Mean grain counts ± S.D. for different cell types

Time (min)	Animal No.	Clara cells	Epithelial and endothelial cells	Type II cells	Macrophages
0.5	1	33.2 ± 10.7	9.5 ± 3.1	2.5 ± 3.1	2.9 ± 5.4
	2	25.1 ± 5.3	9.6 ± 1.4	5.5 ± 3.6	4.4 ± 4.0
1.5	3	52.0 ± 12.7	10.6 ± 4.9	4.9 ± 6.3	2.5 ± 5.5
	4	39.0 ± 15.5	12.5 ± 1.9	3.9 ± 4.1	5.8 ± 7.3
	5	37.1 ± 9.9	15.7 ± 4.2	6.0 ± 2.8	7.8 ± 6.1
4	6	23.3 ± 6.9	17.8 ± 6.5	19.1 ± 9.2	7.4 ± 6.7
	7	6.0 ± 2.2	5.9 ± 2.6	3.4 ± 2.5	7.3 ± 25.7
	8	11.2 ± 5.2	3.5 ± 1.0	6.0 ± 3.8	4.0 ± 4.6
60	9	3.7 ± 2.5	4.1 ± 1.6	23.7 ± 13.7	33.2 ± 18.0
	10	2.9 ± 2.1	2.2 ± 1.0	14.3 ± 8.3	20.9 ± 9.9

the CLARA cells in the terminal bronchioles, which show significantly higher incorporation than any other type of cell ($p < 0.001$ at 30 and 90 sec). There is no difference in labelling between macrophages and type II cells at 30 and 90 sec. The inference is that during the first 2 min, the CLARA cells are actively metabolizing palmitate, and there is no uptake of palmitate from the capillaries by type II cells.

4 min after dosing the labelling of the CLARA cells is reduced, but there is still a significant difference between the degree of labelling of these and the type II cells ($p < 0.001$). Labelling of the CLARA cells and the type II cells at this stage is significantly greater than that of the macrophages ($p < 0.001$).

The alveolar epithelium (type I) and endothelium show comparable degrees of labelling over the 60 min period. The intensity was significantly greater than that seen in the macrophages and type II cells up to 4 min ($p < 0.001$) but never achieved that of the CLARA cells. This activity presumably indicates a moderately active lipid metabolism in these cells though the labelling over the type I cells may be due to newly synthesized surfactant from elsewhere. 1 h after dosing, the distribution of the radioactive source is predominantly in the macrophages and the type II cells. Macrophages show significantly more label than the type II cells ($p < 0.001$) and both kinds of cell are more heavily labelled than all the other cell types ($p < 0.001$ for both cell types).

These results may be interpreted as supporting the hypothesis that pulmonary surfactant, or more precisely, dipalmityl lecithin, is secreted by the CLARA cells and ingested at a later stage by the type II cells. The CLARA cells show at least a 5-fold increase in labelling over other cell types 90 sec after dosing, followed by a reduction in labelling during the succeeding few minutes, which suggests that the turnover of dipalmityl lecithin might be much more rapid than hitherto believed, and that secretion of this material by CLARA cells may be continuous. The type II cells nearest to the terminal bronchioles become labelled a few minutes later, suggesting that labelled material has reached them and has been ingested by them. Many of the silver grains were located over the large clear vacuoles in these cells, which are more likely therefore to be phagocytic vacuoles. Chemical analyses are underway to confirm that the tritiated palmitate remains as such during the first h of the experiment, and also to determine how much of it is incorporated into dipalmityl lecithin.

Résumé. Le taux d'incorporation de l'acide palmitique marqué à l'hydrogène 3 dans les cellules des bronchioles terminales et des alvéoles du poumon de la souris, suggère que le surfactan pulmonaire est sécrété par les cellules de CLARA dans les bronchioles terminales.

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[8] We thank Mrs. SUSAN LONGSHAW for her help.

Teratogenic Effects Induced in Tail of *Bufo arenarum* Tadpoles Following Treatment with Carcinogens

Inducing processes in the early stages of development have in particular attracted much interest. However, in later stages, very few investigations have been performed. Among chemical substances that modify the induction process, several polycyclic hydrocarbons were mentioned by BRACHET. Positive results were described by SHEN[1] in amphibian eggs and by BREEDIS[2] in *Triturus viridescens*, while NEUKOMM[3] and WOERDEMAN'S[4] investigations were not succesful.

In a previous paper we described pseudotumoral nodular formations by subcutaneous application of 20-methylcholanthrene in olive oil (MATOS and LUSTIG[6]). The experiments to be reported here deal with the teratogenic effects induced in tail of *Bufo arenarum* tadpoles following crystal implant of 3 carcinogenetic substances.

Material and method. 20-methylcholanthrene (MC), 7,12-dimethylbenz(a)anthracene (DMBA) and 3,4-benzopyrene (BP) were s.c. implanted in the middle of the tail's length with glass needles. Controls were implanted with fluorene and paraffin. No anesthesia was used. The implanted crystals remained in the site of inoculation, which appeared deficient in pigmentation. The larvae were 20–30 mm long; metamorphosis having just started, the 2 hind leg buds were visible at the time of

treatment. No mortality or retardation in tail growth was observed among the treated tadpoles.

