Arch Dermatol Res 269, 13–20 (1980)

Archives of
Dermatological
Research

The Effect of Rat Serum on the Morphology of Rat Hair Follicles in Tissue Culture

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Summary. Rat hair follicles obtained from the skin of 12-day-old animals were cultured in the presence of 20% fetal calf serum and compared with those cultured in the presence of 20% rat serum obtained from animals aged 12–14, 18–20 days; and 1 year.

Follicles cultured in the presence of fetal calf or 12–14-day rat serum showed no morphological change after 2 days of culture. Follicles cultured in the presence of 18–20 day serum or adult serum for 2 days showed greatly altered morphology, the dermal papilla degenerated, keratinization of hair cells occurred, and an enlarged outer sheath developed. The cells in this sheath contained many filaments which were periodic acid-Schiff positive. These changes are compared with those occurring during the catagen phase of the hair cycle in vivo.

Key words: Hair follicles – Tissue culture – Hair growth cycles – Catagen phase – Rat serum

Zusammenfassung. Haarfollikel von 12 Tage alten Ratten wurden mit 20% fetalem Kalbsserum zur Kultur angesetzt und mit Kulturen verglichen, denen 20%iges Rattenserum von Tieren im Alter von 12–14, 18–20 Tagen und 1 Jahr, zugesetzt wurde.

Follikelkulturen, die mit fetalem Kalbsserum oder 12–14 Tage altem Rattenserum versetzt waren, zeigten keine morphologische Veränderungen nach 2 Tagen kulturellen Wachstums. Follikel, deren Kultur mit Serum von 18–20 Tage alten oder erwachsenen Tieren versetzt waren, zeigten nach 2 Tagen ausgeprägte morphologische Veränderungen. Die dermale Papille war degeneriert. Es entwickelte sich eine Keratinisation der Haarzellen und eine Verbreiterung der äußeren Scheide. Die Zellen in dieser Scheide enthielten viele Filamente, die PAS-positiv waren. Diese Veränderungen wurden mit solchen verglichen, die sich während der Katagenphase des Haarcyclus in vivo entwickelten.

0340-3696/80/0269/0013/$1.60

Schlüsselwörter: Haarfollikel – Gewebskultur – Haarwachstumscyclus – Katagenphase – Rattenserum

The patterns of growth and replacement of hair in mammals has long been a subject of interest to biologists.

The hair growth cycle can be divided into three phases. In a young animal, the hair follicles are growing and producing hair (anagen phase), then growth stops just before weaning begins and the follicles start to degenerate (catagen phase). After a resting period (telogen phase), the follicles begin to grow again, and a new hair shaft grows up the hair canal, pushing out the old remaining hair (2nd anagen).

Further details of these processes can be found in a recent review article (Montagna and Parakkal 1974).

In the present study, a tissue culture method has been used to study the behaviour of hair follicles obtained from young rats.

Successful tissue culture of hair follicles from post-embryonic tissue has been described previously. Generally, the most that one can expect is that the follicles retain their characteristic morphology for a period of several days (Trowell 1959; Murray 1933). Thus, although the follicles do not continue to grow (produce more hair in culture), as they do in vivo, degradation of the cells may be delayed for some time.

Active growth of hair follicles in tissue culture has been achieved in one case however, with the follicles continuing to produce morphologically normal hair for a few days (Frater and Whitmore 1973). All of these methods involve the use of a complex culture medium, supplemented with fetal calf serum, and in one case (Frater and Whitmore 1973) with a tryptic digest of embryonic tissue.

In the experiments to be described, the effect of serum from rats of different ages on the culture of rat hair follicles will be presented.

