that in a few cases these colours are not the same for equivalent quadrants in different parts of one animal. The dyed membrane may then be both red and green in the same quadrant.

The presence of these colours shows that the dye molecules or aggregates are oriented in the living amœba. It seems likely that they are adsorbed on to a labile oriented structure present in the living membrane or cortex. This idea is supported by the fact that the colours vanish at once if the amœba is killed by fixatives, detergents, heat or cyanide. The addition of a weak solution of the detergent 'Teepol' to a dyed amœba in water causes the colours to vanish instantly; but only after a minute or so is there any cytolysis visible under an ordinary microscope. The dye penetrates both living and dead amœbæ, since under an ordinary microscope these appear stained with the normal colour of the dye. Only in the living animal, however, is the dye oriented.

It does not seem possible to reach any detailed conclusions about the orientation of the dyes, or about the structure on which they are presumably adsorbed. The birefringence of dyes has not yet been fully investigated, nor is it known on to what structures, or in what way, are they likely to be adsorbed.

J. M. Mitchison

Zoological Laboratory,
Cambridge.
April 26.

[1] Schmidt, W. J., "Die Doppelbrechung von Karyoplasma, Zytoplasma, und Metaplasma" (Berlin, 1937).
[2] Schmitt, F. O., Bear, R. S., and Ponder, E., *J. Cell. and Comp. Physiol.*, **9**, 89 (1936).
[3] Mitchison, J. M., *Nature* (in the press).
[4] Mitchison, J. M., and Swann, M. M., unpublished observations.
[5] Teitel Bernard, A., *Bull. Acad. Med. Roumanie*, **17**, 96 (1945).
[6] Zocher, H., and Jacoby, O., *Kolloidchem. Beih.*, **24**, 365 (1927).

Preparation of Standardized Actinomycete Colonies

Many soil actinomycetes of the genus *Streptomyces* inhibit the growth of fungi on solid agar media, the inhibitory power exerted by a colony of such an actinomycete on a neighbouring fungal colony being indicated by the width of the inhibition zone which forms between them. Since the width of this zone is affected by the size and relative positions of the two colonies, it is essential to standardize these conditions in order to compare the anti-fungal activity of an actinomycete on different media. Actinomycetes grow slowly by comparison with most fungi; hence, when making an antagonism test of this type, it is convenient to inoculate the actinomycete over an area of the agar surface upon which will arise a colony large enough to give an easily measurable inhibition zone in a short time. Colonies 5–10 mm. in diameter when three to five days old have been found to be quite suitable. The technique of placing aliquot drops of liquid inoculum directly on an agar surface is sometimes not satisfactory, because the drops may spread, particularly when the plate is moved, thereby giving rise to irregular colonies. Moreover, the amount of spreading is affected by the composition of the medium. Again, it is sometimes necessary to inoculate with the actinomycete after the fungus has grown for some time on the plate. In such cases it is difficult to place the drops at a predetermined distance from the fungal growth.

Standardized actinomycete colonies can be obtained easily with the aid of short open-ended porcelain cylinders similar to those originally recommended for the biological assay of penicillin solutions[1]. Cylinders are dry-sterilized in Petri dishes which are then kept at a constant temperature on a hot plate while the cylinders are being removed for use[2]. A temperature of 100–110° C. is optimal for this purpose. A cylinder at this temperature, when placed end-on to an agar surface, will sink in to a depth of about 0·2 mm. to form a cup which, when quite cool, receives one or two drops of inoculum from a Pasteur pipette.

During the first forty-eight hours of incubation, the inoculum-suspending liquid is absorbed into the medium, leaving the spores or mycelial fragments drawn down into the agar within the confines of the cylinder. When all traces of surface moisture near the base of the cup have disappeared (48–72 hr. incubation) the plates may be inverted for microscopic examination, and for making zone measurements. Since some cylinders will fall off when the plates are inverted, it is advisable to remove them all at this time. A sharp tap on the underside of an inverted plate is sufficient to dislodge a cylinder and allow it to fall into the lid from which it may be removed. Neat circular colonies are left in the required positions on the agar surface. Removal of cylinders from the medium with forceps is not recommended, as this procedure frequently leads to a deposition of spores round about the colony. Provided the inoculum used is sufficiently strong to give a confluent growth on the base of the cup, the sizes of such colonies, their rates of growth and their antibiotic properties vary very little on any one medium. Little advantage is gained by using cylinders to place fungal colonies, since a drop of inoculum too small to spread gives rise to a large and uniform colony. A wire loop 1 mm. in diameter is used for placing a loop of fungal inoculum at the required distance from the actinomycete growth.

F. A. Skinner

Soil Microbiology Department,
Rothamsted Experimental Station,
Harpenden, Herts.
April 14.

[1] Heatley, N. G., *Biochem J.*, **38**, (1), 61 (1944).
[2] Jackson, W. Foster, and Woodruff, H. B., *J. Bact.*, **47**, (1), 43 (1944).

Application of the Kozeny Equation to Consolidated Porous Media

Wyllie and Rose[1] have recently directed attention to the fact that the numerical constant (k) in the Kozeny equation may exceed the value of 5, if one applies a tortuosity correction based on the electrical resistance of the system.

During the last few years, work has been carried out in these laboratories on the electrokinetic properties of the interface between the wool fibre and water, as part of which it has been necessary to determine the radius of the fibre *in situ* by permeability measurements. It has been found that, at porosities of the order of 0·8, k may have values of the order of 6·5, and may be even greater at lower porosities.

It has been noticed in the course of the experimental work that, contrary to expectation, the volume flow-rate was not proportional to the applied pressure. A consideration of the criteria for streamline flow showed that the linear flow relation will only be found experimentally under the double condition

©1950 Nature Publishing Group