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The Effect of Chronic Alcoholism on Wound Healing (41110)

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Abstract. This study showed that the initiation of granuloma formation in alcoholic mice was retarded but eventually reached normal levels. The delay appears to be related to a failure of cells to migrate into the sponge as rapidly as they did in control animals. Thus, there is clear evidence to support the clinical impression of poor wound healing in alcoholics. Although repair is eventually accomplished, the initial decrease in the rate of this process may be significant enough to put the alcoholic patient at risk.

Chronic alcoholism is one of the most significant and persistent health problems in the United States, and much effort has been spent studying the psychological causes and effects of alcoholism on various internal organs, especially the liver (1). However, very little research has been directed toward elucidating the biochemical mechanism underlying the clinically observed wound healing deficiencies in alcoholics (2–4). Controlled studies to quantify these clinical findings have not been initiated.

There is, however, biochemical evidence indicating that chronic use of alcohol might effect wound healing. The cellular processes associated with both tissue maintenance and repair may be altered by alcohol. Perin *et al.* (5) reported that the incorporation of amino acids into protein is depressed when liver slices are incubated in medium containing as little as 3 m*M* ethanol. This inhibition appears to result from elevated acetaldehyde concentrations, a product of ethanol metabolism, rather than from the direct action of ethanol. In addition, a specific decrease in the amount of rough endoplasmic reticulum and a corresponding increase in smooth endoplasmic reticulum occur in cells metabolizing ethanol (6, 7). Such a shift in the organelle complement is consistent with a decrease in the synthesis of secretory proteins (8, 9). In addition, defective protein export has been observed in livers of alcoholic animals (10). The synthesis, secretion, and subsequent organization of collagen plays an integral role in wound healing (11–13) and deficiencies in these processes would be expected to impair the healing process.

Poor tissue repair is an important consideration when deciding whether a particular surgical procedure should be attempted in an alcoholic patient, especially when considering an elective procedure. The latter situation often pertains since alcoholics generally suffer from periodontitis (3) which frequently is treated surgically. As in any surgical procedure, the successful outcome of periodontal surgery is largely dependent upon the ability of the individual to repair rapidly the involved tissue, an ability that appears to be reduced in the alcoholic.

These clinical and experimental observations prompted the present study which was designed to investigate the possible differences in collagen synthesis in healing wounds of normal and alcoholic mice. The accumulation of collagen in polyvinyl sponges implanted in mice consuming a 10% ethanol solution as their sole fluid source was monitored. There was a significant lag in the deposition of collagen inside the sponges during the early stages after implantation in the alcoholic animals. At later stages, the sponges from the alcoholic animals contained more collagen than those from control animals. These data suggest that alcohol initially retarded tissue repair but did not inhibit the eventual healing.

Materials and Methods. C57BL/Crgl male mice were obtained from Charles River at 2 months of age. The animals were divided into two groups: one received water, the other 10% ethanol as their sole liquid source. The animals were main-

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tained, 9–10 per cage (19 × 10 × 6 in.), on wood shavings, under a 12-hr light cycle (7 AM–7 PM) and were allowed to feed *ad lib* on standard Purina Laboratory Chow.

After the mice had been on this regime for 6 months, nine animals from each group were randomly selected and weighed. In addition, the fluid and food consumption of two randomly selected cages of animals (nine mice per cage) from each group were measured for 1 week.

In order to determine the breaking strength of the mouse skins, four control and four alcoholic animals without sponge implants were sacrificed by cervical dislocation. The skins were shaved with clippers and then removed. Adhering fatty subcutaneous tissue was removed by sharp dissection. Each skin was cut, using a scalpel and brass template, into four to six hour-glass-shaped strips to ensure that failure would occur in an area of known width (5 mm). The whole skin was used in order to obtain four to six samples per animal. The strips were oriented rostrocaudally. Skin thickness was determined with a spring-loaded micrometer. The skin was bathed frequently with distilled water while the breaking strength was determined (14, 15). Results were computed in terms of force versus cross-sectional area in which the break occurred.

