Origin of Fibroblasts in Wound Healing: *
An Autoradiographic Study of Inhibition of Cellular Proliferation by Local X-Irradiation

HERMES C. GRILLO, M.D.

From the John Collins Warren Laboratories of the Huntington Memorial Hospital, the Robert W. Lovett Memorial Unit for the Study of Crippling Diseases, and the Department of Surgery of the Massachusetts General Hospital and Harvard Medical School

SURGICAL HEALING is considered to have occurred when tissue continuity has been restored, following wounding, to a strength approximating normal tissue strength and when the wound defect has been appropriately surfaced. Restoration of the organism's surface is accomplished by a complex activity of epithelial and connective tissues. Restoration of continuity and strength, whatever the tissue involved, results from fibroplasia. Biochemical understanding of fibrogenesis is increasing rapidly. Mechanisms regulating the proliferation of fibroblasts and their productivity remain largely unknown, however. The origin of these fibroblasts, the basic cells of wound repair, is still controversial. Recent investigations have supported both vascular and local origin of these cells.[2, 36, 44, 49] The present experimental study explored the question of the predominant site of origin of fibroblasts.

Earlier experiments demonstrated the inhibitory effect of x-irradiation on proliferation of new connective tissue varying with time of application of irradiation in relation to time of wounding.[22] In the present work x-irradiation was applied in moderate doses at different times, before and after wounding, to the local area of the wound and proliferation of fibroblasts was studied quantitatively five days after wounding using autoradiography with tritiated thymidine to label the nuclei of cells about to divide.

The tritiated nucleoside, thymidine, is incorporated specifically into replicating deoxyribonucleic acid (DNA) which is formed just prior to cell division. Incorporation occurs promptly after injection and the recently synthesized, labelled DNA remains unchanged in the nucleus unless dilution occurs by further division or until cell death occurs. Autoradiographs prepared from tissues of animals into which tritiated thymidine has been injected accurately identify cell proliferation and permit these cells and their descendants to be followed.[3, 29, 34, 55]

The experimental results were interpreted as demonstrating that the principal site of origin of fibroblasts of wound repair was the connective tissue adjacent to the wound defect, and that most of these cells did not arrive by the vascular route from distant sites.

Methods and Materials

Wounds. Two full thickness wounds measuring 1.0 × 1.0 cm., with the anterior

* Submitted for publication June 5, 1962.

Supported in part by research grant C-3638 from the National Cancer Institute of the United States Public Health Services and A. E. C. Contract AT (30-1)-2643.

This is publication 1103 of the Cancer Commission of Harvard University and 324 of the Robert W. Lovett Memorial Unit for the Study of Crippling Diseases, Harvard Medical School at the Massachusetts General Hospital.

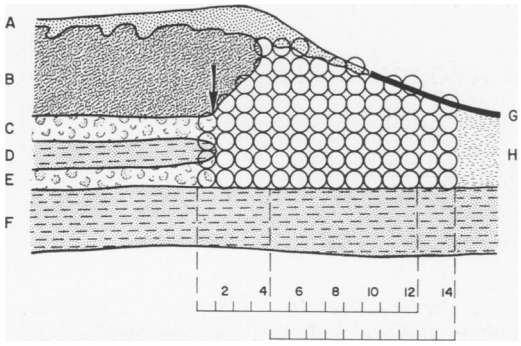

FIG. 1. Diagram of wound margin at 5 days and method of sampling; a, epithelium; b, dermis; c, subdermal connective tissue; d, panniculus carnosus muscle; e, subpannicular loose connective tissue; f, parietal muscle; g, scab and surface exudate; h, proliferating new connective tissue. Arrow designates point of original wound cut. Circles indicate high-power microscopic fields successively counted. Numbers indicate zones. When 10 zones only were counted, zones 5 through 14 were used.

wound placed 2.0 cm. from the posterior wound, were made through skin and panniculus carnosus of the left side of the trunks of male guinea pigs weighing about 240 Gm. They were maintained on a standard laboratory diet of Purina pellets with lettuce and water supplement. The operative field was prepared with electric clippers and a depilatory (Nair). Operations were performed under ether anesthesia. Wounds were not dressed.

Irradiation. X-irradiation was delivered without anesthesia, utilizing tubular plastic holders[22] through a 2.0 × 2.0 cm. portal centered over the anterior wound or the site of the anterior wound. The animals were otherwise shielded by collimation and by 0.8 mm. of lead; 750 roentgens of x-irradiation were delivered in a single dose (10 MA., 100 KV., 0 filter, at a distance of 20 cm., time 1 min. 58 sec.). Thus a zone extending 0.5 cm. away from the wound edge was irradiated. Dose selection has been previously discussed.[22] No systemic effects were noted.

