EFFECTS OF SMALL AMOUNTS OF ELECTRIC CURRENT AT THE CELLULAR LEVEL*

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Recent workers have explored the possible role of bioelectricity in control of such biological processes as bone regeneration,[1,2] limb regeneration,[3,4] stimulation of macromolecular synthesis,[5] and acceleration of wound healing processes.[6] The majority of these studies have had a clinical orientation on human subjects and, therefore, have not been adaptable to theorectical experimentation.

During the 1960's, several laboratories reported that the healing rate of burns[7] and decubitus ulcers[8] could be accelerated by the application of gold leaf sheets over the affected area. It was postulated that the beneficial effects stemmed from an electrostatrice or electrochemical influence, possibly established by the dielectric interface formed between the gold and the wound.[9]

The existence of the "current of injury," that is, a potential electric difference and subsequent current flow between wounded and normal tissue, has long been accepted. Some investigators have attempted to accelerate healing rates through the use of extrinsic current applications.[10-12] Although results of these experiments indicated only limited success, enough convergent data were assimilated to strongly implicate a role for electricity in healing processes.

In 1969, a therapeutic constant-current generator was designed and tested on human patients with ischemic skin ulcers.[13] It was determined that when current levels were maintained between 200 and 800 μA, the ulcers would heal more rapidly. Clinical results from this treatment showed that application of extrinsic current enhanced healing rates by more than 400%.

The majority of studies on electrical enhancement of healing have had a clinical base that utilized human subjects, and, therefore, have not lent themselves well to basic theoretical experimentation.

If the wound healing process can be enhanced by application of external electric current, it is certainly possible that the cell and molecular biological events that occur in the repair process would also be accelerated. If this is the case, such a sequence of events should be detectable through modern techniques. Because mammals have remarkable similar cutaneous characteristics, it may be possible to design experiments that could parallel previous clinical studies and perhaps shed some light on events involved in electrical healing enhancement.

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RESULTS

Cutaneous wound healing is describable as a series of discrete, identifiable events that begin with clot formation, epithelial cell formation, epithelial migration to cover the wound, invasion of the traumatized area by phagocytes, polymorphonuclear leukocytes, and lymphocytes, increase of fibroblasts, and collagen synthesis.[14] These morphological criteria were studied at 24 and 48 hr.

24 Hours

Wounded tissues obtained from control animals show a progression of epithelial cells that migrate from adjacent sides of the cut area toward each other to form a superficial epithelial cap. The dermis has a rather loosely organized appearance. Tissues removed from beneath the positive electrode of experimental animals demonstrative epithelial migration; however, the degree of this migration seems to be not as marked as in controls. The dermis seems better organized and somewhat more dense than that of the control. In tissues taken from beneath the negative electrodes of experimental animals, the migratory process of epthelial cells appears greatly inhibited. No epithelial cap has been formed, but a "polyline,"[15] that is, a linear array of polymorphonuclear leukocytes, has appeared at the interface between the clot and the wound itself. The dermis displays sparse organization.

48 Hours

At this point, wounded tissues from control animals have a well-developed epithelial cap, and epithelial cells appear to be migrating into the dermal region. The dermis is fairly well differentiated.

Tissues from under the positive electrode of experimental animals reveal a well-developed epithelial cap but no dermal invasion by epithelium. The dermis appears to be better differentiated than that seen in control tissues.

Tissues from beneath the negative electrode of experimental animals show no epithelial cap development, and the dermis still appears sparse and poorly organized.

Autoradiographic studies were performed to detect the amount of incorporation of (^{3}H) thymidine, thereby denoting DNA replication. Because the injections of (^{3}H) thymidine were given 5 hr before sacrifice, one can assume that the degree of incorporation is representative of DNA synthetic activity during that period.

At both 24- and 48-hr stages, there were no differences in the degree of incorporation of labeled thymidine in cell types of the control and the two experimental groups. In the regions of the epithelium immediately lateral to the wound edges, all three groups exhibited an index of (^{3}H) thymidine incorporation of 10% (±0.3%) at 24 hr and 13.5% (±0.4%) at 48 hr. No incorporation was seen in the epithelial caps. It is assumed that cells undergoing migration did not synthesize DNA.

None of the cells derived from the blood or reticuloendothelial system demonstrate DNA syntheses, except clusters of lymphocyte-like cells throughout the wounded area. One can identify four to six labeled cells in such clusters.

DISCUSSION

Application of small amounts of electric current in the rat can alter the healing processes, even when the current is applied only during the first 4 hr after wounding. Through 48 hr, it

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DR. A. H. FREY: Would you please provide more detail with respect to how these animals were restrained?

DR. HARRINGTON: Picture a soup can made of Plexiglas®: one end, which would later be the cephalic end, was covered by wire mesh, which the animal would see from inside the container; after the animal crawled in or was placed in the apparatus, a rubber stopper was inserted at the other end. This would restrain him from moving around or getting his head back toward where the electrodes were placed.

DR. FREY: What are the electrical consequences of this procedure: I have done some work with rats in which we put them into a restrained situation, such as that with plastic, and we found some interesting fields caused by their fur rubbing against the inside of the container. We later used the active ingredient of Breck hair rinse to wash the rats every four days to cancel out that effect. If you are concerned with the effects of very low currents, you might have some very interesting electrical field phenomena from such rubbing of fur.

DR. HARRINGTON: We had shaved the hair and then put the electrode jelly on, but I certainly think you don't get right down to the...

DR. FREY: You didn't shave the whole animal?

DR. HARRINGTON: No.

DR. FREY: This is what I am concerned about.

DR. A.M. TOOLE: As an electrical engineer, I am quite appalled to hear for the second time today a current of injury described as a potential. Could you clean up this problem and also explain why you employed current as an electrical parameter when you were discussing potentials, the polarity of the electrodes, since they're in proximity with the tissue you were talking about? Could you explain why you decided upon using that particular parameter?

DR. HARRINGTON: In terms of why we used current? I am a biologist, and many of these terms are almost interchangeable. I do understand the difference between voltage and current.

Some of our prior work on amphibian erythrocytes was performed with currents. At that time, I was working with some physicists and an orthopedic surgeon, and I became accustomed to use the term current. Most of the literature tends to speak in terms of current when, in fact, voltage effects were looked for. With erythrocytes, we concluded that the current itself and the nonuniformity of the current of the electric field were the factors involved.

DR. TOOLE: Could you, in fact, determine the effects caused by the current and whether it was a static voltage effect, a field effect, or something else?

DR. HARRINGTON: That is a very good question. I really don't know the answer. I measured current and probably could use all the other parameters and vary them, I would think, in every experiment.

DR. A.A. PILLA: I think some of the results I discussed yesturday addressed themselves directly to the last question, that is, is it the current, field, voltage, or something else: I think that in common with all past experiments, is the presence of a bulk electric field due to the current passing through it. Whether the effects are localized at the electrodes or in the bulk is another question.

DR. HARRINGTON: I think we are still dealing with a phenomenon at this point and that we are clearly amazed that such changes occur. I think that all of us, certainly those in the biologic area, are just beginning to find out what the discrete causative agents are. I feel as if I am in a phenomenologic observational state. I use current; it became a part of my vocabulary.

DR. C. MINKIN: I think the fantastically complex cellular migrations and differentiations that occur in a wound-healing process superimposed on the complex problems of defining electrical parameters present a problem that will keep us all busy for many years.