a nerve fibre in a muscle fibre is seen in Figure 3, in a histological section.

In recent experiments[16], antagonistic nerve activity on feather follicle was described. In serial sections, never more than a single nerve was found to enter into the muscles of a feather follicle. On the other hand, when the intercostal nerves are observed in the whole mount preparation, their branches directed to the skin flow one into the other and appear to anastomose. Thus, each nerve which ends in feather follicle carries fibres from different source and may cause different effects at the same time. This finding can explain the fact that a wide range of factors influence the feather follicle and feather-skin connection. Among these factors are some which act by local application, such as temperature[17,18] and scalding agents[19]. On the other hand, some drugs act especially when applied parenterally, such as anaesthetics[4,6–8], sympathomimetics[4,5] and parasympathomimetics[16].

The specific action on the different nerve endings and the various muscles needs physiological-pharmacological investigation.

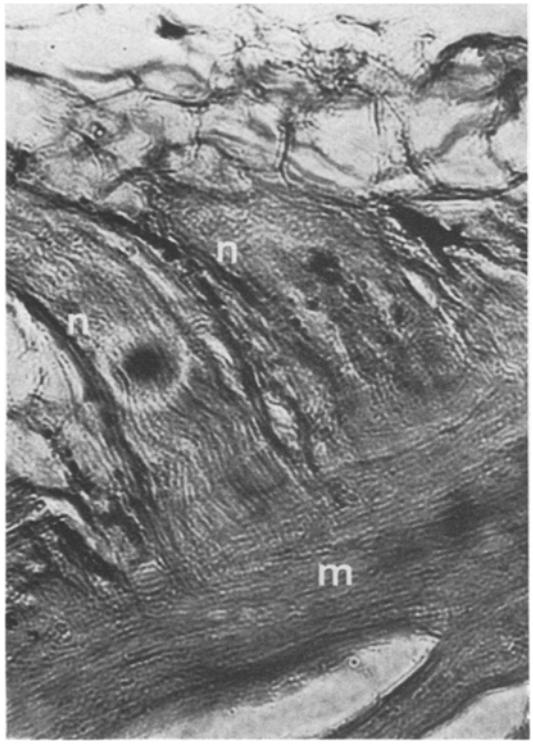

Fig. 3. Section showing the ending of a nerve fibre (n) in a muscle fibre (m) of feather follicle.

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DNA-Mediated Transformation in the Platyfish-Swordtail Melanoma System

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Summary. A genetic marker, the tumor gene *Tu*, which causes the formation of abnormal melanophores, the T-melanophores, in the skin of Xiphophorine fish has been transferred by donor DNA from a *Tu* genotype to recipient embryos lacking *Tu*. Abnormal melanophores which are identical to the T-melanophores of the donor genotype occurred only in recipients treated with *Tu*-DNA and not in those treated with *Tu*-free control DNA.

Genetic transformation – that is, the transfer of a genetic marker from a donor to a recipient organism by DNA and the phenotypic expression of this marker in the recipient – was first achieved in bacteria by Avery, MacLeod, and McCarty in 1944[3]. During the last two decades, numerous researchers have tried to show genetic transformation also in eukaryotic organisms[4]. Some researchers claimed to have succeeded[4,5], but the results they obtained may be open to some other interpretations than genetic transformation[6]. Most of the experiments suffered from the non-availability of a good genetic marker and a sensitive selective system. Both of these conditions are available in the platyfish-swordtail melanoma system[7].

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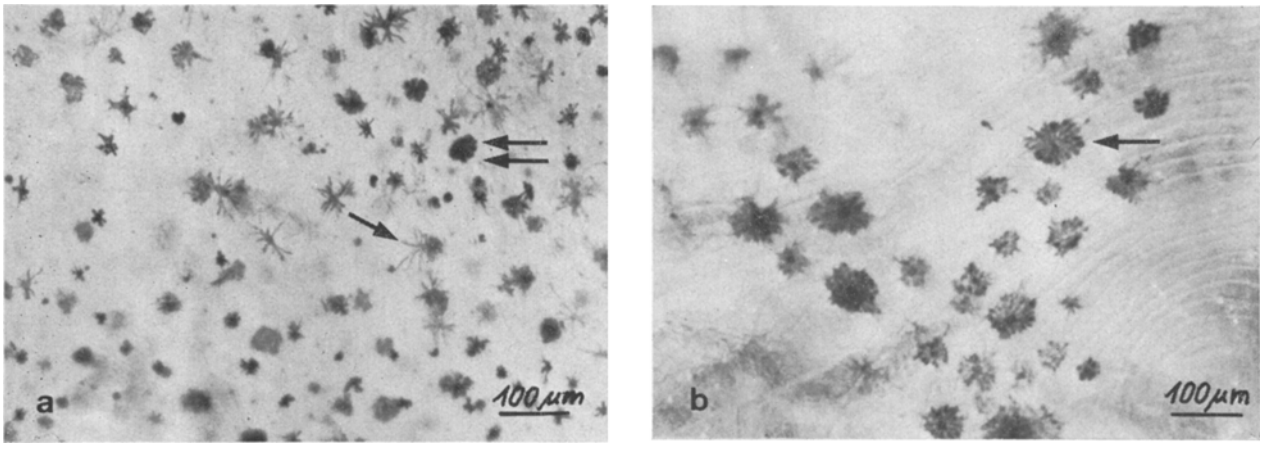

