On the Determination of a Coefficient by which the Rate of Diffusion of Stain and other Substances into Living Cells can be Measured, and by which Bacteria and other Cells may be Differentiated.

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[Plate 3.]

In former papers (3, 4, 5) it has been shown that when blood is spread upon a film of agar jelly which contains Unna's stain and certain salts, the cells will absorb the stain, and that the absorption increases with the temperature and the time during which the cells have been resting on the film. The following facts have also been published:—(1) That *alkalies*, like *heat* and *time*, increase the diffusion of stain into the cells; (2) that *acids* and *neutral salts* delay the diffusion; and (3) that the staining of the nuclei of leucocytes is a sign of death. Soon after death the staining ceases, and the cells rupture or lose their stain.

Evidence has also been given that these phenomena are due to the diffusion of stain into the jelly-like cytoplasm being hastened or delayed, as the case may be, by the agency of these factors, and that death, coincident with the staining of the nucleus, is followed by liquefaction of the cytoplasm and other changes which cause the cells to lose their stain and enter a phase which has been called the condition of achromasia (6).

I have made further investigations in this subject, and have ascertained that if the constituents of the agar film are arranged in a constant manner and the other factors are constant, the staining of the cells will be constant provided that the latter are in the same healthy condition when placed on the agar. It has also been found that when one class of blood cell stains on a given agar film, others do not. By altering one or more of those factors which hasten or delay diffusion of stain into the cytoplasm, that class of cells which previously refused the stain will now absorb it. Therefore the rate of diffusion of stain into the cells differs with the class of cell. Cells other than blood cells, especially bacteria, have also been tried and have been found to be subject to the same conditions; and it has been possible, by altering the arrangement of the factors, to differentiate cells by their rate or coefficient of diffusion. The object of this paper is to give methods by which the coefficient

of diffusion of living cells can be obtained, and the differentiation accomplished.

Scheme of the Method employed.—The cells to be tested are placed on a film of jelly on a slide. The film is prepared from a test-tube which contains 10 c.c. of jelly. The 10 c.c. of jelly contains, besides stain, some of the factors such as *alkalies, acids,* and *salts,* which hasten or delay the diffusion of stain into the cells. In order to simplify matters, I measure these factors in units and I endeavour to arrange them in such a way that 1 unit of any one factor is equal in value, as regards hastening or delaying diffusion, to 1 unit of any of the other factors. The factor *heat,* which hastens diffusion, is applied after the cells have been spread upon the film by keeping the slide at various temperatures for various periods of time. This factor, as well as that of *time,* is also arranged in units, so that 1 unit of either of them is equal in value to 1 unit of the other factors contained in the agar. Hence if 1 unit of a given substance delays diffusion of stain into a cell, the delay it causes can be exactly neutralised by the addition, either to the agar or to the slide, of 1 unit of a factor which hastens diffusion.

Consequently an equation is formulated by means of which the total number of units which, with a certain amount of stain, will cause given, or stipulated, staining of the cells, is equivalent to, or said to be equivalent to, their coefficient of diffusion. Therefore to find the coefficient of a cell, it is necessary to prepare films from a succession of tubes of 10 c.c. of jelly, each tube having a certain, known, number of units added to it, and to examine each film until the stipulated staining is obtained. Since the units are equal in value, it matters little, within reasonable limits, which factor is added, provided that the total number of units is known. Then, after subtraction of those units which delay diffusion, the remainder added to the quantity of stain is the coefficient of diffusion of the cell experimented with.

Conversely, if the coefficient of diffusion of a cell is known, one is enabled, by means of the equation, to know how many units of one or several of the factors it is necessary to add to the jelly, or apply to the slide, to obtain stipulated staining of that variety of cell in a given time. I have mentioned that the staining of healthy cells, only, appears to be constant. When the rate of staining of cells of persons suffering from disease has been found experimentally, the equation indicates in a moment the difference between the coefficients of healthy and diseased cells, and this difference can be expressed in grammes, degrees, or minutes, etc., according to the nature of the factor into which the coefficient of diffusion may be ultimately resolved.

Definitions.—When a film of agar jelly contains stain and other substances,

its Index of Diffusion (*fx*) may be defined as the sum of its constituents which delay diffusion subtracted from the sum of constituents which accelerate diffusion added to the quantity of stain contained in the jelly.

