Acta Dermatovener (Stockholm) 62: 107–112, 1982

EFFECTS OF SEX STEROIDS ON HUMAN SKIN IN ORGAN CULTURE

Raija Tammi

Department of Anatomy, University of Turku, Turku, Finland

Abstract. Histometric and autoradiographic methods were applied to investigate the actions of sex hormones on the cultured epidermis of post-menopausal women. Both testosterone and progesterone at the concentration of $10^{-9}-10^{-7}$ mol/l enhanced the keratinization of the epidermal cells during the 7-day culture. The numbers of the granular cells were increased by both of these hormones and the transit time of the epidermal cells was shortened by progesterone. Progesterone (but not testosterone) influenced cell proliferation, too, as indicated by an increased labelling of the epidermal cells with [^{3}H]thymidine. In a contrast to other sex steroids, 17-β-estradiol (10^{-7} and 10^{-9} mol/l) appeared to be ineffective in short-term organ culture.

Key words: Human epidermis; Organ culture; Estrogen; Testosterone; Progesterone; Histometric analysis

The age and sex related differences in the appearance and function of the human skin suggest that it is influenced by sex steroids (7). It is generally accepted that the skin appendages (hair follicles, sebaceous glands, apocrine sweat glands) are a target of these hormones (7), and it seems that the epidermis is a sex steroid responsive tissue, too, although the area has not yet been settled.

Thus, in both sexes the epidermis atrophies with age (24) concomitant with the declining production of steroid hormones. This aging atrophy of the epidermis may be counteracted by estrogen (8, 11) and testosterone (10, 20, 32) treatments. A slightly stimulatory effect on the senile human skin has been proposed also for progesterone (20). Furthermore, in women, castration is accompanied by an epidermal atrophy (21) which can be reversed by estrogen replacement therapy (21).

However, the experimental work carried out on this subject has given contradictory results. For example, long-term estrogen administration to intact rats (13) and mice (2) was followed by epidermal atrophy. As an explanation, it has been postulated that the high doses of estrogen are antimitotic on the epidermal cells, while the smaller, more physiological doses would be mitogenic (5).

Whether the cited effects of the sex steroids on the epidermis are direct or are mediated via other organs is not known, since the majority of the studies have been carried out in vivo. The actions of testosterone on the epidermis and sebaceous glands may depend on the hypophysis (6). Furthermore, an indirect route of action is suggested by a finding that the epidermal atrophy by the high-dose estrogen administration might, at least partly, be due to the activation of the adrenal glands (3). However, high concentrations of estrogen (10^{-4} mol/l) inhibit the proliferation of the epidermal cells also under organ culture conditions (18).

The aim of the present study was to investigate the effects of sex steroids (17-β-estradiol, progesterone, and testosterone) at small concentrations on the proliferation and differentiation of adult human epidermis. The recent development of the skin organ culture methods (27, 28) afforded a possibility to investigate the direct skin effects of these hormones in the absence of the complex hormonal feedback mechanisms and the systemic effects of the steroids, which have complicated the interpretation of the results of earlier reports.

MATERIAL AND METHODS

Tissue culture

The skin samples were obtained from 12 female patients undergoing mastectomy operations. The ages of the donors ranged from 50 to 78 years. The skin cultures were prepared as described earlier (27). A Trowell-type organ culture was used with Eagle's MEM in Earle's balanced salt solution (Flow Laboratories Ltd, Irvine, Scotland), buffered by 20 mmol/l Hepes and 25 mmol/l bicarbonate and containing 600 mg/l L-glutamine. No serum was added. The cultures were incubated in air-tight plastic chambers under an atmosphere containing 40% oxygen, 55% nitrogen and 5% carbon dioxide at 37°C.

