BIOPHYSICAL AND PHYSIOLOGICAL INVESTIGATIONS ON CARTILAGE AND OTHER MESENCHYMAL TISSUES

I. AN INTERFEROMETER APPARATUS FOR DETERMINATION OF DIFFUSION CONSTANTS IN GELS AND TISSUES*

by

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INTRODUCTION

The purpose of the method described here is mainly to study the diffusion rate of small or medium sized molecules in some mesenchymal tissues, *i.e.* cartilage and nucleus pulposus. From GRAHAM's time diffusion in gels has been studied, but commonly only dilute agar or gelatin gels have been used to prevent convection. In these systems FICK's second law holds, and the diffusion constant in the gel is only slightly less than in water; however, in several kinds of tissues this does not seem to be the case. In the method described here the change of the refractive index is measured and the concentration of the diffusing substance calculated from that. The apparatus is a kind of FABRY-PEROT interferometer, which has much in common with the interference microscope designed by AMBROSE[1].

DESCRIPTION OF APPARATUS

The principal parts of the instrument are shown in Fig. 1. The mercury vapour lamp A burns at a low or medium pressure in order to give mostly monochromatic light. B is a green filter, Wratten 77. The condensing lens C projects an image of A on the aperture plate D, which consists of two variable slits perpendicular to each other. D is situated in the focal plane of the lens E, which is a microscope objective. The two circular glass plates (P_1 and P_2) which form the principal part of the etalon (diameter 6 cm, thickness 0.6 cm-

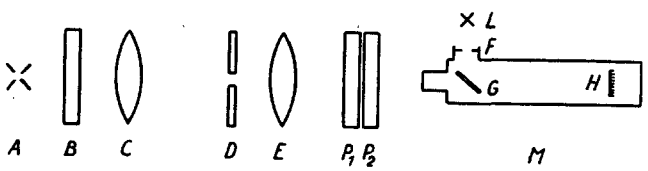

Fig. 1. A sketch of the interferometer

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are polished very accurately. The irregularities of the surface do not amount to more than about 10^{-6} cm. The surface is coated with a transparent aluminium film generally three or four hundred Ångström thick. The plates can be observed through the microscope *M*, which is furnished with an eyepiece scale (*H*). Light from the lamp *L* passes through the iris diaphragm *F*, reflects on the glass plate *G*, and illuminates the scale.

The FABRY-PEROT etalon is reproduced in detail in Figs 2 and 3. The distance between the plate P_1 and P_2 is defined by three small props (*S*), two of which are equal in thickness but the third is a few hundredth mm thinner. Thus the plates form a wedge with a very small wedge angle. It is necessary that the props are so hard that they will not be perceptibly deformed when the plates are pressed against each other, but at the same time they must not be so hard that they could scratch the glass surface. The only metal suitable for use is aluminium, since the others form galvanic cells with the thin aluminium films on the plates. Further, plastics of the type that do not swell in water solutions can be used when the distance between the plates is small. At large distances well polished glass pieces have been successfully applied.

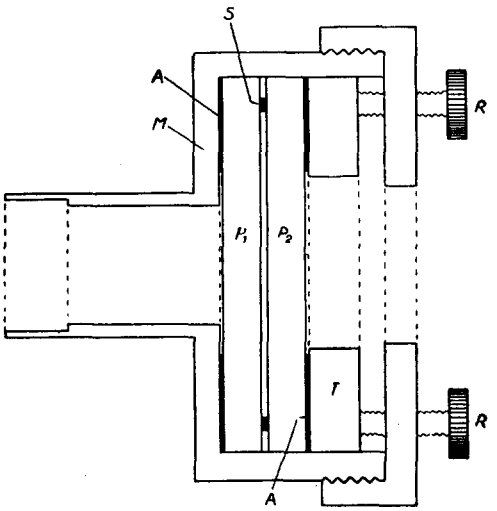

Fig. 2. A vertical section of the FABRY-PEROT etalon.