Fig. 1. a) Normal melanophores in the skin of a fish lacking the *Tu* gene. Note the thin long processes of cells in the dispersed state (arrow) which are not visible in the aggregated state of the cells (double arrow). b) T-melanophores in the skin of a fish carrying the *Tu* gene. Note the broad processes (arrow) and the size of the cells.

In this system, there is a so-called tumor gene, *Tu*, which can be present or absent in the fish genome[8]. Concomitantly, abnormal pigment cells, the so-called T-melanophores, are present or absent, respectively, in the skin of the fish. These cells appear as a result of the action of *Tu* on propigment cells and represent a cell type which can be easily distinguished from the normal melanophores of the skin by their size and morphology (Figure 1, a and b). The number of propigment cells changed into prospective T-melanophores, the rate of cell division, and the time of first appearance of T-melanophores in the skin, depend on various *Tu*-controlling genes. In the absence of these genes, *Tu* acts in almost all propigment cells which develop from the neural crest and causes these cells to divide so often that T-melanophores cover the whole body of the newborn fish.

In order to show genetic transformation in this system, donor DNA from a *Tu* genotype should be applied to the neural crest of recipient embryos which lack both *Tu* and controlling genes. After uptake and integration of a *Tu*-carrying piece of DNA into a propigment cell, the lack of *Tu*-controlling genes in the recipient genome will allow this cell to divide very often. It can be expected that, starting from only one transformed cell, many T-melanophores will develop. Thus, a single transformation event is selectively amplified, and it should be detected easily because of the distinct morphology of the T-melanophores.

The outlined experiment, however, can be only successful, if the donor DNA applied remains in a high-molecular form for a sufficiently long period so that DNA of informative size may be taken up by the cells and integrated into the recipient genome. We have shown in previous experiments that *E. coli* DNA of this size can be demonstrated in recipient embryos during the first 5 h after injection into the neural crest region[9], and that sequences of this donor DNA can be demonstrated in the recipient DNA[10]. These results suggest that there should be a good chance for uptake and integration of a piece of donor DNA carrying the *Tu* information.

The transformation experiment was carried out as follows: Highly purified donor DNA (absorbance ratio 260/280 nm: 1.85; molecular weight: 100×10^6 daltons) was extracted from testes of fish carrying *Tu* and in-

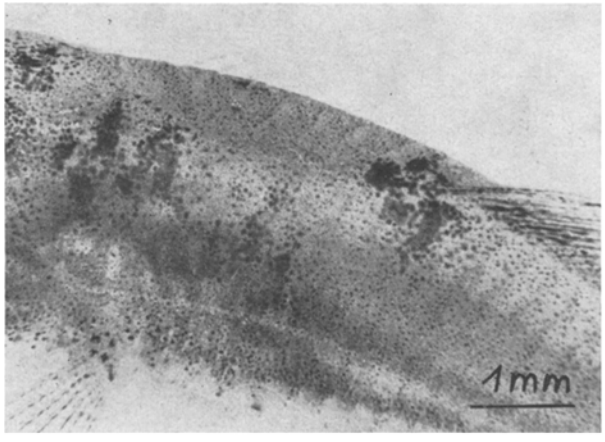

Fig. 2. A 3-month-old *Tu*-free recipient fish which developed several colonies of large abnormal melanophores after treatment with *Tu*-DNA during early embryogenesis.

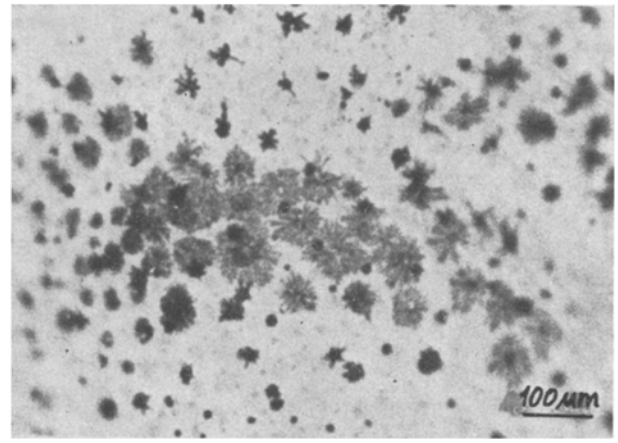

Fig. 3. Colony of large abnormal melanophores which developed in the skin of a *Tu*-free recipient treated with *Tu*-DNA. Note that these cells greatly resemble the T-melanophores shown in Figure 1b.

jected into the neural crest region of 3-day-old recipient embryos lacking *Tu* and controlling genes. As a control, donor DNA from fish of the recipient genotype was injected.

