(2:1); dosage des produits séparés par fluorescence, identification par co-chromatographie dans les solvants: A = eau, B = n-propanol: ammoniaque à 1% (2:1), C = n-butanol: acide acétique: eau (4:1:1), D = n-butanol: pyridine: eau (6:4:3) et par électrophorèse dans les tampons: I = acide acétique, II = acide formique, III = acide borique, IV = acétate de Na (0,05 M; 400 V; 12 min, dans chaque cas); étude des produits de dégradation éventuels.

Résultats. On identifie 2 composés: la riboflavine fluorescente en jaune et l'isoxanthoptérine, fluorescente en violet. Pour la riboflavine: $R_f = 0,49$, 0,42, 0,32 et 0,37, respectivement dans les solvants A, B, C et D et migrations (en mm): −10, −11, −6 et +2, respectivement dans les tampons I, II, III et IV. Pour l'isoxanthoptérine: $R_f = 0,41$, 0,33, 0,21 et 0,14, respectivement dans les solvants A, B, C et D et migrations (en mm): −16, −16, 0 et +10, respectivement dans les tampons I, II, III et IV. Une substance fluorescente en jaune, non-identifiée, est présente dans les exuvies seulement. La comparaison des teneurs des lots B et T (tableau) montre que:

- la riboflavine et l'isoxanthoptérine sont toutes deux présentes dans chaque lot,
- chaque composé est plus abondant dans le tégument avec pigment blanc qu'à l'extérieur de celui-ci,
- la différence essentielle entre les 2 substances porte sur la répartition de leurs concentrations respectives entre les 2 types de tégument: la riboflavine est 11,76 fois plus abondante dans la partie pigmentée, alors que l'isoxanthoptérine ne l'est que 6,88 fois.

Teneurs des différents tissus étudiés en riboflavine et en isoxanthoptérine

Substance et origine		Poids frais de l'échantillon (g)	Concentration en µg par g de tissu frais
Riboflavine	T	3,357	0,34
	B	3,858	4,03
	E.P.E.	6,981	0,25
	E.P.R.	0,194	2,04
Isoxanthoptérine	T	3,357	0,26
	B	3,858	1,84
	E.P.E.	6,981	0,13
	E.P.R.	0,194	1,40
Substance fluorescente jaune	E.P.E.	6,981	0,15
	E.P.R.	0,194	5,95

Discussion. La présence simultanée de la riboflavine et de l'isoxanthoptérine dans le tégument contenant le pigment blanc ne permet pas de considerer l'une des 2 substances plus que l'autre comme responsable de la pigmentation blanche étudiée. Les résultats obtenus souligneraient plutôt le caractère complexe et même composite du pigment blanc. Il convient de rappeler ici l'existence des complexes flavine-proteines signalés chez certains crustacés par Busnel et Drilhon[7]. Des expériences seront prochainement faites pour préciser la forme sous laquelle se trouve la riboflavine au niveau du pigment blanc et étudier le rôle possible de ce composé, en tant que substance photosensibilisatrice[9], dans le caractère photosensible des chromatophores blancs.

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Primary tissue cultures from organ fragments: A simplified method[1]

E.J. Noga[2]

Department of Biological Sciences, Florida Atlantic University, Boca Raton (Florida 33431, USA) 18 May 1978

Summary. Organ fragments washed in Ca^{++} and Mg^{++} - free saline, treated with trypsin and placed directly into culture flasks adhered within seconds to the vessel surface. If the fragments were suspended in culture medium before they were added to the flasks, they did not adhere. This technique permits the rapid attachment and subsequent growth of the primary tissue cultures.

Organ fragments offer distinct advantages over single cell suspensions for initiating primary cultures. Individual fragments are less affected by deleterious culture conditions than are single cells[3]. Prolonged enzyme treatment for the release of individual cells causes the loss of cellular components and lowers cell survival[4]. But trypsin-treated organ fragments which are not separated into individual cells exhibit minimal cell damage and loosened intracellular connections, which aids in cell migration[5].

Since fragments must firmly attach to the vessel surface in order to allow cell growth, various methods have been explored to secure them. These have included plasma clot[6], drying of the tissues onto the vessel surface[7], and the use of collagen films[5]. Fragments have been either left undisturbed to allow attachment[8] or physically held in place by sandwiching them between 2 surfaces[9], but it may often require several days before the fragments will adhere[3]. The method described herein eliminates the need for such procedures and results in fragments which are quickly and securely attached to the vessel surface, thus facilitating cell outgrowth. The entire procedure can be performed in less than 1.5 h. While the following is a description of the method used in the initiation of cultures from a fish, the procedure has also been found to be equally successful with rat kidney, spleen and lung[10].