The plates are situated in a brass socket (*M* in Fig. 2). The screws, *R*, in which are cut threads fairly close, press against the pressure apportionment plate *T*. Either three or four screws (*R*) are used. In the former case each screw is placed just over a prop,

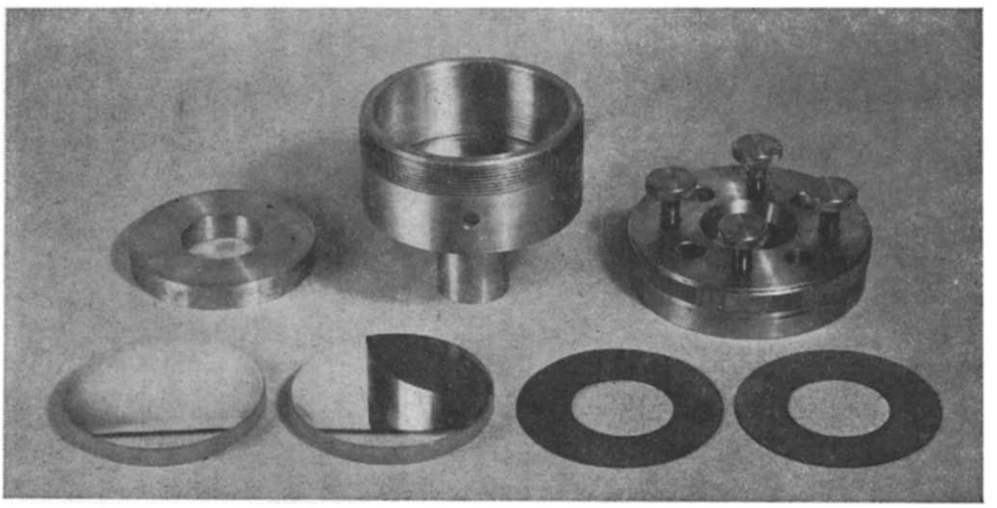

Fig. 3. The constituents of the FABRY-PEROT etalon.

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and in the latter case two of the screws are placed symmetrically on each side of the thinnest prop. Between glass and metal are packings (*A*) made of rubber or plastic.

Before making the thin aluminium film the plates are thoroughly cleaned with soap solution, alcohol and benzene. Then in high vacuum (about $8 \cdot 10^{-4}$ mm Hg) 2.1 mg aluminium is evaporated from an electrically heated tungsten filament at a distance of 4.2 cm from the plate surface. This aluminium film weighs about $9 \cdot 10^{-6}$ g/cm^2. If the film is made a little thicker, it is necessary to use a high pressure mercury vapour lamp (*A*, Fig. 1). To get better firmness the aluminium film is annealed for a day at 80° C and then coated by a protective film made of cellulose nitrate ("Zapon lacquer").

INTERFERENCE FRINGES

If the distance between the plates is d, the refractive index of the medium between the plates n, m a whole number and λ the wave-length in vacuum, light can pass when

$$2 n d = m \lambda \qquad (1)$$

If an optically homogeneous medium is situated between the plates, a series of equidistant interference fringes can be seen in the field of view of the microscope. The higher the reflecting power of the aluminium film, the sharper are the fringes. If a substance diffuses into the medium between the plates perpendicular to the interference fringes, the index of refraction increases and the fringes seem to go in the same direction as the diffusion. From the displacement of each fringe it is possible to calculate the concentration at respective time and distance from the border of the specimen. If the increment of the refractive index can be regarded as directly proportional to the concentration—and this is generally the case—the displacement of a fringe is also proportional to the concentration.

