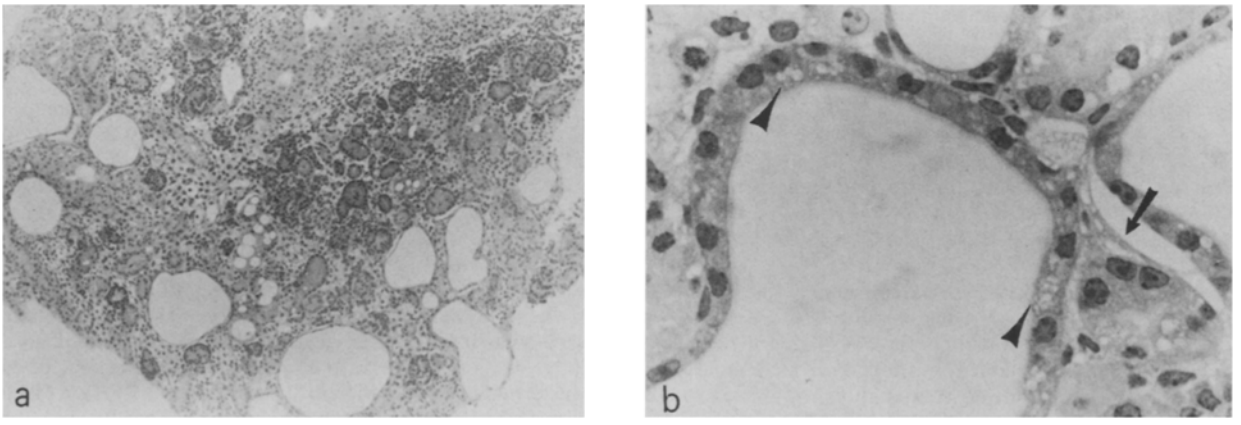

Figure 2. Tissue treated with cortisol (1.4×10^{-5} M) following 144 h of incubation. *a* Cystic tubular changes are present amidst a background of tubular and glomerular elements. Hematoxylin × 54. *b* Flattened epithelial cells of cyst walls show vacuolar changes (arrowheads), and tubular supporting wall structures are retracted behind the bases of cysts (arrow). Hematoxylin × 420.

The precise mechanism by which glucocorticoids induce cystic changes has not been determined. In the organ culture model, cortisol induces cystic abnormalities in developing renal tissue without vascularization, glomerular filtration, or urine formation. Since nephron obstruction and increased intratubular hydrostatic pressure cannot occur in the nonperfused system, the model isolates the role of drug-induced alterations in epithelial cell and tubular supporting wall structure and function for further study. The morphological features of tubular cell flattening, intracellular vacuolization, and retraction of cyst wall supporting tissue suggest diffuse drug effects on intracellular metabolism and extracellular matrix formation with resultant increased tubular wall compliance. This is consistent with known glucocorticoid effects on protein synthesis and extracellular matrix production[9,10]. Studies are currently underway to define the molecular and biochemical events which may mediate glucocorticoid-induced cystic changes in this model.

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Enhancement of muscle regeneration by bone marrow cells in the monkey

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Summary. Transplantation of muscle minces with and without autogenous bone marrow cells was performed in the monkey. The addition of bone marrow cells markedly enhanced muscle regeneration. The findings suggest a possible clinical application of the technique.

Muscle growth and regeneration in health and disease have been the subject of extensive recent research[2-5]. However, results of clinical trials with therapeutic muscle transplantations have generally been unsuccessful because of poor myogenic regeneration, fibrosis of the transplant and inadequate restoration of function[2,3].

Our previous work with muscle cultures[6,7] and transplantation of muscle minces in rats[8] has shown that marked enhancement of myogenesis occurs when autogenous bone marrow cells are added to muscle minces. The procedure was also found to suppress bacterial infection in both the transplant and in culture. The present work was undertaken to see if the effect of adding autogenous bone marrow cells to muscle mince transplants in a young adult monkey would, as in rats, enhance myogenesis.