Irradiation was applied to the anterior wound of one guinea pig 20 minutes prior to wounding, to another 20 minutes following completion of operation, and to a third animal 28 hours after wounding. One animal received no irradiation.

In addition to these wounds prepared for autoradiographic study, wounds were prepared for histologic examination with and without irradiation at periods needed for interpretation of the experimental results. Wounds were studied 20 minutes, 6 hours, 18 hours, and 28 hours after operation, without subsequent irradiation, and at 6, 18, and 28 hours after operation where irradiation had been applied in standard fashion 20 minutes after wounding.

Autoradiography. Five days after wounding each guinea pig was injected intraperitoneally with 1.0 microcurie of tritiated thymidine (specific activity 1.9 curies per millimole) per gram of body weight. Forty-five minutes following injection the wounds were totally excised with full thickness of body wall, pinned on cork to prevent distortion, and fixed in Bouin's fluid. Control samples of normal parietes, liver and small intestine were taken. Wounds were sectioned longitudinally and both cephalad and caudad margins studied. Sections were cut at 5 to 6 micra from chilled blocks with a cold microtome blade to avoid dermal-connective tissue fragmentation. Hematoxylin and eosin staining was used for routine preparations. Autoradiographs were prepared by stripping film technic using Kodak AR. 10 plates. Film was removed under alcohol to prevent flashing.[41] Dried sections were sealed in light-tight boxes in the presence of dessicant and stored at 4.0° to 6.0° C. for four weeks. After exposure, autoradiographs were developed with Kodak D.19b developer, dried thoroughly, stained with Harris' hematoxylin and mounted in synthetic resin (Permount).[13, 18, 54]

Quantitation. Slides were reviewed in detail and quantitative measurement of labelled mesenchymal cells in a selected area of the wound was made as an index of

cellular proliferation of connective tissue. The wound margin was chosen for study because of its previously demonstrated activity [21, 60] and for specificity of comparison. Approximately 11 slide preparations of each wound were examined and counted. For quantitative appraisal all clearly labelled mesenchymal cells in a given area superficial to the deep muscle layer of the abdominal wall and not within the dermis were counted. On the fifth day the cells distributed in these connective tissue zones were principally if not entirely fibroblasts and capillary endothelial cells. Where epithelium or dermis crossed part of a field, cells within these tissues were not included.

