Wound Healing in Normal and Diabetic Chinese Hamsters

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Summary. Wound healing was examined in normal and diabetic, non-ketotic Chinese hamsters by morphological and morphometric methods. Dermal, perforating wounds were made in the ears of the hamsters and the response to injury was evaluated in tissue biopsies. The response in normal hamsters was characterized by vascular and cellular migration and pronounced infiltration of polymorphonuclear leukocytes into the area closest to the wound (zone 1). The transition region (zone 2) between wounded and non-wounded tissue was infiltrated primarily by fibroblasts and capillaries. In wounds from diabetic hamsters, 8 h after injury, there was less cellular infiltration (fibroblasts 49%, polymorphonuclear leukoytes 48% of control) and vascular proliferation (47% of control). In the late phase of healing (16 h after injury) the vascular (87% of control) and polymorphonuclear leukocyte (103%) responses in diabetic wounds were not significantly different from control in zones 1 and 2. Wounds from diabetic hamsters also showed considerable oedema (143% of control) in zones 1 and 2, which was accompanied by vascular degeneration and necrosis. At 16 h the collagen content of diabetic wounds was also decreased (54% of control). Increased oedema with reduced vascular proliferation and cellular infiltration in the early healing period characterises the response to injury in the diabetic Chinese hamster.

Key words: Wound healing, spontaneous diabetes, Chinese hamster, oedema, injury, morphometry.

The diabetic syndrome in the Chinese hamster, *Cricetulus griseus,* results from a homozygous recessive trait [1, 2] and is characterised by hyperphagia [3] and early onset of hyperglycaemia and glycosuria [4].

Strains are classifed as non-diabetic (no glycosuria and no diabetic litter mates for 10–15 generations), non-ketotic diabetic (> 2 g/100 ml glycosuria without ketonuria) and ketotic diabetic (> 2 g/100 ml glycosuria and ketonuria). Morphological studies have revealed pathological changes and lesions consistent with the diabetic state, such as abnormal glycogen deposits in the retina [5], pancreatic islets [6], kidney tubules [5] and lesions in the aortic intima [7]. The progression of the syndrome is accompanied by decreased plasma and pancreatic insulin [8], ultimately leading to an increased mortality among the more severly diabetic animals. The Chinese hamster offers an optimally suitable test animal to examine wound healing in spontaneous insulin dependent diabetes.

Diabetic alloxan-C 57 B 1/6 mice have been shown to have an impaired healing response characterised by increased oedema and decreased cellular and vascular proliferation into the wound [9]. The morphological changes were reversed after insulin treatment of the diabetic mice [10]. These studies in mice suggest that the effect of insulin on growth, thus modulating the cellular and vascular events in wound healing, may be suppressed in diabetes and further, that decreased insulin levels in diabetes may be a cause of the impaired healing response. The present study was designed to examine this postulate in the spontaneously diabetic Chinese hamster.

Materials and Methods

Experimental Animals

Thirty Chinese hamsters (17 normal and 13 diabetic) were studied. Adult male or female diabetic and non-diabetic siblings of the same sex, age and body weight were generously supplied by the Upjohn Colony (Kalamazoo, Michigan) through the courtesy of Dr. G. Gerritsen. The animals were divided into two groups according

Table 1. Fasting and non-fasting blood glucose, body weight, urine glucose and ketone levels of control and diabetic Chinese hamsters

	Blood Glucose (mmol/l)		Weight (g)	Urinary glucose (mmol/l)	Urinary ketones
	fasting	non-fasting			
Control ($n = 17$)	4.94 ±0.06	10.17 ±0.44	29.7 ±0.9	0	0
Non-ketotic diabetic ($n = 13$)	12.67 ±1.22[a]	20.44 ±0.61[a]	29.7 ±0.8	>111	0

Urinary glucose and ketone levels were assessed with Ketostix and Diastix. Values are expressed as mean ± SEM.
[a] Significantly greater ($p < 0.001$) than corresponding value for control hamsters
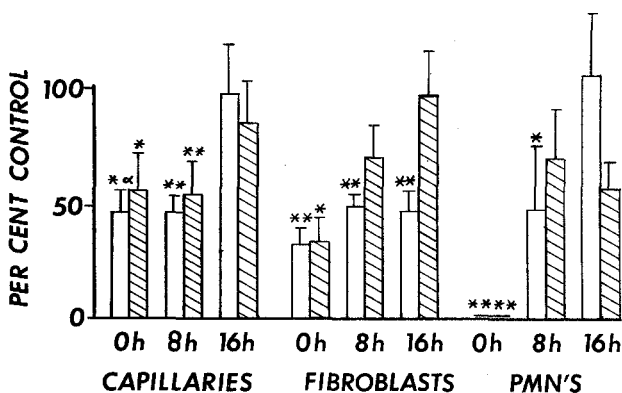

