Histometric Determination of Collagen Fibers in Granulating Wounds of Alloxan Diabetic Rats

The present study was designed to reveal that under the metabolical effect of diabetes the collagen fibres deposition decreases in experimental wounds, and the simple method which is utilized to achieve the results might be used as a rational tool for future investigations.

Materials and methods. Thirty male albino rats of Wistar strain weighing an average of 200 g (range 180–220 g) on the day of operation were utilized in this experiment. Diabetes was produced in animals after they had been fasted for 48 h with 200 mg of alloxan (Merck)/kg body weight administered i.p. After receiving alloxan the blood sugar levels were daily determined by the method of HASLEWOOD and STROOKMAN[1]. Only rats with blood sugar levels greater than 300 mg% were considered diabetic. The normal range of blood sugar levels in this strain was 65–120 mg%. Under ether anaesthesia the animals were shaved and wounded by excising a 2×2 cm of dorsal skin. After the establishment of hemostasia, the wounds were left open. At the sixth, eighth, tenth, twelfth and fourteenth days, they were operated again in order to obtain small pieces of granulation tissue. The pieces were excised from the periphery of the wound, fixed in 10% formol, embedded and cut in paraffin and stained with hematoxylineosin and Masson's trichrome.

The histometric analysis of granulation tissue sections of equal thickness consists of determinating the number of collagen fibres stained by Masson's trichrome. The utilized process was that of CHALKLEY[2] using a Zeiss integrating eyepiece I ($\times$ 8).

The statistical analysis was performed through the establishment of regression lines between the number of hits and periods of wounding. Tests for linearity and for regression lines were then made. The 2 regression lines were subsequently compared for slope and intersection. All tests were made at a 5% level.

Results. The histometric counting of collagen fibres in granulating wounds of diabetes and control rats were analyzed by a variance technique. In Tables I and II the means and individual values for both groups are shown under the appropriate time period. The basis for the variance analysis consisted of each time period. The results for means and individual values are represented by a diagram (Figure). The calculation of regression for control groups gives the equation (a). In Table III, the test of linearity for control groups is presented. This test was shown not to be significant at 5% level. In the same way, the calculation of regression for diabetes groups gives the equation (b). The test of linearity, presented in Table IV, was also shown not to be significant at 5% level.

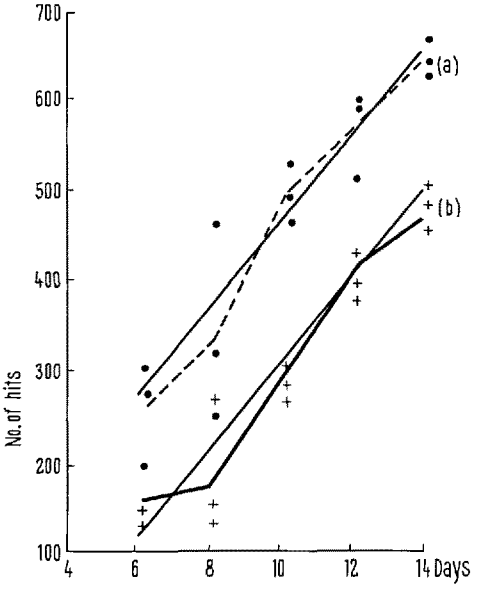
The difference between the regression coefficient was not statistically significant.

Table I. Histometric analysis of the collagen fibres proportion in granulating wounds of control groups. (Hits/eyepiece position)

Days	Control groups			Total	Means
6	276	304	205	785	$X = 261.66$
8	464	322	245	1031	$X = 343.66$
10	543	498	475	1516	$X = 505.33$
12	537	597	601	1735	$X = 578.33$
14	652	648	671	1971	$X = 657.00$
				Total 7038	

Table II. Histometric analysis of the collagen fibres proportion in wound healing of alloxan diabetic groups (hits/eyepiece position)

Days	Diabetic groups			Total	Means
6	135	167	146	448	$X = 149.33$
8	150	149	261	560	$X = 186.66$
10	290	301	285	876	$X = 292.00$
12	401	406	429	1236	$X = 418.00$
14	474	498	514	1486	$X = 495.33$
				Total 4606	



Histometric analysis of collagen fibres in granulating wounds (means, individual values and repression lines) of control and diabetic groups. (a) $\overline{Y}x = 469.20 + 45.71\ (x - 10)$; (b) $\overline{Y}x = 307.06 + 45.86\ (x - 10)$. • Individual values for control groups, + individual values for diabetic groups.

Table III. Test of linearity for the control group

Source of variation	Sum of square	Degree of freedom	Mean square
Within groups	35,159.00	10	3,515.90
Regression	250,728.49	1	
about regression	71,211.51	3	23,737.17
Total	321,940.00	4	

$F_{(10;3)}$ observed $= 0.148 < F_{(10;3)}\ (\alpha = 5\%) = 8.79$ (N.S.).

[1] G. A. D. HASLEWOOD and T. H. STROOKMAN, Biochem. J. *33*, 920 (1939).
[2] W. H. CHALKLEY, J. natn. Cancer Inst. *4*, 47 (1943/44).

On the other hand, there was a statistically significant difference between the α coefficients ($P < 0.005$). The analysis permitted computation of the overall histometric variation of collagen fibres, the histometric variation within the groups and the histometric variation between the groups.

