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EXPERIMENTAL ACUTE RADIODERMATITIS FOLLOWING BETA IRRADIATION

V. Histopathological Study of the Mode of Action of Therapy with Aloe vera

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Continued emphasis of both the peacetime and military application of atomic energy has greatly increased the number of potential victims of radiation damage to the skin. A few cases of serious beta-radiation damage to persons engaged in atomic-energy work have already occurred.[8] At the present time surgical excision with plastic repair of the exposed area is considered by many as the treatment of choice if the lesion is restricted in size. Various ointments are still in use in the treatment of radiodermatitis but, for the most part, have been shown to be relatively useless. However, an effective pharmaceutical means of therapy would decrease the morbidity of radiodermatitis if surgery is either not available or not feasible. The peacetime incidence of this injury alone would seem to direct attention toward the development of such a remedy.

In this connection the relatively recent discovery that the juices of the leaf of the *Aloe vera* plant are efficacious in the treatment of acute and chronic radiodermatitis[3] is of considerable interest. Furthermore, there have been many subsequent successful clinical trials,[6, 9, 15, 19, 24, 26, 27] which have been supported by tests on experimental animals.[20, 21] As a result of these studies several modern texts concerning the therapeutic use of ionizing radiations describe *A. vera* as being useful in treating inflammatory and ulcerative sequelae in the skin following radiotherapy.[14, 17] Interest, however, seems to have declined. In spite of these favorable references, *A. vera* has not been the subject of a clinical or experimental study since 1941.

That *Aloe* contains a healing agent is well documented by medical history. Its use was known in the fourth century B. C., in India where today as "musabbar" it is applied to external inflammations.[1] In China, where it is known as "jelly leek" or "Lu Hui," meaning black deposit, a scholar living between 772 and 842 described its use in treating eczematous rashes and, in the Sung Dynasty, 960 to 1279, it was also used in the treatment of eczema.[2, 22] *Aloe*'s use as a vulnerary or healing agent for wounds by "the ancients" is mentioned in the Dispensatory of the United States of America.[7, 18] The occidental use of "the herbe *Aloe*" "to hele wounds" is first recorded by Turner in 1568, and again by Coxe in 1818. Its use in China in the early twentieth century in the household treatment of thermal burns has been described,[21] and Crewe recently found it active in curing thermal burns in this country. The long history of usefulness in treating dermal inflammations and wounds, however, has led to only one recorded attempt to find the active ingredient or principles of *A. vera*.[22] While the attempt was unsuccessful, it and subsequent tests in rats[21] served to show that the healing caused by *A. vera* was not due to tannin, pectin, vitamin A, vitamin D, urea, or any nitrogenous substances.

The extensive documentation of the remarkable healing effects of *A. vera* seems to justify its inclusion in any investigation of pharmaceutical means of enhancing the rate of healing of experimentally produced ulcerative radiodermatitis. In the previous clinical and experimental studies already cited, only one attempt was made to obtain objective evidence of curative action other than by observation of the gross rate of healing as determined with the unaided eye. In this attempt[20] in which five rats (that "died during different periods of treatment") were examined for histological evidence of effectiveness, "no qualitative variations could be found to distinguish the treated and untreated . . . rats." In view of the well-defined sequence of changes, and the healing deficit that occurs in experimentally produced ulcerative radiodermatitis in rats,[10, 11, 12] an increase in the rate of healing should be reflected in either a speeding up of the sequence of

