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Effect of Intra-Arterial Nitrogen Mustard Infusion on Wound Healing in Rabbits

Formation of Granulation Tissue and Wound Contraction

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MANY technics have been devised for the delivery of high concentrations of nitrogen mustard or other alkylating agents to the site of malignant tumors [5, 7, 8, 11, 28, 29, 30] and frequently these are used in conjunction with excisional surgery. This has raised the question of the effects that high doses of cytotoxic drugs might have on the course of wound healing.

Previous studies suggest that at least some of the agents in current use cause delayed healing, as measured by gain in tensile strength, the effect depending on dosage, the species of experimental animal and route of administration.

Hatiboglu *et al.*[18] report impairment of tensile strength in 7-day-old wounds which were exposed for 10 minutes at the time of wounding to one of a variety of agents applied as a topical solution. These included nitrogen mustard and thio-TEPA. Some effect was also observed with actinomycin, acriflavine and sodium hypochlorite.

Pisesky *et al.*[27] found impairment of tensile strength of wounds in animals treated in this way was reported as being greater than the controls.

Harris and Thomas[17] found that irrigation of the fresh wound in mice with nitrogen mustard solutions produced impairment of tensile strength measured on the eighth day, with no obvious depression of fibroplasia as judged histologically. Using solutions varying in strength from 2 mg./100 ml. to 10 mg./100 ml., these workers report that the depression of tensile strength increased with increasing concentration.

Kaiser *et al.*[20] investigated the effect of isolated perfusion of the dog hind-limb with oxygenated blood containing nitrogen mustard on the healing of incised skin wounds of the thigh. They found histologic changes similar to those following irradiation (fibroblasts of abnormal morphol-

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ogy, delay in endothelial proliferation and collagen fiber formation). These changes were only seen if perfusion was made at the time of wounding.

However, Conn, Leb and Hardy[6] found no effect on the tensile strength of gastric wounds in the dog when either nitrogen mustard, 0.4 mg./Kg., or thio-TEPA, 2.0 mg./Kg., was given in divided doses intravenously for 3 days.

In a study of the tensile strength of abdominal wounds in the rat, Hardesty[16] also found that thio-TEPA, 0.8 mg./Kg., had no demonstrable effect over a 12-day period. Nitrogen mustard, 0.3 mg./Kg. and 0.6 mg./Kg., was found to retard healing approximately in proportion to the dose, but the effect was short-lived. By the twelfth day the tensile strength did not differ from control values. A similar retarding effect of nitrogen mustard was noted after topical application.

Farha *et al.*[13] found a similar effect using nitrogen mustard with rats, but reported that an exactly similar retardation was produced by dietary restriction to give a comparable weight-loss. Their conclusion was that no specific effect of nitrogen mustard on the healing process had been demonstrated in their experiments.

Studies on cyclophosphamide by Desprez and Kiehn[10] yielded a similar conclusion. A dose of 2 mg./Kg. in mice had no effect whether administered systemically or locally, whereas a dose of 15 mg./Kg., while effective in reducing wound tensile strength, also produced marked weight loss and leukopenia.

Krementz, Gidden and Chapman,[21] working with guinea pigs, found that tri-ethylenemelamine, 1 mg./Kg., given intraperitoneally, significantly reduced the bursting strength of gastric wounds tested on the seventh postoperative day. The dosage used was high (equivalent to the LD_{50} for mice) and interpretation of the results, therefore, is uncertain.

Reports are few on the effects of alkylating agents on wound healing in human subjects. Mrazek *et al.*[25] reported on two groups of patients, one with breast cancer and one with rectal cancer. Each patient received 0.4 mg./Kg. of nitrogen mustard intravenously preoperatively and for 2 days succeeding operation. Although the incidence of wound complications was the same in these patients as in untreated controls, the treated groups produced the worst problems in healing. This was reflected in the fact that the average length of stay in hospital for the treated group was 54 days compared with 29 days for the controls.