Material and Methods

Tissue Culture

Pieces of hair follicle tissue were obtained from 12–14-day-old hooded rats. The animals were killed by decapitation and the skin sterilized by washing in a stream of 70% ethanol, followed by rinsing in sterile distilled water. A piece of skin, approximately 1.5 × 0.5 cm was removed from the back and placed into sterile Hanks medium. Any adhering connective tissue was removed, and strips approx. 0.5 cm wide cut from the skin piece. The strips were then turned on their sides, and the lower portion of dermal tissue (containing the hair follicles) carefully dissected off. These pieces were then further cut into small blocks approx. 0.5 mm^3. Several of the tissue pieces (up to 10) were transferred to plastic petri dishes (Falcon) containing 3 ml CMRL medium (Commonwealth Serum Laboratories, Australia), supplemented with 20% fetal calf serum (Comm. Serum Labs.) or 20% rat serum.

Rat Serum

Blood was obtained by cardiac puncture from rats aged 12–14 and 18–20 days, and also from fully grown rats (approx. 1 year old). The blood from several animals was pooled and stored overnight at 5°C, and the serum removed next day. All sera and medium used were sterilized by filtration through 0.45 μ Millipore filters.

The cultures were incubated for up to 2 days in a CO_2 incubator at 37°C and then removed for histological examination.

Histological Methods

The tissue pieces were washed once in phosphate buffered saline and then fixed for 12 h in 2.5% paraformaldehyde buffered with phosphate at pH 7.

For low power examination the pieces were then immersed in Herr's $4^1/_2$ clearing fluid (Perry et al. 1975) for 1 h and then photographed using a Leitz Diavert microscope equipped with interference contrast optics. For more detailed examination, the tissue pieces were dehydrated through a graded series of ethanol/water mixtures (70%–80%–90%–100% v/v) and embedded in Spurr's medium (Spurr 1969). Sections were cut at 2 μm using a glass knife, and stained with alkaline toluidine blue (Alsop 1974).

Results

Fetal Calf Serum

Tissue pieces incubated for up to 2 days in the presence of fetal calf serum had morphologically normal hair follicles. Typical features of a growing hair follicle were well preserved, and, in particular, the dermal papilla and the outer root sheath appear normal (Fig. 1). Examination at higher magnification (Fig. 2) confirmed that little damage had occurred to the follicles during the culture period.

Twelve-day Rat Serum

After 2 days of culture, the follicles appear the same as those incubated in the presence of fetal calf serum.

Eighteen to Twenty-day Rat Serum

In the presence of this serum, the follicles show marked changes after 2 days exposure (Fig. 3). The dermal papilla is no longer apparent, and the outer root sheath appears to have been replaced in the lower part of the follicle by a thick sheath of cells. The inner column of cells (those which in a normal follicle would eventually produce the cuticle, cortex and medulla) appear to have differentiated and a prominent knob of these cells is seen at the base of the column.

A stained section of one of these follicles is shown in Figs. 4 and 5. Figure 4 shows clearly the thick surrounding sheath and the keratinized knob of inner hair cells. At higher magnification (Fig. 5) it can be seen that the surrounding sheath contains cells which are filled with fibrous material which stain with the periodic acid-Schiff (PAS) reagent.

When cultured further (up to 7 days), no further changes occur, but signs of general necrosis are apparent. These necrotic changes are also seen in long-term cultures of hair follicles in the presence of fetal calf or 12-day rat serum.

Adult Rat Serum

After 2 days of culture, identical changes to those seen with 18–20 day serum occur. Longer periods of culture elicit no further changes.