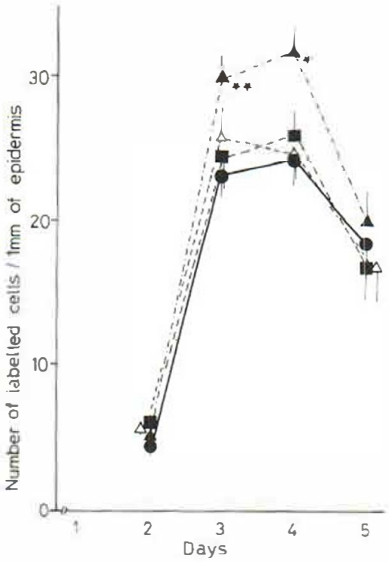

Fig. 1. Effects of sex steroids (10^{-7} mol/l) on the numbers of the [^{3}H]thymidine-labelled nuclei in the epidermis. The means and standard errors of the means (SEM) were calculated by pooling the data of five experiments. The significant differences between the hormone-treated and control cultures on the indicated day after beginning of the cultures are shown as: *=$p<0.05$, **=$p<0.01$, ***=$p<0.001$. ●—●, Control; ■---■, 17-β-estradiol; △---△, testosterone; ▲–·–▲, progesterone.

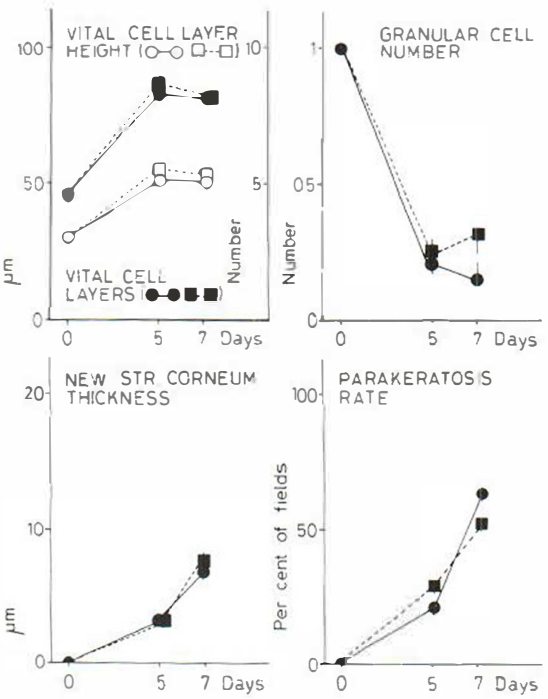

Fig. 2. Effects of 17-β-estradiol (10^{-7} mol/l) on the histometric parameters of the cultured epidermis. Means and SEM were calculated by pooling the data from five experiments. ●—●, Control; ■---■, 17-β-estradiol.

17-β-estradiol (1, 3, 5, (10) estratrien-3, 17-β-diol diacetate; Organon, Holland), testosterone (17-β-hydrosy-4-androsten-3one; Merck, West Germany) and progesterone (4-pregnen-3,20-dione; Orion, Finland) were dissolved in propylene glycol and added to the culture medium 24 hours after the start and at each renewal of the culture medium (every second day). The final concentrations of the hormones were 1×10^{-7} and 1×10^{-8} mol/l and that of propylene glycol, 0.04 % (v/v). The control cultures contained an equal concentration of propylene glycol.

Histological processing and histometry

The skin samples were fixed in formalin, dehydrated in alcohol and embedded in paraffin wax. Serial sections (7 μm thick) were cut perpendicular to the epidermal surface and stained with hematoxylin and eosin. The parameters in Figs. 2–4 were recorded according to the histometric method described in detail elsewhere (28). In each experiment 6–8 explants were analysed from the control and hormone-treated cultures at the beginning and on the 5th and 7th culture days. Data from five experiments, each including samples from one donor, were pooled for the calculations of the means and standard errors of the means.

Autoradiography

Five experiments each including control, 17-β-estradiol, progesterone and testosterone treated groups (all at 10^{-7} mol/l final concentration) were started as described above. Six explants from each group on the 2nd, 3rd, 4th and 5th culture days were transferred for 2 hours into a medium which contained 5 μCi/ml of [^{3}H]thymidine (6-[^{3}H]thymidine, spec. act. 28 Ci/mmol; The Radiochemical Centre, Amersham, U.K.). The explants were then washed with non-radioactive medium and processed for histology as described above. Serial sections (5 μm thick) were cut perpendicular to the epidermal surface and dipped with Kodak nuclear emulsion NTB 3 (Eastman Kodak Co., Rochester, N.Y.). The autoradiograms were exposed for 14 days at −15°C, developed in Kodak D-19 developer for 4 min and stained with hematoxylin and eosin. In each explant, the number of the labelled nuclei was counted per 1 mm of epidermis from six sections at least 30 μm apart from each other. The combined data of five separate experiments were used for the calculations of means and standard errors of the means.