The present study was designed to determine the effect of a high concentration of nitrogen mustard administered over a short period of time by intra-arterial infusion on the composition of granulation tissue and the contraction behavior of a large excised skin wound in the rabbit.

Material and Methods

Male albino rabbits were anesthetized with intravenous Nembutal. Immediately prior to the operative procedure, the abdomen and lateral aspects of both thighs were prepared with electric clippers. No skin antiseptic was employed.

Square wounds, 2 × 2 cm., were marked out on the lateral aspect of each thigh using the tattooing technic previously described.[2] Full-thickness wounds were made by incising through the centers of the tattoo marks down to the panniculus carnosus. The square of skin thus demarcated was dissected carefully off the panniculus and discarded. The wounds were left undressed. Subsequent sepsis was rare, but occurrence led to withdrawal of the animal from the experiment.

Measurements of wound area were made at the time of wounding and at intervals thereafter by tracing the tattoo marks on to cellophane paper. The marks were sub-

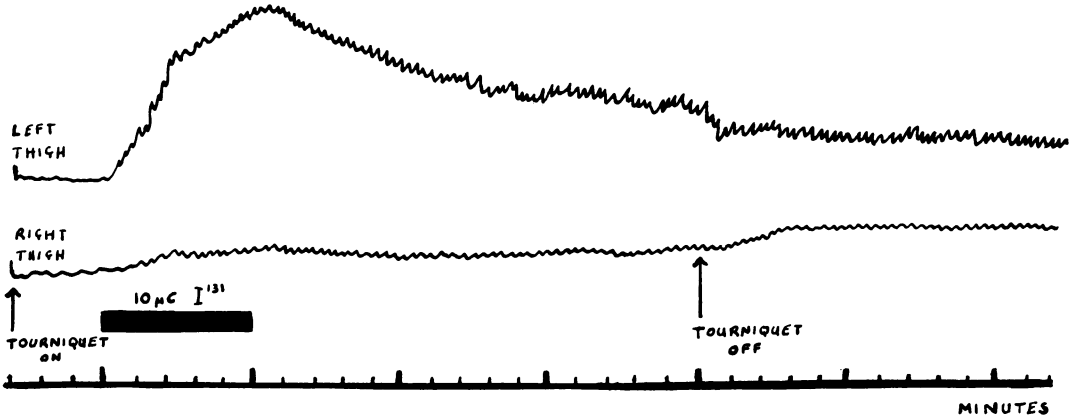

FIG. 1. Simultaneous tracings obtained on the lateral aspect of both thighs during the infusion of 10 μC I^{131} into the abdominal aorta of a rabbit over a period of 5 minutes. The right side was protected by a tourniquet and an occlusive clamp on the right common iliac artery during the period indicated by the arrows.

sequently transferred to tracing paper of standard thickness, the areas outlined being carefully cut out and weighed. From the weight per unit area of the tracing paper, areas of the wound tracings were calculated.[15]

On the tenth day after wounding, the granulation tissue filling the wound was marked out with tattoo dots just within the skin margin and its area measured. The tissue was then dissected off the panniculus and weighed immediately. The specimens were stored at −20° C. while awaiting chemical analysis. Biopsy of granulation tissue was *not* performed in the two subgroups B-LT and BX-LT in which wound area changes were followed up to 28 days after wounding.

Biochemical Technics. The tissue specimens were dried to constant weight at 100° C. and homogenized in 10% trichloracetic acid. Total nitrogen was estimated by the method of Ma and Zuazaga.[22] Collagen was measured by hydroxyproline estimation using the method of Neumann and Logan[26] as modified by Martin and Axelrod.[24]

Hematologic Technics. Blood samples were obtained by needle puncture of the central artery. Hemoglobin levels were determined by the Sahli technic. Estimates of total white blood cell count were performed by a standard technic.[9]

Standard Infusion Technic. The abdomen was opened through a lower midline incision and the bladder emptied by manual expression. The aorta was exposed in the region of its bifurcation. All major branches in this area were ligated, including the internal iliac, inferior epigastric and lumbar arteries.