MANIPULATION

When using the apparatus the first problem is to choose the best value of the distance d between the plates. It is desirable to make d as large as possible, because the error of the measurement is inversely proportional to d and further the error caused by a small change of d during the course of the diffusion gets greater for small values of d. The upper limit of d is determined by the optical inhomogeneity of the medium. For a gelatine gel $d = 0.5$ mm can be used, but for nucleus pulposus it is necessary to decrease d to about 0.05 mm to get measurable interference fringes. Hyaline cartilage demands still lower d values. The specimen is placed in a vessel consisting of the two aluminium-coated plates and a wall of silicon grease between them. The silicon grease is applied to the plate by a syringe. If the distance between the plates is small, it is better to use a benzene solution of silicon grease and then evaporate the benzene. In order to fix the plates in their position, the screws *R* (Fig. 2) are cautiously pulled in. The specimen is so placed, that the interference fringes are perpendicular to the direction of diffusion. The eye piece micrometer is parallel to this direction, *i.e.*, perpendicular to the border of the specimen. As the change of the distance d usually is bigger during the first time after the screws have been pulled in, it is advisable to let the stresses of the etalon be equilibrated for half an hour. Then the vessel is filled with a water solution of the diffusing substance. This is accomplished with a device similar to a simple micro

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buret. As the plates are coated with cellulose nitrate it is not easy to fill the vessel when d is small, but if a very thin cotton fibre is placed vertically between the plates the filling is much facilitated. When the solution reaches the border of the specimen, the diffusion begins and the time $t = 0$. It is necessary to control that the distance d does not change during the time of diffusion, which generally amounts to between 20 minutes and some hours. This is carried out by observing interference fringes in the specimen far from the border where no perceptible amount of the diffusing substance yet has arrived. If the specimen is entirely optically homogeneous it is possible to detect a change $d = 10^{-6}$ cm or less, but if the specimen is a tissue as nucleus pulposus the least perceptible value is two or three times greater.

VALUATION

The interferometer method described here has the following advantages:

a. It is rapid. It is possible to get measurements sufficient to determine a high diffusion constant within 20 minutes.

b. Only rather small amounts of diffusing substance and specimen are consumed.

c. Absolute determinations of the diffusion constant can be made.

On the other hand the apparatus has the following drawbacks:

a. It is not thermostated. As a diffusion constant increases about 3% per degree, the apparatus in its present shape can not be used for precision measurements.

b. It is not easy to keep the distance between the plates constant. On account of that measurements sometimes must be discarded.

c. Only transparent and optically tolerably homogeneous specimens can be used.

THEORY

Diffusion in a liquid generally can be discribed by FICK's second law:

$$\left(\frac{\delta c}{\delta t}\right)_x = D\left(\frac{\delta^2 c}{\delta x^2}\right)_t \tag{2}$$

where c is concentration, t time, x distance and D the diffusion constant. At the border of the specimen $x = 0$ and x is positive in the specimen in the direction perpendicular to the border. In the apparatus described, there is

(i) when $t = 0$: $c = 0$ for all positive x-values.

(ii) when $x = 0$: $c = c_s$ for all t-values.

Under these boundary conditions equation 2 becomes

$$c = c_s\left(1 - \frac{2}{\sqrt{\pi}}\int_0^{\xi} e^{-y^2}\,dy\right) \tag{3}$$

where $\xi = \dfrac{x}{2\sqrt{Dt}}$

In Fig. 4 equation (3) is reproduced for the case $D = 0.25$ cm²/day and $t = 1$ day. Equation (3) is derived for a system consisting of a solution with constant concentration (full convection), in contact with solvent, where no convection subsists. Such a system

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can be realized if the solvent contains a dilute gel, *i.e.*, agar or gelatine gel, but then the diffusion constant has a little lower value than in water. FRIEDMAN AND KRAEMER[2] state that the decrease of the diffusion constant with increasing gel concentration in dilute gels follows the formula:

$$D_{water} = D_{gel}\left(1 + 2.4\frac{r}{R}\right)(1 + \alpha)\left(1 + \left(\frac{g}{d}\right)^{\frac{2}{3}}\right) \qquad (4)$$

where r is the radius of the diffusing molecule, R the mean radius of the pores of the gel, g the number of grams/cm^3 and d the density of the dry substance. $(1 + \alpha)$ is an empirical factor named "correction factor of viscosity" (2). As quoted by FRIEDMAN the factor $(1 + 2.4\frac{r}{R})$ is taken from LADENBURG's correction for the fall of a body in a capillary tube.

