

scope equipped with interference contrast optics. For electron microscopy, small slices of skin were immersed in 2% osmic acid dissolved in a cacodylate-buffered Ringer of 1180 mosm. Thin sections were photographed on a Philips 301.

Results. In isolated skin there is a considerable measure of spontaneous activity of chromatophores: phasic and tonic expansion as well as activity progressively spreading over a distance can be observed up to several hours after the removal of skin samples. This phenomenon offers an excellent possibility for the study of the organization and functioning of chromatophore organs.

The chromatophores are arranged in 4 or 5 different layers¹¹. The lowest one is occupied by very small, faint yellow chromatophores, which have an incomplete set of muscle or none at all; we suppose that these are the youngest members of a population with continuous proliferation and loss, because we regularly find degenerating chromatophores in the top layer¹². Spontaneous activity of chromatophores in isolated skin, and most probably all activity in intact skin, is restricted to the 2 or 3 layers between the presumed germ layer and the degenerating layer. As can be observed under the microscope, discrete groups of chromatophores expand and contract synchronously or almost synchronously while others remain silent.

It was not found, until we used interference microscopy, that those chromatophores, which operate synchronously or nearly synchronously, are all interconnected horizontally by their muscles (figure); frequently the muscles split into 2 or more branches. It is difficult to determine the dimension of individual muscles, but during their contraction it can be seen that they terminate either on other chromatophores or on other chromatophore muscles. They can form elaborate patterns of connectivity between several chromatophores.

Although we were unable to determine all the connections of any particular chromatophore, we estimate that each

one is connected directly with 8–14 other chromatophores, perhaps even more. There is apparently no vertical connection between chromatophores of different layers. All species investigated show chromatophore link.

Discussion. The finding that chromatophores in cephalopods are connected by muscles, does not fundamentally conflict with earlier physiological evidence; however, in some points the picture must be revised:

a) Although the chromatophore muscle is an independent functional entity^{4, 5, 10}, chromatophores cannot be operated individually, because they are linked. As one can observe directly under the microscope, the full expansion of 1 chromatophore involves automatically the partial expansion of approximately 10 neighbouring chromatophores.

b) Spontaneous progressive spread of chromatophore expansion in isolated skin (or dying or denervated skin^{9, 13}) can now be reasonably explained: in living skin there is a physiological barrier to prevent excitation transfer from one muscle to the other⁴. Post mortem, the membrane resistance is lowered and excitation can cross at myomuscular junctions, travelling along the muscular network over considerable areas. (This phenomenon was named 'Wolkenwandern' or wandering clouds by Hofmann.)

c) As suggested by Packard¹⁴, the consistency of colour patterns in *Octopus* may be due to the patterned distribution of chromatophore nerves. However, it cannot be ruled out now that muscular link as well can be a basis for the consistency of colour patterns in cephalopods.

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Métaplasie épidermoïde de l'épithélium mammaire humain en culture organotypique à long-terme

Squamous metaplasia of human mammary epithelium in long-term organ culture

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Summary. Squamous metaplasia of the mammary epithelium was observed in human breast tissue maintained in long-term organ culture. The phenomenon occurred only in the synthetic medium 199 with Earle's salts. Insulin and/or glucose enrichment enhanced its occurrence.

L'apparition de phénomènes de métaplasie squameuse ou malpighienne dans l'épithélium mammaire maintenu en culture organotypique a été rapportée dans différentes observations¹⁻⁵. En ce qui concerne la glande mammaire humaine les conditions dans lesquelles apparaît la métaplasie n'a pas été étudiée. Au cours d'un travail portant sur le maintien en survie à long-terme et l'influence d'additifs hormonaux sur la glande mammaire humaine adulte normale et au repos nous avons observé ces phénomènes dans des conditions de culture précises. Le but de cette communication est de présenter les facteurs physico-chimiques qui interviennent dans la genèse de la métaplasie malpighienne dans la glande mammaire humaine explantée.

Méthodes. Le matériel d'étude a consisté en du tissu mammaire provenant de 13 femmes (âge moyen: 30,5 ans) soumises à une intervention de correction chirurgicale. Nous avons cultivé un total de 5000 explants par immersion dans le milieu synthétique 199, que nous avons utilisé dans les formules de Earle et de Hanks. La diffé-

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Incidence de la métaplasie épidermoïde

Milieu de culture	Pourcentage d'explants
199 (Earle) non-enrichi	2.8
199 (Earle) + insuline 5 µg/ml	25.6
199 (Earle) + glucose 5 mg/ml	17.6
199 (Earle) + insuline 5 µg/ml + glucose 5 mg/ml	14.3

rence principale entre ces 2 préparations concerne leur concentration de NaHCO_3 , qui est respectivement de 2,2 et 0,35 g/l. Certains additifs ont été ajoutés au milieu 199 de base, en l'occurrence de l'insuline (5 µg/ml) et/ou du glucose (concentration finale de 5 mg/ml). Les cultures ont été faites par immersion. Des prélèvements ont été effectués chaque jour au cours d'une période de 25 jours. Le milieu de culture était renouvelé tous les 3 jours.

