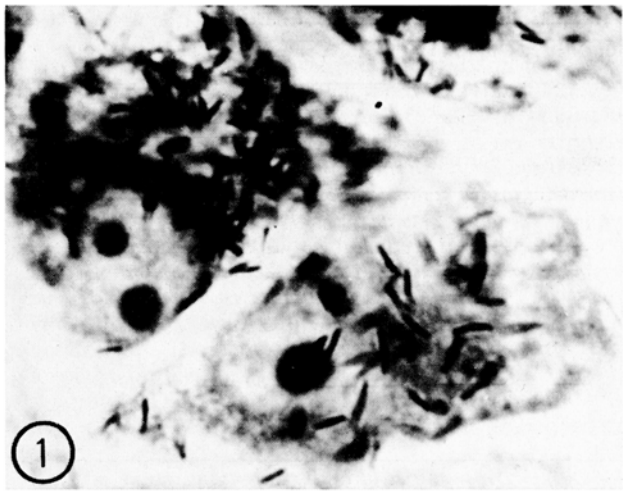

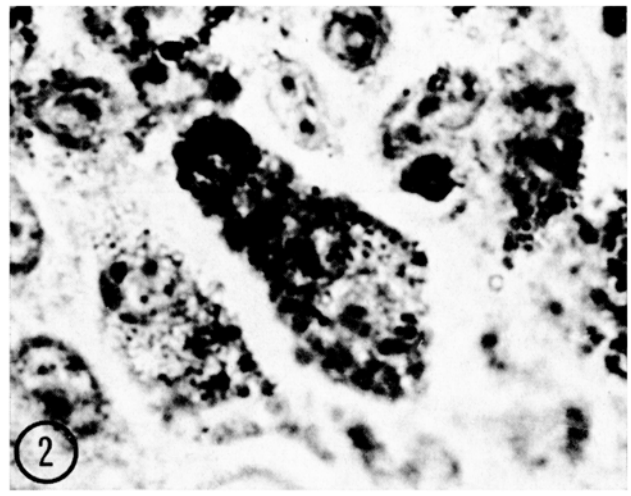

Fig. 1. 7-day chick tapetal cells after 60 h in primary culture. Monolayer coverslip preparation stained in May-Grünwald and Giemsa stains. ×2100.

Fig. 2. 7-day chick tapetal cells grown for 43 days in monolayer culture and transferred to organ culture for 5 days as an aggregated mass. 7-µm section stained in hematoxylin-eosin. ×2100.

When retinal pigment cells of 6½- to 7-day Rhode Island Red chick embryos (HAMBURGER and HAMILTON[5] stages 30,31) were grown for 30 days or longer in frequently subdivided monolayer cultures and then reaggregated into tissue masses and maintained as organ cultures, the granules which appeared in the cells after 4–5 days of organ culture were tiny spherical particles (Figure 2). Even in cells observed after 2 weeks in organ culture, none of the granules were rod-shaped. Similar results were obtained when White Leghorn embryos were used. The culture methods have been described previously[1,2].

The purpose of this communication is to suggest that the granule shape observed in these cultured cells is not caused by a change in the capabilities of the dedifferentiated cells, but by a response of the cells to the culture environment. This hypothesis is substantiated by the behavior of normal chick tapetal cells in organ culture. Tissues from 3- to 3½-day Rhode Island Red embryos (stages 18 and 19) were maintained as organ cultures on Millipore filter rafts in a medium consisting of 1 part Tyrode's saline solution, 2 parts horse serum, and 1 part 12-day chick embryo extract (with 50 IU/ml each of potassium penicillin G and streptomycin sulfate, and 0.005% phenol red). 7 series of these cultures were sectioned and studied histologically after times in culture ranging from 1 to 19 days. The tapetum was unpigmented at the time the cultures were initiated, but became visibly pigmented after 1 to 2 days in organ culture. Spherical pigment granules similar in size to those of the secondarily pigmented cells of Figure 2 were produced in culture.

If spherical granules develop in normal chick retinal pigment cells after a relatively short time in organ culture, there is no reason to believe that the extensive growth (cell divison) and dedifferentiation occurring in monolayer cultures necessarily cause any restriction in the potential range of melanosome differentiation. There is no significant amount of cell division in organ cultures of the young pigment epithelium pieces.

The formation of chick pigment cell melanosomes has been investigated at the ultrastructural level[6], but nothing is yet known about how extracellular conditions can affect granule size or shape. However, the genotype of the pigment cell appears to influence its response to the culture evironment. Under organ culture conditions comparable to those used above, melanocytes developing in neural crest explants of certain chicken breeds have rod-shaped granules, whereas White Leghorn melanocytes develop spherical granules under the same conditions[7]. Possibly culture conditions will eventually be found which will permit completely normal granule development[8].

Zusammenfassung. In Kurzzeitkulturen mit noch intakten embryonalen Retina-Pigmentzellen von Hühnchen sowohl als auch in Langzeitkulturen, in denen die entdifferenzierten Zellen wieder zur Pigmentbildung angeregt wurden, sind die Pigmentgranula kugelförmig im Gegensatz zu den stäbchenförmigen in vivo.

