Discussion. In view of the short reaction time and direct control of kinetics, the described continuous optical procedure for phosphatase estimation presents improved accuracy with respect to discontinuous sampling methods, and appears particularly suitable for kinetic studies. The remarkable simplicity of the determination furthermore enables measurements of high precision and reproducibility to be obtained.

The test can be carried out at all pH values in the alkaline range, provided that the appropriate extinction coefficient for the liberated *p*-nitrophenol is used.

Zusammenfassung. Ein kontinuierliches optisches Messverfahren für die Bestimmung der alkalischen Phosphatase mit *p*-Nitrophenyl-phosphat als Substrat wird ausführlich beschrieben. Das Verfahren wird zur Bestimmung der alkalischen Phosphatase in der Rattenniere angewandt.

F. MELANI and A. GUERRITORE

Istituto di Chimica Biologica dell'Università di Firenze (Italy), April 17, 1964.

Ophthalmoscopy of Pigeons Using Transillumination

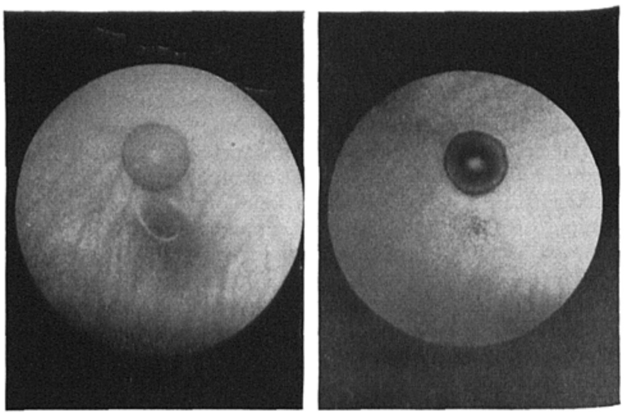
Through the invention of the direct ophthalmoscope by HELMHOLTZ[1], and its subsequent improvement by GULLSTRAND et al.[2], the central areas of the fundus are able to be examined easily. However, only with large pupil sizes are the areas of the peripheral fundus conveniently observed. This is especially true in human subjects whose pupils are dilated conveniently to 6 mm in diameter, or more. However, ophthalmoscopic examination of a bird is not as easily accomplished. The pupil is normally small and, as the iris is skeletal muscle, cannot be enlarged by the usual adrenergic blocking agents. Consequently, poisons such as nicotine or similar ganglionic or neuromuscular blocking agents must be employed with some risk to the life of the animal at concentrations which produce maximal dilation. At best, these produce a pupillary dilation of 5 mm.

Most indirect ophthalmoscopes and cameras are designed for larger pupillary apertures, or even when pupil size is immaterial, as with the Zeiss fundus camera, annoying corneal light reflexes are present. We have developed a different method, which we believe to be useful for both examination and photography of birds and possibly other small animals with eyes on opposite sides of the head. This method is indirect, transverse illumination and is an adaption of those methods used on humans by STEVENSON[3] and others[2] where the illuminating light is placed in the mouth or nose.

Both eyes of the animal are dilated (although this need not be to a maximal degree) by an appropriate cycloplegic agent[4], after which the head is held firmly by some type of holder. Both eyelids are taped open to prevent blinking but the nicitating membrane is left free. A bright light, such as that from an ordinary dissecting lamp, is focused onto the pupil of one eye. (Suitable precautions should be taken to prevent overheating the eye, with subsequent cataract formation.) With this light properly positioned to fall on the pupil, the opposite eye is illuminated by the light passing through the head.

The eye of the observer is brought up to the transilluminated eye and the entire globe can be seen clearly, including that area behind the pecten usually shadowed by the illuminating light from conventional ophthalmoscopes. Also, the light reflex around the fovea is now reversed and the fovea shows up as a dark diffusely granular area.

The examination should be conducted in a darkened room, and a shield to prevent light from the lamp from straying into the observer's eye is also helpful. No practice is necessary; contrary to their first experiences using the ordinary ophthalmoscope, untrained workers can make a good examination of the eye on their first attempt. Conventional fundus cameras will take excellent photographs when transilluminated by a properly triggered flash lamp.



A B

Photographs of a pigeon's eye taken with a Noyori hand fundus camera approximately 40 min after producing a lesion with a Laser flash. In the center of each picture is the fovea with the lesion immediately above it. A was taken using the built-in flash illumination of the camera. B was taken using a flash lamp focused on the pupil of the opposite eye to transilluminate. In A the vessels of the choroidal circulation are quite prominent and there is a light reflex around the fovea. The lesion has very low contrast. In B the choroidal circulation is indistinct, the fovea has no light reflex and shows only as a diffuse dark granular mass, but the lesion has extremely high contrast.

[1] H. v. HELMHOLTZ, *Beschreibung eines Augen-Spiegels zur Untersuchung der Netzhaut im lebenden Auge* (Förstner, Berlin 1851).

[2] For a critical review and history of ophthalmoscopy see W. S. DUKE-ELDER, *Text-Book of Ophthalmology*, Vol. II (Mosby, St. Louis 1937).

[3] N. STEVENSON, Brit. Med. J. *1*, 379 (1893).

[4] For the pigeon, we use several applications of a 10% cocaine solution followed by several applications of a 1% nicotine solution.