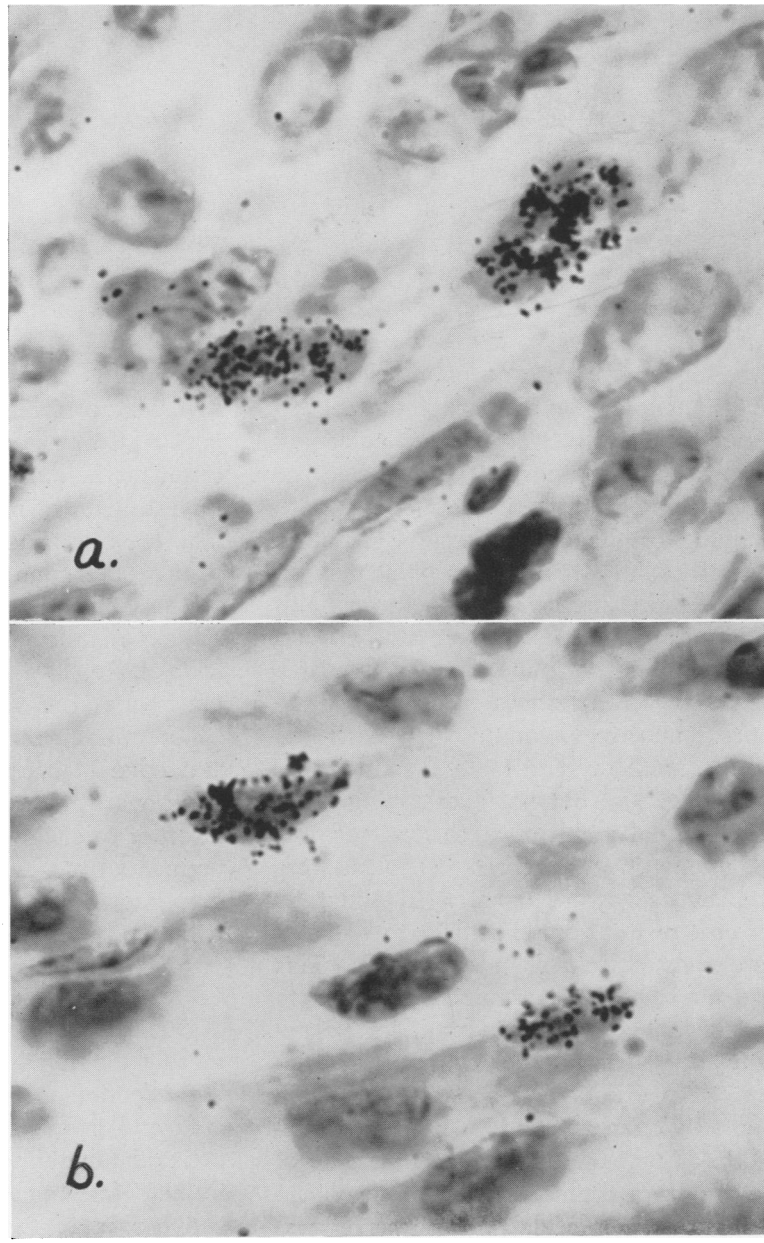

FIG. 2a, b. Photomicrographs of autoradiographs of proliferating new connective tissue in 5 day old wounds, sampled 45 minutes after administration of tritiated thymidine (Harris hematoxylin, × 1,400). a, unirradiated wound. b, wound irradiated 28 hours after operation.

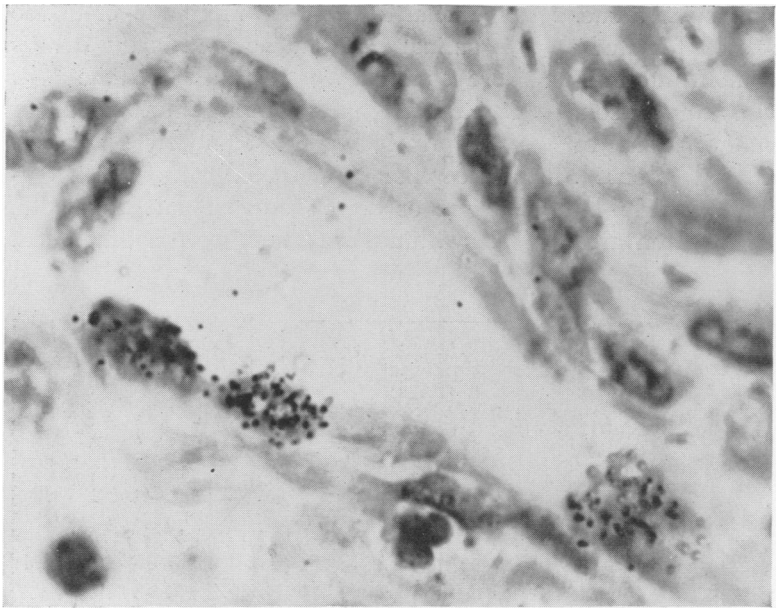

FIG. 3. Autoradiograph of capillary in area of new tissue proliferation (Harris hematoxylin, × 1,400), showing thymidine uptake by cells in capillary wall.

Counting was begun at a point selected to be just beneath the deep edge of the vertically cut dermis. This point is no longer sharp in some wounds at five days because the process of contraction has begun, with rounding of the dermal edge[21, 22] but in general identification of this point may be made clearly. Successive high power fields (1,250 × magnification) were studied and counted under oil immersion. Twelve such successive vertical zones encompassing the entire connective tissue thickness described were examined. These fields and zones were tangential but not overlapping (Fig. 1). The total width of area examined was 4.8 mm.; since microscopic fields were circular, cells were counted in about 80 per cent of this zone. From 65 to 75 fields were thus examined in each slide. This large an area was examined in order to average out variations in proliferative activity found in small segments of granulation tissue. About 700 or 800 fields were counted in each wound. A random distribution of cephalad and caudad margins of wounds were evaluated in each case. The average number of labelled mesenchymal cells in a high power field was calculated. In order to minimize a possible effect of postirradiation edema in lowering the cell count per field, a value was also calculated for the average number of cells in an 0.8 mm. *average* zone of each classification of wound, the zone extending from the top of the connective tissue to the underlying muscle. Also, calculations both of cells per field and cells per zone were repeated in ten zones after discounting the first four zones initially measured beneath the dermal edge in each case and counting two additional zones toward the wound's center in order to check the validity of the measurements by effectively *sliding* the area of sampling in each case and comparing the two results.

