, should assure the use of such methods in all experiments involving the measurement of colour intensity or turbidity of fluids.

The estimation of the number of organisms in a broth culture by methods in common use is laborious and subject to gross error. These methods have been summarised and criticised recently by Gardner (1931), and the errors found vary between 10 and 50 per cent. The time consumed in a single estimation also varies greatly; and, when organisms are counted directly in a counting chamber, is considerable.

During the past few years experiments have been carried out to devise a method whereby such estimations could be performed accurately and instantaneously, in a growing culture, without the intervention of the factor of personal error. For this purpose many types of light-sensitive cells, and many variations in technique and arrangement have been investigated. These will be summarised, and the final arrangement, which has been entirely satisfactory, will be described in detail.

The principle was in all cases the same. It involves the use of a light-sensitive cell as a nephelometer. Such a system demands a constant light source, a cell of great sensitivity, of linear response to varying light intensities, and no "lag" or "tiring" properties. In addition, a sensitive recording instrument is essential.

Light-sensitive cells respond either (*a*) by variation in conductivity, *e.g.* the selenium cell; (*b*) by the liberation of electrons from metals, *e.g.* the potassium

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cell; or (*c*) by the actual production of a current, *e.g.* the Becquerel effect. Of these, selenium is of little use, as its temperature coefficient, and its lag, are great. The potassium or alkali metal cell can be adapted for this purpose, but the current produced is very small, of the order of micro-amperes, and leakage is a large factor of error. It can, however, be used with a Lindeman electrometer, and experiments performed in this way indicated that very small variations in the number of organisms could be detected, *i.e.* 1000 organisms per c.c. The conditions, however, which are necessarily fulfilled if accurate results are required are rigid, and the sensitivity is of an order far beyond what is required in most investigations.

By amplifying the current, a less sensitive unipivot galvanometer can be used; but methods of amplification involve errors due to the characteristics of the thermionic valves used. These are reduced to a minimum by means of the pentode valve circuit shown in Fig. 1, and this method is simple and satisfactory to a degree. When potassium cells are used, the best type was found to be either the vacuum potassium cell made by the General Electric Company, or the Nava Vacuum Sodium Cell made by Tungsram Electric Lamp Works. The galvanometer was the unipivot type manufactured by the Cambridge Instrument Co.

The system employed at present involves the use of an oxide cell, utilising the Becquerel effect, which produces a maximum current of 5 milliamps in sunlight. This cell, which is not yet in general use, has no lag, never tires, and produces a linear response with varying light intensities. It requires no amplification and has now been in use for a year without showing any evidence of deterioration.

The system may be understood by reference to Fig. 2. A light source *A* shines through a filter *B*, a lens *C*, and a culture chamber *D*, to the cell *E*. All this part of the system is in an incubator. The current which is produced by the cell is fed into the circuit arrangement shown in Fig. 3, which is so adjusted that the mirror galvanometer gives a zero deflection. This is obtained by adjustment of the rheostat on the Cambridge potentiometer. As the organism grows, and turbidity increases, the changes in light intensity are measured on the calibrated rheostat scale. A curve of opacity can thus be drawn, and these opacities are calibrated by readings of known numbers of organisms, estimated by direct counting in a counting chamber.

Certain details may be considered separately.

1. *The incubator* should be of large capacity, and electrically heated. The heat of the light source may otherwise raise the temperature above 37° C. Apart from the undesirability of this in bacteriology, there is a temperature coefficient of 0·06 per cent. per ° C. in the cell. Errors from this cause may be obviated by the use of a thermo-couple in series with the photocell circuit, and this has in fact been used, although for general purposes it is not necessary. The cell is embedded in plasticine to prevent sudden changes in temperature on opening the incubator door.