Fig. 1. Vascular and cellular response to injury in wounds from diabetic Chinese hamsters. Percentage wound volume occupied by vascular and cellular components (capillaries, fibroblasts, polymorphonuclear leucocytes, PMN). Values are expressed as percentage of control. * $p < 0.05$; ** $p < 0.01$ compared with control hamsters. ☐ = Zone 1 ▨ = Zone 2
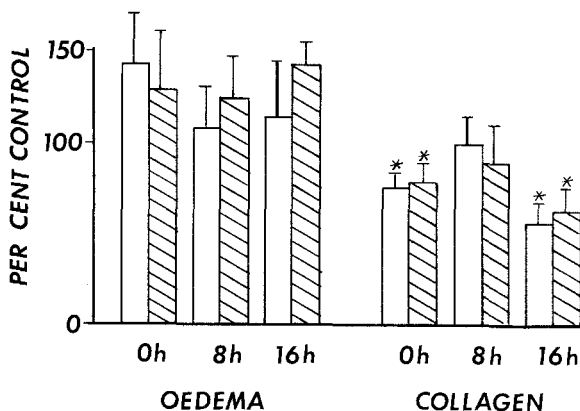

Fig. 2. Response of extracellular components to injury in wounds from diabetic Chinese hamsters. Percentage wound volume occupied by collagen and oedema fluid. Values are expressed as percentage of control. * significantly different from control $p < 0.05$. ☐ = Zone 1 ▨ = Zone 2

to blood glucose, urine glucose and urine ketone levels. Group 1: normal hamsters ($n = 17$), 8–15 months of age, consistently aglycosuric and derived from aglycosuric lines for the past 10–15 generations. Group 2: non-ketotic diabetic hamsters ($n = 13$) 9–12 months of age, non-ketonuric with consistent hyperglycaemia and >2 g/100 ml glycosuria. The animals were housed individually and fed freely on Purina mouse breeder pellets (lipid content 12%) and water. Fasting blood glucose was determined (autoanalyser-Hoffman method) on each animal at the Upjohn colony before shipping, and non-fasting blood glucose was determined (YSI glucose analyser, Yellow Springs, Ohio) before wounding on samples obtained by orbital puncture. Urinary glucose and ketones were determined using Ketostix and Diastix (Ames, Elkart, Indiana) (Table 1). The animals were matched according to weight and divided into experimental groups before wounding.

Wounding of Experimental Animals

Before wounding the hamsters were lightly anaesthetised with ether. Each ear was then sponged with 70% (v/v) ethanol and placed over a light source to locate the vascular bed. Care was exercised to avoid damage to any major vessel during the wounding process. Two circular, perforating wounds were made in each ear with a 1.0 mm dermal trephine. The wounds were biopsied 0 (time of injury), 2, 4, 8 and 16 h later and processed for light and electron microscopy as previously described [9]. Morphological analyses of the wounds from normal and diabetic Chinese hamsters were made on samples taken from a pool of 300 blocks from the 30 experimental animals (five blocks per animal).

Morphometry

The relative volumes occupied by the major cellular and extracellular components present in the wounds from the 0, 8 and 16 h biopsies were quantitated by a modification of the methods of Weibel et al. [11] and Wahl et al. [12]. A 10 mm square reticle with 1.0 mm line intervals was inserted into the eyepiece of an American Optical series 110 microscope and structures lying under the points of intersection were counted. Half-micron tissue sections were cut on a DuPont-Sorvall MT2-B ultratome, stained with methylene blue-Azure II and examined under oil at a magnification of ×1200. The wound area quantitated was divided into two zones; zone 1: 100 µm area of wound including the wound edge and zone 2: transition zone 100 µm wide from zone 1 to uninjured tissue. The tissue components quantitated were capillaries, polymorphonuclear leukocytes, fibroblasts, oedema and collagen. Oedema was defined as that area devoid of visible tissue components but within the defined zones. Fifteen slides from 50 different tissue blocks were randomly selected and quantitated per time point in a double-blind design and the mean ± SEM was determined by a multiple group T-test (Tukey's test).