The Coefficient of Diffusion (*cf*) of a cell is that index of diffusion *plus* the time and temperature required to cause staining of the nucleus, or staining of the cytoplasm in unnucleated cells (*e.g.*, red corpuscles), when the specimen is prepared by a standard method.

Standard Method of Preparation.—This consists in: (1) Mixing the cells with a neutral solution containing 3-per-cent. sodium citrate and 1-per-cent. sodium chloride.* If blood is experimented with, it is mixed with an equal volume of the solution. In the case of bacteria and other cells, the mixture is made as convenient.† (2) The mixture is then placed on a cover-glass, which is inverted and allowed to drop flat on a film of agar jelly containing Unna's stain and salts, and which, after boiling, has been allowed to set on a slide. Since the surface of the film is convex, the solution spreads to the periphery of the cover-glass, leaving the cells gently pressed out between the glass and jelly, and this affords an excellent means of examination by the microscope.‡ Only the cells in the centre of the preparation should be examined.

It is stipulated that the jelly contains stain, but the amount of stain added to the agar may be variable. The chemical nature of methylene blue may or may not affect diffusion. The point is difficult to determine accurately, but in this procedure it is of little importance, because the stain employed is always the same, namely, Unna's polychrome methylene blue (Grubler). On the other hand, it is obvious that the more concentrated it is—that is, the more stain there is in the 10 c.c. of jelly, the more rapidly, *ceteris paribus*, will the cells stain. It is also obvious that the effect of an increase of the concentration of the stain can be neutralised by the addition of one or several factors which delay diffusion. Consequently I also measure the stain in units, so that an increase of its concentration by 1 unit can be neutralised by the addition of 1 unit of a factor which decreases diffusion.§

* Merck's reagents have been used throughout these researches.

† The mixture of the cells with this solution is merely used as a vehicle to keep them alive. As the solution spreads to the periphery of the cover-glass, it does not materially influence the diffusion of stain from the agar, a point which has been tested experimentally by rendering it alkaline. The cells, however, should not be kept in it longer than necessary.

‡ The suggestion of mixing stain with the jelly was made to me, as already noted elsewhere, by my brother, Prof. Ronald Ross.

§ Unna's polychrome methylene blue is only supplied in solution, which is standardised. It cannot be made in a powder.

Convenient Method of Preparing the 10 *c.c. of Jelly.*—I have found it more accurate and simple if the factors which hasten or delay diffusion are added to the jelly from standard solutions, and I make the tubes of 10 c.c. of jelly as follows:—50 c.c. of a 2-per-cent. solution of powdered agar in water, filtered and sterilised, is prepared. This solution has such a consistency that another 50 c.c. of water could be added to it without preventing it from setting on a slide when cold.

Since I have shown that blood cells will not live on agar jelly unless it contains a combination of sodium citrate and sodium chloride (3, 7), I add to the 50 c.c. of jelly 1 gramme of sodium citrate and 0·8 gramme of sodium chloride, and accurately neutralise to litmus with citric acid. The whole is then rendered acid by the addition of 0·083 gramme of citric acid. The reason for this will be given in the next paragraph but one.

The molten jelly is then decanted into test-tubes, each of which contains 5 c.c., so that it is possible to add the stain and certain quantities of the standard solutions which contain the factors to these tubes, and provided the total quantities of the several solutions added do not cause a tube to contain more than 10 c.c. of jelly, its ultimate setting on a slide is assured. The standard solutions are so arranged that their total quantity required in an experiment never does exceed 5 c.c. On the other hand, if the amount is less than 5 c.c., the balance up to the maximum in the tube of 10 c.c. is made up with water. In other words, a test-tube originally contains 5 c.c. of jelly which is acid and holds a certain quantity of salts in solution. The stain and quantities of the standard solutions, which correspond to the number of units of factors which it is intended to try, are added. The total content of the tube is then made up with water to 10 c.c. and boiled. So that a test-tube never contains more nor less than 10 c.c. of jelly when a film is prepared from it, though it may contain a variety of units of stain and standard solutions.