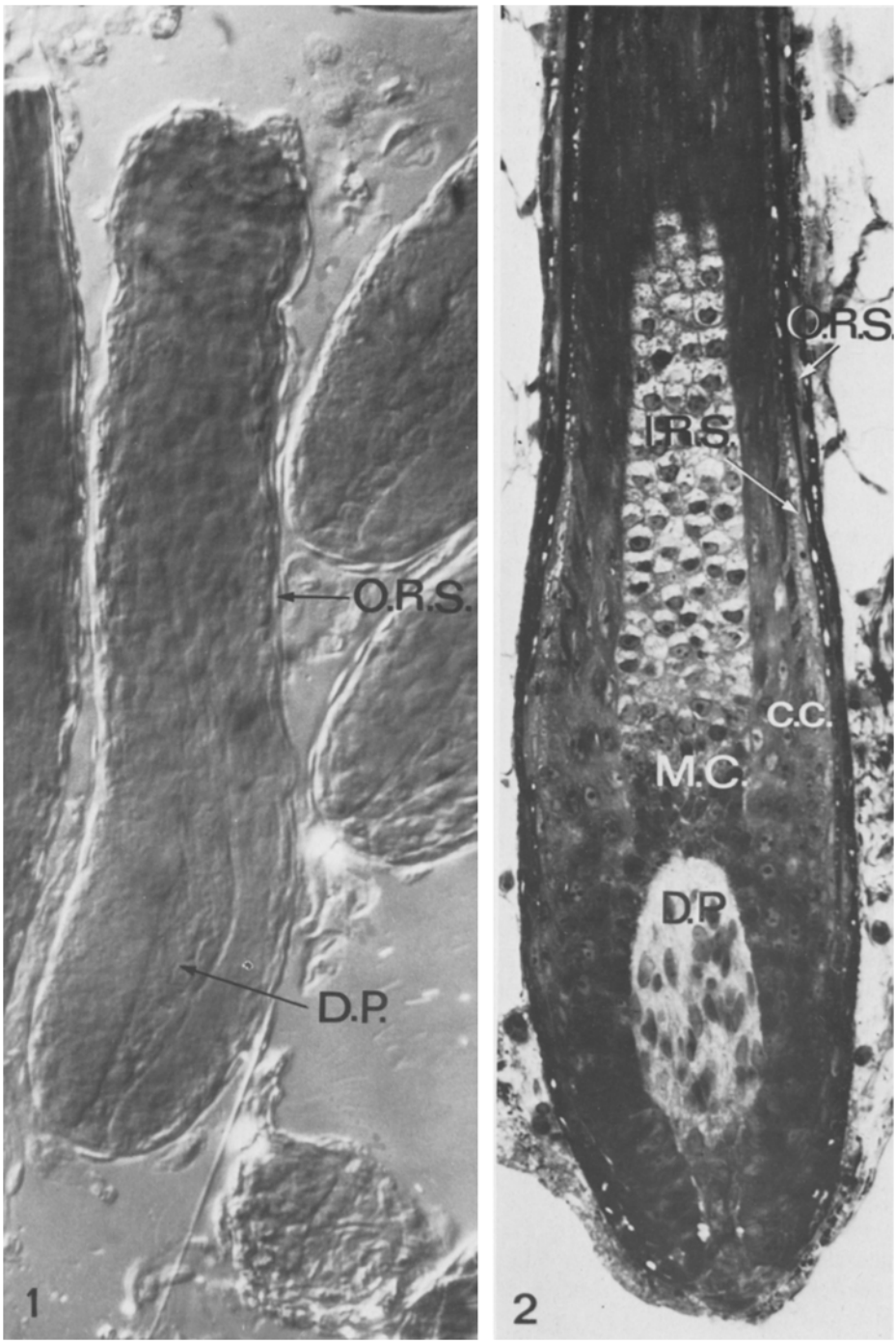
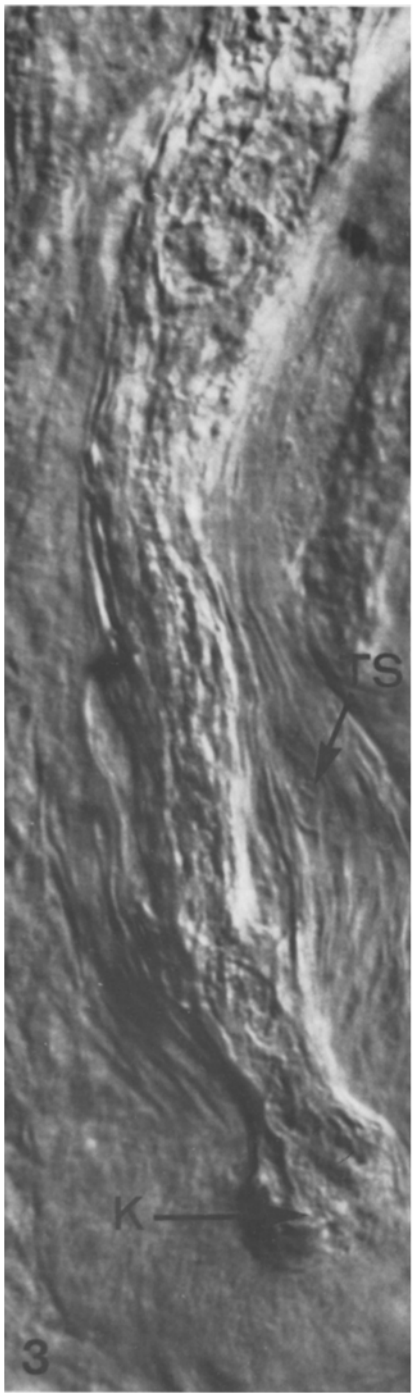


