

Biochemical study of collagen formation in healing wounds under the effect of Serotonin (5-Hydroxytryptamine).*

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Introduction :

THE gap in a wound is filled by a process of repair. Every stage of process of wound healing is intimately concerned with normal structure and chemical composition of the wound contents (Dunphy, 1956).

The process of repair of wound healing has been divided into the various biochemical phases.

Many factors have been reported to influence the wound healing as measured in the form of collagen (Hydroxyproline) content of the repairing tissues.

Bendit in 1955 extracted serotonin from mast cells.

Erspamer (1954) reported that serotonin originates in serum during coagulation, but not much work has been reported on the role of serotonin on healing process.

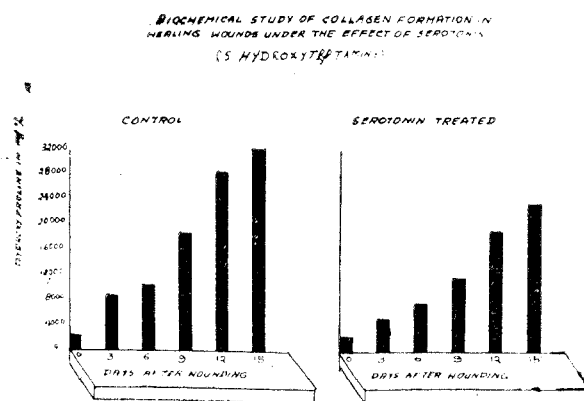
Aims and objects :

The present study was aimed at studying the effect of serotonin on collagen content (measured as hydroxyproline) in albino

rats at various stages of wound healing. Since the healing of wounds in different members of the animal kingdom are common to all its members and collagen provides structural support (Harkness, 1961) to all organs and tissues and because hydroxyproline is the specific component of the collagen. It was measured quantitatively as hydroxyproline by calorimetric method.

Methods and Material :

Healing of normal wounds was studied in a set of albino rats weighing between



GRAPH Showing Collagen Content in healing wounds (Control and Serotonin treated groups) on different days of wound healing.

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100—200 gms. The animals were divided into two groups. One consisting of control and the other of serotonin treated animals. Serotonin was injected intraperitoneally into rats at the various stages of wound healing and the wound area excised on 0 day, 3rd day, 6th day, 9th day, 12th day and 15th day. The tissue was hydrolysed in 4-NHCL for 3 hours in boiling water bath at 105°C according to the method of Forscher, 1957. The hydrolysate was neutralised and then the oxidized solution was first put in boiling water and then in ice bath. Sulphuric acid was added and colour developed with 5% Erhlic's reagent. Solution was compared with standard and hydroxyproline content of unknown sample was estimated with the use of junior Colman photo Spectrometer by the method of Neuman and Logan (1950) and Axeford (1953).

Review of Literature :

Dunphy and Udupa in 1955 reported chemical and histochemical sequences in normal healing of wounds. These workers studied normal healing of wounds in healthy rats. According to these workers the early part of wound healing (precollagen stage lasting 4-5 days) is marked by precipitation of precollagen precipitated by mast cells. Precollagen starts maturing from 5th day onwards till the completion of healing process (collagen phase starts at about the 5th day). It has been reported that between 24-48 hrs capillary loops sprout from damaged blood vessels and fibroblasts start proliferating along these loops of blood vessels (Sandison, 1928 and Stearns, 1940).

In the earliest phase, wound is invaded

by polymorph—lymphocytes, plasma cells endothelial cells and mast cells (Irvine, 1965). Fibroblasts lay down the intercellular matrix (immature collagen). Mast cells are (Railey, 1953-1959) present in large numbers in the connective tissue and are related to the maturation of collagen which is stimulated by Heparin, Histamine and Serotonin.

Effect of serotonin on collagen content of healing wounds has been discussed in this series.

Collagen contains many aminoacids characteristically hydroxyproline and Hydroxylysine, the latter in a tissue is therefore index of the collagen content of the tissue.

Serotonin is a neurohormone and naturally occurring amine (Lewis, 1964). Serotonin is present in platelets and mast cells of vascular endothelial lining. It is a vasoconstrictor involved in the mechanism of haemostasis by the local regulation of vascular tone. It is believed to act as an anti diuretic hormone. It regulates motor and secretory activity of GIT and it inhibits the gastric secretion (Leslie, 1968). Serotonin when injected locally causes oedema (Bendit, 1955).