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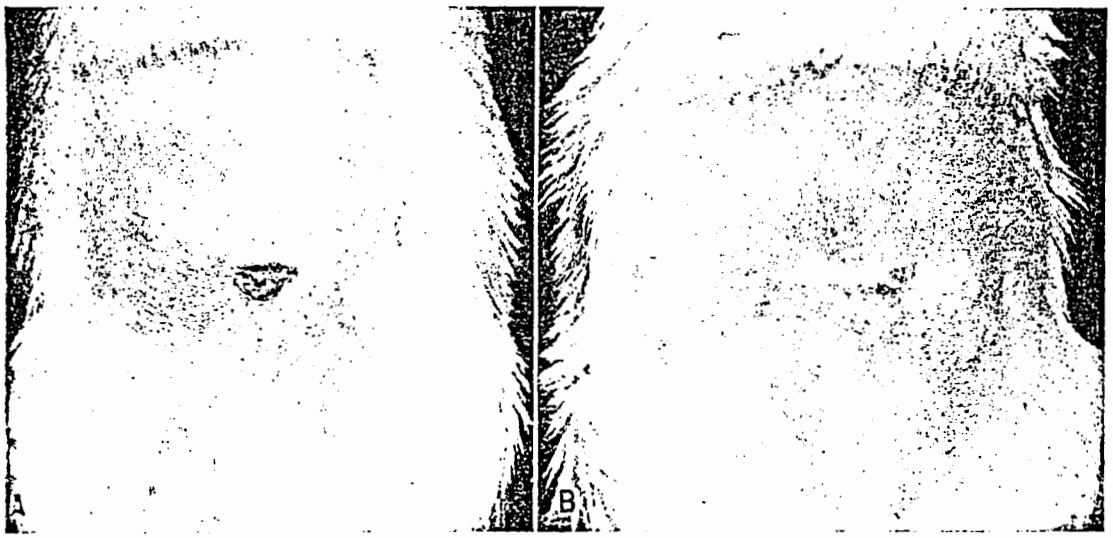


FIG. 1. Life-size photographs of the shaved abdomens of two albino rats exposed locally to 14,000 rep of beta radiation eighteen days previously. A, No local treatment. B, Treated locally with daily applications of *Aloe vera* ointment.

events as they occur in the untreated animal or in a modification of the events themselves. The following experiments, therefore, were undertaken to determine objectively whether the fresh leaf of *A. vera* and a commercially available ointment made from these leaves are useful in the treatment of acute radiodermatitis.

EXPERIMENTAL PROCEDURES

Animals. Several preliminary experiments were carried out with rats to determine whether it was possible to treat radiation ulcers in this species under rigidly controlled conditions so that objective studies could be made. It soon became apparent that the rat was too active an animal for this purpose. When restrained in a harness designed to prevent removal of the bandages and the therapeutic material, many rats refused to eat or strangled in their attempts to escape. During these experiments, however, an occasional more tractable animal that allowed the *A. vera* leaf or ointment to remain in contact with the irradiated area showed a significantly more rapid rate of healing than did the untreated rats. This observation encouraged continuation of the investigation. An example of the difference between eighteen-day-old ulcerative radiodermatitis with and without treatment with *A. vera* ointment is shown in Fig. 1.

Rabbits were finally selected as the animal of choice for the therapeutic trials because they usually remained quiet and did not require restraining. Twenty albino rabbits, weighing 2 to 4 Kg., were used in the experiments described here. They were of undetermined sex and age.

Method of Radiation. An aluminum applicator plated with Sr^{90} was used as the source of beta radiation.[11, 13] The source was 5/8 in. in diameter and delivered 99 rep per second to the skin. Two doses, 14,000 and 28,000 rep, were delivered to the surface of the shaved skin.

Aloe vera. Fresh whole leaf of the *A. vera* plant and a commercially available ointment made from the juice of the leaf in an aquaphor base were the two therapeutic agents tried. The fresh leaf was applied by removing the rind from the flat side of a 2-by-1 in. piece of leaf and placing the exposed inner jelly-like substance in contact with the irradiated area. The rind on the outside of the piece aided in holding the jelly in place because it provided a stiff surface to which adhesive tape could adhere. The ointment was rubbed liberally into the irradiated area and a copious amount left on the surface, which was then covered with wax paper and gauze and held in place with adhesive tape.

Typical Therapeutic-Trial Experiment. The backs of four rabbits were shaved with a fine-toothed electric clipper just before exposure to radiation. The shaved area was divided into four quadrants. In the center of each quadrant two areas ½ in. apart were exposed to beta radiation: the caudal area received 28,000 rep and the cephalad area 14,000 rep. The surface

of the radiation source was placed in contact with the skin and no effort was made to prevent scatter. The two areas in each quadrant were treated under a single dressing. The areas in the upper left quadrant as shown in the illustrations (Figs. 2, 3) were treated with the whole fresh *A. vera* leaf. The areas in the upper right quadrant were treated with the ointment. The