Fig. 1. Photomicrograph of rat hair follicles cultured for 2 days in the presence of fetal calf serum. Interference contrast optics. *D.P.* dermal papilla; *O.R.S.* outer root sheath; Mag. ×200

Fig. 2. Section through Spurr's embedded rat hair follicle cultured for 2 days in the presence of fetal calf serum. *D.P.* dermal papilla; *O.R.S.* outer root sheath; *M.C.* medullary cells; *C.C.* cortical cells; *I.R.S.* inner root sheath. Mag. ×200. Toluidine blue stain



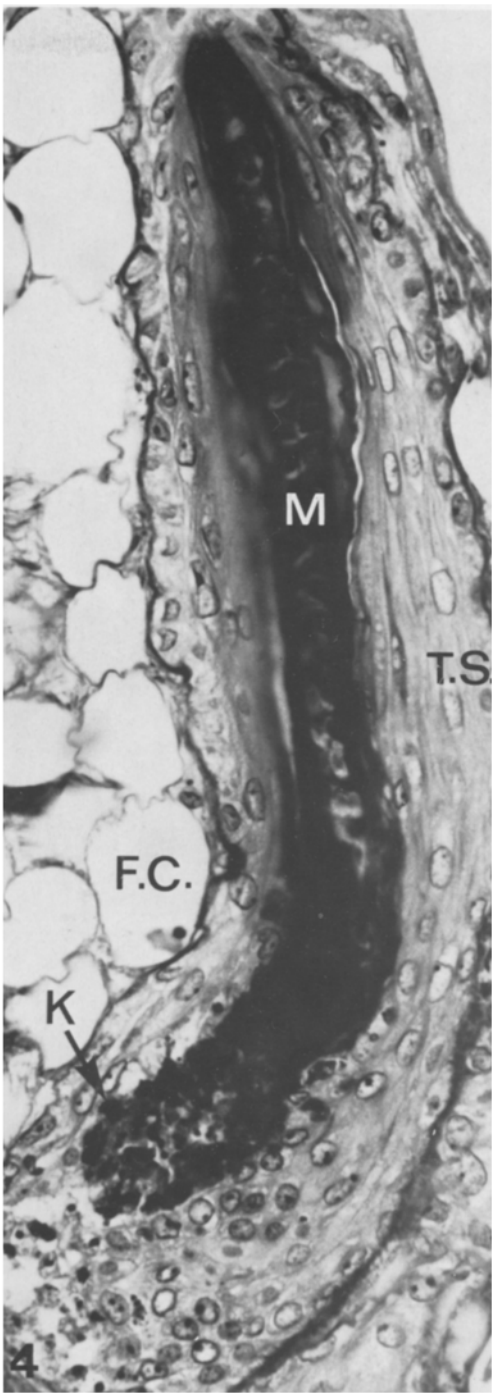


Fig. 3. Interference contrast photomicrograph of rat hair follicles cultured with 18–20-day rat serum. *K* keratinized knob of hair cells; *T.S.* thickened outer sheath. Mag. ×200