Measurement of the transit time

The explants were incubated on the first culture day in a medium containing 5 μCi/ml of [^{3}H]thymidine for 4 hours, washed with non-radioactive medium and transferred to the original dishes. On the 6th day after the [^{3}H]thymidine pulse 5–6 explants were harvested from the cultures, fixed and processed for autoradiography as described above. The position of the labelled cells was

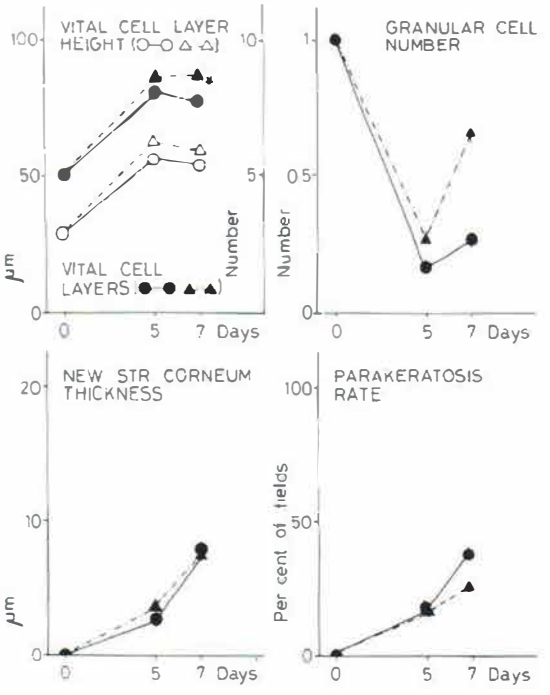

Fig. 3. Effects of progesterone (10^{-7} mol/l) on the histometric parameters of the cultured epidermis. Means and SEM were calculated by pooling the data from five experiments. The significant differences are indicated as in Fig. 1. ●—●, Control; ▲-·-▲, progesterone.

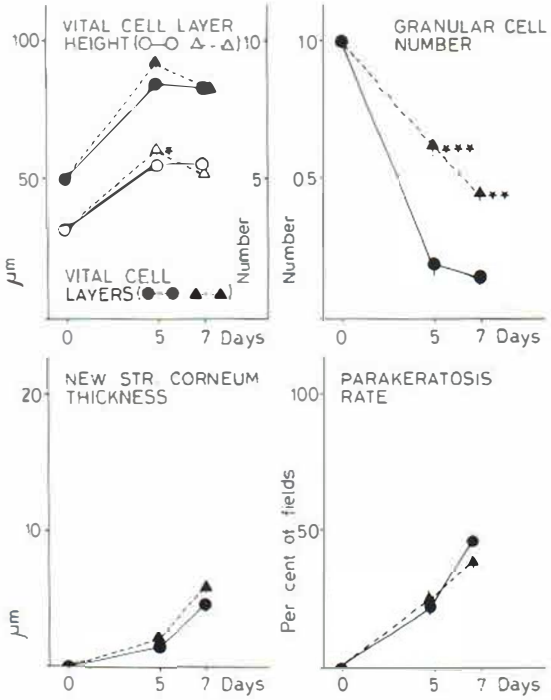

Fig. 4. Effects of testosterone (10^{-7} mol/l) on the histometric parameters of the cultured epidermis. Means and SEM were calculated by pooling the data from five experiments. The significant differences are indicated as in Fig. 1. ●—●, Control; ▲---▲, testosterone.

analysed by counting the numbers of the vital cell layers below and above the labelled cells. In each explant the positions of at least 15 labelled cells were measured. Data from three experiments were pooled and used for the calculations of the means and standard errors of the means.

Statistical methods

The histometric and autoradiographic data were subjected to a three-way analysis of variance of the random effect of the patients, and the fixed effects of the culture periods and hormone treatments (HYLPS program, Univac). The data from the transit time experiments were analysed using a two-way analysis of variance of the effects of the patients and hormone treatments. A simultaneous confidence limit test procedure was applied to the different periods of the treatments to find out which of the means were significantly different (Neuman-Keuls test, 31).

