Ainsi nous avons réparti en courts palliers successifs les chocs thermique (de +5 °C à +18 °C), ionique et osmotique (de pH 8,7 à 7,5) lors de l'homogénéisation des culots obtenus.

Nous précisons que l'emploi des godets en polypropylène permet aux diatomées de conserver la charge électromagnétique de friction qui les maintient agglomérées lors de la décélération et des transvasements: l'utilisation de godets en acier, ou tout matériau conducteur, conduit à un échec.

Discussion. Au cours de nos travaux sur les échanges en lipides existant entre *Chaetoceros simplex calcitrans* Paulsen et son milieu[1-4], les milieux de culture usés récupérés par cette méthode ont été examinés. Au microscope à immersion (×1800–2000) on n'observe aucune diatomée entière, ou fragments cellulaires, pouvant ainsi la bonne séparation. Une autre preuve d'efficacité est donnée par l'analyse des lipides des diatomées et des milieux usés lors des 2 cultures successives. Dans nos travaux sur le pouvoir d'incorporation par la diatomée d'un hydrocarbure[3] ou du cholestérol[4] marqués au ^{14}C, certains lipides extraits de la diatomée sont absents des milieux usés. Ceci montre l'absence de perfusion notoire du contenu cellulaire de l'algue dans le milieu de culture au cours des centrifugations.

Les poids secs de diatomées obtenues à la fin de la première culture (488 mg et 498 mg/5 l), et de la 2e culture (647 mg et 622 mg/5 l), nous ont prouvé la très bonne survie de l'algue après les centrifugations qui n'ont pas freiné sa croissance lors de la 2e étape.

Divers essais de récolte des diatomées avec contrôle de la survie ont été réalisés: centrifugations en continu à 10000×g à +18 °C et à +4 °C, puis à 28000×g (rotor normal) à +4 °C; ces 2 séries ne fournissent pas un rendement satisfaisant du test de survie effectué a posteriori; la 2e série donne seule une bonne séparation. C'est à la suite de la recherche systématique des conditions assurant l'optimation de ces deux paramètres que la méthodologie reportée dans ce travail a pu être précisée.

Applications. Dans les travaux précités, cette méthode de changement de milieu de toute une récolte de diatomées nous a permis d'étudier quels produits de transformation l'algue échangeait avec un milieu neuf après avoir incorporé un précurseur marqué dans un précédent milieu: c'est la première fois, à notre connaissance, qu'un tel travail a été réalisé. Les applications de cette technique sont innombrables et se rapportent essentiellement à toute étude sur des diatomées vivantes et en particulier touchant l'évolution des échanges entre une diatomée et son milieu.

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Scanning-autoradiography - a new method for the demonstration of membrane-surface-associated structures

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Summary. A method for the demonstration of membrane-localized markers and receptors is described which results from the combination of autoradiography and scanning electronmicroscopy.

The demonstration of membrane-localized markers, hormone- and immunoreceptors is an important and difficult task in present research. Although biochemical investigations of isolated membrane components furnish reliable quantitative data, the exact localization of membrane components can be established only if the integrity of the cell membrane is not disturbed. To serve this purpose, investigations making use of isotope, fluorescent dye of other labels are appropriate. A different approach is to examine the cell surface by scanning electron microscopy; however, this method gives only a picture of the fine structure of the surface without revealing the exact localization of the structures in question[1]. The methods were sometimes combined: a substance that specifically binds to membrane structures was labelled with molecules that are easily seen in the scanning electron microscope, as, for instance, synthetics[2-4], metal[5], or immune molecules[6]. A new type of experiment is reported here, where the grains produced by autoradiography are demonstrated by means of scanning electron microscopy.

In pilot experiments, thin sections were covered and developed like in ordinary autoradiography, and then examined by transmission and scanning electron microscopy in the visual area. This was possible because, with the JEOL 100 B electron microscope, the grid has not to be changed when switching from transmission to scanning. It was essential to place the grid with the section face down into the microscope in order to enable the scanning beam to reach the emulsion layer. It could be shown in these experiments that the grains can be seen and identified in scanning electron microscopy due to their ability to emit secondary electrons (figure 1, a and b).

In the following experiments, ^{125}I-albumin was used for unspecific labelling of the cell membrane, and ^{125}I-triiodothyronine for specific labelling.

The thymus of newborn rats was grown in tissue culture for 3 days, fixed with 1.25% glutaraldehyde for 1 h, washed with PBS (phosphate-buffered saline) and incubated for 30 min in 2 ml of 2% human albumin labelled with 40 μCi ^{125}I (spec. act. 5 mCi/mg). After washing 3 times, the material was postfixed for 1 h with 1% OsO_4, dehydrated, dried (using CO_2 and the critical point drying procedure), and coated by evaporated C+Au. The specimens were then dipped into Ilford L 4 emulsion diluted 1:4, and 1 week later developed for 4 min in Microdol X. When examined in a JEOL 100 B electron microscope using the scanning option, the screw-shaped grains are easily recognized at the cell surface (figure 2).

Since the cell surface is not everywhere as smooth as in thymus cultures, the use of a different method seems required which demonstrates the radioactivity in spherical shape. This was especially important in the case of *Tetrahymena pyriformis* GL, the 2nd of our experimental objects, where the structure of the cilia is often screw-like.