Results

Qualitative Observations. We have previously described the general histology of the experimental wounds, both unirradiated and irradiated.[22] Generous uptake of tritiated thymidine by fibroblasts occurred in new forming connective tissue in the active area of the wound margin and in

the open wound bed (Fig. 2a). Relatively few dividing cells even for a much smaller fibroblast population were identified in the loose connective tissue a short distance back from the edge of the wound. At the wound edge greater numbers of cells were labelled in a unit area in the region immediately related to the cut edge of the subdermal connective tissue layer than in the active subpannicular layer. Within the mass of granulation tissue small areas varied in frequency of labelling. In general, highest concentrations of labelled cells were encountered surrounding capillaries in the new forming tissues. Thymidine incorporation into cells of resting connective

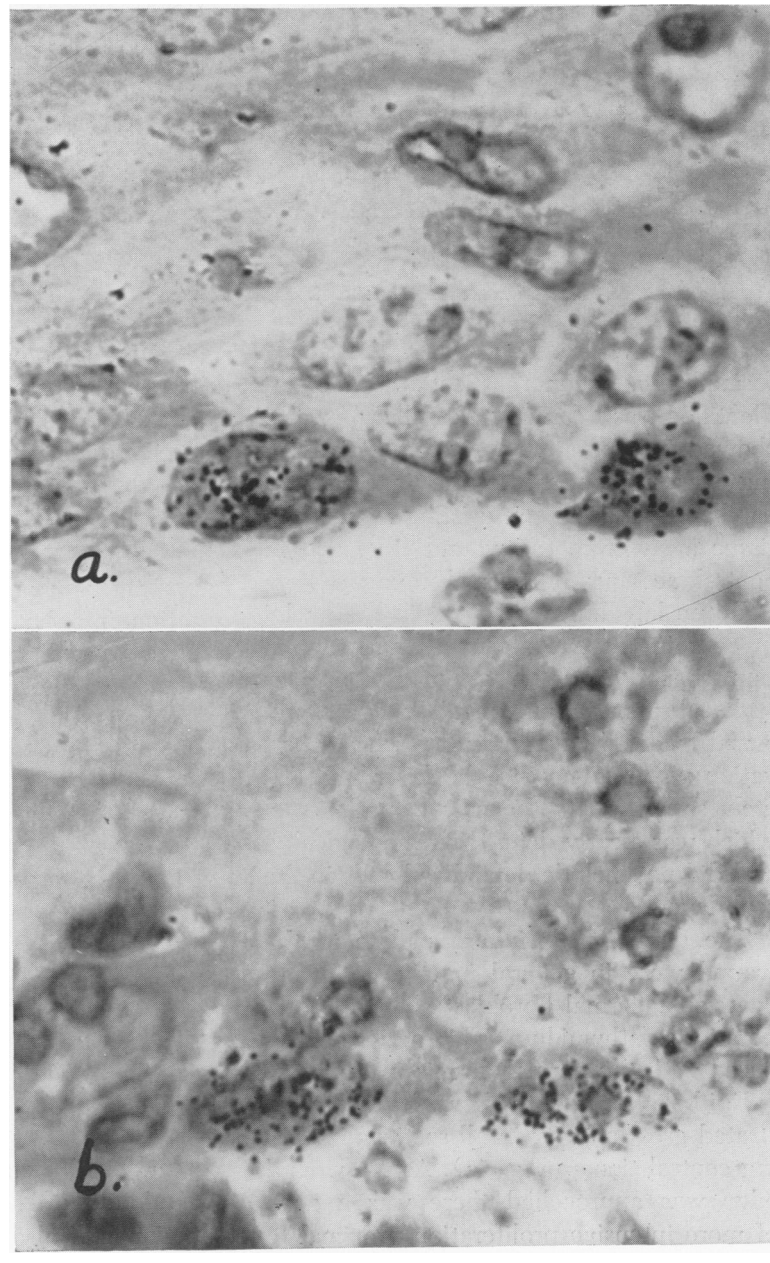

FIG. 4a, b. Autoradiographs of basal layers of epithelium (Harris hematoxylin, × 1,400), showing active uptake by basal cells in both, a, unirradiated and b, irradiated wounds. These areas lie in the zone of swollen epithelium just at the edge of the dermis, but proximal to the thin distal tongue of epithelium migrating over the wound surface.