Material and methods. 2 separate sets of experiments were performed with a 10-month interval between, on an adult baboon weighing 10 kg. The monkey was anesthetized with nembutal 4 ml and pentothal 3 ml i.m. Surgery was performed under strictly sterile conditions. 2.0 ml of bone marrow was aspirated from the sternum using a 14 gauge needle and mixed with

0.3 ml of 3.85% sodium citrate. The bone marrow contained about 30 million nucleated cells per ml.

Pieces of muscle weighing approximately 1 g (constituting about ¼ of the relevant muscle mass) were excised from the triceps, biceps and brachioradialis muscles of each side through separate incisions. Each piece of muscle was minced as finely as possible; muscle from the right limb was then placed in the excision site of the corresponding muscle of the left limb and vice versa. 100,000 units of crystalline penicillin and 150 mg streptomycin were added at each site.

To the muscle minces placed into the right side, 0.5 ml of bone marrow was added, while the left side served as the 'untreated' control. The minces were secured in place by closing the fascia over them with chromic 3–0 sutures. The skin was closed with nylon 3–0 sutures. A further 600,000 units of crystalline penicillin and 750 mg of streptomycin were given i.m. during surgery.

In experiments repeated 10 months later transplants were taken from the same muscles, but sufficiently distant from the original surgery site to avoid possible interference.

This second set of experiments was performed in order to see whether the results obtained were reproducible.

Post-operatively the monkey moved about freely in a cage and received 0.5 g each of methicillin and ampicillin in 2 divided doses daily for 5 days.

The muscle transplants, together with a small area of surrounding host muscle, were removed after 2 weeks from both triceps, after 4 weeks from the biceps and after 12 weeks from the brachioradialis muscles. Specimens taken from the central and peripheral parts of the transplant were fixed in glutaraldehyde and processed for electron microscopy. The rest of the excised tissue was fixed in formalin, and multiple sections from paraffin blocks were stained with hematoxylin and eosin and Van-Gieson stain.

Results. The findings in both sets of experiments were identical. Macroscopically, the difference in appearance between the 2 sides was not marked, though there was an impression of more fibrosis on the left side where the transplant appeared less bulky and more pale than on the right side with the bone marrow cells. This was particularly evident at the 12 week time-point.

Microscopically, muscle regeneration could be seen on both sides. The 2- and 4-week specimens showed many myotubes and separate myocytes. This early myogenesis is known to occur in muscle minces even without the addition of bone marrow cells[2,4,5]. However on the left side (control) the extent of myogenesis was limited, and by 12 weeks the bulk of the muscle mince consisted of connective tissue, new blood vessels and fibroblast-like cells (fig. 1). Only very few myocytes could be seen at this time.

On the right side (minces with added bone marrow cells) active myogenesis was seen throughout. At 12 weeks (fig. 2) the specimen consisted mainly of small new myofibers, myotubes and long polar cells which on electron microscopy proved to be myogenic (fig. 3). Connective tissue elements were minimal, and the difference between the 2 sides was quite remarkable.

Discussion. The work presented here shows that in the monkey, as in rats[7], the addition of bone marrow cells to transplanted muscle minces markedly enhances muscle regeneration. The mechanism of this enhancement remains obscure but it is possible that phagocytic, secretory and inductive properties of the bone marrow cells[9] contribute to the processes involved.

Our previous work[7,8] has shown that after addition to cultures or to transplants, bone marrow cells break down myofiber basement membranes thereby liberating satellite cells which proceed to myogenesis. The old muscle sarcoplasm is then phagocytosed and rapidly degraded by young myogenic and non-myogenic cells. These processes, probably associated with other complex cell-to-cell interactions[4], appear to enhance myogenic cell line development while suppressing fibroblast differentiation in the transplant.

Confirming that the enhancing effect of bone marrow on myogenesis occurs in primates as well as in other animals has brought us that much closer to the eventual goal of successful clinical muscle transplantation.

