The mussel plasma does not contain fibrinogen, and incubation of a mixture of plasma and thrombin at 37 °C was not seen to produce clots even after 1 h. Absence of fibrinogen in the plasma confirms that the mussel is not in possession of a blood clotting mechanism comparable to vertebrates.

The mussel plasma also does not contain any human agglutinins, since it was found unable to agglutinate washed human red blood cells of A, B and O groups.

Résumé. Le sang de *Lamellidens corrianus* est alcalin et incolore. Il contient peu de pigment respiratoire et de proteines. Le plasma contient peu de glucose et pas de fibrogène ou d'agglutinines humaines. Une activité des enzymes amylase, GOT et des phosphatases a été constaté.

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The Effect of Alloxan Diabetes on Skin Collagen Metabolism

The effects of alloxan induced diabetes on impaired wound healing was studied using granulation and skin tissue in the attempt to elucidate possible alterations in the metabolism of collagen. Although it is well recognized that diabetes results in defects in various connective and vascular tissues[1,2], the results reported here suggest that in the rate of formation and maturation of collagen may not be a significant factor.

Materials and methods. Diabetes was induced in 2 groups of male Sprague-Dawley rats weighing 40–50 g and 100–125 g by alloxan injection (120 mg/kg). Alloxan was administered either i.p. (40–50 g rats)[3] or by i.v.-injection (100–125 g rats)[4]. The presence of diabetes was indicated by marked glucosuria (> 0.5%), increased fluid intake and loss of body weight. Rats were maintained on a commercial stock diet through out the experimental period.

The effect of treatment on amino acid uptake by granulation and skin tissue was determined using a sponge implantation technique (40–50 g rats)[5] and the in vitro skin biopsy method of UITTO (100–125 g rats)[6]. In the implantation studies, polyvinyl sponges $1.5 \times 0.5 \times 0.3$ cm) were inserted in the subcutaneous space along the dorsum 10 days after initiation of alloxan treatment. The sponges remained 6 days before removal and decapitation. The sponges were then cut into small pieces and incubated in phosphate-free Krebs-Ringer solution (3 ml, pH 7.6) containing 20 m*M* N-2-hydroxyethylpiperazine-N^1-2-ethane sulphonic acid (HEPES), 20 m*M* glucose and 0.5 μCi U-^{14}C-lysine[6]. Incubations were carried out for 12 h at 37 °C in a metabolic shaker. After incubation, the sponges were completed, homogenized and aliquots were taken for counting and DNA analysis[7]. Dorsal skin biopsies (1 cm diameter, 100–150 mg) were taken 20 days after alloxan injection (100–125 g rats). The tissue was minced and incubated in the Krebs-HEPES solution (10 h, 37 °C) except U-^{14}C-proline was added at 0.1 μCi in place of radiolysine. The uptake of radioproline was linear for at least 18 h (Figure 1). Both tissues and sponges were dialyzed exhausively before radioactivity was determined by scintillation counting.

The diameters of wounds after biopsy were also measured daily until complete closure as an indirect index of wound healing (Figure 2). At the end of this period, a second biopsy was taken and total hydroxproline determined[8]. Urinary hydroxyproline was monitored at various time intervals with rats receiving alloxan injection. In addition, skin samples were taken from these animals and collagen solubility in neutral NaCl solution (1 *M*) was determined[9]. The extracted collagen was purified, electrophoresed on acrylamide gel, and the ratio of monomer (α) to dimer (β) collagen subunits was calculated after staining with amido black and scanning with a densitometer[10].

Results. The uptake of radiolysine and radioproline as cpm into granulation tissue from sponge implants and skin biopsy material is indicated in Tables I and II, respectively. In both cases, alloxan treatment stimulated

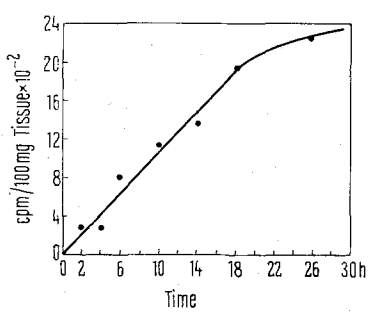

Fig. 1. The uptake of U-C^{14}-proline into rat skin. Radioproline was present at p. 1 μCi in Krebs-HEPES solution (3 mls)[7].

