coccus hemolyticus, Streptococcus viridans, Pneumococcus types I, II and IV, *B. pertussis* and *C. diphtheriae* remained viable after 24 weeks under liquid paraffin. Control culture tubes sealed with rubber stoppers were dead within 1 to 4 weeks at 37° C.

The morphological structure of organisms and colonies, as well as the biochemical and serological reactions remained essentially unchanged throughout the entire period of survival. Virulence of all the 4 types of *Pneumococcus* were greatly reduced. Toxigenicity and virulence of *C. diphtheriae* were not greatly altered after 24 weeks' subculturing under liquid paraffin, and pellicle-formation remained the same as before the incubation under liquid paraffin. Hemolytic and toxigenic principles of the *Streptococcus hemolyticus* isolated from erysipelas and scarlet fever sources were not appreciably altered by this method of preserving the cultures.

Viability of bacterial cultures for many months under liquid paraffin at 37° C. doubtless is due to prevention of drying as well as protection against the harmful action on bacteria of oxygen, which Phelon, Duthie and McLeod[6] showed lead to the early death of organisms by the rapid development of alkalinity in the medium. This simple method of keeping delicate bacteria alive for months is exceedingly practicable and labor-saving in laboratories entrusted with large stock culture collections.

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THE CULTIVATION OF NYCTOTHERUS OVALIS AND ENDAMOEBA BLATTAE

Nyctotherus ovalis from the hindgut of the cockroach, *Blatella germanica,* can be easily cultured in a modified Smith and Barret[1] medium. This medium was used by the discoverers for *Endamoeba* (*Entamoeba*) *thomsoni,* and according to Lucas[2] it is suitable for the cultivation of neither *Endamoeba blattae* nor *N. ovalis.* The medium as used by Smith and Barret[1] consists of 19 parts of 0.5 per cent. NaCl to one part of inactivated human blood serum. By substituting non-inactivated rabbit serum for the human serum a medium is produced in which *N. ovalis* lives and multiplies freely. Dividing forms are common, and occasionally precystic and cystic forms are met with. Three cultures have been maintained for 40 days and at the last examination the organisms were as normal in appearance as those found in their native habitat. Subculturing is done at weekly intervals, and the cultures are maintained at room temperature.

The cultivation of *E. blattae* has been less successful than *N. ovalis.* Two cultures out of 12 attempts were maintained for 29 days. At the end of this time the organisms were few in number, but entirely normal in appearance and movement. One 2- and one 8-nucleate form were seen, the latter with nuclei of different sizes and evidently precystic. The next examination was negative. This gradual dwindling in number does not necessarily indicate an unfavorable environment, but rather that division is not frequent enough to permit weekly subculturing, without gradually diminishing the number of organisms to the point of extinction. Longer intervals between subcultures result in an overgrowth of bacteria and the small flagellate *Monocercomonas orthopterorum.*

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SOME NOTES ON EMBRYOLOGICAL TECHNIQUE[1]

IN the course of some varied experiences with the sectioning of a wide variety of vertebrate eggs and embryos I have worked out a few tricks of technique that have been of value in handling difficult material. I am offering these in the hope that they may be of use to other embryologists. The two points which have been of most importance in getting good results are fixation and dehydration, and I shall take up each of these briefly.

FIXATION

Bouin's has proved to be the best fixative for general use on vertebrate material. Except for certain special purposes, such as the study of lipoids, I have found no fixative to compare with it in its faithful preservation of cellular relations without shrinkage or distortion. The length of fixation must, however, be carefully regulated according to the animal, for what is satisfactory for one is entirely wrong for another. In general, delicate tissues should be fixed a lesser time. For example, mammalian blastocysts fix in thirty minutes to an hour; chick embryos of three days or less in an hour; 10 mm pigs in two hours or more. There are other differences, however, which are absolutely unpredictable. Armadillo embryos and ovaries may be stored in Bouin's for days or weeks without injury; carnivore or rodent material will not stain properly if fixed more than about four hours. The optimum time must be determined

[6] H. V. Phelon, G. M. Duthie, and J. W. McLeod, *Jour. Path. and Bact.,* 1927, xxx, 133.

[1] N. M. Smith and H. P. Barret, "The Cultivation of a Parasitic Amoeba from the Cockroach," *Jour. Parasit.,* 14: 161–175, 1928.

