findings, although the ratio of satellite cells in control EDL muscle in this study is higher than previously reported. The difference in sample size may be a factor for such controversy. The ratios of satellite cells in the EDL-first-graft and EDL-second-graft are similar to each other and to SOL muscles suggesting that the grafts retain the satellite cell frequency of the original muscle, rather than being transformed to a frequency found in EDL muscles. However, this similarity should not be viewed as absolute and be interpreted that the satellite cell content in these two types of grafts and SOL muscles were comparable for several reasons. First, the sample size may not be large enough. Snow[11] has quantified 4711 nuclei (total nuclei) in the SOL muscle of adult mouse and has suggested large sample size is needed to assure statistical significance of the ratios. What optimal sample size would be considered large has not been determined. In the present study, a total of 1617 nuclei for the second-grafts and 1669 nuclei for the first-grafts were counted. These numbers are obviously small compared to 4711. Secondly, the degree of innervation in grafts may be related to the number of satellite cells. Denervation has been shown to increase the number of satellite cells in the muscle[12]. In the graft, the innervation is never as complete as that of normal muscle[1,7]. Therefore, some regenerated muscle fibers are in the denervated state which may contribute to the variation of satellite cell numbers. Thirdly, there are satellite-like cells which were included in the quantitative analysis by previous workers[13,14] but not included in the present study due to its ambiguity. Finally, the study of ratios of satellite cell nuclei per total nuclei has to consider the factors such as nuclear dimensions (width, length, volume), cross-sectional areas of muscle fibers, nuclear density etc. as indicated by recent communications[15,16] using stereological analysis so that morphometric comparison is accurate and meaningful. Though the above-mentioned uncertainties make the frequency of satellite cells in the regenerated muscle fibers less precise, the present investigation showed that satellite cells were present in grafted and regrafted muscles indeed. The persistance of these satellite cells means that they are not depleted in the first-grafts, and that second- and third-grafts are possible.

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Morphological effects of serotonin and ketanserin on embryonic chick skin in vitro

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Summary. Cell and organotypical cultures are used to study the direct effect of serotonin and of ketanserin, a serotonin antagonist, on dermal and epidermal cells of embryonic chick skin. Ketanserin stimulates the increase in cell number and inhibits the differentiation, whereas serotonin stimulates differentiation and inhibits the increase in cell number.
Key words. Serotonin; ketanserin; fibroblast culture; organotypical culture.

Ketanserin, a newer 5-hydroxytryptamine$_2$ (and also α_1) antagonist in vascular muscle is used as an antihypertensive drug[1-4]. Recent clinical observations[5] suggest that ketanserin promotes the healing of chronic skin ulcers. This healing effect could be due to the indirect effect of vasodilatation. To study a possible direct effect of serotonin (5-HT) and ketanserin on dermal and epidermal cells, we have used cell and organotypical cultures of embryonic chick skin[6].

Methods. Organotypic culture. The skin of the dorsal region of 6.5-day-old embryonic chicks was dissected and fragments of 0.3 cm diameter were punched out. These fragments were transferred to minimal Eagle's medium with Hanks' salts, supplemented with 10% fetal calf serum and antibiotics. This medium served as a control medium. Test media were prepared by adding serotonin or ketanserin, in concentrations similar to those used in experiments for testing the antihypertensive effect of ketanserin[7]: 10 µg/ml serotonin and 5 µg/ml ketanserin. The skin fragments were explanted on the medium described above, made semisolid by adding 0.5 mg/ml agar, or on the bottom of a Falcon plastic dish submerged in the fluid medium. Twelve series of organotypical cultures were incubated at 37 °C. On days 1, 2, 5 and 6, eight series of cultures were photographed in order to measure the area covered by the skin fragment and its outgrowths. After an incubation of 2, 4, 7 or 12 days, the skin fragments were fixed in a Bouin-Hollande solution. Some of the organotypical cultures were stained in toto with Giemsa. Seven series of cultures were prepared for 6-µm paraffin cross-sections and stained with Hematoxylin-Eosin or with a specific keratin stain following Ayoub-Shklar[8].