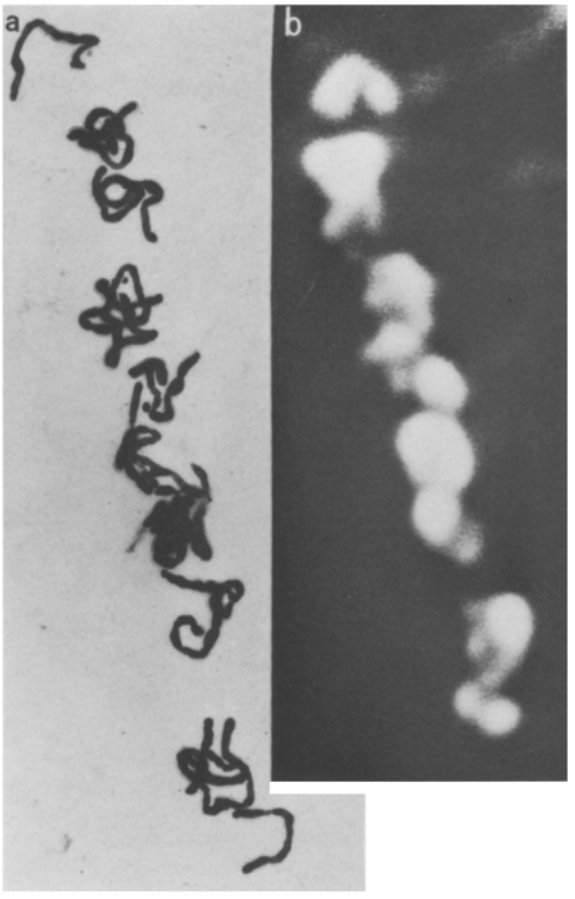

Fig. 1. Unspecific grains above a thin section observed *a* in transmission electron microscopy, ×24,000; *b* in scanning electron microscopy, ×20,000.

Some *Tetrahymena* membrane component specifically binds T_3[7], and this was used as a model for specific binding.

Tetrahymena cells grown for 2 days were fixed in 4% glutaraldehyde for 1.5 h. After washing, the cells were incubated for 30 min in 2 ml containing 20 μCi of triiodothyronine (T_3, Amersham, spec. act. 50 μCi/mg). After washing 3 times, the material was postfixed for 1 h with OsO_4, dehydrated and critical-point dried. The specimens were then coated (C+Au), and covered with Ilford L 4 emulsion diluted 1:4. After 1 week, development was carried out for 1 min with the developer described by Haase (composition: 1.5 g ascorbic acid, 0.25 g fenidon, Reanal, 1-phenyl-3-pyrazolidone, 0.6 g potassium bromide, 1.3 g potassium carbonate, 6.0 g potassium rhodanid, 20.0 g of water-free sodium sulfite, filled up with water to 100 ml; to be prepared freshly). The spherical grains are easily seen (arrows) at the surface of cilia (figure 3).

The method reported here seems useful for the localization of structures that bind to the cell membrane and that can be labelled with isotope, thus for the demonstration of the sites for hormone receptors, markers etc.

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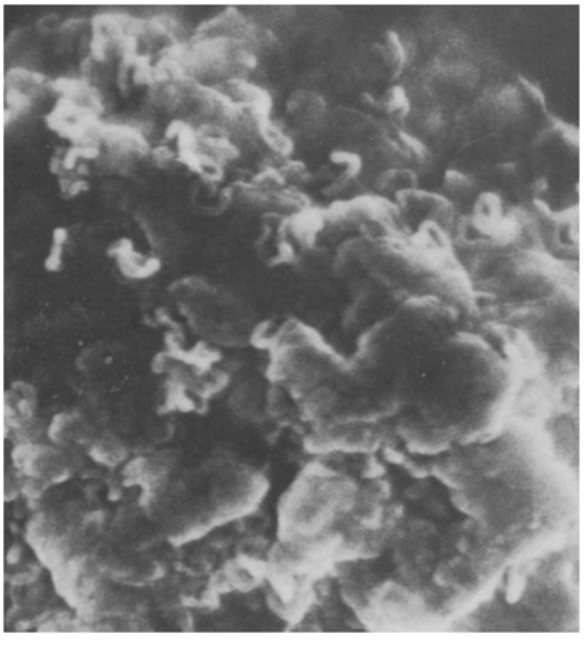

Fig. 2. Newborn rat thymus cells as seen by scanning autoradiography. The screw-shaped grains indicating ^{125}I-albumin are well seen at the cell surface. ×30,000.

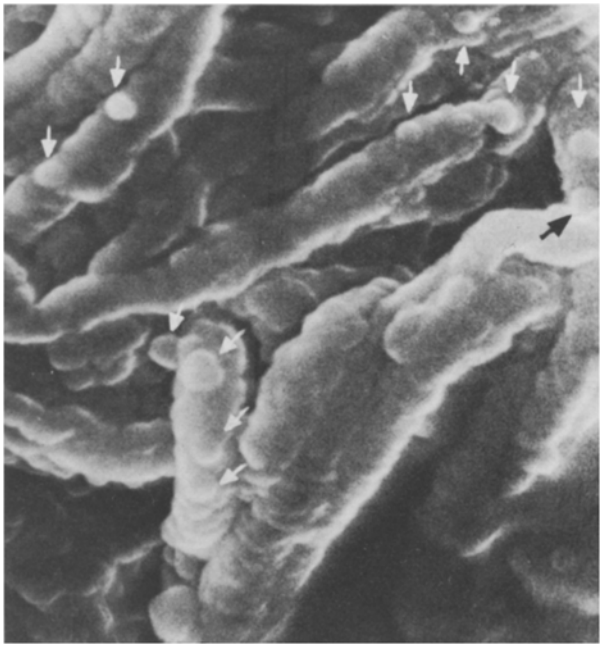

Fig. 3. Spherical-shaped grains (arrows), after special development, are easily seen at the cilia of *Tetrahymena* treated with ^{125}I–T_3. ×30,000.