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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in a final concentration of 0.5 μCi/ml. DNA synthesis was estimated by measuring H^3-thymidine incorporation into DNA as described previously[9,11]. The data were analyzed statistically by Randomized Block Test. Parameters were further tested by Dunnet's Test (one sided) and Orthogonal Contrast Test.

Autoradiographic analyses were performed on a portion of the cultured explants. Autoradiographs were prepared by the method of MESSIER and LEBLOND[12] as modified by WALKER[13]. A dip-coating technique with Kodak NTB-2 liquid emulsion (Eastman Kodak, Rochester, NY.) was used. The labelling index was based on counting 1000 tumor cells, within 8 or 9 fields of each microscopic slide (15–20 slides per group). Cells showing a minimum of 3 silver grains overlying the nucleus were scored in each group. Autoradiographic studies were statistically analyzed by 2-way Analysis of Variance with unequal numbers and Sheffe Test.

The study was divided into 2 separate experiments. In experiment 1, 360 explants derived from 3 rat mammary tumors (120 explants per tumor) were randomly distributed into 3 groups. Groups II and III received ovine PL and ovine GH, respectively. Group I served as a control. This experimental procedure was performed 4 times, using a total of 12 rat mammary tumors. In experiment 2, 480 explants derived from 3 rat mammary tumors (160 explants per tumor) were randomly distributed into 4 groups. Group I served as a control; Groups II and III received ovine PL and human GH, respectively; Group IV received a combination of these hormones. This experimental procedure was performed 3 times, using a total of 9 rat mammary tumors.

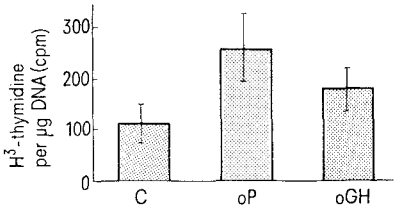

Fig. 1. Effect of ovine growth hormone (oGH) and ovine prolactin (oP) on H^3-thymidine incorporation into DNA of 5-day organ cultures of DMBA-induced rat mammary carcinomas. Each value represents the mean of 4 experiments. Corticosterone and insulin were added to the media of each group. $P < 0.05$: C vs oP; p = N.S.: C vs oGH, oP vs oGH.

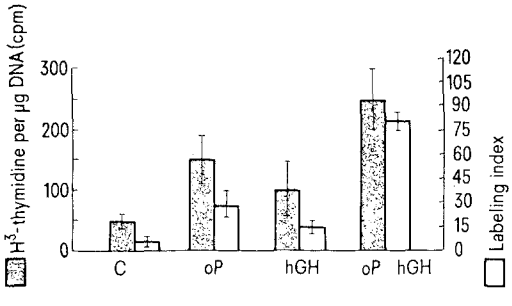

Fig. 2. Effect of ovine prolactin (oP) and human growth hormone (hGH), alone and in combination, on H^3-thymidine incorporation into chemically extractible DNA and labelling (H^3-thymidine) index in 5-day organ cultures of DMBA-induced rat mammary carcinomas. Each value represents the mean of 3 experiments. Corticosterone and insulin were added to the culture media of each group. Labelling index was determined by counting the number of cells labelled per 1000 cells. H^3-thymidine/μg DNA (cpm): $p < 0.01$: C vs oP + hGH; $p < 0.05$: C vs oP, hGH vs oP + hGH; p = N.S.: C vs hGH, oP vs hGH, oP vs oP + hGH.
Labelling index. $p < 0.001$: C vs oP, C vs oP + hGH, oP vs oP + hGH, hGH vs oP + hGH; p = N.S.: C vs hGH, oP vs hGH.

Results. When PL was added to synthetic media containing explants of mammary carcinoma, a significant ($p < 0.05$) increase (>125%) in H^3-thymidine incorporation into DNA was observed when compared to the control group (Figure 1). There was, however, no significant difference between the ovine GH treated group and the control group nor between the PL treated group and the ovine GH treated group in the mean incorporation of the isotope into DNA. Ovine GH appeared to slightly increase DNA synthesis when compared to controls, as 3 of the 4 cultures treated with the hormone showed a slight increase in uptake of H^3-thymidine into DNA. However, the 4 separate studies, when combined, did not show statistically significant differences.

Figure 2. shows the effects of PL and human GH, alone and in combination, on DNA synthesis of 5 day organ cultures of DMBA-induced rat mammary carcinoma. Results indicate that the PL treated group showed significantly ($p < 0.05$) increased (>200%) H^3-thymidine incorporation into DNA in comparison to the control group. However, the human GH treated group did not differ significantly when compared with the controls or the PL treated group. The combination of human GH and PL significantly ($P < 0.01$) increased H^3-thymidine incorporation into DNA when compared to the control group, or to the human GH treated group ($P < 0.05$). There was no significant difference between the combined hormone treated group and the PL treated group.