Cell culture. In addition to the organotypic cultures, ten series of cell cultures of dermal fibroblasts were also prepared. The dermal stroma of the skin fragments, mechanically separated from the epidermal layer, was placed in a trypsin

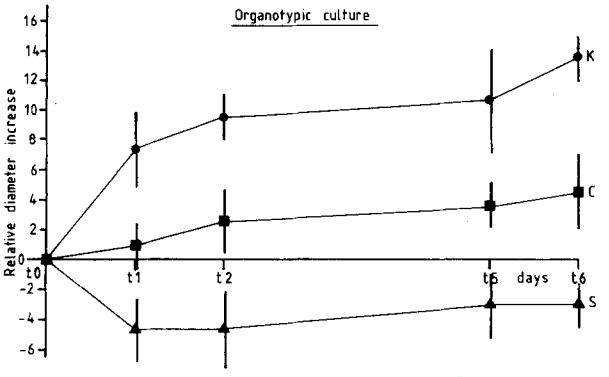

Figure 1. The relative increase in diameter $\left(\frac{\Delta\phi}{\text{original }\phi}\right)$ of organotypical cultures, incubated in control-, ketanserin-, or serotonin medium, measured on days 1, 2, 5, 6, shown as a function of time. (Mean value of 8 series.)

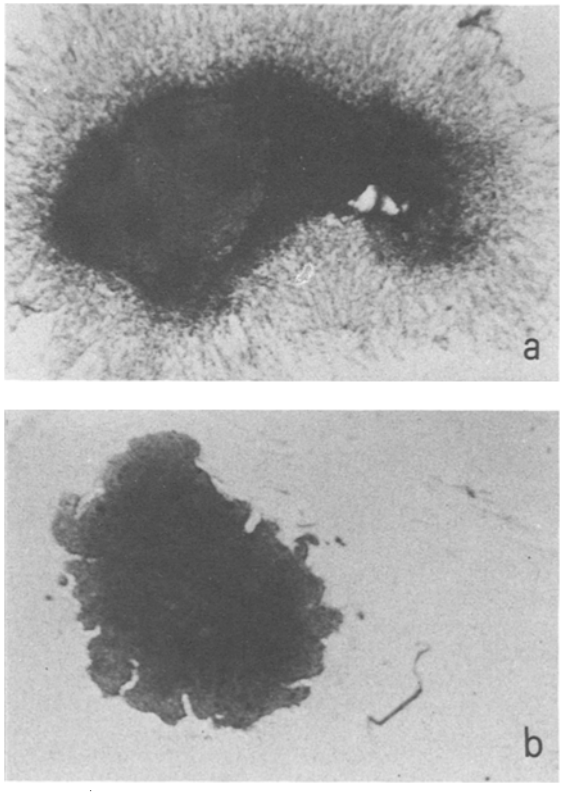

Figure 2. Overall picture of an in toto staining with Giemsa of organotypical cultures incubated for 12 days in medium containing ketanserin (2a) or serotonin (2b).

solution and stirred for 20 min at 37 °C to obtain a single cell suspension. This cell suspension was seeded in a culture flask at a concentration of 2.10^6 cells/ml and cultured in the medium described above, at 37 °C. Cell numbers/unit area were counted every 24 h in five series of cell cultures.

At confluency, the fibroblast cultures were fixed in a Bouin-Hollande solution and stained with Giemsa for morphological analysis.

Results. Organotypic culture. The diameter of the area covered by the skin fragments, whether cultured in fluid medium or in semisolid medium, changed during incubation. Figure 1 shows shows that the diameter increase in the ketanserin-treated cultures was more pronounced than in the control cultures. Serotonin, on the other hand, caused an initial decrease in diameter, followed by a steady state from the second day onwards.

The examination of the organotypic cultures stained in toto confirms the observed changes in diameter. The ketanserin-treated cultures as well as the control cultures show an obvious outgrowth at the edge of the fragments, formed by a meshwork of stellate and spindle shaped cells (fig. 2a). This phenomenon of growing out starts from the first day onwards with a stretching of the peripheral cells. In contrast, serotonin-treated fragments show a clearcut margin without any outgrowth. The initial decrease in diameter observed is probably due to the bending down of the epithelium at the edge of the fragment (fig. 2b).

