

CICATRIZATION OF WOUNDS.

XII. FACTORS INITIATING REGENERATION.

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The nature of the factors which initiate cicatrization after an injury is not as yet exactly known. The resumption of cell proliferation in the wounded tissues of an adult animal may be attributed to the removal of resistance to growth, in consequence of the defect resulting from loss of tissue.¹ In other words, the removal of the products of growth, that is of a portion of the tissues, immediately reinitiates the growth process, just as the removal of the products of a balanced chemical reaction at equilibrium immediately reinitiates the forward reaction.² This means that regeneration, being a direct consequence of the injury, is started by forces within the organism. But the same phenomenon may also be logically attributed to the action of an external factor. According to this hypothesis, the cells would be directly stimulated to growth and multiplication by forces without the organism, acting on tissues deprived of their natural protection by the injury.

I.

Effect of Protection against Irritation.

If regeneration is a direct consequence of the loss of tissue and initiated by an internal factor, the cicatrization of a wound protected against all external irritation must take place normally. But if this hypothesis be not true, the wound should not begin to cica-

¹ Welch, W. H., *Science*, 1897, v, 813.

² Robertson, T. B., *Principles of biochemistry for students of medicine, agriculture and related sciences*, Philadelphia, 1920, 482.

trize. It was observed in 1908 that the latent period of cicatrization of a wound dressed with dead connective tissue or plasma clot was abnormally prolonged. This fact suggested that regeneration was not initiated directly by the loss of tissue and that, if the surface of the wound were effectively protected against mechanical, chemical, and bacterial irritations, the setting in motion of the process of cicatrization would be indefinitely postponed. In order to ascertain in what measure the onset of regeneration could be delayed by adequate protection of the surface of the wound, five experiments were performed. Two circular wounds of equal size were made on the dorsal region of dogs, according to a technique previously described.^{3,4} The control wound was covered with a paste containing chloramine-T in a concentration which had been shown to be non-irritating for the tissues, and to keep them in a sterile condition.⁵ The experimental wound was dressed with subcutaneous connective tissue, excised from the lumbar region of a dog and kept in cold storage. Circular flaps, slightly larger than the wound and about 0.5 cm. thick, were prepared and fixed to the surface of the experimental wound by a few stitches. Both wounds were protected by a pad of dry gauze, sutured to the skin. Then the dressing was completed by a few other gauze pads, a large amount of cotton, a bandage, and a shirt. The animals were examined after a period of time varying from 13 to 25 days. As the examination involved the removal of the stitches holding the inner dressing to the skin, and also of the stitches fixed to the connective tissue placed on the wound, necessitating a considerable disturbance of the wound, the experiment was stopped after the second or third dressing.

The results of the five experiments are summarized in Table I. Experiment 1 was unsuccessful because the gauze pads slipped from the surface of the wounds and infection occurred. 14 days after the operation, no difference was found in the condition of both control and experimental wounds. Experiments 2 and 3 succeeded partly. The protection given to the wounds by the connective

³ Carrel, Alexis, and Hartmann, A., *J. Exp. Med.*, 1916, xxiv, 429.

⁴ All operations were performed under ether anesthesia.

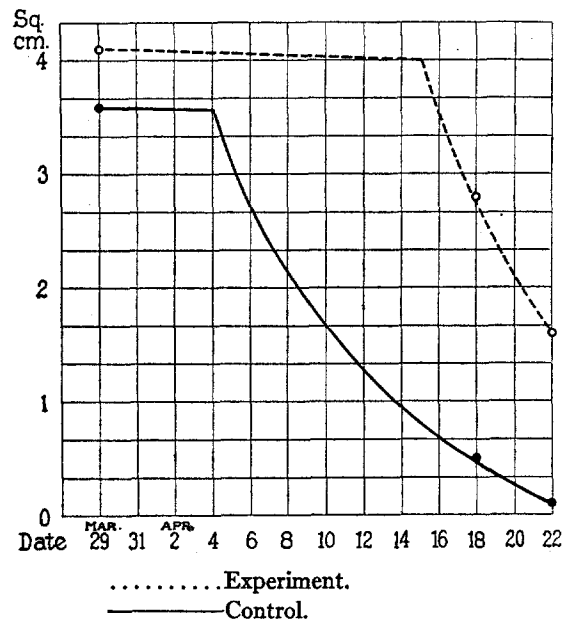
⁵ Carrel, Alexis, du Noüy, P. L., and Carrel, Anne, *J. Exp. Med.*, 1917, xxvi, 279.