During the infusion, circulation to the wound under test was unimpeded. On the side randomly selected as the control, the wound was protected by a tourniquet placed around the thigh above the level of the wound. In addition, the common iliac artery was temporarily occluded on that side by a nontraumatic bulldog clip.

The measured dose of nitrogen mustard (Mustargen hydrochloride, Merck, Sharp & Dohme), diluted to a final volume of 10 ml. with normal saline, was injected into the lowest part of the abdominal aorta at a constant rate of 2 ml. per minute. Thus, the total infusion time was exactly 5 minutes. After allowing a further 15 minutes for dispersion and destruction of the circulating nitrogen mustard, the bulldog clip and the tourniquet were removed. After

TABLE 1. *Wet Weights and Water Content, with Standard Errors, of Granulation Tissue Removed at Biopsy*

Group	No. Animals	Side	Wet Weight (mg.)	Wet Weight per Unit Area (mg./mm.^{2})	Water Content (mg./Gm.)
A	10	Treated	44.2 ± 5.6	1.12 ± 0.22^{++}	836.5 ± 5.8
		Control	46.7 ± 4.4	1.17 ± 0.09	835.1 ± 5.1
AX	5	Side 1	44.4 ± 7.2	1.50 ± 0.24	831.1 ± 24.1
		Side 2	44.0 ± 8.4	1.32 ± 0.19	855.5 ± 18.6
B	10	Treated	13.0 ± 0.9	0.21 ± 0.03	781.6 ± 19.1
		Control	39.0 ± 5.7	0.63 ± 0.14	824.2 ± 8.4
BX	5	Side 1	46.8 ± 6.2	1.04 ± 0.25	842.7 ± 23.2
		Side 2	55.9 ± 8.1	1.25 ± 0.28	829.1 ± 18.1
C	6	Treated	32.7 ± 6.3	1.00 ± 0.24	838.8 ± 5.6
		Control	34.9 ± 3.5	1.06 ± 0.21	839.3 ± 16.1
D	5	Treated	41.6 ± 4.5	0.83 ± 0.19	807.6 ± 23.9
		Control	39.0 ± 12.0	0.54 ± 0.21	827.4 ± 33.4

another period of 15 minutes to allow the reactive hyperemia to subside, the procedure was repeated using normal saline and occluding the opposite limb. In conducting the experiment, the order in which the two injections were administered was in fact randomized.

The efficacy of this technic in preventing leakage of the agent to the protected limb was tested in five animals, using I^{131} in the infusate. A tracing from one of these animals is shown in Figure 1. The time-course of the slight rise in activity over the thigh of the protected limb exactly parallels the large rise on the opposite side. It is therefore more probable that this is due to the inadequate screening of the protected limb rather than an actual leak past the tourniquet.

Experimental Design. The animals were subdivided into eight treatment groups.

Group A (10 animals). Standard thigh wounds were made as described. Immediately afterwards the aorta was exposed and the infusion procedure carried out. The dose employed was 0.5 mg./Kg.

Group AX (5 animals). These received exactly the same treatment as for Group A, but normal saline was substituted for nitrogen mustard.

Group B (10 animals). Standard thigh wounds were made as in the previous two groups. The infusion procedure was then delayed until the third post-wound day. The dose used was 0.5 mg./Kg.

Group B-LT (5 animals). Treated as in Group B, but not biopsied on the tenth day after wounding. Analysis of variance showed that data for wound area, animal weight, hemoglobin levels and total leucocyte count in this subgroup were homogeneous with that for Group B at each stage of healing up to the tenth day. In Tables 3 and 5, therefore, these groups are combined.

Group BX (5 animals). Treated as in Group B, but with normal saline substituted for nitrogen mustard. Results are tabulated under the headings Side 1 and Side 2, indicating the order in which each side was subjected to saline infusion.