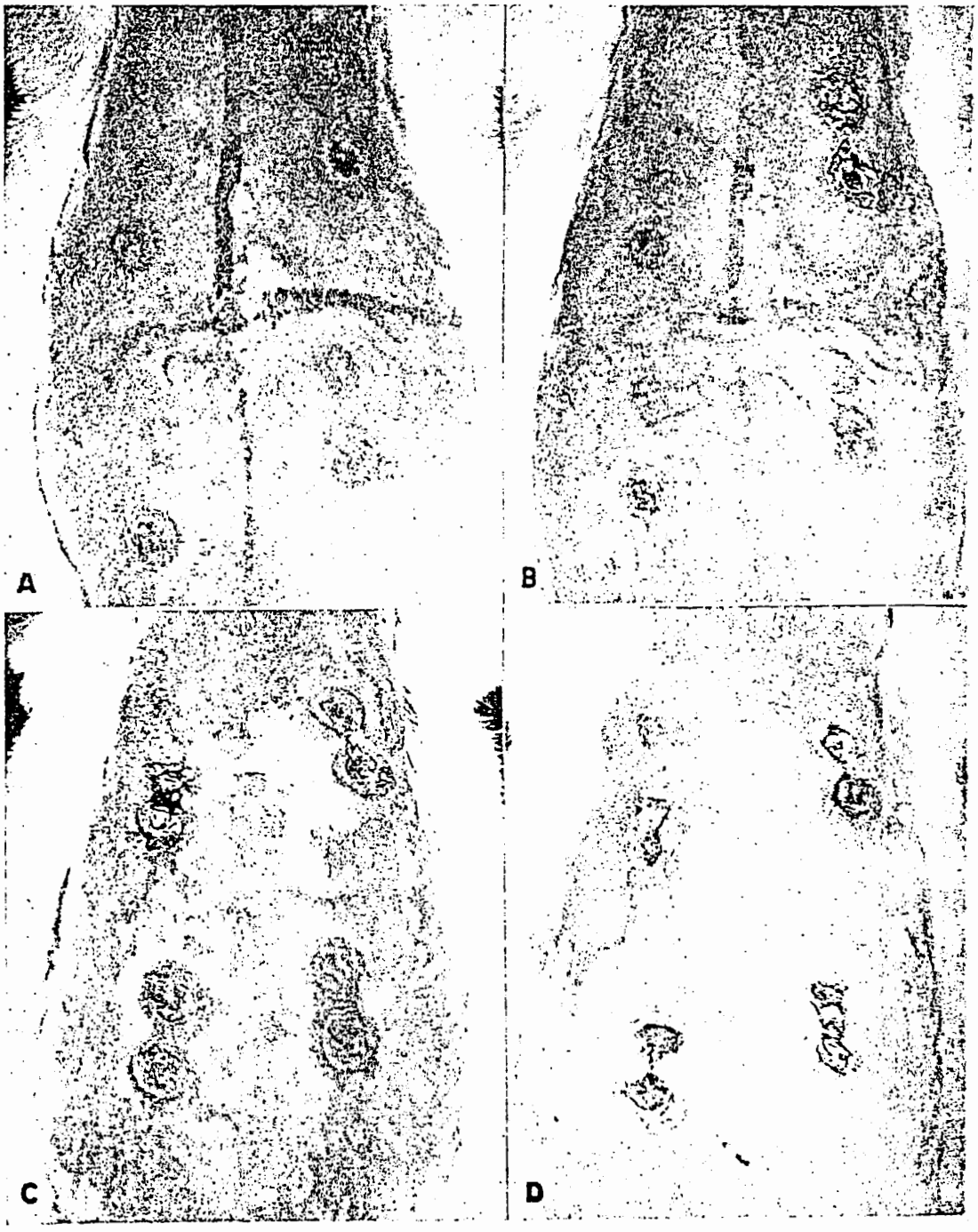


FIG. 2. Sequential photographs of the back of a rabbit that was exposed to 14,000 and 28,000 rep of beta radiation from Sr^{90} in each of four quadrants. The uppermost lesions in each quadrant is the result of the smaller dose. Treatment in each quadrant was as follows: upper left, *Aloe vera* leaf; upper right, *Aloe vera* ointment; lower left, dry bandage; and lower right, none. The photographs were taken (A) seven, (B) twelve, (C) twenty, and (D) thirty-one days after exposure to radiation. Treatment was started the same day and repeated once daily except for Sundays.