TABLE I.

Action of Connective Tissue Dressing on the Latent Period.

Experiment No.	Animal No.	Date.	Control wound.		Experimental wound.		Remarks.
			Area. sq. cm.	Bacteria per field.	Area. sq. cm.	Bacteria per field.	
1	1	1921 Apr. 5	4.0		4.6		Connective tissue was heated at 56°C. for 3 hrs.
		" 19	1.9	16	1.4	1	Inner dressing slipped from both wounds.
2	2	Mar. 29	3.6		4.1		Text-fig. 1.
		Apr. 18	0.5	0	2.8	0	Inner dressing slipped from both wounds. Connective tissue dressing partially disappeared.
		" 22	0.1	0	1.6	0	"
3	3	Mar. 31	4.0		3.9		Inner dressing slipped. Infection of both wounds.
		Apr. 19	0.7	∞	2.8	∞	
4	4	Mar. 31	3.5		4.7		Text-fig. 2. No displacement of dressing.
		Apr. 7	3.0	0			
		" 19	2.0	0	4.7	0	Dry gauze.
		" 25	1.7	0	5.1		" "
5	5	" 7	5.2		4.2		Text-fig. 3. Connective tissue heated at 56°C.
		" 20	3.3	0	4.0	0	No displacement of dressing.
		" 25	3.0	0	4.5	0	Appearance of granulation tissue.

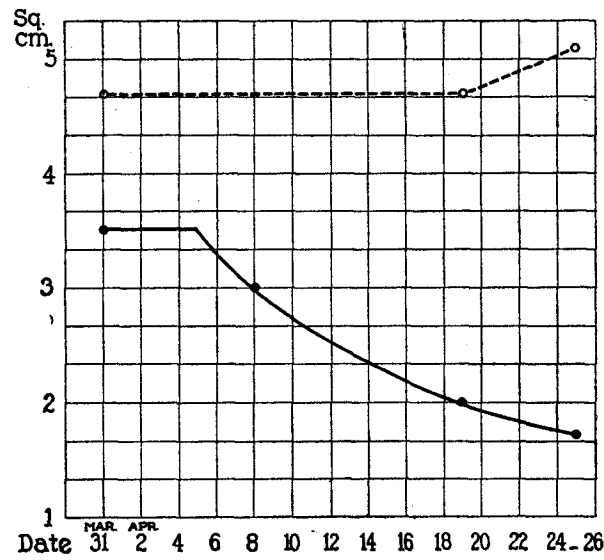
tissue dressing was incomplete. When the wounds were inspected for the first time, 19 and 20 days respectively after the operation, the experimental wound was no longer covered by the connective tissue dressing, and cicatrization had started. In Experiment 2, the curve expressing the progress of regeneration showed that the latent period had lasted very much longer in the wound protected by connective tissue than in the control wound (Text-fig. 1). The duration of the latent period was probably 17 days, while in the control it was 6.



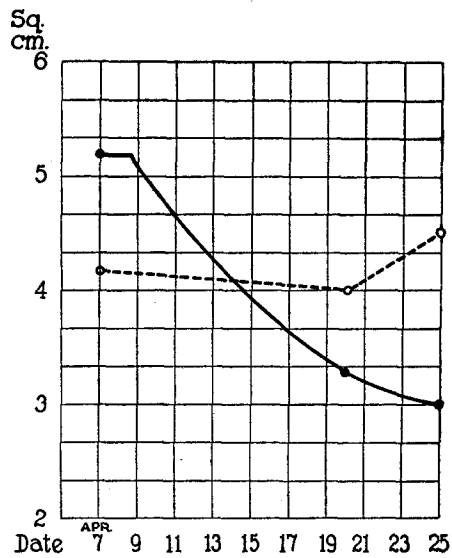
TEXT-FIG. 1. Experiment 2, Table I.