Histological sections of the chick skin before incubation demonstrate a double layered epithelium, overlying the dermal stroma[9]. This dermal layer is formed by a mesh-like arrangement of mesenchymal cells. Dermal papillae have not yet been formed (fig. 3a).

During incubation a progressive change is observed in each of the 7 series of control, serotonin and ketanserin treated cultures.

Sections of control cultures on day 7 show an epidermis, about 3-cell-layer-thick and covered by a small margin of keratin and by remnants of shed necrotic epithelial cells. In the stroma zone, fibroblasts are homogeneously distributed. A small number of dermal papillae are formed (fig. 3b).

Ketanserin cultures on day 7 consist of a multilayered epidermis. The cells of the top layer are still nucleated and even the outermost zone scarcely stains for keratin. The stromal zone is rich in cells, especially immediately under the basement membrane, where cells pile up. The basement membrane is not well defined. Numerous epidermal pegs are present (fig. 3c). Sections of serotonin-treated cultures show a thin epidermis, consisting of 2–3 cell layers and covered by a thick border which stains darkly for keratin. At the top of this keratin layer we notice a large amount of necrotic and swollen shed epithelial cells. The stromal region is poor in cells; these cells are separated by broad intercellular spaces. Papillae are rare or even absent (fig. 3d).

Cell culture. In the cell cultures, cell numbers/unit area increase daily. This increase is higher in the presence of ketanserin than in control cultures or serotonin-treated cultures (fig. 4).

Differences in morphology between the fibroblasts cultured in the presence of serotonin or ketanserin can be observed in all of the Giemsa-stained cultures. In the presence of ketanserin, fibroblasts are stellate or spindle shaped. Neighboring cells form an intermingling meshwork by their long and thin extensions (fig. 5a). Serotonin-treated fibroblasts are flattened and widely spread. They have broad but short extensions (fig. 5b). Control cells are spindle shaped with mostly only two but rather long extensions.

Discussion. Using cell cultures and organotypical cultures, we observed a direct effect of serotonin and ketanserin on the cell number and on the differentiation of embryonic chick skin. Ketanserin causes a greater increase in diameter of the organ fragments; the epidermis becomes multilayered; the dermis is rich in cells and the cell cultures show a larger number of cells/unit area in comparison to control cultures. Serotonin-treated cultures, on the other hand, retain their clearcut margin; the epidermis is very thin and the dermis is poor in cells. The number of cells/unit area does not increase at the same rate as the control cultures. Preliminary results of ^{3}H-thymidine incorporation confirm these data. Hence,