To study collagen accumulation, four polyvinyl sponges were implanted subcutaneously in each mouse. The discoid sponges (7 mm diameter by 4 mm depth) were sterilized by boiling in 0.9% NaCl for 30 min. The animals were anesthetized with ether, the incision site was washed with ethanol and shaved with clippers. The sponges were implanted aseptically and placed symmetrically under the dorsal skin through a single incision approximately 2 cm long. The skin wound was closed with 9-mm wound clips. All animals were returned to their established fluid sources. A few animals that developed infections were eliminated from the study.

At 5, 10, 20, 30, or 43 days after implantation, the animals were sacrificed and the sponges were removed. A minimum of 16 and a maximum of 60 sponges were used for each time point. The surrounding connective tissue capsules were not removed. Each sponge was placed in 1 ml of 5% trichloroacetic acid (TCA) and brought to 90° for 30 minutes. The sponges were squeezed to remove excess TCA, then placed in 1% sodium dodecyl sulfate (SDS) in 0.1 *N* sodium hydroxide and heated to 90° for 15 min. The sponges were removed from the SDS and squeezed to express the solvent.

Samples from the TCA and SDS extracts were combined and assayed for protein (16). An aliquot of the combined extract was hydrolyzed in 6 *N* HCl for 24 hr at 105°, evaporated to dryness, and the hydroxyproline content was determined (17).

In addition, some sponges were removed and immediately placed in 10% neutral-buffered Formalin. These sponges were dehydrated in ascending concentrations of ethanol, cleared, embedded in paraffin, and sectioned at 6 μm either tangent to the long axis of the sponge or in cross-section through the middle of the sponge. The sections were stained with hematoxylin and eosin. One sponge per animal was used for light microscopic examination. Two independent observers rated the sponges for cell infiltration and collagen deposition. The rating was based on an arbitrary scale from +1/2 to +4. The slides were number coded and arranged randomly to ensure an unbiased evaluation.

Results. Alcoholic mice ate 10% less food than control mice (Table I) but this decrease in food consumption was not sufficient to cause malnourishment (18). In addition, the mice imbibing alcohol drank significantly less (Table I) but the animals did not appear to be affected. The alcohol in their water served as a calorie source for these animals and even after being maintained on this diet for 6 months, the alcoholic mice had the same average weight as did the control animals (Table I).

The tensile strengths of the skins from normal and alcoholic mice were the same (Table II). The thickness of the alcoholic mice skins was not statistically different from that of control mice (Table II).

The total protein in the sponge extracts for both alcoholic and normal mice is shown in Fig. 1. The content was the same

TABLE I. FOOD, WATER, AND ETHANOL INTAKE OF CONTROL AND ALCOHOLIC MICE

	Control	Alcoholic	*P*
Weight (g)	32.5 ± 0.6	33.1 ± 1.6	N.S.
Fluid consumption (ml/mouse/day)	4.2 ± 0.8	3.6 ± 0.6	<0.05
Ethanol ingested (g/kg/day)	—	8.7 ± 1.2	—
Food (g/kg/day)	100.9 ± 11.8	87.0 ± 8.2	<0.01

Note. After the mice had been maintained, either on water or 10% ethanol for 6 months, 18 mice from each group were weighed. Then the fluid and food consumptions of three cages, each containing 9 mice, from each group were monitored over 7 days. The data represent the average daily intake per mouse.

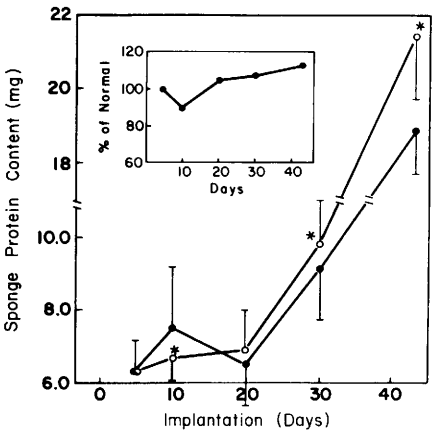

FIG. 1. Total protein content of sponges from normal and alcoholic mice. Sponges were removed from the animals at the times indicated and extracted with TCA and NaOH dissolved in SDS. Aliquots of both extracts were assayed for protein. Each point represents the mean of at least three sponges per animal and a minimum of four animals per group (control mice ●; alcoholic mice ○). Brackets represent one SEM. An * indicates values which were statistically different from controls ($P < 0.05$). The inset shows the protein content of sponges from alcoholic mice as a percent of the corresponding value from the normal mice.

for both groups of animals except 10 and 43 days after implantation. At the earlier time, the sponges from control animals had significantly more protein than those from alcoholic mice. Conversely, at the later time, sponges from alcoholic mice contained significantly more protein than those from control animals. The inset shows the results from the alcoholic mice as a percentage of those from the control.