Results

Morphometric Studies

The vascular and cellular response to injury is illustrated in Table 2 and Figure 1. In the diabetic, capillary volume was significantly less than the control at the time of injury in both zones 1 and 2. This volume remained decreased up to 8 h after injury. By 16 h, the capillary response approached control levels. Similar

Table 2. Percentage volume occupied by wound components from control and diabetic Chinese hamsters

Time		Capillaries (%)	Fibroblasts (%)	Polymorpho-nuclear leukocytes (%)	Oedema (%)	Collagen (%)
0 h						
Zone 1	Control	6.5 ± 2.8[a]	9.7 ± 1.1[b]	0.5 ± 0.32[b]	23.7 ± 3.6	44.6 ± 3.1[a]
	Diabetic	3.1 ± 0.8	3.1 ± 0.8	0.0 ± 0.0	33.8 ± 6.5	32.8 ± 3.2
Zone 2	Control	7.5 ± 1.6[a]	10.9 ± 2.3[a]	0.4 ± 0.22[b]	21.8 ± 2.6	45.1 ± 3.2[a]
	Diabetic	4.3 ± 1.4	3.8 ± 0.9	0.0 ± 0.0	27.7 ± 6.4	34.5 ± 3.6
8 h						
Zone 1	Control	5.9 ± 0.9[b]	8.3 ± 1.2[b]	7.4 ± 2.9[a]	24.1 ± 4.3	33.1 ± 2.9
	Diabetic	2.8 ± 0.4	4.1 ± 0.4	3.5 ± 2.1	25.8 ± 5.6	33.6 ± 4.5
Zone 2	Control	7.0 ± 1.1[b]	6.5 ± 0.9	2.6 ± 1.2	30.3 ± 4.5	28.3 ± 3.4
	Diabetic	3.9 ± 0.9	4.6 ± 1.0	1.8 ± 1.7	37.3 ± 7.5	24.8 ± 5.6
16 h						
Zone 1	Control	5.4 ± 0.4	6.9 ± 1.4[b]	9.6 ± 2.5	18.6 ± 7.6	37.8 ± 5.0[a]
	Diabetic	7.1 ± 1.5	3.2 ± 0.5	11.8 ± 2.4	21.1 ± 5.7	20.4 ± 4.7
Zone 2	Control	5.1 ± 2.2	5.3 ± 0.8	3.5 ± 1.1	25.3 ± 9.3	41.3 ± 7.0[a]
	Diabetic	4.4 ± 0.9	5.8 ± 0.9	2.0 ± 0.4	36.2 ± 2.9	26.2 ± 4.9

Results expressed as mean values ± SEM.   [a] $p < 0.05$ diabetic versus control;   [b] $p < 0.01$ diabetic versus control.

observations were made on examination of the cellular response. Fibroblasts were significantly decreased in zones 1 and 2 of wounds in the diabetic hamsters at injury when compared with controls and remained lowered up to 8 h after injury. At 16 h, there was an increase in fibroblasts in zone 2, most distant from the injury, but no change in zone 1, the immediate site of injury.

In the diabetic hamsters, polymorphonuclear leukocytes were not detectable at injury, but increased during the first 8 h. However, leukocytes in zone 1 of diabetic wounds at 8 h were significantly less than observed in zone 1 of control wounds. In the adjacent area (zone 2) more leukocytes were detected at 8 h. By 16 h more leukocytes were present in both zones.

The volumes occupied by oedema fluid and collagen in the wound are shown in Figure 2. The wounds from diabetic hamsters at the time of injury showed more oedema than the controls and oedema volume remained elevated in the diabetic wounds up to 8 h after wounding. In contrast, the volume of collagen in the diabetic wounds was significantly ($p < 0.05$) reduced at injury when compared with control. A slight increase was observed at 8 h, but by 16 h the value was still significantly below control. Overall, collagen was therefore reduced in the diabetic animals and did not respond to the injury stimulus.

Morphological Studies

Normal and Diabetic Hamsters. At the time of wounding (0 h) skin biopsies from control hamsters revealed a dense supporting network of collagen surrounded by capillaries, macrophages, fibroblasts and small nerve bundles (Fig. 3a). By contrast, biopsies from diabetic hamsters at 0 h had much extracellular oedema surrounding dermal connective tissue which was comprised of few cellular elements (capillaries, fibroblasts, macrophages) and collagen (Fig. 3b).

Within 4 h after injury, the control wounds were heavily infiltrated with polymorphonuclear leukocytes, fibroblasts and macrophages. In many of the fibroblasts the cytoplasmic organelles were organised with a central Golgi zone surrounded by mitochondria and rough endoplasmic reticulum (Fig. 4a).