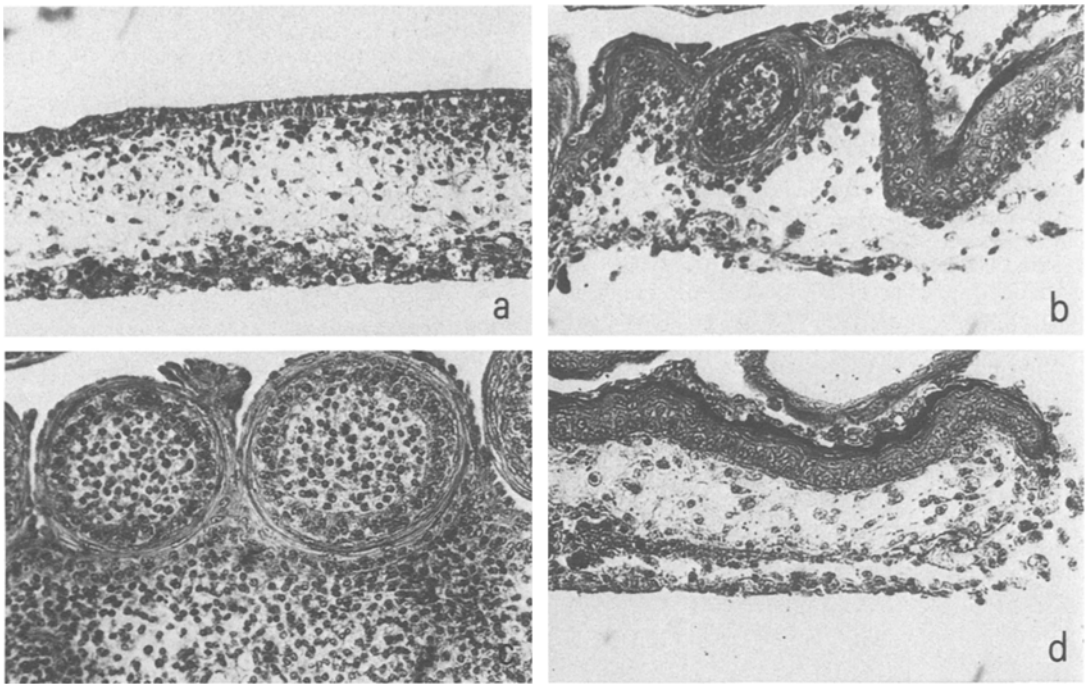

Figure 3. Light micrographic pictures of paraffin sections of skin cultures, *a* before incubation, *b* after 7 days of incubation in control medium, or *c* in a medium containing ketanserin or *d* serotonin (keratin stain).

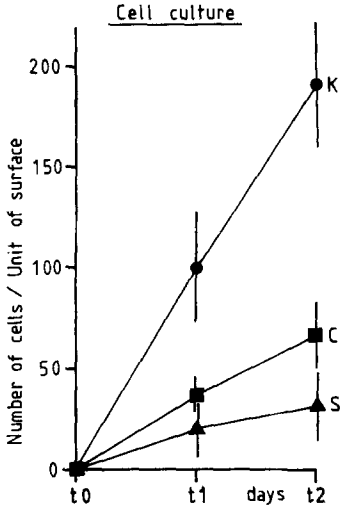

Figure 4. Number of cells/unit area in cell cultures incubated in control-, ketanserin-, or serotonin medium, counted after 24 and 48 h, shown as a function of time. (Mean value of 5 series.)

ketanserin seems to enhance the number of cells, whereas cultures treated with serotonin do not show the same increase in cell number as the control culture. This last conclusion contradicts the results of other authors who postulate that serotonin has a stimulatory effect on the proliferation of fibroblasts [10]. However, these authors have used concentrations and incubation times completely different from ours. As the concentration of serotonin used has proved to be very important in the domain of nervous tissue [11], the contradiction could be explained by a dose- and/or time dependent change of the effect of serotonin on fibroblasts. This supposition should be confirmed by further experiments. Histolog-

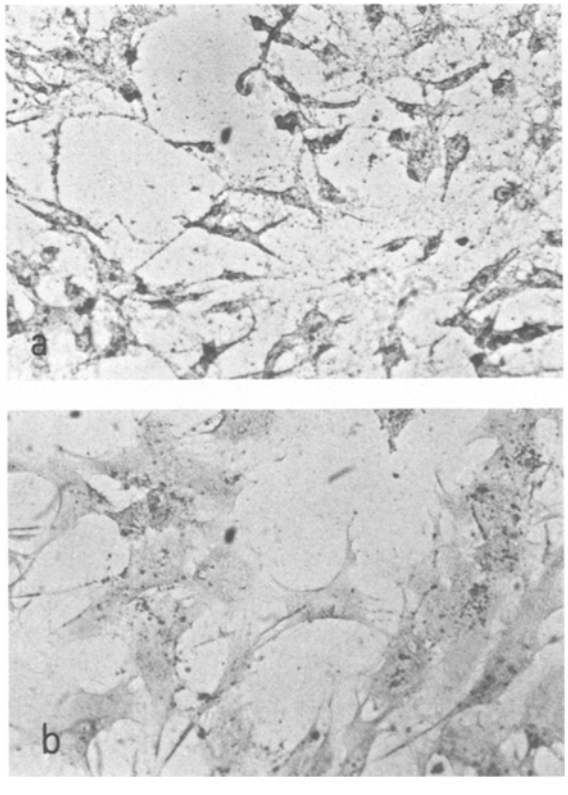

Figure 5. Light microscopic pictures of a Giemsa staining of cell cultures, treated *a* with ketanserin, or *b* with serotonin at the moment of confluence of the fibroblast culture.

ical analysis of paraffin sections of serotonin and ketanserin-treated cultures reveals another difference. The epidermis of the serotonin-treated cultures is covered by a thick border of keratin and shed epithelial cells, whereas the ketanserin-treated cultures hardly show any keratinization. As keratinization and shedding of epithelial cells can be seen as aspects of differentiation, our results indicate that serotonin enhances differentiation, whereas ketanserin inhibits differentiation.