TABLE 2. *Collagen and Non-Collagenous Nitrogen (NCN) Content, with Standard Errors, of Granulation Tissue Removed at Biopsy*

Group	Side	Total Collagen (mg.)	Collagen Conc. (mg./Gm.)	Total NCN (mg.)	NCN Conc. (mg./Gm.)
A	Treated	1.50 ± 0.20	34.1 ± 2.0	1.28 ± 0.17	23.6 ± 1.1
	Control	1.57 ± 0.21	33.6 ± 1.8	1.31 ± 0.11	23.4 ± 1.4
AX	Side 1	1.42 ± 0.34	31.9 ± 2.6	0.92 ± 0.16	20.0 ± 0.82
	Side 2	1.40 ± 0.28	31.7 ± 3.2	0.83 ± 0.14	19.1 ± 0.91
B	Treated	0.33 ± 0.04	25.3 ± 2.1	0.45 ± 0.05	34.7 ± 2.2
	Control	1.30 ± 0.18	34.5 ± 3.0	0.98 ± 0.12	26.8 ± 2.5
BX	Side 1	1.55 ± 0.36	33.1 ± 3.2	1.03 ± 0.12	21.1 ± 1.2
	Side 2	1.77 ± 0.38	31.4 ± 4.4	1.21 ± 0.15	23.6 ± 1.4
C	Treated	1.06 ± 0.30	21.9 ± 2.5	0.94 ± 0.20	23.7 ± 4.7
	Control	0.88 ± 0.13	23.0 ± 3.3	0.89 ± 0.32	24.3 ± 6.2

Group BX-LT (5 animals). Treated exactly as in Group BX, but not biopsied on the tenth day. Analysis of variance showed that up to this time the data for wound area, animal weights, hemoglobin levels and total leucocyte count could be combined with that from Group BX. Subsequently, wound area was followed up to the twenty-eighth day after wounding.

Group C (6 animals). Treated as in Group B, with the dosage of nitrogen mustard reduced to 0.25 mg./Kg. given on the third day after wounding.

Group D (5 animals). Treated as in Group B, except that the dose of 0.5 mg./Kg. on the third post-wound day was infused into the inferior vena cava instead of the aorta. In all other respects the technic was identical.

Results

Biochemical Composition of the Granulation Tissue

In Table 1 the reuslts are given for wet weights and water content of granulation tissue excised on the tenth day after wounding. Data on collagen and non-collagenous nitrogen components of the tissue are summarized in Table 2.

It is clear that administration of nitrogen mustard, 0.5 mg./Kg., within 1 hour of wounding (Group A) has no effect on the amount of granulation tissue formed or on its chemical composition. Values do not differ significantly whether side-to-side comparisons are made between the treated and control wounds within this group, or whether the mean values are compared between this group and corresponding saline controls (Group AX).

In Group B there were marked differences between the treated and protected wounds. Not only is the total amount of tissue excised greatly reduced (13.0 ± 0.9 mg. compared with 39.0 ± 5.7 mg, $p < .001$), but its *collagen concentration* of 25.3 ± 2.1 mg./Gm. in the treated side is significantly less than the mean value, 34.5 ± 3.0 mg./Gm. for the protected wounds of this group ($p < 0.01$).