In diabetic wounds at 4 h the most striking change was in the capillaries where degenerative changes were marked by the appearance of myelin figures in the nucleus, and disruption of the capillary wall. Similarly many fibroblasts contained cytoplasmic irregularities in the form of dense bodies and large clusters of lysosomes (Fig. 4b), features not compatible with protein secreting cells. A decrease in polymorphonuclear leukocytes, fibroblasts and macrophages at 8 h after injury was accompanied by a increase in tissue oedema.

By 16 h, oedema was the striking feature in diabetic wounds, with many disrupted and degenerated cellular elements observed around the dissolving capillary structures (Fig. 5b). Wounds from diabetic hamsters did not contain any immature capillary structures at any of the time points examined.

Cellular infiltration and collagen density were the prominent feature at 8 h after injury in wounds from
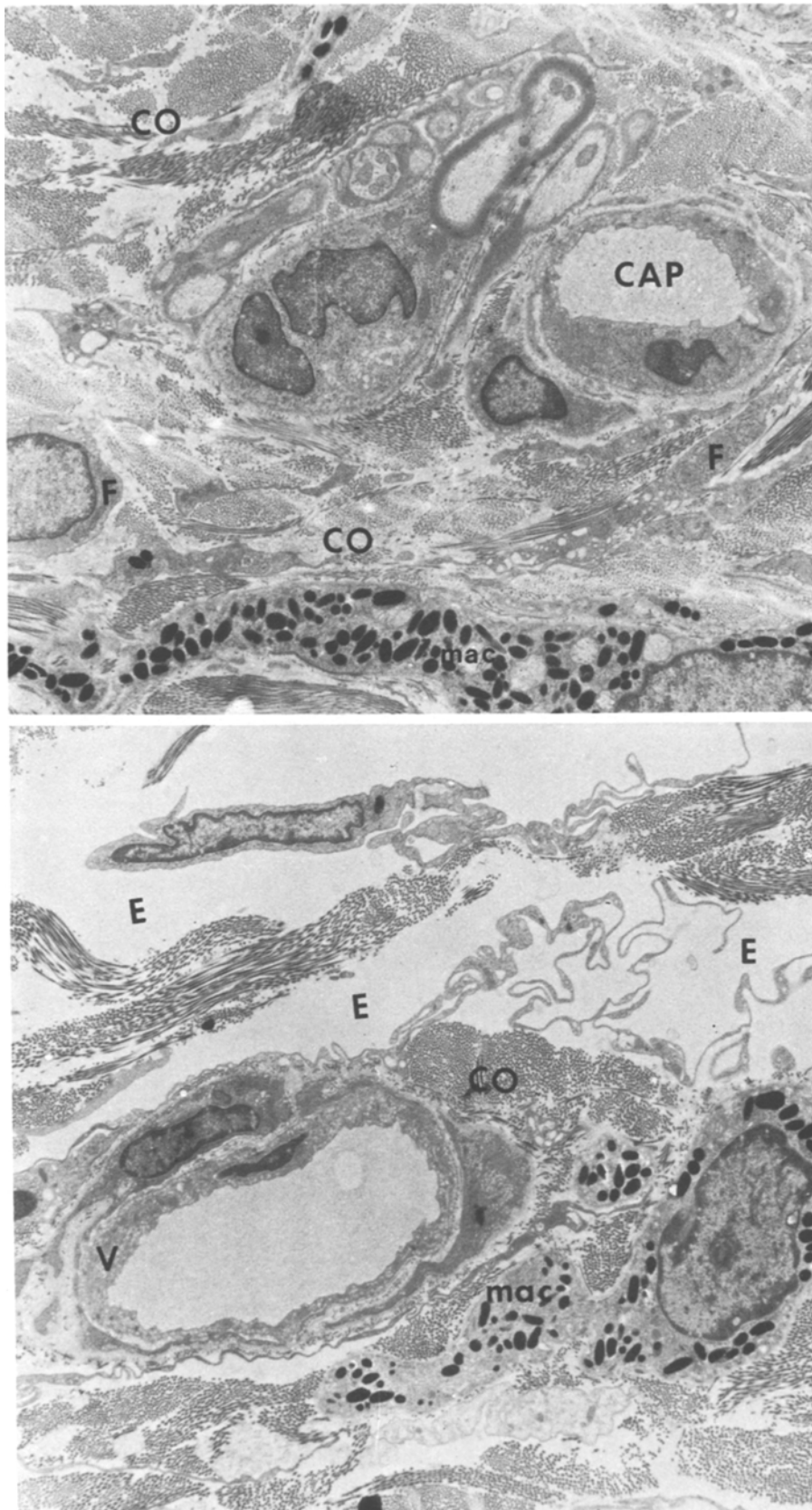
Fig. 3. a Wound edge from a control hamster at the time of injury (0 h). Dermal tissue contains collagen *(CO)*, capillary *(CAP)*, macrophage *(mac)* and fibroblast *(F)*. × *8500*. **b** Wound edge from a diabetic hamster at injury (0 h). Dermal tissue contains small