reas in the lower left quadrant were covered with a dry gauze bandage and the areas in the lower right quadrant were left uncovered and untreated. The treatment was begun immediately after irradiation and was renewed once each day except Sundays. It usually took about three hours for the rabbits to remove the bandages. The progress of the lesions was observed at the time the bandages were changed and at intervals of seven, twelve, twenty, thirty-one, and fifty-eight days after irradiation and subsequent treatment, and photographs were made of the back of each rabbit.

Histological Experiment. Ten rabbits were used in this experiment. The shaved back was divided into four quadrants and the central area of each quadrant received 14,000 rep of beta radiation from the strontium source. Two of the four areas were treated daily with the *A. vera* ointment. The other two areas were left unprotected and untreated. To obviate the possibility of differences in rate of healing of the various areas of the back, the treated lesions were varied in location on the different rabbits. Under nembutal anesthesia an untreated and a treated lesion were removed from two of the ten rabbits at intervals of five, ten, sixteen, twenty-one, twenty-seven, thirty-two, and forty days after the beginning of the experiment. The rabbits were chosen at random for this procedure. The pieces of skin containing the lesion were stretched on cork and fixed in Zenker's acetic acid fixative. Sections were made of a strip of skin obtained along a diameter of the fixed lesion with normal skin at both ends. The paraffin sections were stained with hematoxylin and eosin, azure-eosinate, Verhoeff's elastic-tissue stain, and Heidenhain's Azan stain. The stained sections were examined microscopically and the treated and untreated lesions compared.

RESULTS

The results of the typical therapeutic experiment just described are shown in Figs. 2 and 3. During the first week hyperemia and edema appeared gradually in the untreated areas, becoming most obvious on the sixth day. The treated areas were markedly edematous and hyperemic on the first day. The areas treated with the ointment had a large surrounding area of hyperemia and edema several times the diameter of the exposed area. This subsided during the first week. On the fifth day necrosis began in the area exposed to 28,000 rep and subsequently treated with the ointment. In the corresponding untreated area necrosis did not begin until about the tenth day (Fig. 2,A). The necrosis in areas treated with the ointment ulcerated rapidly so that by the twelfth day these areas showed ulceration (Fig. 2,B). The changes in areas treated with the whole leaf lagged behind those treated with the ointment but occurred more rapidly than in the untreated areas. Epithelization in the areas treated with the ointment was macroscopically well advanced by the twentieth day but had not similarly advanced even by the thirtieth day in the untreated areas (Fig. 2, C and D). The effect of the fresh whole leaf was again intermediate. Complete healing was obtained by the ointment at the end of two months and was not obtained by the end of four months in either of the untreated areas exposed to the 14,000 and 28,000 rep (Fig. 3, A, B, C, and D). When examined four months after the beginning of the experiment, the unhealed, untreated areas still had small hard scabs in their centers surrounded by telangiectasia, while the areas treated with the ointment did not show either scabbing or telangiectatic vessels.

The increased rapidity of development and healing seen grossly after application of the ointment was also found histologically. At five days, when the first biopsy specimen was obtained, the untreated lesion was characterized by elongation of the epithelial cells along an axis parallel to the surface, vesiculation of the nuclei, formation of prominent chromatin particles, slight edema of the superficial dermis, and infiltration of the rete pegs by a few polymorphonuclear leukocytes. The treated lesion, in contrast, was much further advanced. In addition to these early cytological changes, there was sloughing of keratinized and necrotic epithelium, formation of blebs filled with leukocytic debris, marked edema of the superficial dermis, and a heavy infiltration by polymorphonuclear leukocytes, lymphocytes, and macrophages (Figs. 4, 5).