I have stated that the agar is rendered acid at the outset; this is done to reduce the number of factors. Acids and alkalies delay and accelerate diffusion respectively. Since they neutralise each other, the neutral point would also have to be taken into consideration. As this would complicate the equation, I render the agar acid at the outset, so acid that I cannot get any cell to stain on it with 1 unit of stain, and deal only with alkali. The neutral point I ignore, although by knowing the initial acidity of the agar, and that the units of all the factors are equal, the point can be readily determined by referring to the equation. I therefore deal with one factor, alkali, instead of two and a neutral point.

To recapitulate shortly: 50 c.c. of a 2-per-cent. solution of agar is prepared

which contains 1 gramme of sodium citrate and 0·8 gramme of sodium chloride. It is neutralised and rendered acid with 0·083 gramme of citric acid. It is then collected in quantities of 5 c.c. In order to determine the *cf* of a cell, a tube of 5 c.c. is melted and certain quantities of stain and standard solution of alkali added. The content of the tube is then completed with water up to 10 c.c. Consequently the tube contains 1 per cent. sodium citrate and 0·8 per cent. sodium chloride in addition to the acid, stain, and alkali, and this content of salts allows leucocytes to live on the jelly. The whole is then boiled until it froths up the tube and a film prepared from it by pouring a drop on a slide and allowing it to set. The cells are placed on to the film and the slide is kept at a convenient temperature for a period of time. If the nuclei or cytoplasm are not yet stained, a higher temperature may be tried combined with a longer period of time, or a fresh tube prepared with more units of alkali added, and so on until staining is obtained. Should the contents of a tube cause the cells to stain very deeply, or if they soon become achromatic, a fresh tube is made containing less stain, or more salts, or less alkali, or acid may even be employed, and so on. But provided the arrangement of the contents of the tube which just causes staining of the nuclei is known, and if the time and temperature are also known, the equation will give the *cf* required.

Units.—In preparing these units I have mainly considered their practical application in the endeavour to curtail the procedure as much as possible. In the instance of alkali and salts, I give the actual amount in grammes which 10 c.c. of jelly should contain as 1 unit. I also give a convenient standard solution and the amount of it in cubic centimetres to be contained in the 10 c.c. of jelly to constitute 1 unit.

Alkali, Sodium Bicarbonate, hastens diffusion.—Unit, 0·005 gramme. Standard solution 5 per cent., unity being 0·1 c.c. It is convenient to remember that this solution is neutralised by a 4·175-per-cent. solution of citric acid, and that 1 unit of alkali is neutralised by 0·1 c.c. of such a solution. Since the agar at the outset is acid to the extent of 0·083 gramme to 50 c.c., a tube of 10 c.c., made up as described, must contain 0·0083 gramme of acid. This is exactly neutralised by 0·2 c.c. of the standard alkali solution; that is, the agar at the outset, before any stain or other factor is added, delays diffusion to the extent of 2 units. Or, the addition of 2 units of sodium bicarbonate will render the agar neutral.

Sodium Citrate, delays diffusion.—Unit 0·03 gramme. Standard solution 10 per cent., 0·3 c.c. being unity. Since 50 c.c. of agar contains 1 gramme at the outset, the 10 c.c. of jelly may be said to contain about 3 units.

Sodium Chloride, delays diffusion. Unit 0·08 gramme. Standard solution

10 per cent., unity being 0·8 c.c. The 10 c.c. of jelly contains this from the outset.

Heat, hastens diffusion.—Each unit 5° C. 10° C. is unity, 15° C. is 2 units, 20° C. 3 units, etc. For practical purposes I call 37° C. 7 units.

Time, increases diffusion.—10 minutes being 1 unit, 20 minutes 2 units, and so on.

Stain, Unna's polychrome methylene blue (Grubler), behaves as if it increased diffusion.*—Unit 0·1 c.c.

Equations for ascertaining the Coefficient of Diffusion.—The nuclei of polymorphonuclear leucocytes recently shed from a healthy person, just stained in 10 minutes when resting on a film of agar, 10 c.c. of which contained 0·2 c.c. of stain, 1 per cent. sodium citrate, 0·8 per cent. sodium chloride, and 6 units of sodium bicarbonate. The slide was kept at a temperature of 37° C. What was their coefficient of diffusion?