venule *(V)*, macrophage *(mac)*, collagen *(CO)* and large area of interstitial oedema *(E)*. × *8000*
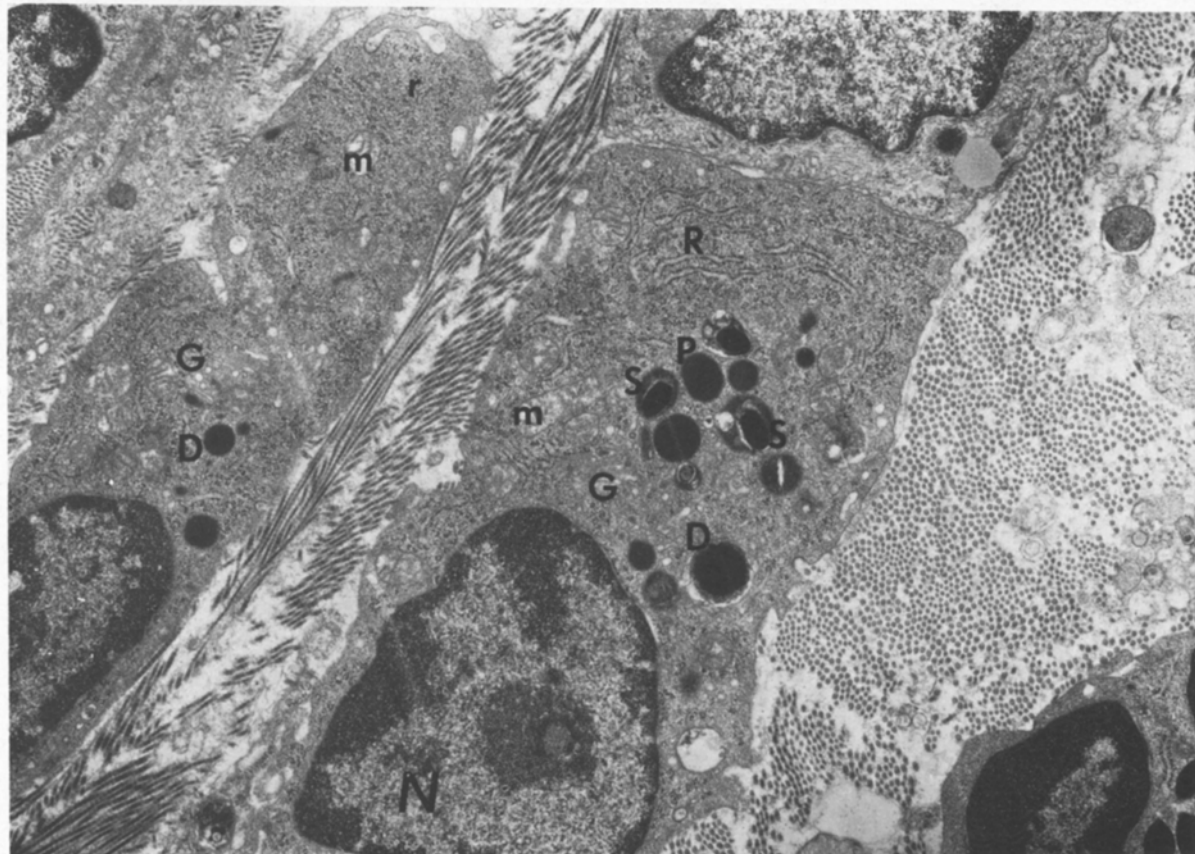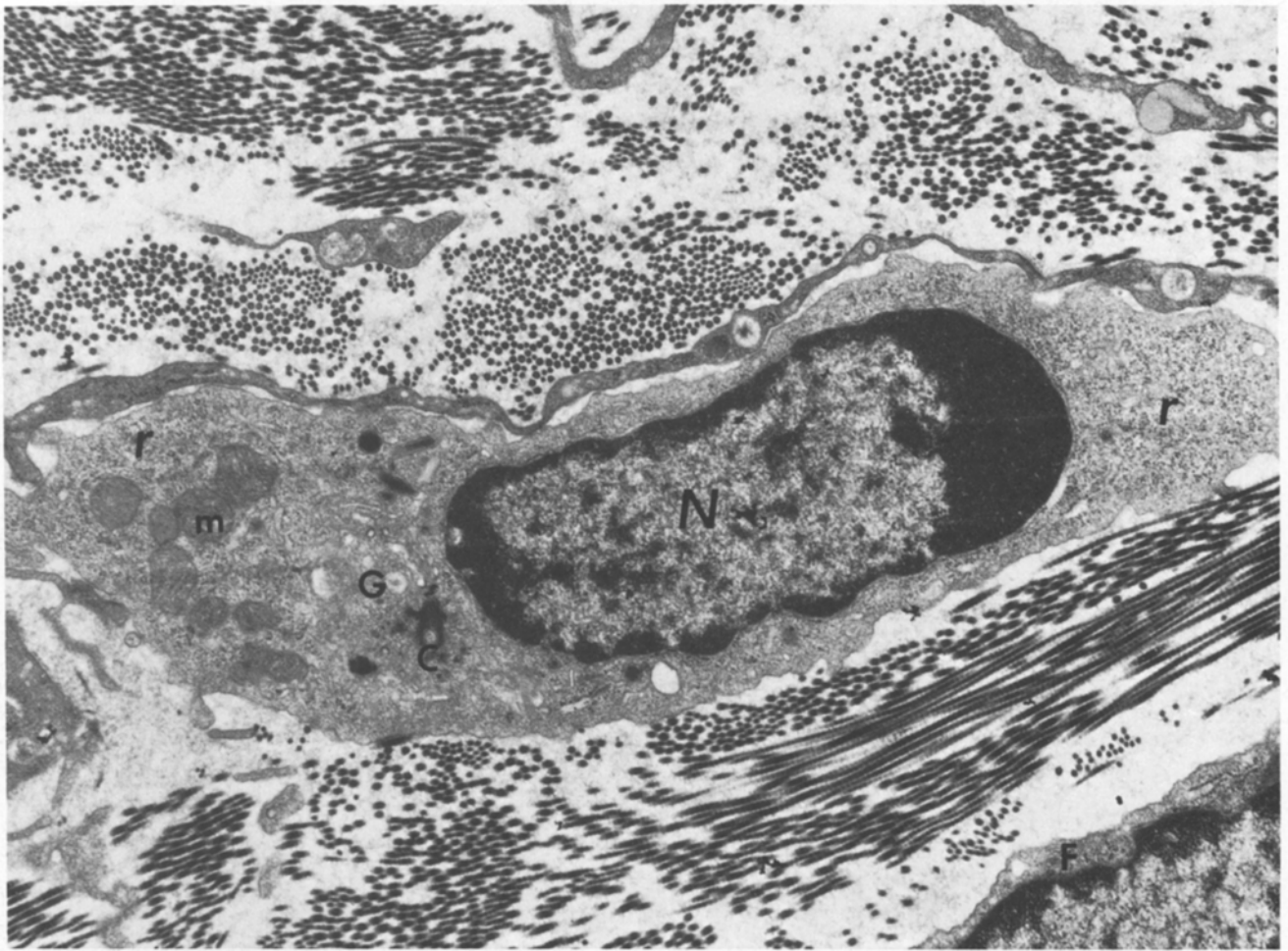
Fig. 4. a Fibroblast observed in the wound edge from a control hamster 4 h after injury. Cytoplasm contains mitochondria *(m)*, free ribosomes *(r)*, Golgi material *(G)* capping the nucleus *(N)*, centriole *(C)*, and rough endoplasmic reticulum. Sections of adjacent fibroblasts *(F)* are seen. × 10,700. **b** Fibroblasts in the wound from a diabetic hamster 4 h after injury. Cytoplasm contains free ribosomes *(r)*, rough endoplasmic