That this is not simply due to edema is clear from the data in Table 1 on the water content of granulation tissue specimens. In fact, the treated wounds of Group B show a higher relative solid component than do the protected wounds. The same trend is reflected in the figures for non-collagenous nitrogen in Table 2. In neither case are these differences significant in the

TABLE 3. *Mean Sizes in Cm.², with Standard Errors, of Areas Marked by Skin Tattoos*

Group	No. Animals	Side	Initial Area (Before Skin Incision)	Treated Area (After Skin Incision)	3 Days	7 Days	10 Days
A	10	Treated	3.94 ± 0.16	5.72 ± 0.18	2.20 ± 0.14	2.05 ± 0.12	1.02 ± 0.14
		Control	4.06 ± 0.12	5.52 ± 0.24	2.19 ± 0.10	2.06 ± 0.08	1.09 ± 0.05
AX	5	Side 1	3.91 ± 0.19	6.05 ± 0.49	2.84 ± 0.13	2.22 ± 0.27	1.07 ± 0.08
		Side 2	3.86 ± 0.22	5.92 ± 0.20	2.84 ± 0.25	2.30 ± 0.24	1.03 ± 0.08
B, B-LT	15	Treated	4.09 ± 0.07	5.89 ± 0.20	2.67 ± 0.14	2.37 ± 0.10	1.73 ± 0.10
		Control	4.15 ± 0.04	6.25 ± 0.18	2.52 ± 0.15	2.37 ± 0.15	1.62 ± 0.11
BX, BX-LT	10	Side 1	3.93 ± 0.07	6.04 ± 0.24	2.69 ± 0.07	2.41 ± 0.13	1.10 ± 0.06
		Side 2	4.02 ± 0.10	5.76 ± 0.23	2.58 ± 0.19	2.26 ± 0.16	1.07 ± 0.08
C	6	Treated	4.33 ± 0.11	5.70 ± 0.17	2.48 ± 0.30	2.17 ± 0.18	1.16 ± 0.11
		Control	4.57 ± 0.29	6.69 ± 0.31	2.87 ± 0.42	2.49 ± 0.26	1.34 ± 0.13
D	5	Treated	4.65 ± 0.08	7.11 ± 0.37	2.78 ± 0.13	2.54 ± 0.13	1.28 ± 0.09
		Control	4.53 ± 0.24	6.98 ± 0.52	3.20 ± 0.40	2.75 ± 0.20	1.92 ± 0.29

numbers of animals used, but their direction emphasizes that there is a marked impairment of collagen synthesis in the treated wounds of Group B.

In none of the parameters so far considered do the figures for protected wounds of Group B differ from those for Group BX, given saline alone. However, the figures in the last row of Table 1 show that while the mean weight per unit is markedly reduced (0.21 ± 0.03 mg./mm.²) on the treated side when compared with the protected side (0.63 ± 0.14 mg./mm.², $p < 0.01$), this in turn is significantly less than the 1.17 ± 0.09 mg./mm.² for the corresponding wounds in Group A ($p < 0.05$). Evidence is presented later that this is due to impairment of wound contraction in the *control* wounds of Group B.

Reducing the dosage of nitrogen mustard to 0.25 mg./Kg. on the third day after wounding (Group C) abolishes the differences in composition between the two sides. Thus there appears to be a threshold infusion concentration between 0.25 mg./Kg. and 0.5 mg./Kg. below which no effect on granulation tissue composition can be detected under the conditions of the experiment.

Increasing the dosage to 1.0 mg./Kg. was attempted in a pilot series. However, the mortality was so high that no useful data were obtained.

When the infusion was given into the inferior vena cava (Group D), wet weights and water content of tissue samples did not differ on the two sides (Table 1). However, values for wet weight per unit area suggest that granulation tissue is thinner on the protected side. Although the difference is not significant in this small group, it will later be shown that wound contraction is indeed selectively impaired on this protected side in this group.

Collagen concentrations of both treated and protected wounds of Group D are significantly lower than those of any other group except the treated wounds of Group B (Table 2). It seems unlikely that during the venous infusion, the unprotected wound should have been exposed to a greater concentration of the agent than were corresponding wounds of Group C. Neither is it easy to see how the protected wounds could have received more of the agent when given by this route than did the corresponding wounds of Group B. At pres-

TABLE 4. *Mean Sizes in Cm.², with Standard Errors, of Areas Marked by Skin Tattoos in Wounds Not Subjected to Biopsy at 10 Days*

Group	No. Animals	Side	14 Days	21 Days	28 Days
B-LT	5	Treated	0.95 ± 0.08	0.64 ± 0.03	0.72 ± 0.02
		Control	0.99 ± 0.19	0.70 ± 0.05	0.71 ± 0.06
BX-LT	5	Side 1	0.88 ± 0.05	0.81 ± 0.04	0.82 ± 0.03
		Side 2	0.82 ± 0.07	0.78 ± 0.04	0.78 ± 0.05

ent, therefore, this observation remains unexplained.

