The above results as well as results of the preliminary joint research show inconsistencies following removal of limb-bud ectoderm by means of versene, and these inconsistencies centre chiefly around the presence or absence of the refractile layer on the treated mesoblast. It has been observed that the refractile layer may cover all or part of the mesoblast and not necessarily only that portion which underlies the ridge. Grafts which develop distal parts appear to come from limb buds which have been treated in such a way that the refractile layer may be preserved; but how much is required and precisely where it must be located has not yet been determined. Evidence is not yet sufficient to decide whether the refractile layer is cellular or acellular; in the latter case, whether it may consist of basement membrane, intercellular matrix or both. It seems, therefore, that limb development may proceed in the absence of an apical ectodermal ridge as a morphological entity and that distal outgrowth in some experiments depends on the presence of a 'refractile' layer which may be non-cellular.

The observations described above do not invalidate the earlier experiments on the role of the ectoderm in limb development. For example, surgical removal of the ectodermal ridge definitely produces limbs which lack distal structures. This was first described by Saunders, has been confirmed by Amprino and Camosso[6], by Zwilling in unreported experiments and by others. The earlier findings must now be evaluated in the context of recent observations.

The authors plan future joint and independent investigations which will be designed to elucidate the nature of the refractile layer as well as its possible role in limb development.

EUGENE BELL
Biology Department,
Massachusetts Institute of Technology,
Cambridge, Mass.
JOHN W. SAUNDERS, jun.
Biology Department,
Marquette University,
Milwaukee, Wisconsin.
EDGAR ZWILLING
Biology Department,
Brandeis University,
Waltham, Mass.
Aug. 1.

[1] Saunders, J. W., *J. Exp. Zool.*, **108**, 363 (1948). Saunders, J. W., Cairns, J. M., and Gasseling, M. T., *J. Morphol.*, **101**, 57 (1957). Saunders, J. W., Gasseling, M. T., and Cairns, J. M., *Dev. Biol.*, **1**, 281 (1959).
[2] Zwilling, E., *J. Exp. Zool.*, **128**, 423 (1955); *Amer. Nat.*, **90**, 257 (1956).
[3] Hampé, A., *C. R. Soc. Biol.*, **150**, 1671 (1956).
[4] Bell, E., *Amer. J. Phys. Med.*, **37**, 184 (1958). Bell, E., Kaighn, M. E., and Fessenden, L. M., *Dev. Biol.*, **1**, 101 (1959).
[5] Amprino, R., and Camosso, M., *J. Exp. Zool.*, **129**, 453 (1955).
[6] Amprino, R., and Camosso, M., *C. R. Assoc. Anat.*, **42**, 197 (1955).

Glaucoma Species in the Central Nervous System of the Carp

THE free-living ciliate *Glaucoma pyriformis*, or at least a close relation, has been found to occur as a facultative parasite both in arthropods[1-6] and in the central nervous system of fishes[7] and of amphibians[8,9]. This parasitism may be rendered possible by the fact that this protozoan parasite is quite tolerant of carbon dioxide[10].

Recently we observed a *Glaucoma* infection in young larvæ of the carp *Cyprinus carpio*, in which about 8 per cent was found to be infected. The parasites were present in the cerebrospinal fluid, the brain, the spinal cord and the meningeal fluid.

On the strength of the morphological character the parasite could with certainty be identified as belonging to the genus *Glaucoma*: flask-like body with a sub-cylindrical anterior and tapering gradually to a rounded extremity, approximately 20 longitudinal rows of cilia with large basal bodies in the ectoplasm, wide cytostoma surrounded by larger cilia and containing two undulating membranes, large cytopharynx, single spherical macronucleus and very minute micronucleus, both situated in a separate clear area.

The average dimensions of the parasites were: length 59 μ, breadth 24 μ, depth 16 μ. The average diameter of the rounded parasites, which were found in the tissues of the brain and of the spinal cord, was 30 μ. Cyst formation was not observed.

Just as in the former cases of *Glaucoma* infection in fish[7] and amphibians[8,9] the infection probably took place after hatching. Subsequently the parasites could find their way from the skin of the intestine to the tissues of the central nervous system.

A. STOLK
Histological Laboratory,
Free University,
Amsterdam.
Sept. 12.

[1] Penard, E., 'Etudes sur les infusoires d'eau douce' (Génève, 1922).
[2] MacArthur, W. P., *J. Roy. Army Med. Corps*, **38**, 83 (1922).
[3] Treillard, M., and Lwoff, A., *C. R. Acad. Sci.*, **178**, 1761 (1924).
[4] Lwoff, A., *C. R. Acad. Sci.*, **178**, 1106 (1924).
[5] Wenyon, C. M., "Protozoology" (London, 1926).
[6] Janda, V., and Jirovek, *Mém. soc. zool. tchék de Prague*, **5** (1937).
[7] Epstein, H., *Arch. Russes de Protist.*, **5**, 169 (1926).
[8] Shumway, W., *Biol. Bull.*, **58**, 283 (1940).
[9] Stolk, A., *Naturwiss.* (in the press).
[10] Kofoid, C. A., *Bull. Ill. State Lab. Nat. Hist.*, **8**, 1 (1908).

Value of a Simple Observational Technique for Determining the Fat Content of Algal Cells

DURING the period 1953–55 an investigation was made of the survival of freshwater algæ during dry periods[1]. Some attention was paid to the accumulation of fat in the cells of diatoms and other algæ though details of this work have not yet been published. The method used to investigate fat accumulation was a simple one in which cells were measured in length and in width in girdle view. For some cells the width in valve view was also measured. Fat globules, stained with sudan black, were measured and a note made of the number of fat globules within each particular size range, present in each cell. On the basis of these results, determinations could be made, for comparative purposes, of the mean relative amount of fat per cell in sample populations. The results suggested that fat accumulation was invariably associated with drying in those species investigated which included *Pinnularia viridis*, *P. appendiculata*, *Nitzschia palea*, *N. frustulum* in addition to *Microspora stagnorum*.

Since late 1957 attempts have been made to check the accuracy of the observational technique by biochemical methods and to repeat some of the earlier work. From a sample of *Navicula pelliculosa* (Breb.) Hilse, kindly given to me by Dr. G. E. Fogg of University College, London, pure, unialgal cultures were set up. So far as possible, the method of culture described by Collyer and Fogg[2] was adopted. For most cultures, the medium used was Chu No. 10[3]. Determinations of dry weight, fatty acids and unsaponifiable lipoid were made in duplicate upon each sample by the methods described or referred to in the account by Collyer and Fogg[2].