Although the connective tissue dressing did not remain at the surface of the wound for a long time, its effect was, however, manifest.

More significant results were obtained in Experiments 4 and 5. The connective tissue dressing remained exactly where it was applied and the surface of the wound was really protected against all outside irritation. The examination of the wounds was made 25 days after the operation in Experiment 4, and 18 days after the operation in Experiment 5. The period of contraction had not yet started and the area at that time was as large as at the time of the operation (Text-



TEXT-FIG. 2. Experiment 4, Table I.



TEXT-FIG. 3. Experiment 5, Table I.

figs. 2 and 3). It was a striking fact that a wound, effectively protected by a non-irritant dressing, did not show any evidence of cicatrization 25 days after the operation.

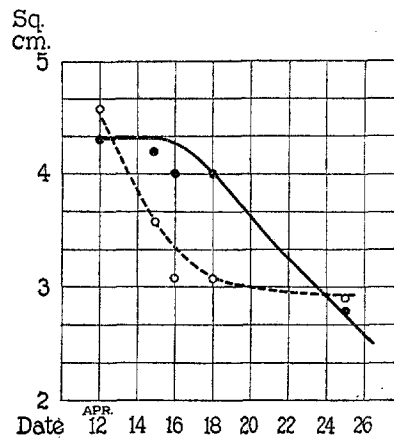
II.

Effect of Irritants.

In a second series of experiments, it was investigated whether the application of mild irritants on the surface of the wound would

TABLE II.
Action of Turpentine on the Latent Period.

Experiment No.	Animal No.	Date.	Control wound.			Experimental wound.			Remarks.
			Area.	Bacteria per field.	Dressing.	Area.	Bacteria per field.	Dressing.	
6	6	1921							Text-fig. 4. No displacement of dressing.
		Apr. 12	4.3		Chloramine-T.	4.6		Turpentine.	
		" 15	4.2	0	Dry gauze.	3.6	0	Dry gauze.	
		" 16	4.0	0	" "	3.1	0	" "	
		" 18	4.0	0	" "	3.1	0	Turpentine.	
" 25	2.8	0		2.9	1				



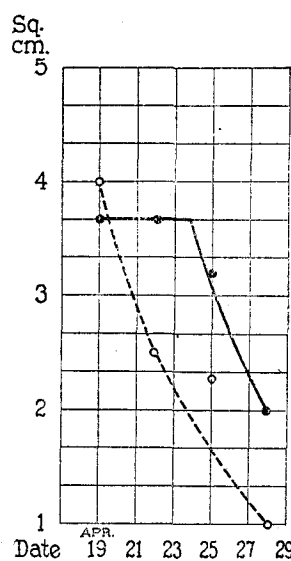
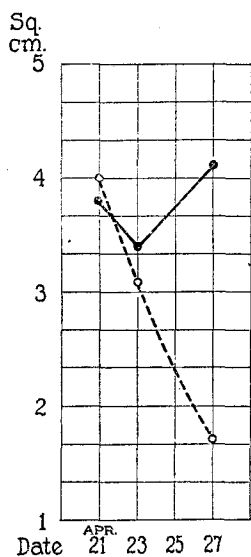
TEXT-FIG. 4. Experiment 6, Table II.

shorten the latent period. The experimental wound was covered by a gauze pad, soaked in turpentine and fixed to the edges of the skin by a few stitches, while the control wound was dressed with chloramine

paste. Both wounds were protected by gauze pads, sutured to the skin. The latent period of the experimental wound was very much shortened, lasting less than 2 days, while the latent period of

TABLE III.
Action of Chick Embryo Pulp on the Latent Period.

Experiment No.	Animal No.	Date.	Control wound.		Experimental wound.		Remarks.
			Area.	Bacteria per field.	Area.	Bacteria per field.	
7	7	1921 Apr. 21	3.8		4.0		Text-fig. 5.
		" 23	3.4	0	3.1	5	
		" 27	4.1	0	1.7	0	
				Chloramine-T.		Chick embryo pulp.	
				"		Dry gauze.	
				"		" "	



TEXT-FIG. 5. Experiment 7, Table III. TEXT-FIG. 6. Experiment 10, Table IV.

the control wound lasted for about 5 or 6 days (Table II and Text-fig. 4).