reticulum *(R)*, mitochondria *(m)*, Golgi *(G)*, and dense bodies *(D)* associated with primary *(P)* and secondary *(S)* lysosomes. Nucleus *(N)*. × *11,750*
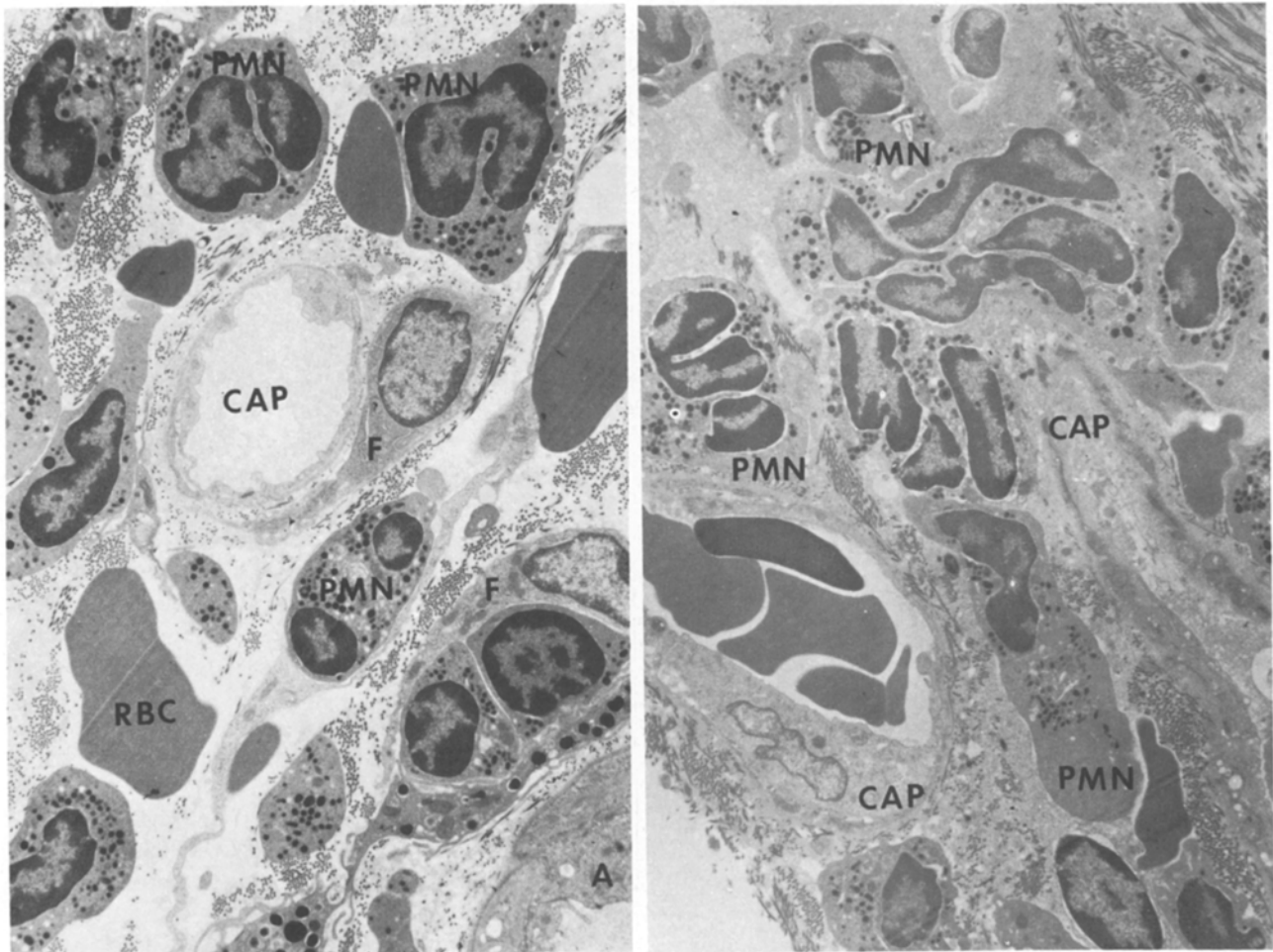
Fig. 5. a Wound from a control hamster 16 h after injury. Dermal tissue is infiltrated with polymorphonuclear leucocytes *(PMN)*, fibroblasts *(F)*, erythrocytes *(RBC)* and vascular elements, arteriole *(A)* and capillary *(CAP)*. × 5,770. b Wound from a diabetic hamster 16 h after injury. Degenerating capillaries *(CAP)* are surrounded by polymorphonuclear leukocytes *(PMN)* in various stages of degeneration. × 6,160

