thought" experiences of the Würzburgers may appear as such in consciousness simply because of the limitations of introspective observation, and that muscular activity might actually be present all the while without being introspectively detectable.

The foregoing applies only to muscular factors in thinking, and not to imaginal data of other sensory modalities. While the writer's electromyographic records tend to show that the traditional kinesthetic image is really a proprioceptive awareness of existing muscular contraction, they do not as yet yield a decisive answer to the question of whether all modalities of imagery are fundamentally muscular in nature: If the latter hypothesis (first propounded by Dunlap in 1907) should be proved valid, the present result concerning the comparatively high threshold of myoesthetic sensibility would serve to explain "imageless thought" in its entirety.

This finding also provides a simple physiological answer to an objection raised against the peripheral or motor theories of thinking;—the objection, namely, that if the motor theory is valid, the thinker should experience a kinesthetic awareness of the peripheral muscle-contractions, whereas actually such awareness is frequently lacking. Dunlap meets this objection by stating that the muscle-patterns, like those of the ocular muscles in depth-perception, act merely as unperceived "signs" whose meanings alone enter into consciousness. Rexroad has subsequently offered a somewhat similar explanation. In the light of our experimental results, however, a less involved explanation would be that such muscle-patterns remain unperceived simply because they are below the threshold-intensity for myoesthesis.

LOUIS WM. MAX
LABORATORY OF PHYSIOLOGICAL PSYCHOLOGY,
NEW YORK UNIVERSITY,
MARCH, 1932

SCIENTIFIC APPARATUS AND LABORATORY METHODS

PRESERVATION OF BACTERIAL CULTURES UNDER LIQUID PARAFFIN

NUMEROUS attempts have been made to obviate frequent and essential transfers of bacterial cultures in order to keep stock strains alive. Swift[1] was able to keep alive certain strains of *Meningococcus* for several months by freezing and drying the cultures at low pressure. Hiss[2] kept several strains of *Meningococcus* alive for as long as 14 months in a medium consisting of 1 per cent. glucose broth and 1 per cent. calcium carbonate. Murray[3] succeeded in keeping meningococcic cultures alive for 6 months to 1 year on Dorset's egg medium and the tubes sealed with cotton plugs soaked in molten wax. Other investigators have been less successful with this method. Bruni[4] made the important observation that strains of *Meningococcus* grown on ordinary agar could be kept alive after 80 days at 37° C. if layered with liquid paraffin. Parish[5] confirmed Bruni's observation and extended the study to include besides the *Meningococcus* several strains of *Gonococcus, B. influenzae* and *M. catarrhalis*. These cultures he cultivated on ordinary agar, blood agar and trypagar for 24 to 48 hours at 37° C. when he added approximately 8 cc of previously sterilized liquid paraffin to each tube and replaced the cultures in the incubator. In this manner he succeeded in keeping *Meningococcus* and *B. influenzae* alive for 8 to 12 weeks, *Gonococcus* and *M. catarrhalis* for 16 to 22 weeks. Control cultures in tubes sealed with cotton plugs soaked in molten wax usually died in 2 to 3 weeks.

We have repeated Bruni's and Parish's studies with 2 strains of each of the following organisms: *Streptococcus hemolyticus* (scarlet fever and erysipelas), *Streptococcus viridans, Pneumococcus,* types I, II, III and IV, *Gonococcus, Meningococcus, B. influenzae, B. pertussis* and *C. diphtheriae* (Park 8 A). The bacteria were grown on blood agar slants for 24 to 48 hours at 37° C., when approximately 10 cc of sterile liquid paraffin were added to each tube from a pipette. At this time rubber stoppers were substituted for cotton plugs and the cultures replaced in the incubator. It was apparent that bacterial metabolism was slowed down by the addition of liquid paraffin and that no appreciable increase in size of colonies took place during the subsequent long periods of incubation. It was also noted that the blood agar discolored exceedingly slowly under liquid paraffin, in contrast with the rapid development of chocolate color in the control culture tubes within 2 to 3 days at 37° C.

Subcultures were made onto blood agar plates by removing a small platinum loopful of surface growth, at bi-weekly intervals. Care was taken to allow the liquid paraffin to run off the loop by touching the wire against the sides of the tube. *Meningococcus, Gonococcus* and *B. influenzae* were found to remain viable for 10 to 12 weeks under liquid paraffin. *Pneumococcus,* type III, lived for 16 weeks. *Strepto-*

[1] H. F. Swift, *Jour. Exp. Med.*, 1921, xxxiii, 69.
[2] P. H. Hiss, Zinsser's "Textbook of Bacteriology," 6th edition, p. 436. 1927.
[3] E. G. D. Murray, *System Bact. Med. Res. Coun.*, London, 1929, ii, 291.
[4] E. Bruni, *Ann. di. Med. nav. e. col.*, 1930, xxxvi, ii, 396.
[5] H. J. Parish, *Jour. Path. and Bact.*, 1932, xxxv, 143.

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.

KONRAD E. BIRKHAUG

UNIVERSITY OF ROCHESTER
SCHOOL OF MEDICINE AND DENTISTRY

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.*

HARRY E. BALCH

DEPARTMENT OF ZOOLOGY,
UNIVERSITY OF CALIFORNIA

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.