2. *The light source.* A 32-watt straight filament lamp was used. Variations in this source are either gradual, due to deterioration of the lamp, or sudden, due to changes in the E.M.F. of the supply. It is not convenient to use accumulators, which produce a constant E.M.F., as they quickly run down and are

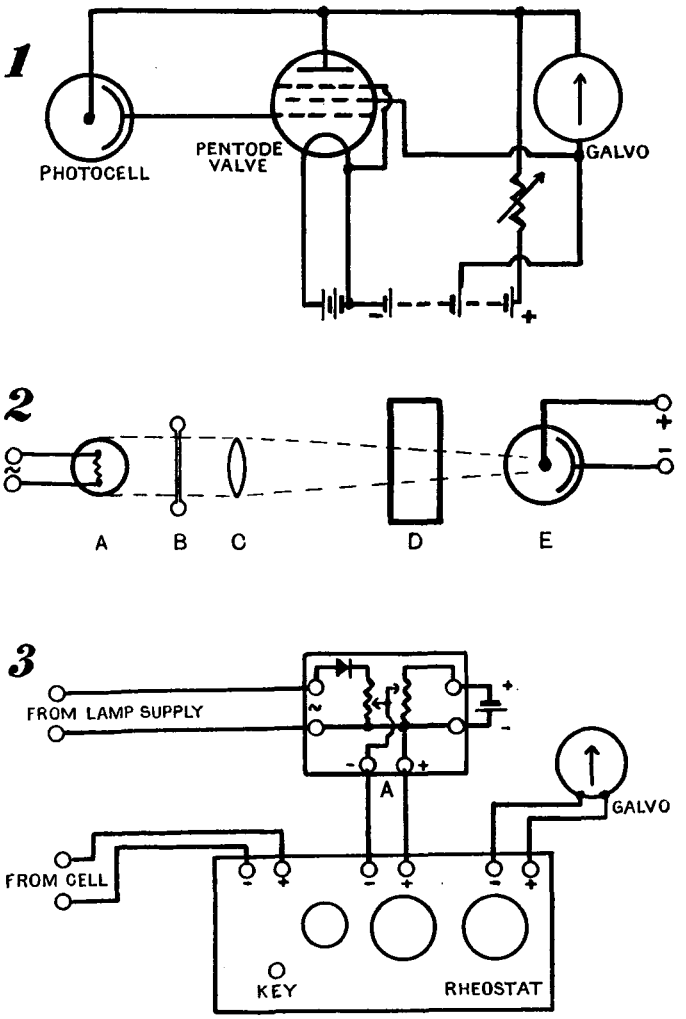


Fig. 1. Diagram of pentode circuit used with potassium cell.
Fig. 2. General arrangement of apparatus.
Fig. 3. Diagram of circuit used with "Sperrschicht" cell.

only accurate on full charge. Mains supply was therefore used, with a suitable step-down transformer.

To compensate for deterioration in the lamp, a neutral filter was inserted in the light beam, and a standard deflection of the galvanometer was attained before each experiment. Lamps deteriorate slowly, and thus each experiment can be calibrated.

Fluctuations in the E.M.F. of the mains were at first a great difficulty, but were entirely eliminated by the new method described later, developed by one of the authors (C. G. L.).

3. *The filter.* While the effect of light of the intensity used is probably negligible in effect on bacterial growth, it was thought wise to exclude all but red rays. A Wratten filter, No. 25, was therefore used. The cell is fortunately very sensitive to red rays.

4. *The culture chamber.* After experiment with several types, a flat-sided container with optically ground glass sides, was generally used. All containers were sterilised by boiling in methylated spirits. The medium must be filtered before use.

It was found, however, that various types of culture chambers enhanced or decreased the accuracy and sensitivity of readings taken during different phases of the bacterial growth-rate curve.

If it was required to determine accurately the shape of the curve at the beginning of the growth, a spherical container having a diameter of 55 mm. was found the best to use. The container acted as a condensing lens, while the medium was clear—and was so placed that the filament of the lamp was focussed on the cell. As turbidity increased, a certain amount of light was absorbed, but in addition to this the filament became out of focus. This enhanced the sensitivity sufficiently to give accurate readings long before any opacity became visual.

To make an accurate graph of the growth rate at the end of the growth curve, and when the maximum opacity is almost reached, a flat-sided container, about 10 mm. thick, proved to be most suitable. Even with this container, a good growth curve could be obtained from the period of inoculation.