control hamsters. Fibroblasts, polymorphonuclear leukoytes, macrophages and capillaries were found within the collagen framework. At 16 h, polymorphonuclear leukocytes were clustered at the wound edge in close proximity to the vascular bed (Fig. 5a). Many of these capillaries were immature in appearance as defined by irregularity of endothelial cell borders and organisation of cytoplasmic organelles [13, 14]. These "immature" capillaries were frequently observed in both zones 1 and 2 of the control wounds.

Discussion

This study presents an evaluation of wound healing in normal and diabetic Chinese hamsters. The wound model employed consisted of a small self-limiting wound, in which the wound edge and tissues immediately encircling the area of injury were examined. This is in contrast to other wound models in which relatively large penetrating incisions or implanted artificial chambers are used. The benefit of this model is the ability to examine the sequence of events in the early phase of healing, particularly the initial responses and interactions of the vascular, cellular and extracellular components of the wound.

The ultrastructural appearance of the capillaries in healing wounds from normal and diabetic hamsters was evaluated at 0, 8 and 16 h after injury. In wounds in normal hamsters, minimal changes were observed in the venules 4–8 h after injury consistent with acute vascular injury [15–18]. Some swelling of the endothelial cells produced a slight increase in vascular permeability probably leading to the mild oedema. There were no alterations in the cytoplasmic organelles of these endothelial cells. By 8 and 16 h, ingrowth of new capillaries appeared through sprouts from the surviving vascular border. Differentiation in

the type and size of these vessels was determined by the blood flow through their lumen [19] and at this stage they possessed the cytoplasmic characteristics of immature capillaries.

On the other hand, wounds from diabetic hamsters had no proliferating capillaries at any time after injury. Many of the mature capilliaries were observed in various states of degeneration 8 and 16 h after injury. Injury to the capillaries was irreversible and was marked by stasis, disruption of endothelial cells and cell junctions, fracturing of basement membrane and finally cell necrosis. Such pronounced vascular injury presumably contributed to the oedema of the diabetic wounds, since there was disruption of both capillaries and venules. Thus not only fluids and plasma proteins but also cellular elements found access to the extracellular space, resulting in an increase of red blood cells and polymorphonuclear leukocytes in the 16 h wounds.

The increased interstitial oedema in the wounds from diabetic hamsters found at the time of injury suggests that accumulation of extracellular fluid may be part of the diabetic syndrome in the Chinese hamster, most likely in response to hyperglycaemia. Evidence to date supports the hypothesis that hyperglycaemia may be associated with sorbitol accumulation and tissue swelling in the lens of the eye, peripheral nerves [20] and smooth muscle and endothelial cells of blood vessels [21]. Swelling of vessel wall components may then contribute to increased vascular permeability followed by loss of fluid into the extracellular space producing more oedema. Although oedema in the tissues of diabetics has not been measured directly, it is clearly a function of increased vascular permeability and plasma volume shifts due to extravasated proteins. The interaction of vascular fragility and permeability and oedema in the impaired response to injury in diabetic tissues is of considerable interest.

It is noteworthy that in some tissues, most notably the cornea and retina, the presence of oedema stimulates a neovascular response which is probably mediated by a diffusable substance in oedema fluid [22]. Increased oedema in wound tissue of diabetic hamsters failed to induce any detectable neovascular response. In fact, by 16 h after injury, many capillaries and venules had undergone extensive degeneration and necrosis. The endothelial cells appeared to be unresponsive to extracellular vasculogenic factors, perhaps due to lack of receptors for these factors, decreased insulin levels (growth effect of insulin), or persistent hyperglycaemia. In the diabetic wounds an increased plasma shift to the extracellular space may stress the compromised vasculature leading to degeneration and necrosis.

In addition to capillaries and leukocytes, fibroblasts were observed by electron microscopy in the wounds from normal hamsters. No significant increase in fibroblasts was observed consistently in the wounds during the healing period, although the ultrastructural appearance of these cells changed over the 16 h. Many fibroblasts became hypertrophic with an increase and distinct organization of cytoplasmic organelles suggesting protein synthesis and secretion. By contrast, the fibroblasts in healing wounds from diabetic hamsters contained irregularities in the cytoplasm (lysosomes and dense bodies), which are indicative of cells undergoing internal degeneration. This was probably in response to an altered extracellular environment, for example, high glucose, oedema, and low insulin. The appearance of the fibroblasts concomitant with the decrease in collagen in diabetic wounds suggested that the fibroblasts were not responding to injury as observed in control hamsters, but instead were undergoing progressive degeneration. This has been confirmed in other studies where the fibroblast was compromised and unable to synthesise collagen [23–25] and from reports examining the activity of fibroblasts *in vitro* from diabetic patients [26]. In every instance there was an impaired response to injury with decreased collagen synthesis *in vivo* and *in vitro* and shorter life span *in vitro* [27]. The milieu of the diabetic environment which affects the fibroblasts morphologically and functionally remains to be defined.

The impaired healing response to injury in the spontaneously diabetic Chinese hamster is characterised by a reduction in the vascular, cellular and fibrous elements of wound healing accompanied by increased interstitial oedema. The syndrome in the Chinese hamster shares a common feature with human insulin dependent diabetes, that is, insulinopaenia. Decreased insulin levels not only affect glucose metabolism but probably also suppress a permissive effect of insulin on growth which may lead to impaired wound healing. The results of this study support the hypothesis that insulin is a necessary component for wound healing.

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