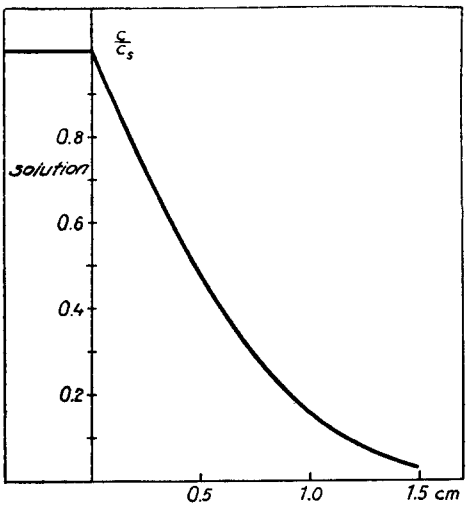

Fig. 4. The ratio between the concentration (c) and the constant concentration (c_s) in the solution as a function of the distance from the border

If the gel is a little more concentrated it can be presumed to contain pores whose radii differ widely, and the smallest of them are not bigger than the diffusing molecule. Then the concept of measured diffusion constant has to be reconsidered. Regard first the diffusion in each pore as independent of the same process in the surrounding pores. From equation (4) it can be seen that the diffusion proceeds faster the bigger the pore is. Calculate for a given time t and distance from the border x the concentration (equation 3) in each pore in the gel. If the distribution of the pore radii is known, first an average concentration and then an integral diffusion constant can be calculated. This quantity, however, is fundamentally distinguished from the diffusion constant in liquids, for it increases with the distance from the border x. It is difficult to accomplish this calculation on a real gel, but that this integral diffusion constant depends on x can be shown with the following simple model. Assume a gel containing only two sorts of pores, small pores ($D_{gel} = 1$) and big pores ($D_{gel} = 2$). Each sort occupies equally large transverse sections. Then at the time $t = 1$ the integral diffusion constant increases from 1.4 when $x = 1$, to 1.6 when $x = 4$. It should be pointed out, that the concentration dependence of the diffusion constant observed in liquid systems also tends to increase the integral diffusion constant with increasing x values.

The integral diffusion constant, which can be calculated from measurements with the interferometer described above, increases with increasing distance from the border. This has been supposed from theoretical reasons here, and it has also been practically shown for the diffusion of several substances into nucleus pulposus. These measurements will be published later.

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SUMMARY

An interferometer of FABRY-PEROT type is described. With this apparatus the diffusion rate of a solute in gels or tissues can be determined. Some deviations from FICK's second law in systems with pores whose radii differ widely, are discussed.

RÉSUMÉ

Nous décrivons un interféromètre du type FABRY-PÉROT. A l'aide de cet appareil la vitesse de diffusion d'une substance dissoute dans un gel ou autre tissu peut être déterminée. Nous discutons quelques déviations de la seconde loi de FICK dans les systèmes à pores de rayons très différents.

ZUSAMMENFASSUNG

Ein Interferometer Typ FABRY-PEROT wird beschrieben. Mit Hilfe dieses Apparates kann die Diffusionsgeschwindigkeit einer gelösten Substanz in Gelen oder anderen Geweben bestimmt werden. Einige Abweichungen von dem zweiten FICK'schen Gesetz in Systemen mit sehr verschiedener Porengrösse werden erörtert.

REFERENCES

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[2] L. FRIEDMAN AND E. D. KRAEMER, *J. Am. Chem. Soc.*, 52 (1930) 1294.

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