Discussion. A simplified semiquantitative histological method was used to study the relative frequency of collagen fibres in wound healing of alloxan diabetic rats.

Table IV. Test of linearity for the alloxan diabetic group

Source of variation	Sum of square	Degree of freedom	Mean square
Within groups	10,208.00	10	1,020.80
Regression	252,376.75	1	
about regression	5,798.25	3	1,932.75
Total	258,175.00	4	

$F_{(10;3)}$ observed $= 0.528 < F_{(10;3)}$ ($\alpha = 5\%$) $= 8.79$ (N.S.).

The values obtained for the rate of fibroplasia, have been proved to increase continuously in a linear fashion for both groups. On the other hand, the contents of total mature collagen fibres decreased throughout healing development in alloxan diabetic rats when compared to control animals. In the same way, the relative frequency of collagen fibres in an appropriate period of time seemed to be more reduced in diabetes than in control groups. On the other hand, the calculation for testing the difference between the regression coefficient showed that for each simple period the gross increase in collagen fibres was the same for both groups.

Résumé. La fréquence de la fibrillogenèse collagène dans le tissu de bourgeonnement augmente continuellement et sous une forme linéaire pour les groupes de contrôle et diabétique. D'autre part, le contenu de fibrilles de collagène dans une période de temps déterminée, semble plus réduite dans le groupe diabétique que dans le groupe de contrôle.

S. A. Catanzaro-Guimaraes

Department of Oral Pathology, University of São Paulo, School of Dentistry, Bauru (São Paulo, Brazil),
9 May 1968.

Immunofluorescent Technique and Long Acting Thyroid Stimulator in Human Serum

Recently Blum et al.[1] have developed a rapid, highly sensitive assay for the detection of long acting thyroid stimulator (LATS), in human serum based on the immunofluorescent technique of Coons[2]. These authors have incubated guinea-pig thyroid sections first with human serum and then with fluorescein-labelled rabbit anti-human IgG γ-globulin. They have considered that a positive reaction, shown by a cytoplasmic fluorescence, was characteristic of the presence in the serum of an IgG γ-globulin with a particular affinity for the thyroid cell cytoplasm. They have established a correlation based on clinical and experimental grounds between a positive staining and the presence of LATS, detected by the McKenzie technique. They have differentiated this cytoplasmic reaction from the fluorescence due to the complement fixing antibody by the persistence of the staining after fixation of the tissues before assay, either in ethyl or methyl alcohol, fixation which destroys in human thyroids the complement fixing antigen[3].

The present study reports analyses performed on the sera of 22 thyrotoxic patients and 23 patients suffering from atrophic asymptomatic thyroiditis[4]. LATS was detected by the slightly modified McKenzie bioassay[5] and the complement fixing antibody by the indirect fluorescent Coons test[6]. The selected sera of thyrotoxic patients gave a bright cytoplasmic fluorescence with the Coons technique and displayed positive results in the LATS bioassay. The sera of the patients with atrophic thyroiditis were positive for the fluorescent detection of complement fixing antibody; in this latter group, no LATS assay was performed, but, in a previous work, we found only 4 positive reactions on 46 cases[7]. Thin sections (4–5 μ) of thyrotoxic human thyroid were fixed in methanol at 56 °C for 3 min, then incubated with the serum for 30 min, and afterwards for the same time with fluorescein labelled rabbit antihuman IgG γ-globulin[8]. Each incubation was followed by a washing with phosphate buffer at pH 7.2. The tissues were examined by UV-microscopy.

Neither in thyrotoxic nor in asymptomatic atrophic thyroiditis serum could any cytoplasmic fluorescence be detected.

A correlation has been established in thyrotoxic sera between the presence of LATS detected by bioassay and that of a positive cytoplasmic fluorescent reaction with unfixed human thyroid sections. However, a lot of sera showed diverging results when tested by both methods[9]. These data suggest that these 2 techniques do not detect the same antibody in the serum.

The disappearance of any cytoplasmic fluorescence after methanol fixation of the tissues agrees with the data

1 A. S. Blum, F. S. Greenspan, J. H. Hargadine and J. M. Lowenstein, Metabolism *16*, 960 (1967).
2 A. H. Coons and M. H. Kaplan, J. exp. Med. *91*, 1 (1950).
3 I. M. Roitt, N. R. Ling, D. Doniach and K. G. Couchman, Immunology *7*, 375 (1964).
4 P. A. Bastenie, P. Neve, M. Bonnyns, L. Vanhaelst and P. Chailly, Lancet *1*, 915 (1967).
5 M. Bonnyns, Revue fr. Étud. clin. biol., in press (1968).
6 E. J. Holborow, P. C. Brown, I. M. Roitt and D. Doniach, Br. J. exp. Path. *40*, 583 (1959).
7 P. A. Bastenie, M. Bonnyns and L. Vanhaelst, in *Proceedings of Thyrotoxicosis Symposium* (Ed. W. J. Irvine; Livingstone, London 1967), p. 40.
8 B. M. Balfour, D. Doniach, I. M. Roitt and K. G. Couchman, Br. J. exp. Path. *42*, 307 (1961).
9 M. Bonnyns and L. Vanhaelst, J. Endocr., in press (1968).