Observations and Discussion :

A study of the observations listed in this review lead to one obvious conclusion, that the whole phenomenon of wounds, as observed in a mammal like rat is a complex one. It is quite evident therefore that effect of various factors on wound healing must be noted and discussed in an attempt at the explanation of wound healing.

For the first 3-6 days after causing wound the amount of measurable collagen formed was very little. The wound margins fell apart when the suture were removed in both groups of animals (control as well as serotonin group). The initial stage corresponds to the lag phase (Udupa, 1955).

Following the initial phase collagen content of the wounds increased between 6-12th day from 10.4 gm% to 28.5gm% in the control group, while it increased from 7.5gm% —19.0 gm% in serotonin treated group (difference of 2.9 gm% at the sixth day and 9.5 gm% on the 12th day). Collagen formation in the later group being very slow.

The third phase showed very little collagen formation between the 12th and 15th day. Collagen formation was 9.5 gm% less in Serotonin treated rates. Collagen increased only by 4.3gm% between 12th and 15th day in Serotonin group. This was related to the maturation of the scar.

In this series wounds in control group of animals were clean with little oedema or oozing of blood while in Serotonin treated group the wounds were oedematous, infection often occurred. Wounds in later group would disrupt on removal of sutures. This decrease in the collagen formation in serotonin treated animals is believed to be due to avascularity oedema, leading to infection caused by Serotonin. This is supported by Douglas (1963). Serotonin and similar other materials release in the wound are responsible for inflammatory response. Furthermore there appears to be a disparity between the blood supply needed for simpler cellular existence and that required for tissue repair (Douglas, 1963). The theoretical explanation for the failure of wounds to heal in ischaemic tissues is presumably that the production of new tissue in collagens requires local increase of energy which can only be supplied by an increase in oxygen. For this, the capacity to increase blood flow must be present. Further healing

Master Chart

Mean values of collagen (Hydroxyproline) content in normal (control) and Serotonin treated wounds.

S. No.	Control group			Serotonin treated group			
	No. of animals	Day after wounding	Collagen content in gram per-cent	Number of animals	Day after wounding	Collagen content in gram percent	Average
1.	Five	3rd day	8.7. gm%	Five	3rd day	5.2 gm%	3.5 gm%
2.	Five	6th day	10.4 gm%	Five	6th day	7.5 gm%	3.2 gm%
3.	Five	9th day	18.6 gm%	Five	9th day	11.7 gm%	6.9 gm%
4.	Pive	12th day	28.5 gm%	Five	12th day	19.0 gm%	9.5 gm%
5.	Five	15th day	30.0 gm%	Five	15th day	23.3 gm%	8.7 gm%

can be disturbed by malnutrition (Doughlas, 1963) resulting in postoperative disruption of wounds. There are several possible ways in which serotonin could be mediator in gastric inhibition induced by duodenal acidification (Leslie, 1968) thereby interfering with digestion of nutritive materials especially proteins thereby interfering with laying down of collagen.

Serotonin treated rats are thin, listless, rough haired and irritable during handling while normal control rats are sleek, active and easily handled, control group of rats show well healed mature wounds were cleaned with little oedema or oozing of blood while serotonin treated wounds were oedematous. Infection often occurred. These wounds contained little and immature collagen and lagged behind in healing. The wounds had tendency to disrupt on minimum handling or removal of suture. This is probably because of ADH (anti diuretic hormone) like behaviour of serotonin causing oedema thereby resulting in delayed healing. This effect in the normal wounds is blocked by serotonin antagonists and various other antigen antibody complexes released by eosinophils (Udupa, 1967).

Histamine released is believed to prevent the conversion of serotonin precursor 5-hydroxytryptophan (Lewis, 1964) into serotonin.

Summary and conclusions

The change in the concentration of a component of biochemical interest was studied over a period of 15 days under the effect of serotonin.

The component studied was hydroxyproline (Collagen), study was made on two sets albino rats, one comprising of normally healing wounds and the other serotonin treated wounds.

Study was made on 3rd, 6th, 9th, 12th and 15th postoperative days. Wound area included skin, subcutaneous tissue and the underlying muscle. Equal amounts of tissue were excised on either side of the wound. Serotonin was injected intraperitoneally into the rats labelled as 'treated on 2nd, 5th, 8th, 11th and 15th postoperative days.

Hydroxyproline content was estimated in excised tissue.

Difference in the hydroxyproline content of normal healing wounds and those treated with serotonin is discussed.

It has been reported in this review that there is net diminution of collagen content in serotonin treated wounds expressed as amount in grams% of dry tissue.

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