Area Changes

These are summarized in Tables 3 and 4. Analysis of variance among all groups shows homogeneity of data for each set of observations up to and including the seventh day after wounding.

Immediately after the wound is made, its area expands by about 50 per cent due to the elastic pull of the surrounding skin. By the third day its area is reduced to less than half this initial area. Between the third and seventh days the area is only slightly reduced.

Between the seventh and the tenth days certain differences are seen in changes in the wound area in the various treatment groups.

In Groups A, C, AX and BX/BX-LT, the wound area, having contracted by about one half by the third day, contracts by a similar factor between the seventh and tenth days. The combined data for Groups B and B-LT, however, show marked impairment of wound contraction during this period, both in the treated and control sides. In the former, the area is slightly reduced, 2.37 ± 0.10 cm.² to 1.73 ± 0.10 cm.², and in the latter 2.37 ± 0.15 cm.² to 1.62 ± 0.11 cm.² In both cases this difference is significant ($p < 0.05$). However, in both cases the wound areas on the tenth day are significantly greater than the corresponding figures for either Group A (treated on the day of wounding) or Group BX (treated with saline alone).

This impairment of contraction is only of short duration, as evidenced by data from the long-term groups B-LT and BX-LT (Table 4). From the fourteeenth to the twenty-eighth day, no significant difference was found between the wound area of the two groups.

To investigate the nature of this temporary slowing of wound contraction occurring in both the protected and the treated wounds of Group B, the dose of nitrogen mustard was reduced to 0.25 mg./Kg. The wound areas did not differ significantly from those obtained with saline controls. This result made it unlikely that the effect on the protected wounds of Group B was due to leakage past the tourniquet during infusion.

The effect of administering the drug by infusion into the inferior vena cava was then tested. Results are given in the last column of Table 3. There is impaired contraction in the control, i.e., protected, wounds, but not in the treated. In the latter, the wound area of 1.28 ± 0.09 on the tenth day differs significantly from the area of 2.54 ± 0.13 cm² recorded on the seventh day ($p < 0.01$) and from the area of 1.92 ± 0.29 cm.² ($p < 0.05$) of the control wounds at 10 days. The area changes through the 10-day period in the *treated* wounds of Group D do not differ from those of the control Group BX. The area changes in the *protected* wounds of Group D do not differ significantly from either the treated or control wounds of Group B. As already noted, there was no difference in

TABLE 5. *Changes in Weight Hemoglobin and Total Leucocyte Count Before and After Wounding*

Group		Weight (Kg.)	Hemoglobin (Gm./100 ml.)	Total Leucocyte Count ($\times 10^3$/mm.^{3})
A	Initial	2.88 ± 0.13	11.9 ± 0.6	3.5 ± 1.0
	10th day	2.73 ± 0.08	11.3 ± 0.4	4.2 ± 0.4
AX	Initial	3.10 ± 0.24	12.9 ± 0.9	6.4 ± 1.5
	10th day	3.07 ± 0.21	11.1 ± 0.8	4.2 ± 1.8
B, B-LT	Initial	2.80 ± 0.16	12.2 ± 0.7	7.5 ± 0.9
	10th day	2.47 ± 0.18	10.9 ± 1.1	6.2 ± 1.5
BX, BX-LT	Initial	3.02 ± 0.21	11.0 ± 0.6	4.5 ± 2.2
	10th day	3.00 ± 0.22	10.1 ± 0.8	3.2 ± 1.6
C	Initial	2.70 ± 0.16	12.1 ± 1.1	5.6 ± 2.4
	10th day	2.45 ± 0.20	11.0 ± 1.2	4.4 ± 1.8
D	Initial	3.15 ± 0.12	12.5 ± 0.9	6.6 ± 2.0
	10th day	2.84 ± 0.21	11.4 ± 1.0	5.6 ± 2.4

the amount or composition of the granulation tissue on the two sides in Group D.