Then cf = that $fx+h+t$ which just causes staining of the nuclei,

but $fx = (s+a)-(c+n)$;

$$\therefore\ cf = (2s+6a)+(7h+t)-(3c+n) = 16-4 = 12.$$

Where s is the unit of stain, a the unit of alkali, h the unit of heat, t the unit of time, c the unit of sodium citrate, and n the unit of sodium chloride.

The 10 c.c. of agar in this case was made up as follows:—5 c.c. from the original 50 c.c. of agar which contained sodium citrate 1 gramme, sodium chloride 0·8 gramme, and citric acid 0·083 gramme. The jelly was melted and the following quantities of standard solutions added: 0·2 c.c. stain, 0·6 c.c. 5-per-cent. solution of sodium bicarbonate, and 4·2 c.c. water. Total 10 c.c.

The eosinophiles, however, did not stain under quite the same conditions for it was found in the foregoing experiment that they were either achromatic or ruptured. A fresh tube was made with 1 unit less alkali, when it was found that the eosinophiles would just stain in 10 minutes. What was their cf?

$$cf = (2s+5a+7h+t)-(3c+n) = 11.$$

The lymphocytes, large and small, required 0·2 c.c. of alkali more than the polymorphonuclear cells, the other factors being as before, what was their cf?

$$cf = (2s+8a+7h+t)-(3c+n) = 14.$$

The foregoing tubes contained a very low content of stain, the chromatin

* As already pointed out, stain should not be a unit of diffusion, for it is doubtful whether it affects diffusion. It contains salts, and is alkaline when made, and alkalies and salts are antagonistic. However, for reasons already given, it may be included in the category.

network stained better if its concentration was increased. In order to obtain staining of the nuclei of lymphocytes in 10 minutes on agar from a tube which contained 4 units, instead of 2 units, of stain, the amount of alkali added was of course less in proportion to the increased concentration. With 4 units of stain, the other factors, except alkali, being as before, the equation now stood as :—

$$cf = (4s+6a+7h+t)-(3c+n) = 14.$$

The red corpuscles appear to have a very high *cf.* I caused them to stain on agar which contained 1 c.c. of stain (10 units) and 11 units of alkali, in the presence of 1-per-cent. sodium citrate and 0·8-per-cent. sodium chloride at 37° C. in 10 minutes. This was the equation :—

$$cf = (10s+11a+7h+t)-(3c+n) = 25.*$$

Examples.—A growth of staphylococci had a *cf* of 16.

How much alkali must 10 c.c. of jelly contain to cause the germs to stain in 10 minutes if the jelly already contains 5 per cent. of stain, 1·5 per cent. sodium citrate, and 0·8 per cent. sodium chloride, when the slide is incubated at 37° C. ?

$$a = (16cf+4{\cdot}5c+n)-(5s+7h+t),$$

$a = 8{\cdot}5$ units, or 0·0425 gramme of sodium bicarbonate.

A strain of typhoid bacilli had a *cf* of 21. A tube of agar contained 6 units of alkali solution and the usual quantities of sodium citrate and chloride. How much stain should be added to the tube to produce staining of the bacilli in 20 minutes at 37° C. ?

$$s = (21cf+3c+n)-(6a+7h+2t),$$

$s = 10$ units of stain, *i.e.*, 1 c.c.

* I do not think this is strictly accurate, for it depends on the coloration of the stroma. Nucleated red cells have a comparatively low *cf*, resembling that of the polymorphonuclear cells, though I am also doubtful of this point, because I have only been able to obtain these nucleated cells from persons suffering from disease, and, as I have already shown (8), all the blood cells in chronic illnesses, especially phthisis, malaria, and Hodgkin's disease, have so far shown a general fall in their coefficient of diffusion. Again, attention may be drawn to the comparatively low *cf* of the granular erythrocytes which constitute about 1 per cent. of all the red cells in healthy blood as demonstrated by this method of examination (1). The rate of staining of the granules is about the same as those of the polymorphonuclear leucocytes, but the rate of staining of the stroma of these granular cells is much higher than that of their granules, yet lower than the rate of staining of the stroma of ordinary erythocytes. This fact is the more interesting, because it is almost in these granular red cells alone, which have a low *cf*, that "red spots" are to be seen, although I have on three occasions seen them in ordinary red cells, and the spots appear to be dependent on diffusion (4). These cells also become achromatic more readily than ordinary erythrocytes. The granules have been described (1) as the remains of a nucleus. The question, therefore, of the *cf* of the red cells I will leave for the present as indicated.