Fig. 4. Section through Spurr's embedded rat hair follicle cultured in the presence of 18–20-day rat serum. *T.S.* thickened outer sheath; *K* keratinized knob of hair cells; *M* medulla; *F.C.* fat cells. Mag. ×200. Toluidine blue stain

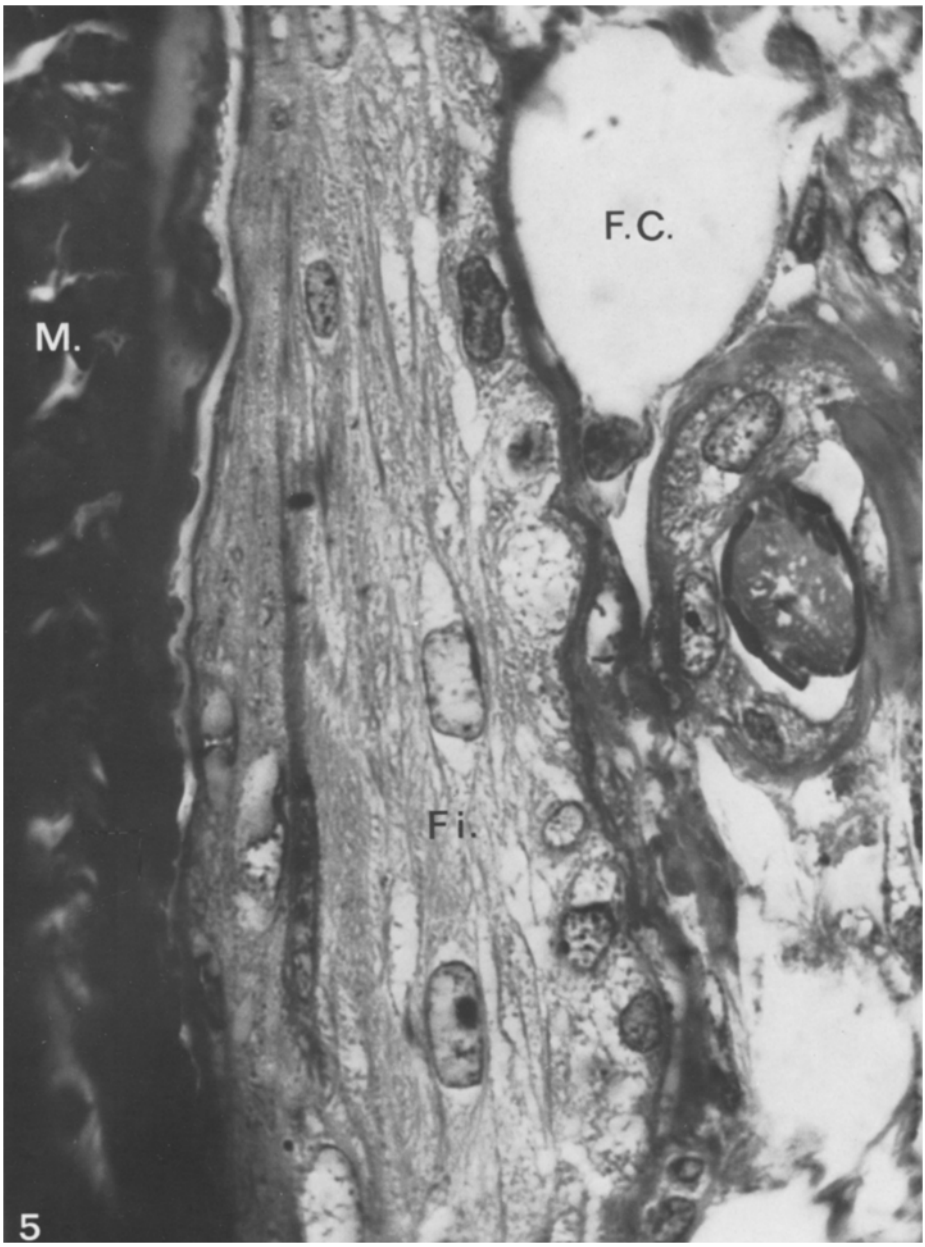


Fig. 5. High power photomicrograph of section shown in Fig. 4. *M.* medulla; *F.C.* fat cell; *Fi.* filaments of P.A.S. positive x material. Mag. ×600. Toluidine blue stain

Discussion

The changes which occur in hair follicles incubated in the presence of either 18–20-day or adult rat serum are very similar to those occurring during the early phase of

catagen in vivo. The degeneration of the dermal papilla, changes in the outer root sheath and the connective tissue sheath, and keratinization in the column of hair cells proper are all characteristic features of catagen. In addition, the thickening of the connective tissue sheath during catagen in vivo is accompanied by the appearance of fine, fibrous, PAS-positive filaments within the cells. Such filaments also are to be found with the cells of the outer sheath in the cultured follicles.

One feature of catagen in vivo was not apparent in the cultured follicles. This is the thickening and wrinkling of the hyaline membrane between the degenerating outer root sheath and the connective tissue layer (Kligman 1959). It was not possible to see this membrane in the cultured follicles, and it may be that it has become incorporated into the cells of the outer sheath.

The observation that neither 12-day rat serum of fetal calf serum cause changes in the cultured follicles, but that 18–20-day or adult rat serum did is of some interest.

A possible explanation is as follows: the growth of hair follicles in young rats ceases when the animals are 17–18 days old, and catagen begins soon after this (Butcher 1934). The follicles remain quiescent until about day 32, and then a new anagen phase begins in some of the follicles and slowly spreads over the whole of the animal. However, before all of the follicles are once again in anagen, a second wave of catagen begins. Hence, at any one time, there will be some follicles in anagen and some in catagen (or telogen).