In Experiment 7, chick embryo pulp was used instead of turpentine (Table III). 6 days after the operation, the contraction of the

control wound had not started. On the contrary, in the experimental wound contraction began after a very short time, less than 2 days

TABLE IV.
Action of Staphylococcic Infection on the Latent Period.

Experiment No.	Animal No.	Date.	Control wound.		Experimental wound.		Remarks.	
			Area. sq. cm.	Bacteria per field.	Area. sq. cm.	Bacteria per field.		Dressing.
8	8	1921 Apr. 19	5.0		4.0		1:100 dilution of staphylococcus suspension.	Mild infection; no edema; slight discharge.
		" 21	8		78		Dry gauze.	
		" 22	4.0	0	3.5	45	" "	
		" 25	3.2	0	3.3	24	" "	
		" 28	2.8	0	1.8	13	" "	
9	9	" 19	4.0		3.7		1:10 dilution of staphylococcus suspension.	Text-fig. 6.
		" 21	4		∞		Dry gauze.	
		" 22	3.7	0	3.4	85	" "	
		" 25	3.4	0	3.9	1	" "	
		" 28	2.8	0	1.7	20	" "	
10	10	" 19	3.7		4.0		1:50 dilution of staphylococcus suspension.	Text-fig. 6.
		" 22	3.7	0	2.5	50	Dry gauze.	
		" 25	3.2	0	2.3	0	" "	
		" 28	2.0	0	1.0	41	" "	
11	11	" 21	4.0		3.5		Pure staphylococcus cultures.	
		" 23	2.6	0	2.4	6	Dry gauze.	
		" 25	2.5	0	2.2	0	" "	
		" 28	2.3	0	0		" "	

(Text-fig. 5). 6 days after the operation, the area of the experimental wound was about 50 per cent smaller than that of the control wound.

In four experiments (Table IV), the wounds were infected with staphylococci. Varied dilutions of a 24 hour culture of staphylococci in bouillon were used for inoculation. The control wound was dressed with chloramine paste, while the experimental wound was inoculated with 0.05 cc. of the dilution of staphylococcic culture, and dressed with dry gauze. The wounds remained in a condition of slight infection without swelling of the edges or abundant suppuration. The duration of the latent period was decreased, and often reduced to less than 2 days, as shown in Experiment 10 (Text-fig. 6).

III.

SUMMARY.

As long as the wounds were protected by a connective tissue dressing against mechanical, chemical, and bacterial irritations, no evidence of cicatrization was found. The complete or partial failure of four experiments was due to the slipping of the inner dressing from the wound, mechanical irritation by the gauze, and infection. In the two experiments in which the connective tissue was maintained at the surface of the wound, there was no beginning of cicatrization, although 25 and 18 days respectively had elapsed since the operation, while in the control wound the duration of the latent period did not exceed 5 or 6 days. The experiments were interrupted after the second or third inspection, on account of the technical impossibility of again applying to the wounds a non-irritant dressing. It is probable that the wounds could have been kept for a much longer time in a condition of quiescence. While it is not known whether cicatrization could be prevented for an indefinite period, there is no doubt that the mechanism of regeneration is not set in motion at the usual time, when all external irritations are suppressed. It appears, therefore, that under ordinary conditions, cicatrization is not initiated by an internal factor.

On the contrary, the application of turpentine, chick embryo pulp, and staphylococci decreased markedly the length of the latent period, which was often reduced to less than 2 days. This fact demonstrated the importance of external factors in the initiation of cicatrization. It seems that the mechanism of regeneration has

become adapted to the ordinary conditions of life of the animals. A small wound will begin to cicatrize sooner if slightly infected, as practically always happens, than if it were thoroughly protected by a non-irritant dressing.

IV.

CONCLUSIONS.

1. It may be concluded that, under the conditions of the experiments, a wound, protected by a non-irritant dressing, shows no granulation tissue or beginning of contraction for 25 days at least.
2. Local application of certain irritants, such as turpentine, chick embryo pulp, and staphylococci, reduces the duration of the latent period to less than 2 days.
3. Regeneration is apparently initiated, not by an internal, but by an external factor.