Precautions.—As regards Life and Death: In a previous paper (3) it has been shown that the staining of the nuclei of leucocytes, when examined by this method, is a sign of death, and that the nuclei of dead cells will stain, *ceteris paribus,* before those of living cells. Consequently all the experiments given in the present paper have been made with fresh normal cells, and in the case of micro-organisms with cultures not more than 48 hours old. It may also be mentioned that the liquefaction of the cytoplasm which occurs after death materially alters the conditions of staining of leucocytes, and that the *cf* of living blood cells falls gradually after the blood has been shed. The fact has already been mentioned that in chronic wasting diseases the coefficient of blood cells may be very low.*

As regards Excess of Alkali causing rapid death and liquefaction of the cytoplasm with consequent prevention of staining (achromasia): The addition of excess of alkali may cause death, staining of the nuclei liquefaction, and the loss of stain on the part of the cells (3, 6). This may occur before a preparation can be focussed, in which case the cells appear unstained and will refuse to stain, no matter how much more stain or alkali are tried. Therefore it is better to begin with a low index of diffusion and to try tube after tube, each containing a little more alkali, until staining is obtained. Further, the amount of sodium bicarbonate should not exceed 20 units, because, as has already been pointed out in a former paper (3), if added to excess, it may act as a neutral salt and delay diffusion.

As regards Deficiency of the Salts sodium citrate and sodium chloride: If the jelly contains no salts, the blood lakes and the leucocytes are killed outright. If it contains sodium chloride only, the cells are killed rapidly, and the same may be said if sodium citrate only is employed (7). In examining blood, therefore, the combination is essential.

As regards Excess or Deficiency of Heat: A temperature above 40° C. may allow the cells to diffuse through the agar (2). A temperature below 15° has not been experimented with, because, even at a temperature of 20° C., it requires a minimum of 3 units of stain to cause staining of the nuclei of leucocytes in spite of the addition of a large amount of alkali, for the alkali is not sufficient, *per se,* to cause the cells to absorb sufficient stain to colour the nuclei unless the stain is concentrated.

As regards excess of Time: A period of more than half an hour has not been employed for fear of death and liquefaction of the cytoplasm, for the

* I have found that the life of leucocytes of persons suffering from some chronic diseases, when bathed in their own plasma, is considerably shorter than the life of healthy persons' leucocytes bathed in their own healthy plasma (8). I have reasons for believing that there is an association between this and a low *cf.*

cells may die and become achromatic before there has been time for sufficient stain to diffuse into them to cause staining of the nuclei, in which case, of course, the cells will never stain.

As regards Excess of Stain: More than 10 units of stain may cause precipitation of the agar as the film cools on the slide, and the precipitate carries some of the stain down with it, vitiating the results, for it has been shown that agar is not very soluble in cold stain (3).

As regards Examination: The observation of cells floating near a bubble under the cover-glass should be avoided. The fact that blood cells in such a situation will stain before others has already been noted (3). I consider this to be due to these cells floating in a small quantity of alkaline citrated plasma collected round the bubble.

Consequently the experiments have all been made within the compass of the above restrictions. So far no cells, whether blood, bacteria, or other cells, have been met with which would not give a coefficient of diffusion by this method. It may also be advised that when unnucleated cells contain granules in their cytoplasm, the staining of the granules gives a more constant rate than the staining of the cytoplasm. By this means it is seen that the *cf* of the blood-plates is identical with that of the polymorphonuclear cells.

The Construction of other Units.—It may be necessary to add other substances to the jelly to test their effect on cells. For instance, it may be useful to try other salts, in which case their rate of antagonism to diffusion must be found and a unit made. This may be done by comparing their action with that of a unit of one of the other factors, after which the new unit may be added to the equation. In the case of sulphate of atropine, it was found that a tube of 10 c.c. of agar, which had a correct *fx* to cause staining of lymphocytes in 10 minutes, but which also contained 0·013 gramme of sulphate of atropine, required the addition of 1 more unit of alkali to cause the nuclei to stain in 10 minutes. Consequently the unit of atropine sulphate may be said to be 0·013 gramme.