For general use, however, a square glass box was used, having a thickness of 50 mm. The glass used was optically ground in order that the container could be removed and replaced during an experiment without introducing any error. This container gives very good results at all phases of the growth rate.

Technique.

Oxide cells. A very recent addition to the realm of photo-electricity is the property of metal to metal-oxide films producing an electromotive force when illuminated—known as the Becquerel effect—and this type of cell was used.

The "Boundary Layer" or "Sperrschicht" photo-electric cell, as this type of cell is called, comprises a nickel-iron base upon which a silver-selenium layer is deposited. The current produced is sufficient to operate a reasonably sensitive galvanometer without the aid of any amplification or polarising voltage.

Experience showed that the use of the oxide cells removed all difficulties associated with variation of battery voltage and characteristics of valve amplification.

Elimination of the variabilities of the supply to the exciter lamp was the next procedure. This was eventually accomplished in the following manner.

In the circuit diagram Fig. 3 is the scheme of connections which was finally used.

It can be seen that a potential difference exists between the leads from the photo-electric cell, due to excitation from the lamp; this P.D. is then fed into a Cambridge potentiometer and balanced out by an opposing E.M.F. from source *A*. Zero current then flows through the galvanometer.

Source *A* is obtained from a 2-volt accumulator connected across a resistance, and across this resistance is fed another voltage in parallel. This secondary voltage is obtained from a Westinghouse metal rectifier connected to the source of current supplying the exciter lamp.

In effect we have, therefore, at source *A*, a steady P.D. from an accumulator and another P.D. from the exciter lamp.

If the 2-volt accumulator was used solely as the opposing E.M.F., any decrease in the intensity of the exciter lamp, due to mains fluctuation, caused the galvanometer to move in a negative direction.

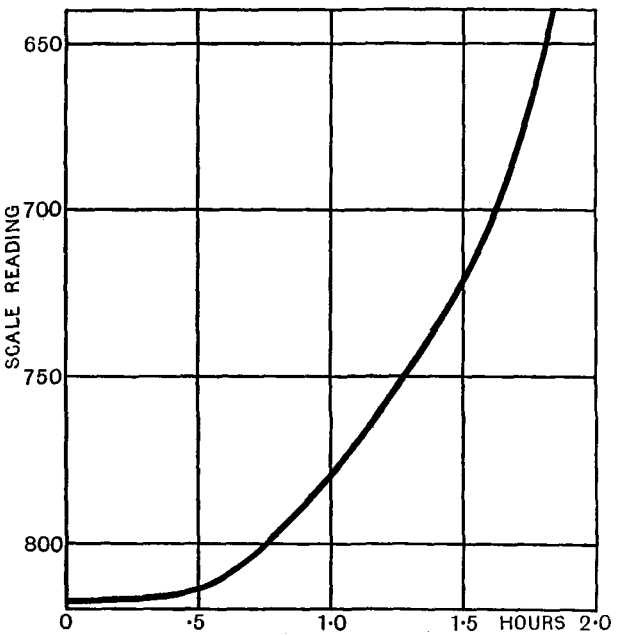
When the rectified A.C. voltage from the exciter lamp supply is used as the opposing E.M.F., a positive movement of the galvanometer was observed upon a decrease in intensity of the lamp. This, of course, is due to non-linearity of illumination to lamp voltage.

The two resistances in source *A* combine part of each of the accumulator P.D. and rectified A.C. P.D., so that a variation of voltage across the lamp and consequent variation of intensity did not affect the galvanometer in the slightest degree. Once the component P.D.'s were adjusted, the galvanometer would remain absolutely stable for any length of time, irrespective of changes in the supply voltage.