Résultats. La métaplasie pavimenteuse de l'épithélium mammaire n'a été observée que lors de l'utilisation du milieu 199 préparé selon la formule de Earle (NaHCO_3 : 2,2 g/l). L'incidence globale du phénomène atteignait $15,5 \pm 5,7\%$ (DS) des explants. Son apparition la plus précoce fut notée dès le 4^e jour de culture; en moyenne la date d'apparition la plus fréquente était située aux environs du 12^e jour. L'influence des additifs au milieu (tableau) montre que l'apport d'insuline et/ou de glucose favorise de façon hautement significative l'apparition de la métaplasie par rapport aux conditions basales en milieu non-enrichi. Les pourcentages entre les 3 milieux enrichis ne diffèrent pas de façon statistiquement significative.

Discussion. La glande mammaire humaine explantée n'est pas la seule à présenter des phénomènes de métaplasie; la glande de rongeur réagit de cette façon en présence de 7,12-diméthylbenz(a)anthracène⁶. Dans l'épithélium bronchique explanté le déficit en vitamine A est un facteur étiologique⁶; l'addition de vitamine A empêche le phénomène⁷. Nous pensons que ce facteur n'intervient pas dans la glande mammaire puisque le milieu 199 contient 0,1 mg/l de vitamine A; or cette concentration suffit à empêcher la métaplasie dans les bronches⁷.

Si nos observations permettent de penser que le pH joue un rôle, il faut toutefois envisager l'influence des mécanismes de réparation dont la métaplasie pavimenteuse est considérée comme le témoin⁸. La métaplasie et la kératinisation impliquent un taux de mitoses suffisant pour produire assez de cellules capables de constituer un système de formation de kératine⁹. Dans la trachée la synthèse d'ADN est notablement accrue dans les foyers de métaplasie en culture. Il est actuellement bien établi que l'insuline joue un rôle dans la synthèse d'ADN et la division cellulaire des tissus en culture. Nos observations confirment le rôle stimulant de l'enrichissement insulino-glucosé sur l'apparition de la métaplasie dans l'épithélium mammaire explanté. Toutefois, le pH du milieu constitue un facteur déterminant puisque dans les mêmes conditions d'enrichissement insulino-glucosé la métaplasie n'apparaît jamais en milieu 199 de Earle.

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Effect of indole-3-acetic acid on the kinetics of horseradish peroxidase catalyzed scopoletin oxidation

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Summary. The kinetics of horseradish peroxidase catalyzed scopoletin oxidation were observed to be sigmoidal. The apparent K_m s for scopoletin is 0.9 mM. Inhibition of scopoletin oxidation by indole-3-acetic acid (IAA) appears to be non competitive. Non competitive inhibition by IAA suggests a more significant role of the peroxidase protein matrix in regulating the activity of the heme moiety.

The interest in physiological substrates for peroxidase includes scopoletin, a naturally-occurring coumarin which has been shown to inhibit growth in tobacco seedlings². Although Andreae³ first demonstrated scopoletin oxidation by crude IAA oxidase preparations of potato tissue in 1952, it was the work of Imbert and Wilson⁴ on the modulation of growth hormone degrading activity (IAA oxidase) by scopoletin that has led to several studies of the interaction of scopoletin and/or IAA with peroxidases. Sirois and Miller⁵ postulated mechanisms to explain the non-linear competitive nature of inhibition of IAA oxidase activity by scopoletin and demonstrated the oxidation of scopoletin in the presence of IAA by horseradish peroxidase. Sigmoidal kinetics were shown for the oxidation of scopoletin in the absence of IAA by tobacco tissue culture isoperoxidase A_3 ⁶, but after more extensive investigation, this enzyme was shown to contain only

a single subunit, although many of the characteristics of substrate cooperative effects or 'allosterism' were observed⁷. The kinetics of scopoletin oxidation by horseradish peroxidase were studied and the effects of IAA upon these kinetics were evaluated to probe the mecha-

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