Materials and methods. Kidney, gonad and liver from adult walking catfish (*Clarias batrachus* (L.)) were placed into separate plastic petri dishes. Approximately 3 ml of Puck's saline G[6] without calcium or magnesium (CMF) were added and the organs minced into 1-mm^3 pieces with a pair of scalpels. The fragments were transferred to 16 × 125 mm test tubes, 4 vol. of CMF added, the fragments allowed to settle and the fluid removed. Washing was repeated until the supernatant fluid was clear. 4 vol. of 0.25% trypsin (Difco 1:250; Difco, Detroit, Michigan) in CMF were added and each tube was mixed by pipetting. A cloudy supernatant was normally produced by this enzymatic treatment, except in the case of gonadal tissue. The super-

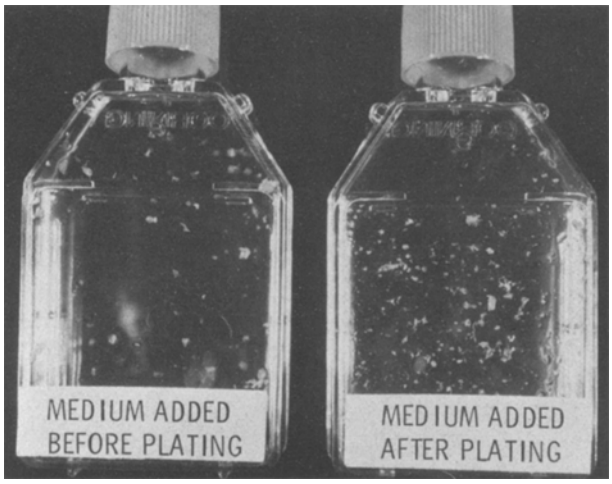

Fig. 1. The adherence of trypsin-treated organ fragments with complete culture medium added either before or after the fragments were decanted into the flask.

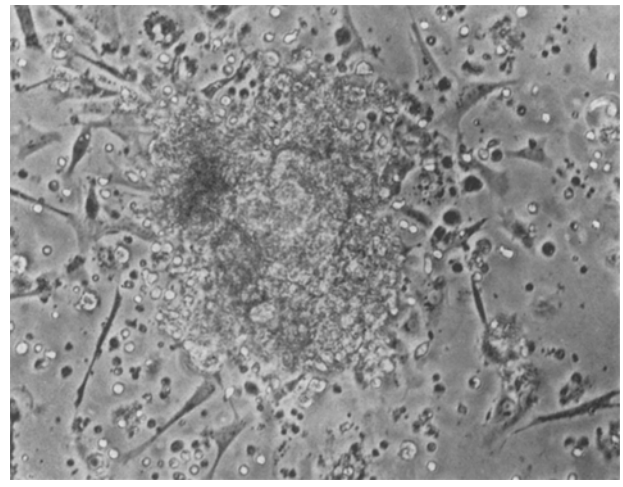

Fig. 2. Outgrowth from a walking catfish kidney explant initiated 1.5 days previously. × 50.

natant was then decanted and fresh trypsin added to the organ fragments; this was repeated 3–4 times. After the last trypsin treatment, the supernatant was decanted and the fragments placed into plastic culture flasks (Corning Glass Works, Corning, New York).

Culture medium consisted of a modified Ham's F-12 in which all components were present at 52% of the standard recommended concentration. The medium contained 23 mM HEPES, 25 mM sodium bicarbonate, 9% fetal bovine serum, penicillin G (200 units/ml), streptomycin sulfate (200 µg/ml) and amphotericin B (5 µg/ml). During initial attempts to establish cell cultures, fragments were mixed with culture medium and decanted into the vessel. The flasks were slowly tilted into an upright position and left for 2 h so that the fragments remained on the growth surface without medium[7].

Results and discussion. The procedure as described above was of limited success, as most of the fragments usually failed to adhere to the surface of the culture flask. Fragments which had apparently adhered usually detached even though the medium was carefully added. If the trypsin-treated fragments were added to the flask without first adding culture medium, more than 90% of all the organ pieces adhered within seconds (figure 1). Under these conditions, the fragments remained firmly attached after the addition of culture medium.

The cultures could be immediately examined without dislodging the fragments. Cells began migrating from the explants within 36 h (figure 2). The attachment was so tenacious that fragments were difficult to remove once they had contacted the surface of the flask for only a few sec. Cultures initiated in this manner were confluent within 1–2 weeks, depending on the organ used. 2 of these cultures, from gonad and kidney, have been passaged in excess of 70 times.

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GABA stimulates the rabbit corneal endothelial fluid pump[1]

R. Vidal[2], A. Wendel[3] and S. Dikstein[4]

Cell Pharmacology Unit, School of Pharmacy, Hebrew University of Jerusalem, P. O. Box 12065, Jerusalem (Israel), 14 June 1978

Summary. GABA and its analogues were shown to activate the rabbit corneal endothelial fluid pump in the micromolar range. The stimulation was abolished by bicuculline and chlorpromazine, but not by picrotoxin.

The standard technique of study of the rabbit corneal endothelial fluid pump is to pre-swell the cornea from its normal thickness (less than 400 µm) to about 550 µm and to follow the extent of deturgescence as a function of time at 37 °C. It was shown[5] that the deturgescence depends on the presence of sodium and bicarbonate ions and is improved by reduced glutathione[5]. Later on oxydized glutathione and cystine were shown to improve pumping and survival as well[6]. In such solutions, the survival time is about 3 h, but the deturgenscence is always only partial[5,6]. In the presence