Systemic Effects of Nitrogen Mustard

As already noted, Farhat *et al.*,[13] although observing a retarding effect on wound healing following the administration of nitrogen mustard to rats, found that dietary restriction to produce a similar weight-loss had the same effect.

Accordingly the weight, hemoglobin levels and total leucocyte counts were measured before wounding and at set intervals afterwards, until the time of biopsy.

Results are shown in Table 5. No significant fall occurred in any of these parameters during the period of study. Moreover, any such systemic effect might have been expected to be most marked in Group A, rather than Group B, since the drug was given 10 days before sacrifice in the former group, and only 7 days before in the latter.

It seems highly unlikely that our results can be explained on the basis of bone-marrow depression or nutritional disturbance.

Discussion

These results indicate that the effect of nitrogen mustard on wound healing depends upon three factors: dose, route of administration and time of treatment in relation to wounding.

The standard dose employed in this study was 0.5 mg./Kg. This appears to have no effect on either the biochemical composition of granulation tissue or on the process of wound contraction if administered at the time of wounding by intra-arterial infusion.

The same dose given by the same route on the third day after wounding has a profound effect on both these modalities on the treated side. In addition, there was a selective temporary retardation of wound contraction on the protected side in this group of animals, with no concomitant effect on granulation tissue formation.

Reducing the dosage to 0.25 mg./Kg. abolishes the effect both on granulation tissue composition and wound contraction on the treated side. The data indicate that there may be some retardation of contraction on the control side in this group, but

in the numbers of animals studied this difference was not significant.

It is well known that the rapidly dividing cells of the bone-marrow and gastrointestinal tract are highly sensitive to the action of cytotoxic drugs, and it is for this reason that isolated perfusion and arterial infusion technics have been developed.

Since the formulation of granulation tissue involves intense cellular activity in which the fibroblast plays a key role,[14] it seems probable that nitrogen mustard can retard the proliferation and metabolism of these cells if given in high enough dosage at a time when they are particularly susceptible.

Studies on the effect of X-irradiation on wound healing support this view. Grillo and Potsaid,[14] using a dose of 750r locally applied to excised skin wounds of guinea pigs, reported a maximal retarding effect on wound contraction at 36 hours after wounding. An effect, however, was detectable up to 5 days after wounding.

Although the present study does not give information on the time of *maximal* sensitivity to nitrogen mustard, it is clear that a marked effect is observed when the drug is given on the third day after wounding which does not occur if given at the time of wounding. If the hypothesis[3] that the fibroblasts in healing wounds are derived from the monocytes of the bloodstream is substantiated, then a possible explanation would be that the drug will not affect the cells while they are in the circulation. Only when they have become fixed in the region of the wound and start to proliferate, do they become susceptible to local unfusions of cytotoxic drugs. If, on the other hand, fibroblasts of granulation tissue are derived not from circulating monocytes but from fixed tissue cells in the immediate neighborhood of the wound,[23] they may be relatively resistant to X-rays and cytotoxic drugs while in the resting state and only become sensitive when stimulated into rapid multiplication. In either event the ultimate result is severe retardation in the formation of granulation tissue with special emphasis on collagen synthesis.

The effect on wound contraction is complex. Up to the seventh day after wounding, changes in wound area follow a similar pattern in all groups. It seems likely that the sharp reduction in wound area occurring in the first 3 days is due to the dense scab which forms over these undressed wounds. This then appears to act as a splint preventing further reduction in wound area until it has separated on the seventh day. Thereafter, the normal process of contraction is free to proceed. However, contraction was retarded at this point not only in the treated wound of Group B but also in those protected by tourniquet throughout the period of infusion.