Results and discussion. In a few days papilomas and linfomas in 90% of animals treated with MC, BP and DMBA, moreover an incidence of accessory growths, was observed. The tumoral-like cells invaded the normal structures of the tail, leaving out the newly formed tissues. General controls showed a brief period of perinotochordal stimulation and an apparently non-specific inflammatory reaction around the inoculated mass.

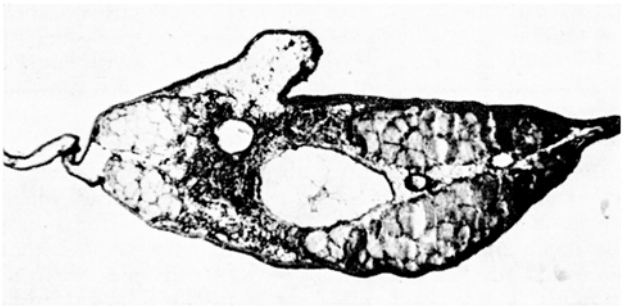

Fig. 1. An accessory tail fin induced by MC crystals implanted 2 weeks previously.

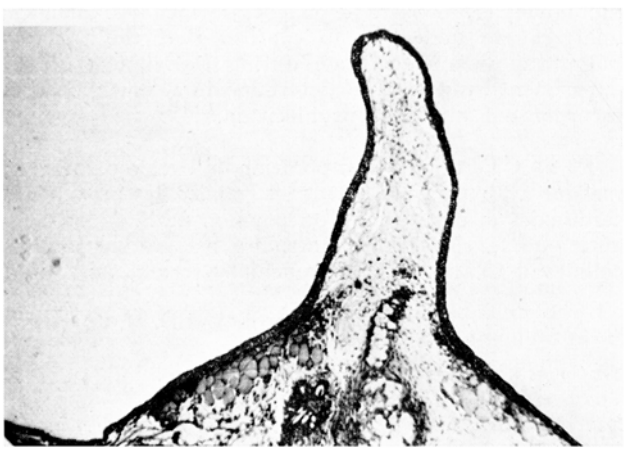

Fig. 2. A complete third tail fin in a tadpole with BP crystals implanted 26 days before. The histological structure looks like a normal tail fin.

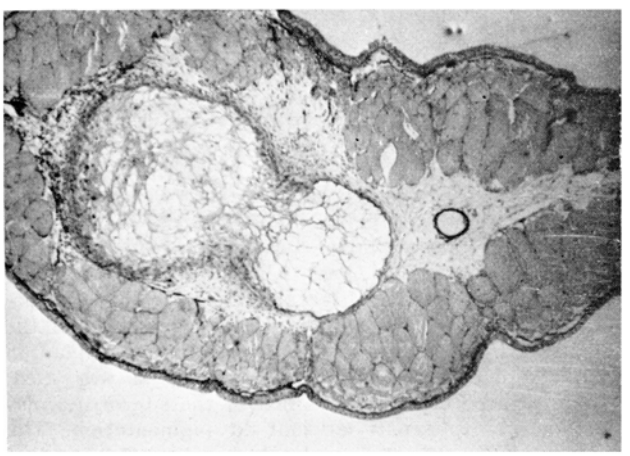

Fig. 3. The normal and the accessory notochord are confluent.

One animal receiving MC crystals developed an accessory tail fin within 16 days, and another implanted with BP, 26 days later. The tadpoles receiving DMBA crystals showed, after 20 days, 4 cases of complete accessory tail fin, and 2 cases with an accessory notochord parallel to the normal which appeared on the 4th week when the degeneration of striated muscle was accomplished by the pseudotumoral mass. The variety of accessory tissues is illustrated by representative photographs in Figures 1, 2 and 3.

When tadpoles taken from their natural habitat were examined with care, no single accessory tail fin or reduplicated notochord were found. The present experiments suggested that:

1. Anura larvae respond, like urodele newts, with reduplication of normal structures following treatment with carcinogens. 2. Fluorene and paraffin are inactive as inductors of abnormal growth in tadpoles. 3. MC, BP and DMBA induce a marked change in morphogenesis of tail fin.

In BREEDIS[2] experiments BP, which was an active inductor in our experiments, failed to induce accessory limbs. Therefore our accessory growth appeared earlier than those obtained by BREEDIS (40–300 days after treatment) with a complete mixture of carcinogenic substances. Moreover, we did not observe an inflammatory reaction as described by the same author.

FAUTREZ[7] observed a chordalization process of neighboring organs as an effect of addition of urea to the medium of amphibian eggs, and he concludes that the process probably consists in a diffusion of organ specific substances elaborated by the differentiating notochord. In our experiments, the notochord differentiation process is not in progress but definitively accomplished. The main stimulus for the production of supernumerary outgrowth may not be the mechanical trauma, because the non-carcinogenic substances are inactive; probably the treatment with chemical agents known to alter nucleic acid synthesis interfere with the amphibian's tail embryogenesis in a pre-metamorphosis period related to the onset of competence in the tail tissue[8].

Riassunto. Si descrivono gli effetti teratogeni indotti nella coda del girino del *Bufo arenarum* in uno stadio premetamorfosico, come conseguenza dell'impianto di cristalli di idrocarburi cancerigeni. Tanto il MC come il BP o il DMBA producono alette sopranumerarie che ripetono le strutture istologiche delle alette normali; ed inoltre il DMBA induce la formazione di una seconda notocorda parallela alla normale.

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[8] The authors wish to thank Dr. E. WEISBURGER for supply of BP, DMBA and fluorene.
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