Hydroxyproline content of the sponges was determined and used to estimate collagen deposition. The hydroxyproline content of the sponges in both groups of animals increased from a minimum after 5 days to a maximum after 30 days of implantation (Fig. 2). By the time the sponges had been implanted for 43 days, their hydroxyproline content had declined indicating that the granulomas had entered the remodeling phase.

Although the pattern of granuloma formation was similar for both groups of mice, the rates of hydroxyproline accumulation were not the same (Table III). Between the 5th and 10th days of implantation, hydroxyproline accumulated in granuloma tissue from alcoholic mice at only 68% of the rate that it did in tissue from normal mice. This resulted in a significantly ($P < 0.05$) lower hydroxyproline content in sponges from alcoholic mice 10 days following implantation (Fig. 2). During the next 10-day period the accumulation rate in the sponges

TABLE II. THICKNESS AND TENSILE STRENGTH OF SKIN[a]

	Thickness (mm)			Tensile strength (g/mm²)		
	Normal	Alcoholic	*P*	Normal	Alcoholic	*P*
	0.351	0.343		175	187	
	0.368	0.251		128	224	
	0.306	0.323		234	158	
	0.363	0.295		194	186	
Average ± SD	0.347 ± 0.028	0.303 ± 0.040	N.S.	183 ± 44	189 ± 27	N.S.

[a] Each tensile strength value represents one animal and is an average computed from four to six trials per animal. Tensile strength was calculated by measuring the breaking strength of strips of skin of known width (5 mm) and thickness and dividing that breaking strength by the product of the width and thickness. Thickness was measured with a spring-loaded micrometer. Each value represents one animal and is an average of four to five samples of skin per animal.

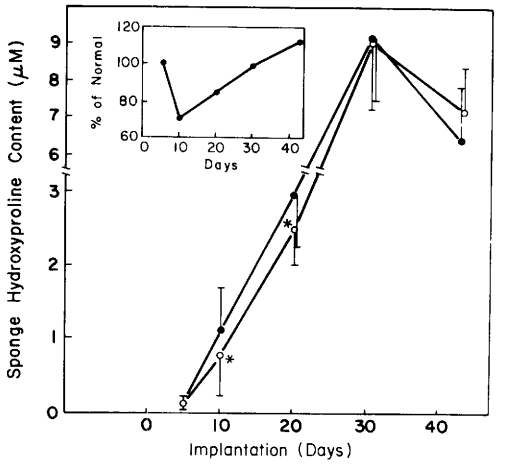

FIG. 2. Total hydroxyproline content of sponges from normal and alcoholic mice. Aliquots of the extracts, prepared as described—Fig. 1, were hydrolyzed in 6 *N* HCl at 105° for 24 hr, dried, and then assayed for hydroxyproline content. Each point represents the mean of at least three sponges per animal and a minimum of four animals per group (control mice ●; alcoholic mice ○). Brackets represent one SEM. An * indicates values which were statistically different from controls ($P < 0.05$). The inset shows the hydroxyproline content of sponges from the alcoholic animals as a percentage of the content of the sponges from the normal animals.

from both groups were very similar but the total hydroxyproline content of sponges from alcoholic mice remained significantly less than in sponges from control mice after 20 days of implantation. In the following 10 days, the rate of hydroxyproline accumulation was higher in sponges from alcoholic mice than normal mice and at the end of this period (30 days) there was no difference in the hydroxyproline content of the sponges from the two groups of animals. The inset in Fig. 2 shows the results from the alcoholic mice as a percentage of those from the control.

Observations made by microscopic examination of sponges removed 10 and 30 days after implantation confirmed the biochemical results. Ten days following implantation the sponges were surrounded by a thin connective tissue capsule that was present in both groups but more pronounced in sponges from control animals. Occasional loci of an acute inflammatory cell infiltrate were observed in all capsules.

The amount and arrangement of connective tissue within the sponges significantly differed between groups. Sponges removed from alcoholic animals included many areas that were devoid of cells. The connective tissue that was present within these sponges consisted of infrequently spaced fibroblasts, and a delicate, irregularly arranged fibrillar network.