RESULTS

Because the light microscopic structure of the human epidermis cultured in the present conditions was described recently (27), only the most salient features are described here. No signs of degeneration were evident in the epidermis before the end of the first week of the culture. Few cells were labelled with [^{3}H]thymidine on the 1st and 2nd days in culture, but the numbers of the labelled cells increased thereafter and attained a maximum on the 4th day (26) (Fig. 1). At the same time, the epidermis became thicker and accumulated more vital cell layers (Figs. 2–4). The cornification continued in the culture, but the numbers of the granular cells were greatly reduced and the newly formed stratum corneum was partly parakeratotic (Figs. 2–4). The propylene glycol concentration (0.04%) introduced into the medium had no significant effect on the proliferation rate or the structure of the epidermis.

The addition of the sex steroids to the medium did not change the vitality or the overall structure of the epidermis. All the steroids studied tended to stimulate the migration of the epidermal cells (10–40% increase in the length of the epibolus), but this effect varied from one experiment (skin donor) to another and did not reach statistical significance.

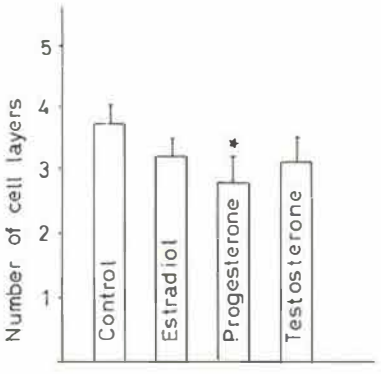

Fig. 5. Effects of sex steroids on the transit time of the epidermal cells in culture. Range bars indicate mean (+SEM) numbers of vital cell layers above the labelled cells 6 days after a [^{3}H]thymidine pulse on the first culture day. The data were pooled from three experiments. Significances as in Fig. 1.

17-β-estradiol (10^{-7} mol/l) caused no significant alteration in the proliferation rate of the epidermis as indicated by the [^{3}H]thymidine labelling of the cells (Fig. 1). Nor did the hormone influence any of the histometric parameters of the cultured skin (Fig. 2). Thus, neither the thickness of the vital and cornified parts of the epidermis, the number of the granular cells, nor the quality of the newly formed stratum corneum (parakeratosis rate) differed from the same parameters in the control cultures. The cornification rate (transit time) was not changed, either, as was demonstrated by the disappearance of the vital cells from a layer above the keratinocytes labelled at the beginning of the culture (Fig. 5). The diminution of the hormone concentration to 10^{-9} mol/l gave the same results.

In contrast to 17-β-estradiol, progesterone (10^{-7} mol/l) significantly stimulated the labelling of the epidermal cells with [^{3}H]thymidine (Fig. 1). The stimulation of the proliferation rate was paralleled by an increase in the number of vital cell layers (Fig. 3) and the overall thickness of the vital epidermis was slightly increased, too (Fig. 3). The cornification rate of the epidermal cells was accelerated, as there were on average fewer vital cell layers above the label in the progesterone-treated explants than in controls on the 7th culture day (Fig. 5). By this time also, the first labelled cells reached the stratum corneum in the hormone-treated cultures, but not in control cultures. The amount and quality of the stratum corneum was not affected, but on the 7th day a thick, multi-layered stratum granulosum was observed in some of the progesterone-treated cultures (Fig. 3). The histometric analysis which was done following the treatment with a lower concentration of progesterone (10^{-9} mol/l) revealed trends similar to the effects of the higher concentration, i.e. a slight thickening of the vital epidermis and an accentuation of the stratum granulosum at day 7.

Testosterone (10^{-7} mol/l) did not significantly influence the epidermal proliferation rate in the cultured female skin (Fig. 1). However, a transient increase in the thickness of vital epidermis and in the number of vital cell layers was observed on day 5 (Fig. 4). The cornification rate of epidermal cells was not affected by testosterone, as indicated by the average position of the labelled cells 6 days after a [^{3}H]thymidine pulse (Fig. 5). The transit time, when expressed as the period to the appearance of the first (labelled) basal cells in the stratum granulosum, was unchanged, too. Testosterone did not affect the amount or quality of the stratum corneum built in vitro but, instead, significantly increased the number of the granular cells, both on the 5th and on the 7th day (Fig. 4). The results appeared similar in two experiments where a concentration of 10^{-9} mol/l of testosterone was used in the cultures.