Until now, 46 out of 1150 individuals treated with *Tu*-DNA during embryogenesis showed abnormal melanophores which occurred as single cells during late embryogenesis, and as large cell colonies in 2- to 4-month-old fish (Fig. 2). These cells were of the same size and morphology (Figure 3) as the T-melanophores shown in Figure 1b. 1 to 2 weeks after their occurrence, they were attacked and removed by macrophages. In the 930 control individuals treated with *Tu*-free donor DNA, no abnormal melanophores were observed. This difference is highly significant ($\chi^2 = 38.41$ for 1 df; $p < 0.001$).

The characteristic morphology of the abnormal melanophores observed after treatment with *Tu*-DNA led us to conclude that these cells are identical with T-melanophores. This view is supported by the fact that the cells are attacked by macrophages like the T-melanophores of a *Tu* genotype[11]. The results suggest that a *Tu*-carrying piece of donor DNA had been taken up and integrated into a recipient propigment cell and that the *Tu* information had been inherited and expressed by the daughter cells. Concerning the high ratio of 46 transformants out of 1150 embryos treated with *Tu*-DNA, it should be considered that, in each embryo, about 1000 cells of the neural crest and an unknown number of their descendants have been treated with the donor DNA. The frequency of a transformation event can be calculated, therefore, to be about 1 in 25,000 cells, which is comparable to that published for *Ephestia*[12].

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Effects of Prolactin and Growth Hormone on DNA Synthesis of Rat Mammary Carcinomas in vitro[1]

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Summary. Explants derived from mammary carcinomas of DMBA-treated female Sprague-Dawley rats were cultured for 5 days in Medium 199 containing insulin and corticosterone. The addition of ovine prolactin to the culture media resulted in a consistent significant increase in H^3-thymidine incorporation into DNA. DNA synthesis of explants treated with either ovine or human growth hormone was intermediary to prolactin-treated cultures and control cultures. A combination of prolactin and human growth hormone often increased DNA synthesis above either hormone alone, suggesting a possible growth synergism between these peptides.

In recent years, prolactin (PL) has been identified as a critical stimulatory hormonal factor responsible for the development and/or growth of rat mammary tumors in vivo[4–6]. The use of organ culture to test the direct action of hormones on mammary tumor growth has provided evidence relevant to the hormonal action in vivo. Thus, recent studies[7–9] have demonstrated an important role for PL in stimulating DNA synthesis in organ cultures of 7, 12-dimethylbenzanthracene (DMBA)-induced rat mammary carcinomas.

Growth hormone (GH) has been reported to have no significant effect on growth of DMBA-induced mammary carcinomas in adrenalectomized-ovariectomized rats[5,6]. On the other hand, Li and Yang[10] recently reported that GH was nearly as effective as PL in promoting tumor growth in hypophysectomized rats bearing DMBA-induced mammary tumors. Thus, the purpose of these studies was to further investigate the direct and comparative effects of these two peptides on growth of DMBA-induced rat mammary carcinoma in vitro.

Materials and methods. Mammary carcinomas were induced in female Sprague-Dawley rats by the administration of DMBA, as described previously[4]. Tumors of approximately 1.5 cm in diameter were cut into 1 mm^3 explants. Explants were placed at random in small (10 × 30 mm) Falcon disposable petri dishes containing 2 ml of Medium 199, Earle base. Medium 199 was supplemented with penicillin G (50 IU/ml), insulin (5 μg/ml) (bovine pancreas, 22.5 IU/mg), and corticosterone (1 μg/ml). Ovine GH (5 μg/ml) (NIH-S9), human GH (5 μg/ml) (Upjohn, lot number 8717), and ovine PL (5 μg/ml) (NIH-S8 or S9) were added to the experimental groups. The petri dishes were placed in a small gassing chamber housed in an incubator at a temperature of 37 °C. The chamber was continuously infused with gas (95% O_2: 5% CO_2) during the incubation period. Explants were cultured for 5 days. Media were changed on days 2 and 4. On day 5, 4 h prior to termination of culture, sterile H^3-thymidine (NEN, 6.7 Ci/m*M*) was added to the explants

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