Certain features are seen more easily using this method than with conventional ones. We have been interested to observe lesions produced by photocoagulation. Such lesions are much more visible by transillumination. Figure A is a photograph of the retina showing the fovea and a lesion taken by the conventional direct illumination method; Figure B is a picture of the same field using the same camera, but taken by indirect illumination. Although certain features, such as the choroidal vessels, are not quite as clear in the latter photograph due to their decreased contrast, the lesion itself is much more easily visible, not only because of its own higher contrast with respect to the other features, but also because there is no light reflex around the lesion to mask its characteristics.

We believe that this method may be useful in teaching and general laboratory work since it allows the easy observation of the interior of the eye and the pecten. Little training and equipment are required. This method can also be used to supplement ordinary ophthalmoscopic examinations for the detection of retinal lesions or features ordinarily shadowed by the pecten.

Résumé. Une nouvelle méthode d'examen visuel facile du fundus des oiseaux est décrite ici. Elle utilise la transillumination de la tête. Cette méthode rend possible la photographie avec des appareils conventionnels. Elle n'exige pas une dextérité speciale, et grâce à elle, certaines caractéristiques peuvent être mises en evidence d'une facon plus nette qu'avec d'autres techniques.

M. L. Wolbarsht, P. E. Stopp, and J. E. Goodson

Naval Medical Research Institute[5], *Bethesda (Maryland USA), March 31, 1964.*

[5] The opinions or assertions expressed herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Navy Department or the Naval Service at large.

Nouveau procédé original de préparation de cellules vivantes par dissociation du foie de rat

Dans des notes antérieures[1–3], nous avons décrit les principaux résultats des recherches que nous avons entreprises sur les méthodes d'isolement de cellules «libres» à partir du foie de rat.

Dans une première série de travaux[1], nous avons effectué une étude critique des différentes méthodes qui avaient été décrites jusqu'à présent et montré que, seul, le procédé de Longmuir et Rees[4] (dissociation dans une solution tamponnée de phosphates à pH 5) répondait au premier critère que nous avions fixé: fournir rapidement et en grandes quantités des cellules parfaitement dissociées. Malheureusement, la méthode ne satisfaisait pas à un second critère concernant l'intégrité morphologique et biochimique des cellules isolées. En effet, l'observation au microscope électronique montre de profondes altérations des noyaux et des mitochondries (Figure 2). En outre, bien que les cellules respirent (Figure 1), elles sont incapables de survivre dans le milieu nutritif TC 199 de

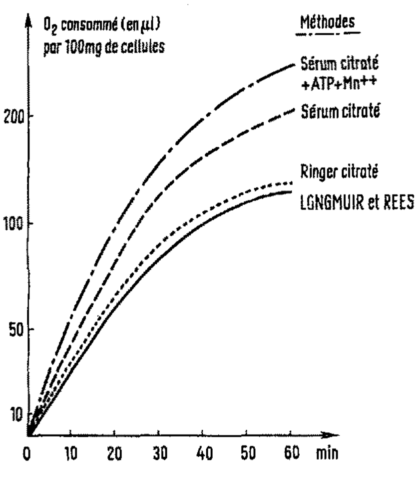
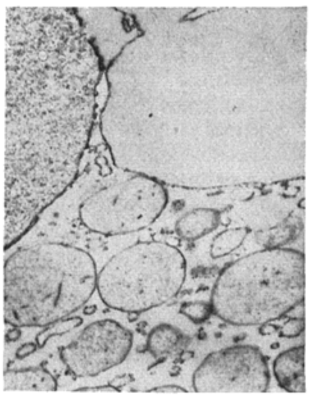


Fig. 1. Courbes de respirations données par des quantités égales de cellules isolées à partir du foie de rat par différentes méthodes. Le milieu d'incubation est 40 m*M* en succinate de sodium.



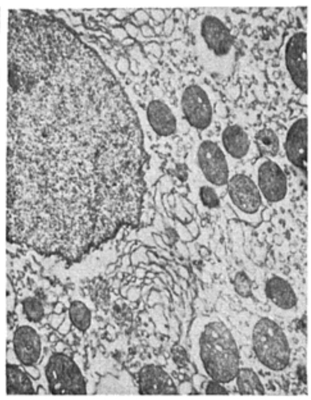


Fig. 2. Aspect morphologique au microscope électronique Siemens Elmiskop-I d'une partie de cellule de foie de rat isolée par la méthode de Longmuir et Rees[4].

Fig. 3. Aspect morphologique au microscope électronique Siemens Elmiskop-I d'un fragment de cytoplasme d'une cellule isolée par la «méthode au Krebs-Ringer citraté-hyaluronidase».

[1] E. Segard, J. Montreuil, A. Colbeau, A. Dupont, A. Demaille, C. Mangez, L. Adenis et J. Driessens, C.R. Soc. Biol. *156*, 1102 (1962).
[2] E. Segard, J. Montreuil, A. Colbeau, A. Dupont, A. Demaille, L. Adenis et J. Driessens, C.R. Acad. Sci. *255*, 1479 (1962).
[3] E. Segard, J. Montreuil, A. Colbeau, A. Dupont, A. Demaille, C. Mangez, L. Adenis et J. Driessens, C.R. Soc. Biol. *157*, 130 (1963).
[4] I. S. Longmuir et W. Rees, Nature *177*, 997 (1956).