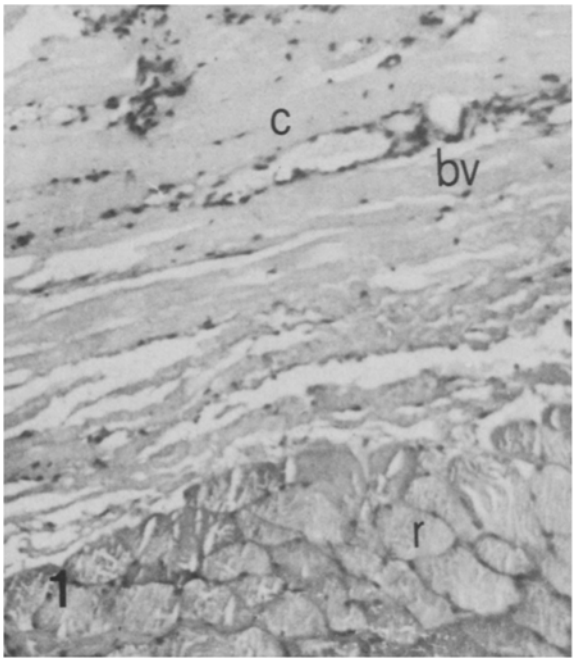

Figure 1. Left brachioradialis 12 weeks after transplantation of minced muscle without additional cells. Most of the transplant is composed of connective tissue (c) and blood vessels (bv). No new muscle cells are seen in the donor mince. Normal myofibers of the recipient muscle bed (r) are seen in lower part of figure; H and E, × 200.

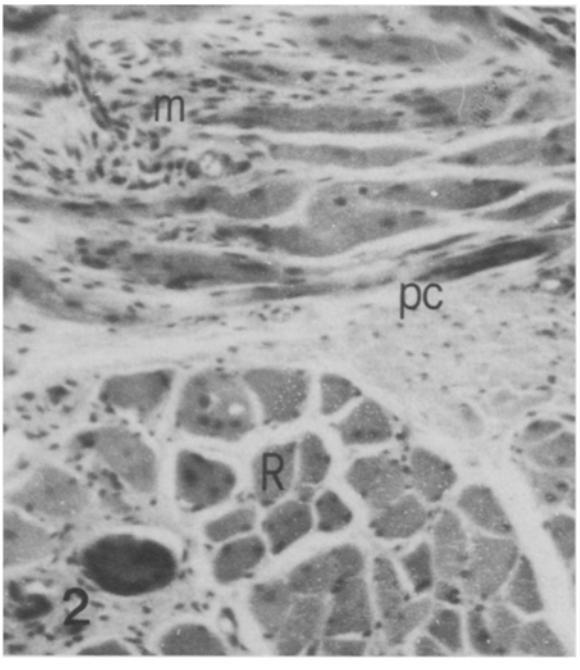

Figure 2. Right brachioradialis 12 weeks after transplantation of minced muscle with added autogenous bone marrow cells. Active myogenesis is in progress with many myotubes, myocytes (M) and long polar cells (pc). Recipient muscle bed (R) is seen in lower part of figure; H and E × 200.