To a greater extent, the interstices of sponges removed at 10 days from normal animals were filled with connective tissue that was both more cellular and fibrillar than that of alcoholic animals. When rated by two independent observers for cell infiltration and collagen content, the 10-day postimplantation sponges from the normal animals received an average rating of +2½ whereas those from the alcoholic animals had an average of +1. These differences can be best appreciated by examining low-magnification photographs (Fig. 3) which give an overall impression of the amount of cellular infiltration.

By 30 days, sponges from both groups were filled with a loose connective tissue that contained a well-developed microvascular network. Sponges from both groups received an average rating of +3. However,

TABLE III. RATE OF HYDROXYPROLINE ACCUMULATION IN POLYVINYL SPONGES

	Normal (μmole/sponge/period)	Alcoholic (μmole/sponge/period)
0–5 days	0.11[a]	0.11
5–10 days	1.00	0.68
10–20 days	1.87	1.74
20–30 days	6.19	6.49
30–43 days	−2.69	−1.77

[a] The change in sponge mean hydroxyproline content during the periods indicated. The data were generated from the same numbers used in Fig. 2.

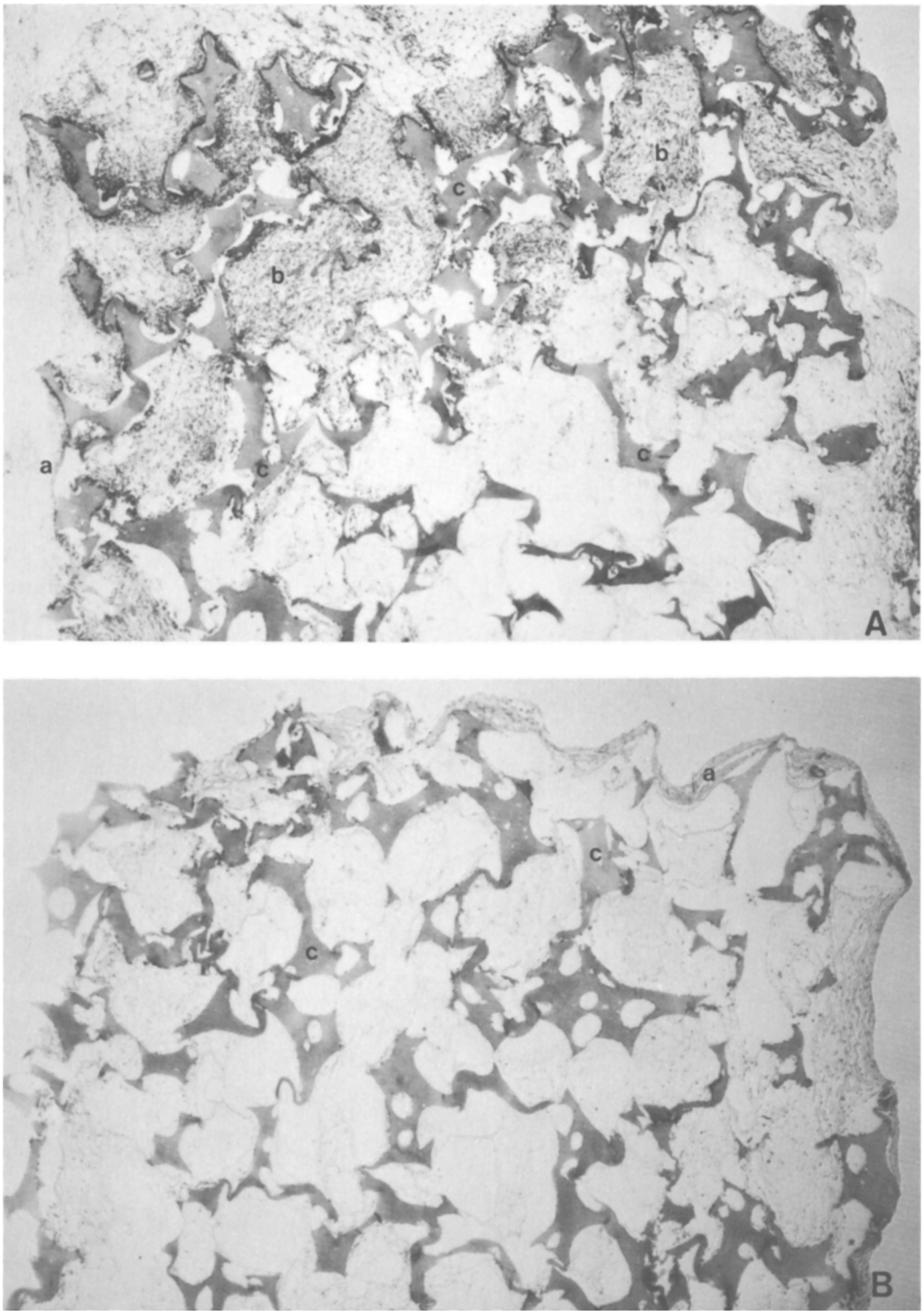

FIG. 3. Sponges from normal and alcoholic mice 10 days after implantation: (A) control; (B) alcoholic. (a) connective tissue capsule, (b) connective tissue, (c) sponge matrix (30×).