The Determination of the Coefficient of Diffusion of Leucocytes involves Death.—Since the staining of the nucleus is the moment by which the *cf* of leucocytes is obtained, and since the staining of the nucleus is a sign of death (3), the cells are necessarily dead at the expiry of the time involved in finding their coefficient.

The Reconciliation of their Coefficient of Diffusion to Cells which may be Alive at the Termination of the Time required.—It has been shown that leucocytes will live for a considerable period and show amœboid movement with their granules stained (3, 5). If 1 digit is subtracted from their *cf* and the jelly arranged according to such an equation, the granules but not the

nuclei will stain in the given time. By this means death is not necessarily involved.

Example to show that the Diffusion of Substances other than Stain may be Dependent on the Coefficient of Diffusion of Cells.—Given eosinophiles have a *cf* of 11. They are resting on agar which contains the usual quantities of sodium citrate and chloride, viz., 3 and 1 units respectively, but it also contains 0·02 gramme of sodium bicarbonate (4 units), 0·6 c.c. of stain, and 0·007 gramme of atropine sulphate. The fact that a mixture of atropine and methylene blue will excite a remarkable exaggeration of amœboid movement in leucocytes has already been published (5). How long will it take to produce marked exaggerated movements in the given eosinophiles at a temperature of 20° C. ?

Then, since it is necessary for the given cells to be alive at the expiry of the time required, 1 digit must be subtracted from their *cf*—

$$t = (10cf + 3c + n + 0{\cdot}5z) - (6s + 4a + 3h),$$
$$t = 1{\cdot}5 \text{ units of time or } 15 \text{ minutes.}$$

Where z is the unit of atropine sulphate, 0·013 gramme. A 1-per-cent. solution was found convenient as a standard, and 0·7 c.c. was used.

The Coefficient of Diffusion may be resolved into the Value of any one of the Units.—Since by the foregoing equations any one of the units can be resolved into the value of any one of the other units which go to make the *fx*, and since $fx + h + t = cf$, therefore any *cf* can be expressed in the value of any of the units ; into alkali for instance. But the unit of alkali is 5 milligrammes of sodium bicarbonate. Consequently the coefficient of diffusion of the bacteria contained in the growth of *Bacillus typhosus*, used in one of the examples, may be said to be equivalent to the alkalinity of 105 milligrammes of sodium bicarbonate.

Summary and Suggestions.

The difficulty has been in the construction of the units. So far I have found them to be sufficiently accurate for practical purposes within the compass of these experiments, of which a very large number have been made, extending over a period of several years, involving the use of many varieties of cells.

The determination of the coefficient of diffusion is brought about by allowing living cells to rest on a jelly which contains stain. Several factors, some of which may be contained in the jelly, hasten or delay the diffusion of the stain into the cells, and the coefficient of diffusion is the sum of the factors which causes the stipulated staining of the cells added to the amount

of stain employed. The factors which hasten diffusion are *heat*, *alkalies*, and *time*; and those which delay it are *acids* and *neutral salts*. But the rate of diffusion depends also on the concentration of the stain, for if this is weak a large sum of factors to produce staining is required; but if it is very concentrated the cells may stain even in the presence of acid. Since all these items are variables, I have constructed a formula by which, if some of them are known quantities, the others can be readily determined.

By this means the coefficient of diffusion of a cell can be obtained, and it varies with the class of cell.

I have also stated that the diffusion of substances other than stain may appear similarly to depend not only on their concentration but on the coefficient of diffusion of a cell. But other substances may be alkalies, acids, or salts, and may affect the diffusion of neighbouring substances and be so affected themselves. I have given a means by which this effect can be determined and a unit made. Then, provided the unit of a given substance is known, and provided the coefficient of diffusion of a given cell is known, the comparative rate of effect of the given substance on the given cell can be ascertained by referring to the equation.*

In medicine, for instance, drugs and sera are frequently given with a view to affecting certain cells, yet, as far as I know, no steps are taken either to ascertain the rate of effect of the drug on the cells, or suitably to modify the alkalinity of the blood by treatment, in order to produce maximum results according to the temperature of the patient. The point appears worthy of consideration.