DISCUSSION

The present method of tissue culture in a defined medium, avoiding serum with its unknown biological effectors, yet still providing good skin vitality (27), appears especially suitable for the studies on hormones, as exemplified by the previous investigations concerning hydrocortisone (26, 29). The quantitative recordings of the structures were quite obligatory in order to detect the hormonal changes in the epidermal structure, which were often subtle.

In contrast to most of the earlier *in vivo* studies on human skin (8, 11, 20, 21) it was found that 17-β-estradiol exerted a negligible influence on the epidermal proliferation and thickness (Figs. 1, 2). Since the present results reflect the isolated skin effects of 10^{-7} and 10^{-9} mol/l concentrations of 17-β-estradiol, it is suggested that this hormone does not directly control the proliferation of the human epidermis *in vivo*. An indirect route of the effects, e.g. via an enhanced dermal circulation (18), is of course possible.

In an earlier *in vivo* study it was suggested that progesterone slightly increases the epidermal thick-

ness (20). The present results are in agreement with that finding (Fig. 3) and also indicate that the thickening of the epidermis is probably due to enhanced proliferation of epidermal cells (Fig. 1).

The treatment of the senile human skin *in vivo* with pharmacological doses of testosterone induced an increase in the epidermal thickness in both sexes (10, 20, 32). *In vitro*, this effect (induced by small hormone doses) was very small and transient (Fig. 4) and was not associated with any significant stimulation of the proliferation rate (Fig. 1). It seems improbable that, in females, the epidermal renewal would be markedly regulated by the physiologic testosterone, since the epidermis atrophies with age despite the unchanged secretion of this hormone (19).

Testosterone increased the number of the granular cell layers both *in vitro* (Fig. 4) and *in vivo* (10, 20). A similar effect was observed in some of the progesterone-treated cultures after the longer culture period (7 days) (Fig. 3). The precise role of the keratohyalin granules in the stratum granulosum is not known, but they characterize normal keratinization in human epidermis (1), whereas their excessive accumulation in the infundibular epithelium is associated with the formation of comedones (15, 17).

Because testosterone virtually lacked the stimulatory influence of progesterone on the epidermal proliferation, it appears improbable that the epidermal effects of progesterone stem solely from its conversion to androgenic metabolites, as suggested (20).

It is not known if or to what extent the epidermal effects of these steroid hormones depend on the dermal connective tissue in the present organ culture experiments. This possibility deserves to be elucidated, as the dermal connective tissue may modulate the growth and differentiation of the epidermal cells (23) and the action of steroid hormones on the epithelium in certain other tissues seems to be mediated through the mesenchyme (4, 16).

Whether the present results are relevant to the fertile female or male skin is still an open question, since all skin samples included in the present experiments were donated by postmenopausal women. It would seem, however, that the capacity of the cutaneous tissues to bind and metabolize steroid hormones is not changed with advancing age (22). Furthermore, the site of the skin must also be taken into account when generalizing the present results, since the metabolism of steroid hormones differs from one skin site to another (9, 12, 14, 25, 30).

ACKNOWLEDGEMENTS

I am grateful to Professor Risto Santti for his constructive suggestions on the presentation of the results. This work was supported by the University of Turku Foundation.