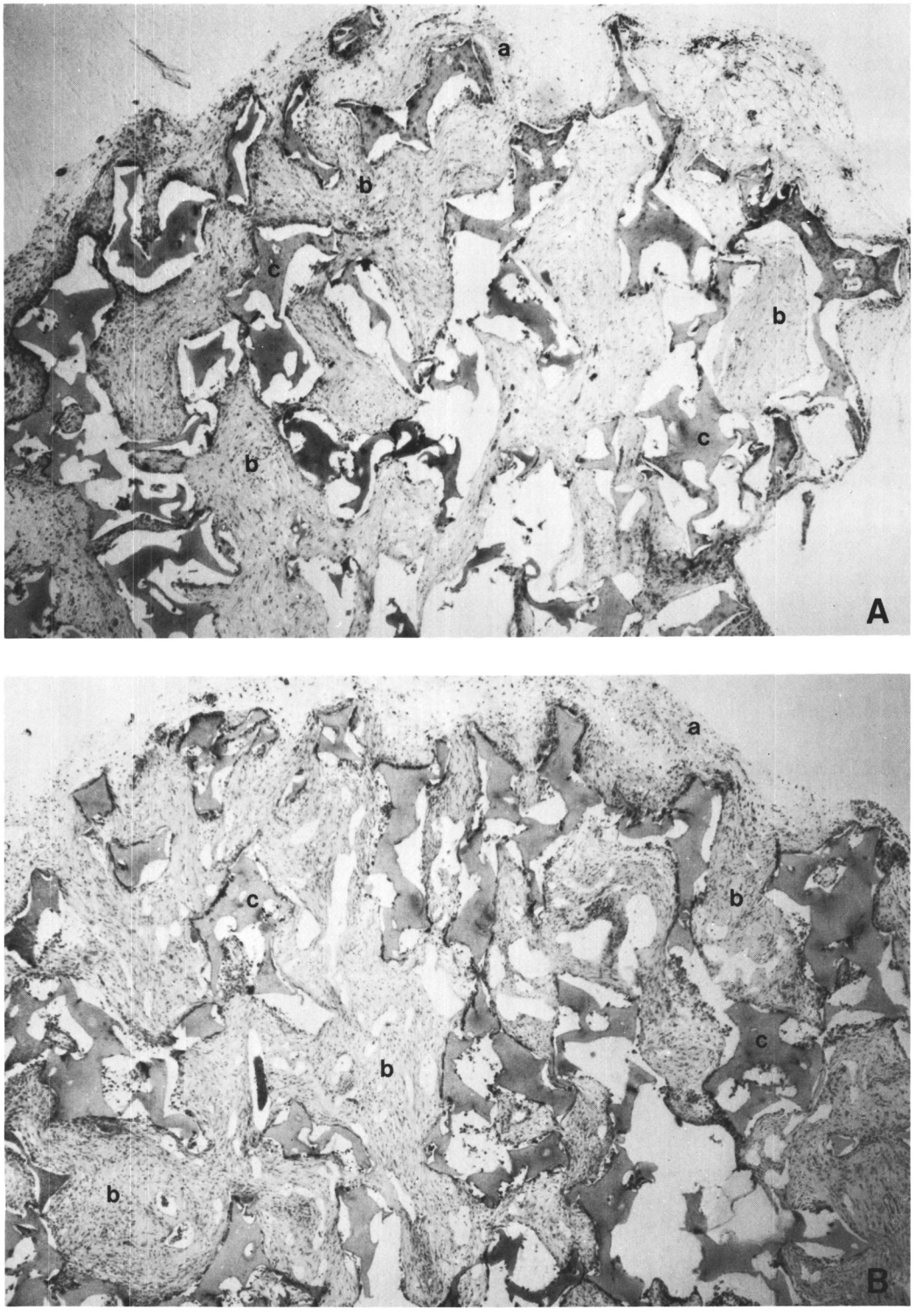

FIG. 4. Sponges from normal and alcoholic mice 30 days after implantation: (A) control; (B) alcoholic (30×).

in some instances more connective tissue was observed in sponges from the alcoholic animals. The photographs in Fig. 4 demonstrate the similarities between sponges from both groups of animals and the vast increase in the amount of cellular infiltration.

Discussion. This study has taken advantage of the C57BL/Crgl mouse which will consume a 10% ethanol solution as their sole liquid source (19) in order to establish an animal population which was alcoholic for more than 50% of its life, a period paralleling that usually experienced by alcoholic humans. The healthy nutritional status of these animals made them ideal for directly studying the effect of alcohol on their physiology without the influence of the nutritional deficit that often accompanies alcoholism in humans. Such a deficiency has been thought to cause a number of physical symptoms and would have severely complicated interpretation of the data.

For example, it has been suggested that alcoholics have, on the average, a greater number of skin lacerations than nonalcoholics. No decreases in the skin thickness, tensile strength, or breaking strength of the skin from alcoholic mice were observed suggesting that skin does not become more fragile as a direct result of alcoholism. Rather, human alcoholics may suffer from a dietary deficiency of ascorbic acid which could easily decrease skin strength.

In contrast, chronic alcohol intake did directly influence the wound healing process. Granuloma formation in imbedded polyvinyl sponges was chosen as a model for wound healing since there are similar metabolic stages in the two processes (20). In addition Valjanto (21) has shown that the deposition of collagen in an implanted sponge correlates directly with the tensile strength of an incised wound.

Biochemical and microscopic examination of the sponges clearly showed that the initial stages of granuloma formation were retarded in alcoholic mice. There was a significant retardation of cell migration into the sponges which was paralleled by a lower content of protein and collagen. Once past this initial lag in cell migration, cells do move into the sponges as evidenced by the rapid rate of synthesis and deposition of collagen. By 30 days after implantation, no apparent differences in either cellular or collagen content could be discerned.