Ten days after exposure the untreated lesions showed polymorphonuclear leukocytes in a developing eschar over areas of epithelial necrosis and sloughing; however, the leukocytic reaction was not so pronounced as in the treated lesions at five days. The dermis of the untreated areas still had few leukocytes and there was little or no loss of the dermal collagen. The treated ten-day-old lesion was covered by a thick eschar composed of leukocytic and cellular debris and the upper layer of the

FIG. 3. Photographs of the backs of four rabbits fifty-eight days after exposure to beta radiation. Treatment was started as explained in the caption for Fig. 2. Figure 3, A is a continuation of the series in Fig. 2. In all instances the lesions in the upper right quadrant, treated with *Aloe vera* ointment, are either healed or further healed than the lesions in the other quadrants. Treatment was stopped after thirty days in the rabbits in Figs. 3, B and 3, D but was continued in the rabbits in 3, A and 3, C.

dermis was becoming involved in the eschar and was heavily infiltrated by leukocytes, lymphocytes, macrophages, and polyblasts. These cells were found in smaller numbers throughout the thickness of the dermis, and fibroplasia was occurring around the larger blood vessels in the depths of the dermis. A few active fibroblasts were found in the upper portions of the dermis also. In striking contrast to the untreated lesion, the epithelium of the margin of the treated lesion contained numerous basal cells in mitosis, was hyperplastic, and had fingers of new epithelium extending centrally.

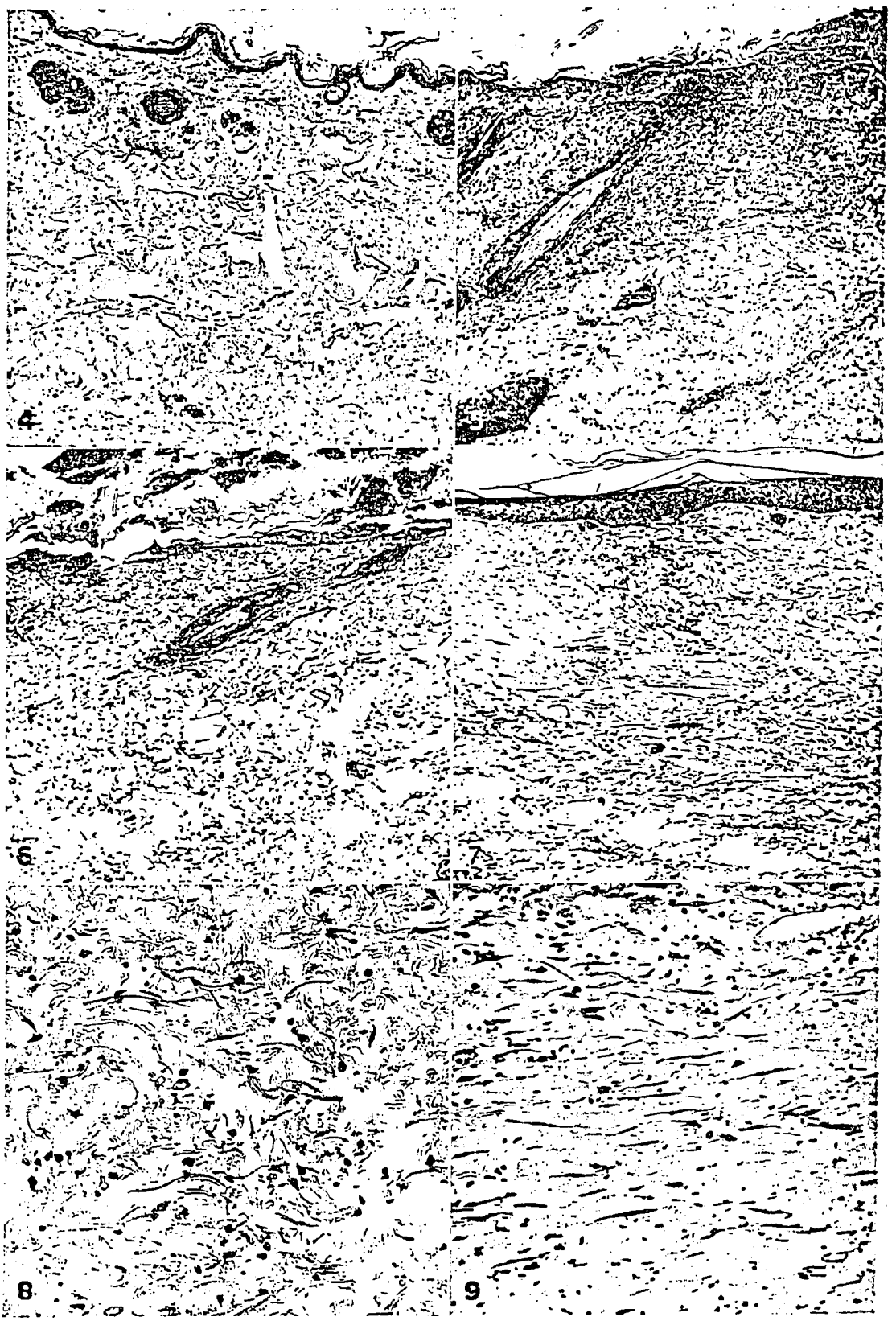
In the biopsy specimens obtained on the sixteenth day, the epithelization had continued to progress in the treated lesion but was still incomplete in the untreated one. Fibroblasts were present diffusely throughout the treated areas but only a few of these cells were found in the untreated ones. There was no new necrosis in the treated lesion and the majority of the eschar had been sloughed, while in the untreated lesion the eschar was continuing to be formed by new necrosis and by the splitting off of degenerating collagen from the dermis. These changes can be seen in Figs. 6 and 7.