There are two possible ways in which this effect on the protected wounds might have occurred. Either the tourniquet protection was incomplete or a significant level of the drug was still circulating at the time the tourniquet was removed.

Against the first possibility is the failure to demonstrate a large leak into the protected limb when I^{131} was substituted for the nitrogen mustard. Also, the fact that a dose of 0.25 mg./Kg. failed to produce an effect on contraction of the *treated* wound strongly suggests that leakage during the infusion is a most unlikely explanation of the observed retardation in the *protected* wounds of Group B. There remains the possibility that the tourniquet provided more effective venous than arterial occlusion, with the result that any drug leaking into the protected limb was trapped. However, radioisotope studies failed to provide any evidence that this was so.

It appears that the effect must be due to residual nitrogen mustard or its degradation products. However, this alone is insufficient to explain the absence of this retardation in the treated wounds of Group

D, which are exposed to a much higher concentration of the agent than are the protected wounds. The explanation may be that in the protected wounds the tissues have been rendered ischemic immediately before exposure to any residual drug. Whether the effect is due to diminished tissue oxygen tension or to reactive hyperemia alone is not known. Ausman and Aust[4] observed during isolated limb perfusion in the dog that diminished oxygen saturation in the perfusing blood increased the uptake of nitrogen mustard, but the precise mechanism of this effect was not determined.

Whatever the mechanism, it has the effect of selectively impairing wound contraction in the protected wounds of Groups B and D without a corresponding impairment of granulation tissue composition. In the unprotected wounds of Group B, both contraction and granulation tissue formation are retarded.

Currently there are two theories concerning the mechanism of the contractile process in healing wounds. One is that granulation tissue is actively contractile and capable of drawing the wound margins together.[2] It has been suggested that the fibroblasts may be the contractile elements of the tissue,[1] and that following contraction a process of resorption takes place, involving all elements of the tissue including collagen.[19]

The other proposal[31] is that the contractile mechanism resides in a specialized zone of tissue immediately beneath the advancing wound margin, which is somehow able to advance over the wound surface. This is popularly known as the picture-frame theory of wound contraction.

The fact that in the present series of experiments wound contraction was retarded in Group B, whether or not there was impairment of granulation tissue formation, might seem to favor the picture-frame hypothesis. However, this would only have been so had contraction proceeded normally despite a gross impairment of granulation tissue formation. This was not the case.

What these experiments have shown is that contraction can be *selectively* impaired without necessarily affecting the amount and composition of the granulation tissue filling the wound. They yield no evidence as to the site of this action. They have also shown that nitrogen mustard may produce a marked, though transitory, deleterious effect on the course of wound repair if administered in sufficiently high dosage after the healing process has commenced.

Relating these results to therapeutic perfusions of alkylating agents in man, it appears likely that this can safely be combined with simultaneous surgical excision of a malignant lesion. But if perfusion is performed as a secondary procedure a few days after an extensive surgical excision, some delay in wound healing may be anticipated.

Summary

Results of this study show that a dose of 0.5 mg./Kg. administered over a 5-minute period has no effect if given at the time of wounding. If the same dose is given 3 days later, there is a marked reduction in the amount of granulation tissue and its collagen concentration and wound contraction is delayed until between the seventh and tenth days.

Wounds protected by a tourniquet during nitrogen mustard infusion on the third day show no impairment of granulation tissue formation or collagen synthesis, but there is a retardation of wound contraction similar in time-course and extent to the unprotected wounds.

Experiments designed to elucidate the mechanism of this selective effect on wound contraction suggest that it is brought about by residual circulating nitrogen mustard, or its degradation products, acting on a

wound previously rendered ischemic by a tourniquet.

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