We conclude that ketanserin is not only a 5-HT antagonist when acting on vascular tissue, but that it probably also has an immediate opposite effect on dermal and epidermal cells of the skin. It might be of some interest to test other 5-HT antagonists in our model. Experiments to demonstrate 5-HT receptors in fibroblasts are in progress. Up to now, the mechanism of action of these drugs on fibroblasts and epidermal cells is not clear. To find out more about the exact mechanism, more experiments will be carried out on organotypic cultures as well as on cell cultures of fibroblasts and of epithelial cells.

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Antibody to tumor necrosis factor (TNF) reduces endotoxin fever [1]

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Summary. Antibody to tumor necrosis factor (TNF), injected intravenously, reduced endotoxin fever in the rabbit. The fever-reducing effect was apparent in the latter half of the febrile response.
Key words. Fever; lipopolysaccharide (LPS); tumor necrosis factor (TNF); monoclonal antibody to TNF.

It has long been postulated that pathogenesis of fever is essentially mediated by a heat-labile protein called endogenous pyrogen (EP) or leucocytic pyrogen (LP) which is synthesized and released by phagocytes in the face of infection by endotoxin [2]. The primary role of EP in fever genesis is to activate the synthesis of prostaglandins which are probably the final mediators acting on the regulatory center for body temperature to initiate fever [3,4]. A product of monocytes, interleukin-1 (IL-1) has recently been found to be identical with EP in its pyrogenicity and metabolic effects [5]. However, febrile responses to infection cannot be simply attributed to a PGE_2 induced by IL-1. Another leucocyte product, for example, interferon-α (IFN-α) can produce fever by its own pyrogenicity [6]. Also, tumor necrosis factor (TNF) has been reported to cause fever independently of its ability to produce IL-1 [7]. In the present experiments, we examined whether the production of TNF is substantially involved in fever caused by bacterial endotoxin, lipopolysaccharide (LPS), using an antibody to TNF.

Experiments were performed between December 1986 and June 1987. Male Japanese white rabbits, weighing 2.0–2.4 kg, were used. During 5 days before the experiments were done, animals had been adapted to settle down in the experimental chambers, in which the neck of the animal was loosely fixed, for at least 4 h a day. By this adaptation procedure, any effects of restraint-stress on body temperature were eliminated. Experiments were performed at an ambient temperature of 24.0 ± 1.0 °C. Rectal temperature, as being representative of body core temperature, was continuously measured with a thermistor probe inserted 10 cm into the rectum. Ear skin temperature was also measured with a thermistor probe. LPS derived from *E. coli* 0111:B4 (Difco Lab., USA) was resolved with sterile saline and injected into the ear vein. The dose of LPS was 100 ng/kg, and injection volume was 0.2 ml/kg.

Rabbit TNF was prepared as described previosuly [8,9]. Antibodies against rabbit TNF were prepared in the following way [10].

BALB/c mice were primed s.c. with 10–60 μg protein of 3000-fold purified TNF, which was emulsified in complete Freund's adjuvant. Mice received doses of 15–30 μg protein i.v. six times over a time period of five months. Three days after the last booster injection, spleen cells from the mice were fused with P3U1 (myeloma cell line) in the presence of polyethyleneglycol. Hybridoma cells which secreted antibodies to TNF were cloned by the limiting dilution method. Ascitic fluids were obtained after i.p. injection of hybridoma cells into BALB/c mice primed with pristane. Monoclonal antibody was purified by ammonium sulphate precipitation followed by ion exchange chromatography on DE52 (Whatman, England). Anti-TNF antibody activity was evaluated by neutralization of the cytotoxic activity of TNF on L929