Five days later, on the twenty-first day, these differences were still prominent. Re-epithelization in the untreated lesion had commenced but was minimal. In the treated lesion formation of fibrils and collagenous tissue in the depths of the dermis around the larger vessels had displaced vessels and fat cells toward the surface as previously described in rats,[22] but in the untreated lesion this process had not begun and the dermis was still characterized by a macrophage and polyblast infiltrate accompanied by a small number of fibroblasts. The contrast was more apparent at the next biopsy period (twenty-seven days) as shown in Figs. 8 and 9, since collagen production had continued in the treated lesion but still had not begun in the untreated one. By the thirty-second day epithelization was proceeding well in the untreated lesion but the epithelium was never found to be so hyperplastic as in the treated ones, which were now almost completely healed. The fibrous reaction in the untreated dermis was still not so diffuse nor so advanced as in the treated lesion by the sixteenth day. In one treated specimen in which the loss of tissue approached the panniculus carnosa, the connective tissue below this layer of muscle showed a large amount of active fibroplasia. Degenerative vascular changes with obliteration of small arterioles seemed to occur in about equal amounts in both the treated and untreated areas. These changes became evident beginning about the thirty-second day and were increasingly more pronounced by the time of the biopsy specimens on the thirty-seventh and forty-second days. These specimens continued to show the healing of the treated lesion and the slow but comparatively ineffectual healing of the untreated one.

Discussion

These experiments show objectively that *A. vera* has a remarkably curative effect upon radiodermatitis in the rabbit. It was found to increase greatly the development of the lesion by apparently doing away with the so-called latent period. Either as a result of earlier development of necrosis and ulceration, or from a specific effect upon the adjacent epithelium, re-epithelization occurred much earlier than usual and was more hyperplastic in character. The inhibition of fibroplasia was also overcome earlier than usual so that new connective tissue was produced throughout the dermis as re-epithelization was occurring. As a result of the enhancement of the healing processes, the damage to the original connective tissue seemed to be restricted and usually did not proceed so extensively as in the untreated lesions. While in occasional treated specimens what appeared to be new capillaries were seen, granulation tissue did not actually develop, and defects were obliterated by fibroplasia and contraction of the connective tissue. No histological explanation could be found for the absence of telangiectatic vessels in the healed treated lesions other than that the treated ulceration, being shallower, might not have led to the exposure and subsequent elevation to the surface of the larger vessels of the deep dermis. Degenerative vascular changes secondary to the radiation appeared to be the same with or without treatment. These experimentally observed beneficial alterations in the course of the radiodermatitis treated with *A. vera* would seem to substantiate firmly previous clinical experiences with this plant in the treatment of human radiodermatitis.

No information was gained from these experiments concerning the mechanism by which *A. vera* produced these changes. Experiments are now in progress to investigate whether the sequence of metabolic and chemical changes found in radiodermatitis are modified by treat-



(For captions see opposite page.)

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