It is interesting to note that a similar defect in collagen accumulation has been observed in sponge-induced granuloma of obese mice (22). These mice serve as an animal model for adult diabetes, another condition that is associated with deficient wound healing. Unfortunately, the granuloma development was followed for only 21 days and it is not known whether the obese mice would ever overcome the initial lag in collagen deposition.

As demonstrated by the decreased collagen content, the granuloma from all animals were in the remodeling stage at the last time point examined, 43 days. It is interesting to note that these data suggest a trend ($P < 0.1$) toward a higher collagen content and therefore a slower rate of remodeling in the tissue from the alcoholic mice. The significance of this trend is reinforced by the observation that the total protein content of the 43-day granuloma tissue is definitely elevated in alcoholic mice.

An alteration in either of the two processes involved in remodeling, the rates of collagen synthesis or degradation, could have caused the decreased rate of remodeling observed in granuloma tissue from alcoholic animals. The elevated rate of collagen deposition seen just prior to 30 days suggests that the synthetic rate may be abnormally high even after this time. It would be of extreme interest to determine whether the difference observed at 43 days is maintained throughout the remodeling phase. If this were the case, then one might infer that every time an alcoholic repaired tissue damage, following an initial lag in collagen deposition, a slightly higher than normal quantity of collagen would ultimately be deposited.

Support for this hypothesis is found in another animal study. Orrego *et al.* (24) have shown that the fibrotic reaction produced by insertion of a surgical suture through the liver parenchyme of rats is clearly elevated in animals on a chronic alcohol diet. In this context, it is interesting

to note that two pathological conditions often observed in alcoholics, liver fibrosis (1) and rhinophyma (2, 23), involve excessive collagen deposition.

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