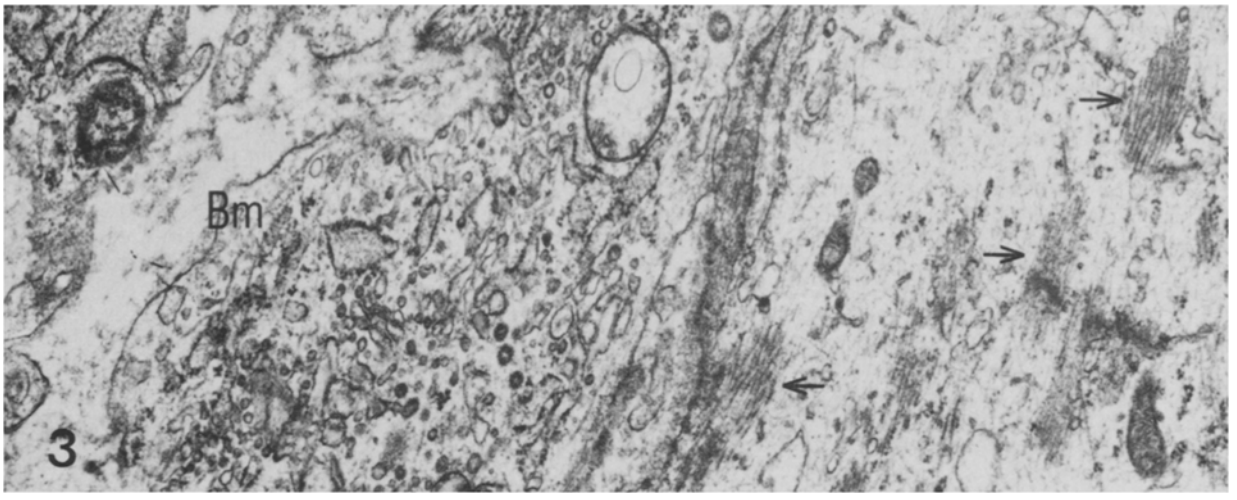

Figure 3. Electronmicrograph from same specimen as in figure 2 showing that the polar cells in the transplant are myogenic, containing organizing myofilaments (arrows). Basement membrane (Bm) of the new cell can be seen; × 16,000.

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Surface morphology of the subfornical organ: Effects of low and high sodium chloride diet

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Summary. Ependymal cells found in the subfornical organ of the rat were counted. Cells covered by small microvilli, small protrusions and smooth cells were frequently found. Also present were cells with long or short cilia, cels with large protrusions and supraependymal cells. High and low sodium diets reduced the number of cells with large protrusions. Microvilli-covered cells increased after a low sodium diet.

The subfornical organ (SFO) has been described as a circumventricular structure placed between the columns of the fornix at the rostral wall of the third ventricle, near the point where the choroid plexuses of the lateral and third ventricles converge[2]. In the rat the surface morphology of this midline elevation shows different types of cells which characterize 3 zones[3]. The structure of some of these cells is modified by water deprivation or hypovolemia[4]. Moreover, factors capable of altering sodium metabolism such as adrenalectomy or sodium-deficient diets affect the size of the neuronal nuclei and produce vacuolization of cytoplasm[5]. Lesion of the SFO increases sodium intake[6]. Sodium excretion after intracarotid infusion of hypertonic saline is prevented by this lesion[7]. This supports a participation of SFO in salt-water homeostasis. To provide more information about SFO as a target of changes in salt intake and especially the participation of the ependymal cells, the surface morphology was studied in rats on a salt-free diet or a hypertonic sodium chloride solution. In order to provide evidences of such changes a quantification of the different types of ependymal cells was made in the above groups and compared to normal animals.

Material and methods. Male Wistar Chbb rats (300–400 g) were housed individually. Control animals (n = 6) were maintained on rat chow (200 mEq Na + /kg) and tap water. High sodium intake rats (8) had 1% sodium chloride solution as drinking fluid. The 'salt free' group (7) had access to a rat chow with less than 4.1 mEq Na + /kg and distilled water. A week after, animals were beheaded, the SFO removed, fixed in 2% glutaraldehyde in Millonig buffer for 2.5 h and post fixed in OsO_4 for 1.5 h in the same buffer. The tissues were dehydrated through increasing concentrations of acetone and finally critical-point dried from CO_2. Tissue blocks were coated with gold-palladium. A picture of each SFO at 150 X and 10–20 pictures at 3000 X were taken of zones 2 and 3 as described by Phillips et al.[2]. The sagittal and medial axes were measured. A reticule containing 20 points was used to count the different types of cells. The point distribution avoided counting the same cells twice. The number of long protrusions per picture was also counted. The means ± SEM of points over each type of cell per 100 cells in each group of animals was calculated but this value was not taken into consideration for the statistical analysis. The results were analyzed by the Kruskal-Wallis one way analysis of variance. The Mann-Whitney U Tests to compare 2 groups were used when the above procedure gave a significant result.

Results. The SFO sagittal axis measured 345 ± 44 μm and the