[2] C. L. T. Lucas, "A Study of Excystation in *Nyctotherus ovalis* with notes on other Intestinal Protozoa of the Cockroach," *ibid.,* 14: 272–273, 1928.

[1] Contribution number 231 from the Zoological Laboratories of Indiana University.

for each form, although there are some, like the armadillo, which give equally good results after almost any period of fixation.

In some forms which have yolky eggs, fixation tends to harden the yolk until it is almost impossible to section satisfactorily. In such cases dilution of the fixative with an equal amount of water may give satisfactory results. This procedure has proved successful with fish eggs and reptile eggs, after ordinary methods had failed completely, and it gives more consistent results with amphibian ova than does the full strength fixative. Mitotic figures in embryos are as distinct and well-fixed following the dilute Bouin's as they are when preserved in the full-strength fluid.

DEHYDRATION

Even after proper fixation, it may prove difficult to get good sections. Many difficulties have been blamed upon the hardening effects of xylol and the paraffin oven. I have come to realize that neither xylol nor paraffin of any reasonable temperature can do any damage comparable to that caused by alcohols of 80 per cent. or higher. To avoid the use of these alcohols I substitute anilin oil as a dehydrating agent. The procedure used is as follows.

Material fixed in Bouin's—35 per cent. alcohol—50 per cent. until excess picric acid is removed—$\frac{1}{3}$ anilin + $\frac{2}{3}$ 70 per cent.—$\frac{2}{3}$ anilin + $\frac{1}{3}$ 95 per cent.—pure anilin until tissue is completely cleared—$\frac{1}{2}$ anilin + $\frac{1}{2}$ xylol (or use more gradual steps if material shows tendency to shrinkage)—xylol at least an hour—xylol + paraffin—paraffin.

I have left material, treated in this way, over night in xylol, and in a paraffin oven for twelve hours at 58°, without making it brittle or hard. The same material, run through absolute alcohol, would have shattered to pieces or turned the edge of the microtome knife. Using this procedure, I can get perfect serial sections of 10 mm mammalian embryos *in situ* within the unopened uterus. Following alcohol, material of this size would become impossibly hard long before infiltration was complete. Similarly, the yolk-laden eggs of teleost and lizard remain soft and workable when the anilin method is used.

There is another advantage of the anilin method that is important in some cases. Tissues become quite tough in $\frac{2}{3}$ anilin and anilin, and at the same time clear much more completely than with xylol. It is accordingly possible to carry out delicate dissections with ease and at the same time with little risk of accidentally injuring the tissues.

The danger-point in the process is in the transfer from anilin to xylol. Even with one or two intermediate steps the diffusion currents set up are so strong that blastocysts or thin-walled cavities of any sort are likely to collapse partially. The remedy lies, of course, in making the transition more gradual, if necessary running in the xylol by the drop method.

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SPECIAL ARTICLES

THE APPARENT EXISTENCE OF EASILY DEFLECTABLE POSITIVES

UP to the present a positive electron has always been found with an associated mass 1,850 times that associated with the negative electron. In measuring the energies of charged particles produced by cosmic rays some tracks have recently been found which seem to be produced by positive particles, but if so the masses of these particles must be small compared to the mass of the proton. The evidence for this statement is found in several photographs, three of which are discussed below.

In one instance, in which a lead plate of 6 mm thickness was inserted in the cloud-chamber, tracks of a particle were observed above and below the lead. The curvature due to the magnetic field was measurable both above and below the lead. There are the following alternative interpretations:

(1) a positive particle of small mass penetrates the lead plate and loses about two thirds of its energy; or

(2) two particles are simultaneously ejected from the lead, in one direction a positive particle of small mass, in the opposite direction an electron; or

(3) an electron of about 20,000,000 volts energy penetrates the lead plate and emerges with an energy of 60,000,000 volts, having gained 40,000,000 volts energy in traversing the lead; or

(4) the chance occurrence of two independent electron tracks in the chamber, so placed as to give the appearance of one particle traversing the lead plate.

In another instance two tracks of opposite curvature appear below the lead. The alternative interpretations are:

(1) a positive particle of small mass and an electron emerging from the same point in the lead; or

(2) a positive particle of small mass strikes the lead and rebounds with a loss in energy; or

(3) an electron of about 20,000,000 volts energy strikes the lead and rebounds with 30,000,000 volts energy; or

(4) the chance occurrence of two independent electron tracks.