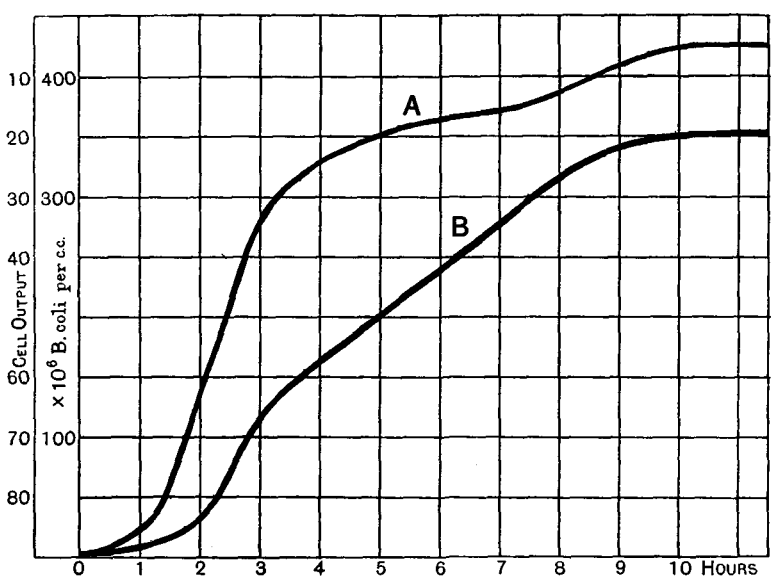
A considerable length of time has elapsed since the apparatus, as described above, was constructed and not the slightest trouble has been experienced with regard to zero shift.

A slight temperature error arose owing to temperature irregularities in the incubator. These were extremely small, as the temperature coefficient of the photo-electric cell is only 0·06 per cent. per ° C. Even this, however, has been eliminated by the simple expedient of incorporating in the incubator one junction of a thermo-couple; this is then connected in series with the output of the photo-electric cell. A temperature change which increases the output of the cell results in an increase in the P.D. developed by the thermo-junction; this, of course, is connected in an opposing manner, and cancels out the increased output of the cell.

The leads from the oxide cell must be encased in lead cable, and all connections must be soldered. This ensures that irregular contacts are eliminated, and that surface leakage due to moisture does not cause any error.



Graph 1. Opacity curve of *B. coli* in beef broth. Readings taken every 7½ min. At the end of the test there were 75 million organisms per c.c.



Graph 2. Opacity curve (*A*) and calibrated growth curve (*B*) of *B. coli* in Lemco broth.

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DISCUSSION.

The type of curve obtained is shown in Graph 1. Readings are taken every $7\frac{1}{2}$ min.

The estimations are, of course, only measures of opacity, as in the case of Brown's tubes, which are in common use. It is obvious that if the organisms double in volume instead of numbers, the opacity will increase in the same ratio. Some observations of interest were made in this connection, and this method in fact offers a simple means of estimating changes in bacterial size, if controlled by an independent count.

Similarly, changes in turbidity or colour of the medium itself will distort the curve. In most cases this is not of much importance; the changes produced by bacterial numbers being extremely great in comparison.

Agglutination or sedimentation of bacteria are greater difficulties, and these cannot be overcome completely. In practice, however, sudden changes in opacity may serve to indicate the onset of agglutination at a very early stage, and this point is being investigated.

The possibility of phototropism of the organisms, leading to their concentration in the light beam, has been investigated, and no sign of it has been found. With certain organisms, however, this factor may be important. The experiments to date show no difference between cultures grown in the light beam, and control cultures poured into the container after incubation in the dark.

The immobility of the galvanometer needle with dead cultures, and the regularity of the curves, are proofs of the accuracy of the apparatus. It has been found, however, that the system takes a short time to acquire equilibrium. In practice, therefore, the apparatus is switched on automatically by a time switch 1 hour before an experiment is begun.

The applications of the method are numerous. It can be used as a sensitive nephelometer for various precipitin reactions, as a colorimeter, and in the investigation of haemolysis.

One of its applications has been in the estimation of the bacteriostatic value of antiseptics, and this will be the subject of a later paper. It is found that the bacteriostatic value of antiseptics is a function of the point in the growth curve at which they are added.

Again, the action of bacteriophage can be studied, and the effect of antiseptics on bacteriophage.

REFERENCE.

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