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.