REFERENCES

1. Breathnach, A. S.: Aspects of epidermal ultrastructure. J Invest Dermatol *65:* 2, 1975.
2. Bullough, H. F.: Epidermal thickness following oestrone injections in the mouse. Nature *159:* 101, 1947.
3. Bullough, W. S.: Stress and epidermal mitotic activity. II. The effects of the sex hormones. J Endocrinol *8:* 365, 1952.
4. Cunha, G. R.: Age-dependent loss of sensitivity of female urogenital sinus to androgenic conditions as a function of the epithelial–stromal interaction in mice. Endocrinology *97:* 665, 1975.
5. Dunaif, C. B. & Finerty, J. C.: The effects of estrogen administration upon epidermal proliferation. J Invest Dermatol *15:* 363, 1950.
6. Ebling, F. J.: The action of testosterone on the sebaceous glands and epidermis in castrated and hypophysectomized male rats. J Endocrinol *15:* 297, 1957.
7. — Steroids and the skin: a general review. Biochem Soc Trans *4:* 597, 1976.
8. Eller, J. J. & Eller, W. D.: Estrogenic ointments. Arch Dermatol Syph *59:* 449, 1949.
9. Frost, P., Weinstein, G. D. & Hsia, S. L.: Metabolism of estradiol and estrone in human skin. J Invest Dermatol *46:* 584, 1966.
10. Goldzieher, J. W., Roberts, I. S., Rawls, W. B. & Goldzieher, M.: Local action of steroids on senile human skin. Arch Dermatol *66:* 304, 1952.
11. Goldzieher, M. A.: The effects of estrogens on the senile human skin. J Gerontol *1:* 196, 1946.
12. Hodgins, M. B. & Hay, J. B.: Factors influencing the interconversion of androstenedione and testosterone in skin. J Steroid Biochem *5:* 318, 1974.
13. Hooker, C. W. & Pfeiffer, C. A.: Effects of sex hormones upon body growth, skin, hair and sebaceous gland in rat. Endocrinology *32:* 69, 1943.
14. Kaufman, M., Straisfeld, C. & Pinsky, L.: Expression of androgen-responsive properties in human skin fibroblast strains of genital and nongenital origin. Somatic Cell Genetics *3:* 17, 1977.
15. Knutson, D. D.: Ultrastructural observations in acne vulgaris: the normal sebaceous follicle and acne lesions. J Invest Dermatol *62:* 288, 1974.
16. Kratochwil, K. & Schwartz, P.: Tissue interaction in androgen response of embryonic mammary rudiment of mouse: Identification of target tissue for testosterone. Proc Natl Acad Sci USA *73:* 4041, 1976.

17. Lavker, R. M. & Leyden, J. J.: Lamellar inclusions in follicular horny cells: A new aspect of abnormal keratinization. J Ultrastruct Res *69:* 362, 1979.
18. Marks, R. & Shahrad, P.: Skin changes at the time of climacteric. Clin Obstet Gynaecol *4:* 207, 1977.
19. Monroe, S. E. & Menon, K. M. J.: Changes in reproductive hormone secretion during the climacteric and postmenopausal periods. Clin Obstet Gynecol *20:* 113, 1977.
20. Papa, C. M. & Kligman, A. M.: The effect of topical steroids on the aged human axilla. Adv Biol Skin *6:* 177, 1965.
21. Punnonen, R.: Effect of castration and peroral estrogen therapy on the skin. Acta Obstet Gynecol Scand (Suppl.) 21, 1972.
22. Sansone-Bazzano, G., Seeler, A. K., Cummings, B. & Reisner, R. M.: Steroid hormone metabolism in skin and isolated sebaceous glands: Preliminary observations on the effects of age. J Invest Dermatol *73:* 118, 1979.
23. Sengel, P.: Morphogenesis of Skin, pp. 68–76, 118–122. Cambridge University Press, Cambridge, 1976.
24. Southwood, W. F. W.: The thickness of the skin. Plast Reconstr Surg *15:* 423, 1955.
25. Stumpf, W. E., Sar, M. & Joshi, S. G.: Estrogen target cells in the skin. Experientia *30:* 196, 1973.
26. Tammi, R.: A histometric and autoradiographic study of hydrocortisone action in cultured human epidermis. Br J Dermatol *105:* 383, 1981.
27. Tammi, R. & Jansén, C. T.: Effects of serum and oxygen tension on human skin organ culture: A histometric analysis. Acta Dermatovener (Stockholm) *60:* 223, 1980.
28. Tammi, R., Jansén, C. T. & Santti, R.: Histometric analysis of human skin in organ culture. J Invest Dermatol *73:* 138, 1979.
29. Tammi, R. & Santti, R.: An ultrastructural study of hydrocortisone action in cultured human epidermis. Br J Dermatol (in press).
30. Wilson, J. D. & Walker, J. D.: The conversion of testosterone to 5α-Androstan-17β-ol-3-one (dihydrotestosterone) by skin slices of man. J Clin Invest *48:* 371, 1969.
31. Winer, B. J.: Statistical Principles in Experimental Design. McGraw-Hill, Tokyo, 1971.
32. Wright, E. T., McGillis, T. J. & Sobel, H.: Action of testosterone on the skin of aging male subjects. Dermatologica *140:* 124, 1970.

Received June 25, 1981

Raija Tammi, M.D.
Department of Anatomy
University of Kuopio
P.O.B. 138
SF-70101 Kuopio 10
Finland