The results of the autoradiographic studies on parallel cultures are illustrated in Figure 2. In general, statistical analysis of the relationships between H^3-thymidine incorporation into chemically extracted DNA (cpm) and the labelling index of cultures indicates parallelism ($P < 0.05$). Autoradiographic data indicate that the responses to PL and human GH, alone or in combination, reflect changes principally in the number of cells engaged in synthesis of DNA. That primarily epithelial (carcinoma) cells and not the connective tissue elements incorporated H^3-thymidine into DNA was also indicated in these studies. The presence of PL in the media resulted in a greater proportion of cells showing silver grains when compared to the control group ($P < 0.001$). Human GH treated cultures were not shown to be significantly different from the controls. The addition of PL and human GH combined resulted in a greater proportion of cells showing silver grains when compared to controls, human GH, or PL treated cultures ($P < 0.001$). However, the difference between PL and human GH treated cultures was not found to be significant based on the proportion of cells in each group bearing silver grains.

Discussion. PL has been considered an important hormone in stimulating the growth of rat mammary carcinomas in vivo[4–6]. The results of this study provide further evidence that PL is a potent stimulatory factor in vitro as well. Although we failed to show a significant difference in DNA synthesis between cultures containing GH and control cultures (combined analysis), the GH (ovine or human) containing cultures have a level of DNA synthesis which was statistically indistinguishable from PL containing cultures. Since PL treated cultures

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consistently showed significantly greater DNA synthesis than controls, it can be concluded that, under the given experimental conditions, GH (ovine or human) does possess slight growth stimulatory effects. This is in accord with the study of LI and YANG[10] who reported that mammary tumor growth of bovine GH treated animals was essentially intermediate to that of controls and PL treated animals. Furthermore, the results of the autoradiographic analyses suggest an additive effect of these hormones as the hormonal combination was significantly greater than either hormone alone. This apparent effect of PL and human GH on DNA synthesis of mammary carcinoma cells is in accord with a previous in vivo study[14] and an in vitro study[15] which have suggested a possible synergism between PL and GH in the regulation of growth of normal[14] and hyperplastic[15] mammary tissue. Thus, the results of these studies further emphasize the key role of prolactin in growth stimulation of carcinogen-induced rat mammary carcinomas, but in addition, provide evidence that growth hormone may also be an influential hormonal factor in this process.

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Muscular and Nervous Systems of the Cubopolyp (Cnidaria)

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Summary. New observations on the morphology, anatomy, asexual reproduction and metamorphosis of the formerly unknown polyp of the tropical Cubomedusae resulted in the conclusion that a new class Cubozoa must be established and positioned between the Scyphozoa and Hydrozoa. This conclusion could be confirmed by the histological investigation of the cubopolyp's muscular and nervous systems by light and transmission electron microscopy.

As the lowest group of true Metazoa, the Cnidaria have always attracted the extensive interest of biologists. In the classes of Scyphozoa and Hydrozoa, there are 2 generations, the sessile asexual polyp and the free-swimming sexual medusa, whereas the class Anthozoa is represented by the polyp generation only. Anatomically, the basic plan of body construction is the tetra-radial symmetry. This is particularly evident in the class Scyphozoa because not only the medusa but also the polyp has a marked tetraradial body plan exhibited by the 4 gastric septa and 4 gastric pockets. Because of their tetramerously constructed body, the Cubomedusae, which are inhabitants of the neritic zones of tropical oceans, have also been grouped by most zoologists with the Scyphozoa, though some authors have pointed to aberrant characteristics by which they differ form 'true' Scyphomedusae (orders Coronatae, Semaeostomeae, Rhizostomeae). The Cubomedusae are famous as 'sea wasps' because of their severe stinging. Some species belong to the most dangerous sea creatures, as they can kill young and sensitive people by the strong venom of their nematocysts.

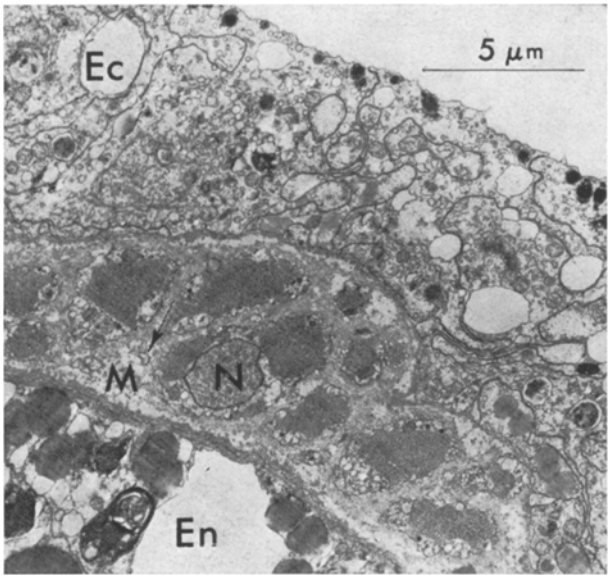

Fig. 1. *Carybdea* spec., transverse section near the base of the polyp. Note how the middle layer is occupied by myocytes. Ec, ectoderm; En, endoderm; M, middle layer; N, nucleus of myocyte; arrow points to a neurite. Fixed in Dorey's chrome-osmic fixative, embedded in Araldite and stained in alcoholic uranium.

New investigations[3–5] have revealed that the systematic position of the Cubomedusae needs to be revised. For the first time, it has been possible to rear the formerly unknown polyp generation to full size, to observe its asexual reproduction by laterally budding off small secondary polyps, and to follow the formation of the medusa, which is unique by the complete metamorphosis of the solitary polyp into one medusa. Through long-term culture experiments, it has been possible to elucidate the complete life cycle of the Caribbean species

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