In the case of bacteria, it is frequently heard that certain bacteria are more resistant to antiseptics and drugs than others. It is possible that this varied resistance may be summed up in the expression "coefficient of diffusion." If antiseptics could be rendered alkaline according to the coefficient of the bacteria which they are intended to kill, and according to the temperature, it might lead to a reduction of the concentration of the antiseptic, with consequent saving of cost and increase of efficiency.

Since the blood fluids affect bacteria, it seems desirable to know the coefficient of diffusion of the bacteria when estimating the effects of the fluids on the cells. Again, since erythrocytes will diffuse bodily into agar jelly and remain suspended in it (2), and since droplets of liquid will diffuse into the colloid cytoplasm of leucocytes and remain suspended in it (red

* The knowledge that heat accelerates the diffusion of substances into cells has already been applied in some researches by Dr. C. J. Macalister and myself in order to demonstrate an excitant for leucocytes in the plasma of cancer patients ('Proc. Roy. Soc Med.,' December, 1908).

spots, 4), it seems possible that germs which proffer the same jelly-like properties as red cells may enter the phagocytes by the process of diffusion and be subject to the same factors which influence it, for the temperature and alkalinity of the blood vary in health and disease. Therefore, it may be important to know the rate of diffusion of leucocytes when estimating phagocytosis.

I have noted that there appears to be a relationship, in leucocytes at least, between the coefficient of a cell and the length of its life as measured by a procedure which I have already published (5, 8). It has also been shown in a former paper (4) that if stain is passed over a jelly such as agar, the rate of the coloration of the jelly depends on its consistency, that is, as to whether it is solid or diffluent. In the same paper it was stated that the effect of stain on cytoplasm also depended in a like manner on its consistency. Since there may be a relationship between the coefficient and vitality, the consistency of a cell may depend to some extent upon its vitality. Therefore, the determination of the coefficient of diffusion may prove important in the prognosis of tumours if the cells can be suitably kept alive, since it may give an indication of the consistency of the cytoplasm, and a lowered coefficient, as occurs in the blood cells in anæmia, may foretell a lowered vitality.

Further experimentation is also required to determine the property on which depends the varying influence of alkalies, salts, etc., in hastening or delaying diffusion.

I hope this method may ultimately prove of value, not only in bacteriology as a means of differentiating bacteria, but also in the investigation of the diffusion of substances into living cells.