Thus, if there were a substance in the serum of rats which causes or initiates catagen, one would expect it to be present from the time when catagen first begins, i.e., when the rats are about 18 days old.

The experiments reported in this paper appear to support this conclusion, and are in agreement with the finding that systemic factors are involved in the control of hair growth waves (Ebling and Johnson 1964).

However, factors in the local environment of the follicles must also be important, as, in the adult rat, anagen follicles can be present also with follicles in catagen, and in other mammals (including man), each follicle appears to have its own independent growth cycle.

Thus, it may be that a growing hair follicle accumulates some inhibitory material, and that some of this material eventually spills over into the tissue surrounding the follicle and is then free to react with the serum substance. Exactly what happens then is a matter for conjecture, but it has been postulated that the catagen process is caused by an activation of dormant macrophages near the connective tissue sheath, and that the lysosomal enzymes so produced are responsible for the degradative and absorptive events which follow (Parakkal 1969). In the follicle culture experiments, damage caused during the dissection process may release the inhibitor, which then will only initiate the observed catagen-like process in the presence of serum from animals aged 18–20 days old and over this. Whatever the substance is in the serum of older animals, it appears to be absent or not expressed in serum from the very young animals.

The appearence of the substance in serum from older rats coincides with the onset of weaning and hormonal changes in the animal, and indeed it has been suggested by several workers that the steroid hormones in particular play an

important role in the control of hair growth cycles (Johnson 1958; Schweikert and Wilson 1974).

Acknowledgement. The author thanks Richard Barry, CSIRO for skilled technical assistance.

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Received February 4, 1980