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the incorporation of the amino acids tested, although not significantly. The incorporation of proline or lysine was not taken as a direct measure of collagen synthesis, but the fact that incorporation into tissue was not decreased with alloxan treatment indicated at least unimpaired potential for synthesis. Total collagen as measured by the amount of hydroxyproline per mg of skin tissue was not altered after alloxan treatment (Table II).

The times required for wound closure from biopsies are given in Figure 2. No differences were noted between groups, although the wounds of alloxan-treated rats appeared more inflamed throughout the healing process and skin thickness was reduced. Alloxan treatment had no affect on collagen solubility or the apparent degree of crosslinking as measured by the ratio of α to β collagens (Table III). Likewise hydroxyproline excretion rates were not significantly altered (Table IV).

Discussion. Insulin appears to accelerate primary wound healing. Conversely, diabetes often results in decreased vascularization and retarded wound healing[1]. Defective collagen metabolism at least during the onset of alloxan diabetes does not appear a significant factor. Of particular significance is the observation the urinary hydroxyproline levels, collagen solubility and subunit distribution are not altered significantly with treatment. A decrease in salt soluble collagen sometimes indicates rapid degradation of newly formed collagen, and an increase often represents impaired maturation[11, 12].

The suggestion that the rate of mature collagen fibril formation in granulation tissue from alloxin animals is the same of control rats has also been offered by CATANZARO-GUIMARAES[13], who used histochemical techniques. With respect to skin, the studies reported here also collaborate such findings.

Table I. Effect of alloxan on the uptake of U-^{14}C-lysine into sponge implant granulation tissue

Treatment	cpm/sponge	µg DNA/sponge	cpm/µg DNA
Alloxan	4338±1370	583±123	7.40±2.1
Control	3156±1156	708±150	4.46±1.21

Average of 5 samples ± S.E.M.

Table II. Effect of alloxan on the uptake of U-^{14}C-proline and the hydroxyproline content of skin biopsies

Treatment	Days[a]	cpm/100 mg of tissue	mg HyPro/100 mg of tissue
Alloxan	20	1428±256	—
	45	—	4.4±0.3
Control	20	1170±178	—
	45	—	4.0±0.2

[a] Days after initial alloxan injection.

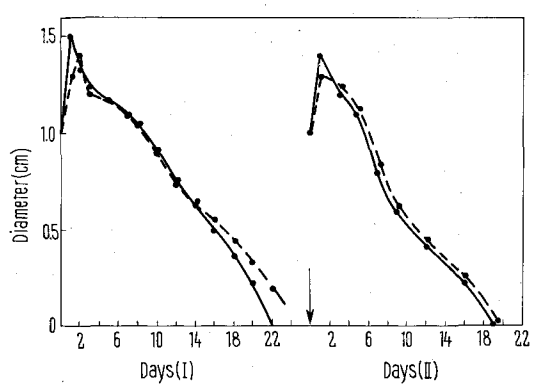

Fig. 2. Diameters of open wound areas after biopsy of dorsum skin. Biopsies were taken 20 and 45 days after the initial alloxan treatment. Blood glucose levels at 30 days were > 200 mg/100 ml, alloxan and < 100 mg/100 ml, controls. Each point represents the average from 4–6 rats (alloxan, broken line; control, solid line).

Table III. The effect of alloxan on the solubility and distribution of α and β collagens in NaCl extracts

Treatment	mg Hypro extracted/100 mg tissue	α/β Ratio
Alloxan	0.33±0.07	2.0±0.2
Control	0.34±0.06	1.8±0.2

Average of 6 samples ± S.E.M.

Table IV. The Effect of alloxan on the excretion of hydroxyproline

Treatment	Days[a]	µg Hypro excreted/day
Alloxan	25	361±32
	33	374±25
Control	25	406±27
	33	410±25

[a] Days after initial s.c. injection. Average of 5 samples.

Résumé. On a étudié chez des rats rendus diabétiques par l'alloxane le métabolisme du collagène de la peau et la guérison des blessures. Le métabolisme du collagène fut évalué par la lysine et la proline dans des biopsies de la peau, par la solubilité du collagène, par l'excrétion journalière de l'hydroxyproline, et par le degré apparent de «crosslinking» du collagène. Chez les diabétiques, on observe souvent une dépression et une vascularisation de la peau, mais la vitesse de guérison des blessures et de formation des fibrilles du collagène a paru normale.

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