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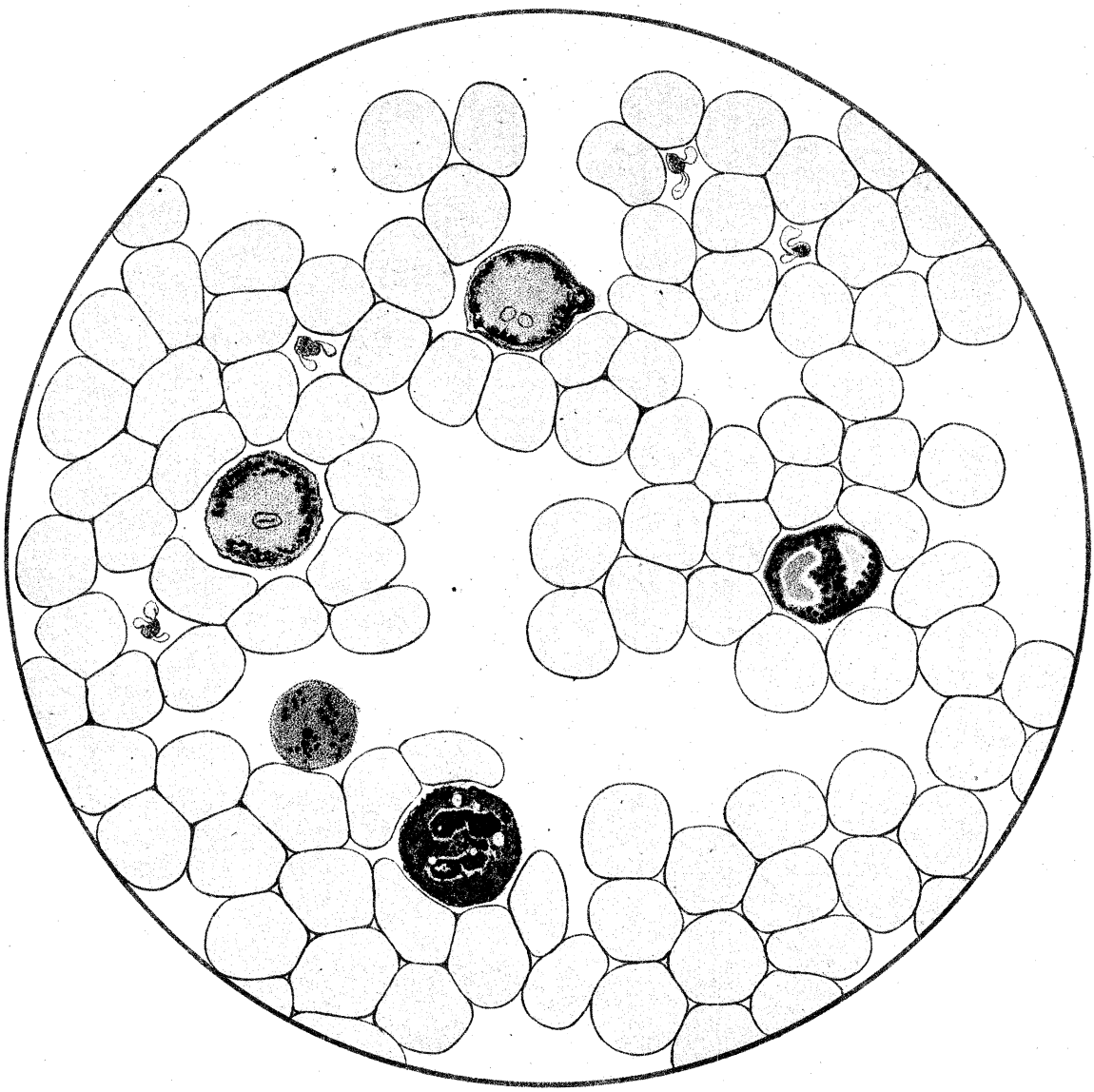

DESCRIPTION OF PLATE 3.

Coefficient of diffusion. Drawn by P. Nairn from a preparation of fresh blood cells which have been resting for 10 minutes at 37° C. on an agar film with an index of diffusion of 4. The nucleus of one polymorphonuclear leucocyte has just stained and the cell is showing three small red spots. The nuclei of two large lymphocytes have not yet stained, one cell is showing 1 centrosome and the other 3 centrosomes. The film also demonstrates an eosinophile leucocyte which is becoming achromatic, *i.e.*, its nucleus has lost its stain; and one granular red cell which contains two red spots. 2 mm. apochromatic objective, No. 4 eye-piece, 250 mm. draw-tube, 1 amp. Nernst lamp.

The Origin and Destiny of Cholesterol in the Animal Organism. Part III.—*The Absorption of Cholesterol from the Food and its Appearance in the Blood.*

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In his 'Text-Book of Physiology' Schäfer has suggested that the constant presence of lecithin and cholesterol in the bile may well be associated with the destruction of the red blood corpuscles which contain relatively large amounts of these substances, the latter, according to Hepner,* being present in the free state and not in the form of esters. This idea has recently received strong support from the investigations of Chasoburō Kosumoto† on the influence of toluylene diamine on the output of cholesterol in the bile. This reagent was found by Schmiedeberg to produce icterus, and Stadelmann, working in Schmiedeberg's laboratory,‡ observed that at the beginning of the action of the drug an increased production of bile took place. This, however, was only temporary, and soon the normal physical properties of the bile underwent an alteration; it became sticky, darker, and more concentrated.

Afanassiew§ showed that the effect of the drug is to destroy the red blood

* 'Pflüg. Archiv f. d. Ges. Physiol.,' 1898, vol. 73, p. 595.

† 'Bioch. Zeit.,' 1908, vol. 13, p. 354.

‡ 'Arch. f. experim. Pathol. u. Pharmak.,' 1881, vol. 14, pp. 231, 422.

§ 'Zeit. f. klin. Med.,' vol. 6.