The results described above show that the proliferative potential of CFU_f is extremely great and that the progeny of CFU_f preserve the properties of osteogenic precursors during cell multiplication. During culture CFU_f give rise to cell populations which can form much more bone tissue than if present in the body. On the basis of all these features osteogenic medullary CFU_f must be regarded as self-maintaining, i.e., as osteogenic precursor stem cells. Osteogenic stem CFU_f account for not less than 4% of all clonogenic bone marrow stromal cells.

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EARLY CHANGES IN CELL AND LYSOSOMAL MEMBRANE PERMEABILITY IN THE RAT TESTIS AFTER LOCAL HEATING OF THE SCROTUM

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The mechanism of the severe disturbance of spermatogenesis caused by the action of heat has not yet been explained. It has been shown that a suspension of spermatogenic epithelial cells, exposed to a high temperature (37°C), secretes more protein into the incubation medium than if incubated at 32°C. Such a suspension of spermatogenic cells, if incubated at 37°C, exhibits increased ability to be stained by trypan blue [5]. These observations show that the action of heat on a suspension of spermatogenic cells causes destabilization of the cell membranes. It must also be noted that lysosomes isolated from testes of sexually mature rats and incubated at 37°C secrete more hydrolytic enzymes than lysosomes obtained from liver cells [6]. This fact is evidence of the thermolability of the lysosomal membranes of testicular cells of adult animals. Increased acid phosphatase (AP) activity has been shown to be demonstrable histochemically in the abdominal testes of rats 24 h after operation [3], further evidence of destabilization of the lysosomal membranes. A biochemical study of AP activity in homogenates of abdominal testes revealed a decrease in activity of the enzyme, parallel to the degree of loss of sex cells. Free cathepsin D activity increased under these circumstances, irrespective of the time elapsing after the operation of artificial cryptorchidism [3]. An increase in AP and leucine-aminopeptidase activity was found in a histochemical study of the testes of rats whose scrotum was immersed in water at a temperature of 42°C for 30 min [4].

The aim of the present investigation was to investigate the disturbance of permeability of cell and lysosomal membranes in the rat testis after short-term heating of the scrotum at 41°C followed by incubation of fragments of the testis at 33°C.

EXPERIMENTAL METHOD

The scrotum of rats weighing 220-270 g was subjected to local heating by immersion of the lower part of the body in water at 41°C for 30 min. During heating the animals were kept in

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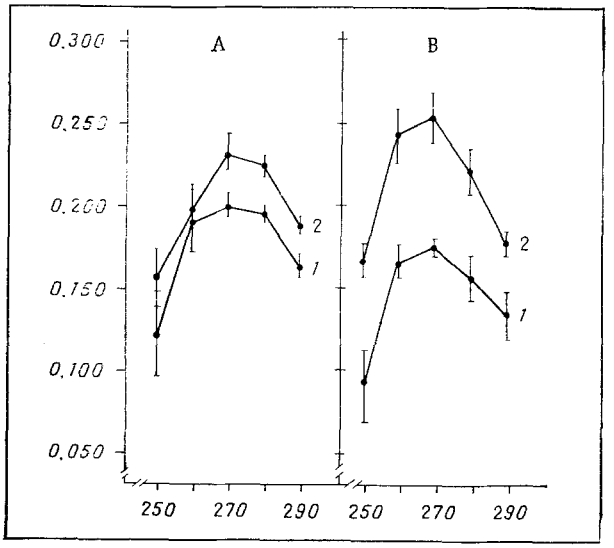

Fig. 1. Optical density of incubation fluid containing testes of rats whose scrotum was heated. Abscissa, wavelength (in nm); ordinate, optical density (in relative units). 1) Control (incubation of testes of intact animals); 2) experiment (incubation of testes of rats exposed to heat). A) Immediately after heating of scrotum; B) 12 h after heating of scrotum.

TABLE 1. Effect of $CaCl_2$ and of Triton X-100 on Optical Density (D) at 270 nm of Incubation Fluid Containing Testes of Rats Whose Scrotum Was Heated

Experiment conditions	D, relative units
Intact animals	0,188±0,006
Heating	0,228±0,05
Heating + $CaCl_2$ (12.5 mM)	0,191±0,008
Heating + Triton X-100 (0.05%)	0,482±0,016

special slings. After the end of heating the rats were decapitated. The testes were freed from the tunica albuginea, cut into four or five pieces, and incubated in Krebs–Ringer solution at 33.0 ± 0.2°C for 1 h with constant stirring. The ratio between the weight of the testicular fragments and the volume of fluid (1 g:10 ml) was strictly maintained. Initially a gas mixture consisting of 95% O_2 and 5% CO_2 was passed through the flasks containing Krebs–Ringer solution for 5 min. After centrifugation and precipitation of the proteins with TCA, the optical density of the incubation fluid was determined at 250-290 nm. In other experiments, the protein concentration [7] and activity of marker lysosomal enzymes – AP [2] and cathepsin D [1] – in the incubation medium were investigated. To determine cathepsin D activity the incubation fluid was mixed with a 1% solution of crystalline hemoglobin. The mixture was incubated for 1 h at 37°C. After precipitation with TCA, the optical density of the fluid was measured at 280 nm. Testes of rats which were not heated were analyzed simultaneously by the same methods. The histological structure of the testes of the experimental and control rats also was investigated (fixation in Carnoy's fluid, embedding in paraffin wax, sections stained with hematoxylin and eosin).

EXPERIMENTAL RESULTS

The histological analysis showed that immediately after the end of heating of the scrotum no visible changes in the structure of the seminiferous tubules could be found in the testis. Eosinophilia of the cytoplasm and pycnosis of the nuclei of late spermatocytes, with some disorganization of the spermatogenic epithelium, were found 2 h after the end of heating. All these changes progressed with an increase in the time elapsing after heating of the scrotum.

It will be clear from Fig. 1 that the optical density at 270 nm of the incubation fluid containing the testes of the experimental rats was significantly higher than the optical density of fluid in which testes of the control animals were incubated. This difference in optical density could be found quite early — when testes were incubated immediately after the end of heating of the scrotum. The increase in optical density was particularly great when testes were taken for investigation 12 and 24 h after heating. These observations indicate that during local heating of the scrotum an increase in permeability of the cell membranes of the testes arises very early. This conclusion is supported also by the fact that addition of $CaCl_2$ to the incubation medium in a final concentration of 12.5 mM led to a decrease in optical density of the incubation medium measured at 270 nm, whereas addition of the detergent Triton X-100 in a final concentration of 0.05% increased the optical density considerably (Table 1).

Determination of cathepsin D and AP activity in the incubation medium showed that the testis heated *in vivo* secretes an increased quantity of these two enzymes into the medium. This phenomenon was found immediately after heating of the testis, although it was not statistically significant. A significant increase in enzyme release into the incubation medium by the heated testis was obtained 12 h after the end of heating of the scrotum. In the control, for instance, AP activity in the incubation medium was 14 ± 2.82 pmoles phosphorus/min/mg protein, compared with 26.08 ± 2.91 units in the experimental group ($P < 0.05$). Whereas cathepsin D activity in the control was 0.79 ± 0.11 units, in the experimental group it was 1.13 ± 0.09 units ($P < 0.05$). After 24 h no increase in secretion of the two enzymes by the heated testis compared with the control could be detected.

The results indicate that the harmful effect of a raised temperature on spermatogenic function may be based on destabilization of cell and lysosomal membranes in the testes.

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