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Tumour-Promoting Activity of Fatty Acid Methyl Esters in Mice

In our previous studies on carcinogenic properties of lipids, a preliminary screening was made by the newt test. The most potent inducer of epithelial hyperplasia was methyl 12-oxo-trans-10-octadecenoate[1]. Methyl hydroxyoctadecadienoate was also highly active. The 2 compounds have now been tested for carcinogenic and tumour-promoting activity on mouse skin. Methyl oleate which was inactive in the newt was also tested.

Tests of methyl esters of fatty acids for carcinogenic and tumour-promoting activity by skin application on mice

Treatment Initiator (dose/mouse)	Promoter	No. of mice	Survivors at 12 months	Cumulative No. of mice with Skin tumours Papilloma	Carcinoma	Sarcoma	Lymphoma	Days to first skin tumour	Papillomas per tumour-bearing mouse
None	None	25	16	0	0	0	0	–	–
None	MO[a]	35	29	1	0	0	0	318	1
None	MOO[b]	35	30	1	0	0	0	320	1
None	MHO[c]	22	13	0	0	0	0	–	–
DMBA[d] 50 µg	None	20	11	0	0	1	0	254	–
DMBA 250 µg	None	20	4	14	0	0	16	59	3.4
DMBA 50 µg	MO	30	5	6	2	3	2	170	1.5
DMBA 50 µg	MOO	30	11	20	1	0	9	80	2.9
DMBA 50 µg	MHO	23	15	11	0	0	1	146	2.1
DMBA 50 µg	CO[e]	20	6	14	2	0	12	41	3.4

[a] Methyl oleate 20% in acetone (v/v). [b] Methyl 12-oxo-trans-10-octadecenoate 20% in acetone (v/v). [c] Methyl hydroxyoctadecadienoate 20% in acetone (v/v). [d] 7,12-Dimethylbenz[α]-anthracene. [e] Croton oil 0.5% in acetone (w/v).

The experimental animals were male and female ST/a mice fed a commercial pelleted stock diet.

Methyl 12-oxo-trans-10-octadecenoate was prepared from ricinoleic acid by the method of NICHOLS and SCHIPPER[2] and was 85% pure. The impurity was methyl 12-oxo-trans-9-octadecenoate. Methyl hydroxyoctadecadienoate was prepared by reduction of methyl linoleate hydroperoxide with dimethyl sulphide and was essentially pure.

Methyl oleate, croton oil and 7,12-dimethylbenz [α] anthracene were from Sigma Chemical Company, St. Louis, Missouri.

All test substances were dissolved in acetone (pro analysi, Merck) in suitable concentrations and a volume of 50 µl was applied by means of a syringe to the interscapular region of each animal. The applications were started when the animals were $6^1/_2$ to 8 weeks old within a resting phase of their hair cycle.

Initiation was accomplished by a single application of 50 µg dimethylbenzanthracene. A control group of animals was given the effectively carcinogenic dose of 250 µg.

Promotion was started 2 weeks after initiation; the test substances were given 3 times weekly for 1 year. Croton oil was used as positive control promoter. Animals to which no promoter was applied served as a negative control group. Further control groups were given the same treatments with respect to promoters but without previous initiation.

The animals were inspected weekly. Papillomas were counted when at least 1 mm in diameter and observed for 3 weeks in succession.

Dead mice were autopsied and tumours examined histologically. Carcinomas were of the squamous-cell type. The sarcomas could be classified as fibroblastic. The diagnosis lymphoma includes lymphocytic neoplasms, generalized or localized, plasma cell neoplasms, generalized or localized, and one case of reticulum cell sarcomatosis. The results after 1 year of treatment are presented in the Table.

From the Table it is seen that no safe evidence of any proper carcinogenic effect of the fatty acid esters was found, but all the 3 substances exhibited some degree of tumour-promoting activity.

Some papillomas and lymphomas and a relatively high incidence of malignant skin tumours were observed after painting with methyl oleate. The result is surprising, since oleic acid occurs as a normal component of animal lipids. However, a promoting effect of oleic acid has been reported by other investigators[3,4].

The most potent inducer of skin papillomas among the esters was methyl oxooctadecenoate followed by hydroxyoctadecadienoate. Oxooctadecenoate also induced the largest number of lymphomas. It was found in the previous experiments to be the most potent inducer of epithelial hyperplasia in the newt out of a series of products of the autoxidation of unsaturated fatty acids.

The results present an example of the usefulness of the newt test as a short-term test for the preliminary screening of substances for carcinogenic properties. Newt-positive substances may be promoters as well as complete carcinogens. The findings are in accordance with the theory that hyperplasia plays a major role in the process of promotion[5–8].

Zusammenfassung. Es wurden die Methylester der 12-Oxo-trans-10-octadecensäure und Hydroxyoctadecadiensäure (Produkte der Autoxidation ungesättigter Fettsäuren), sowie Methyloleat auf Mäusehaut appliziert und auf cancerogene Eigenschaften geprüft. Keiner der Ester wirkte als komplettes Carcinogen, hingegen zeigten alle Promotorwirkung. Am wirksamsten war Oxooctadecensäuremethylester, welches die Bildung von Hautpapillomen wie auch von Lymphomen förderte.

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