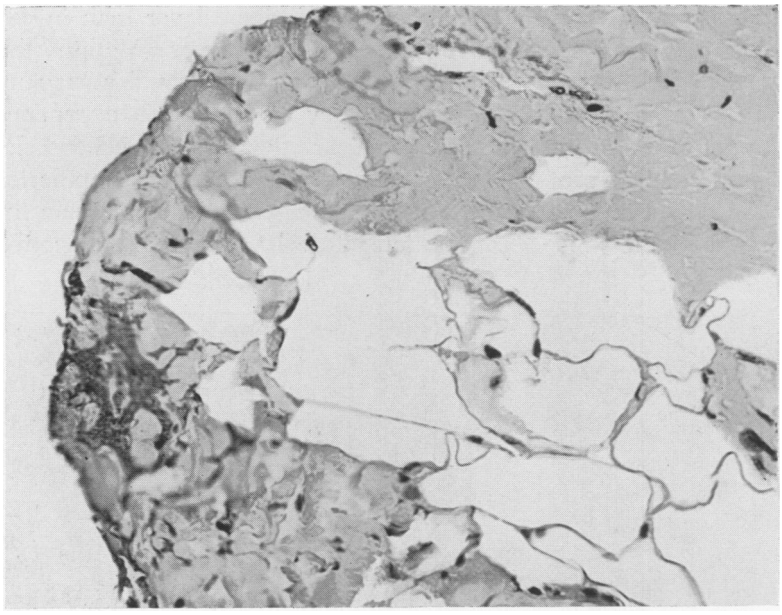

FIG. 5. Photomicrograph of wound edge 20 minutes after operation (hematoxylin and eosin, × 375). The cut edge is at the left. Dermis lies above, subdermal fatty and connective tissue layer beneath, and upper edge of panniculus carnosus muscle is seen beneath. The connective tissue layer will soon be infiltrated with cells. At this time, the time when irradiation was done in these experiments, no infiltration has yet begun.

tissue was extremely rare. In the newly formed tissue numerous endothelial cells, identified as forming capillary walls, showed incorporation (Fig. 3). This finding was in contrast to the inactivity of capillary walls in pre-existing connective tissue away from the wound edge and in control sections of normal tissue. Essentially no labelling was noted in the nuclei of cells in the exudate overlying granulation tissue, either in polymorphonuclear leukocytes or in mononuclear cells, presumably chiefly lymphocytes. This observation was confined to the layer immediately superficial to the new wound tissue, which was clearly defined morphologically, and did not include devitalized cells in the scab layers.

The dermal cells in areas close to the wound were in general inactive. Only an occasional dermal fibroblast showed incorporation of thymidine. This activity compared with the normal dermis in quantity.

Epithelial cells in the basal layer demonstrated great activity in all sections including control tissue (Fig. 4a). As the wound edge was approached there was a zone of more intensive proliferation proximal to the zone where epithelial tongues were seen to be spreading over the granulation tissue. The cells in these tongues showed little frequency of incorporation.

Sections of wound sampled 20 minutes after operation showed no cellular infiltration (Fig. 5). At six hours there was an apparently equal amount of cellular migration into both an unirradiated wound and a wound irradiated 20 minutes after wounding; the cells were largely polymorphonuclear with some mononuclear cells present (Fig. 6a, b). By 28 hours the cellular infiltration still contained many polymorphonuclear cells but mononuclear cells were present in both unirradiated and irradiated wounds. There appeared to be somewhat less exudate and fewer cells in the irradiated wound (Fig. 7a, b).

Quantitative Measurements. X-irradiation of a wound 28 hours after operation resulted in a marked reduction in mesenchymal cellular proliferation (by 53%) when measured on the fifth day following wounding. When irradiation was applied 20 minutes after operation, significant reduction was also evident on the fifth day

(45%). Irradiation 20 minutes prior to operation also appeared to reduce cellular proliferation slightly when measured on the fifth day (Table 1, Fig. 8).

When the number of dividing cells per field was calculated by sampling as described and then compared with calculations after *sliding* the area of sampling, results were generally closely comparable. Calculations based upon *zones* rather than fields, made to minimize possible effects of edema, were also closely comparable (Fig. 9a, b). The number of cell divisions per field in unirradiated wounds was nearly identical in anterior and posterior wounds (3.96 and 4.01) in a single animal so that

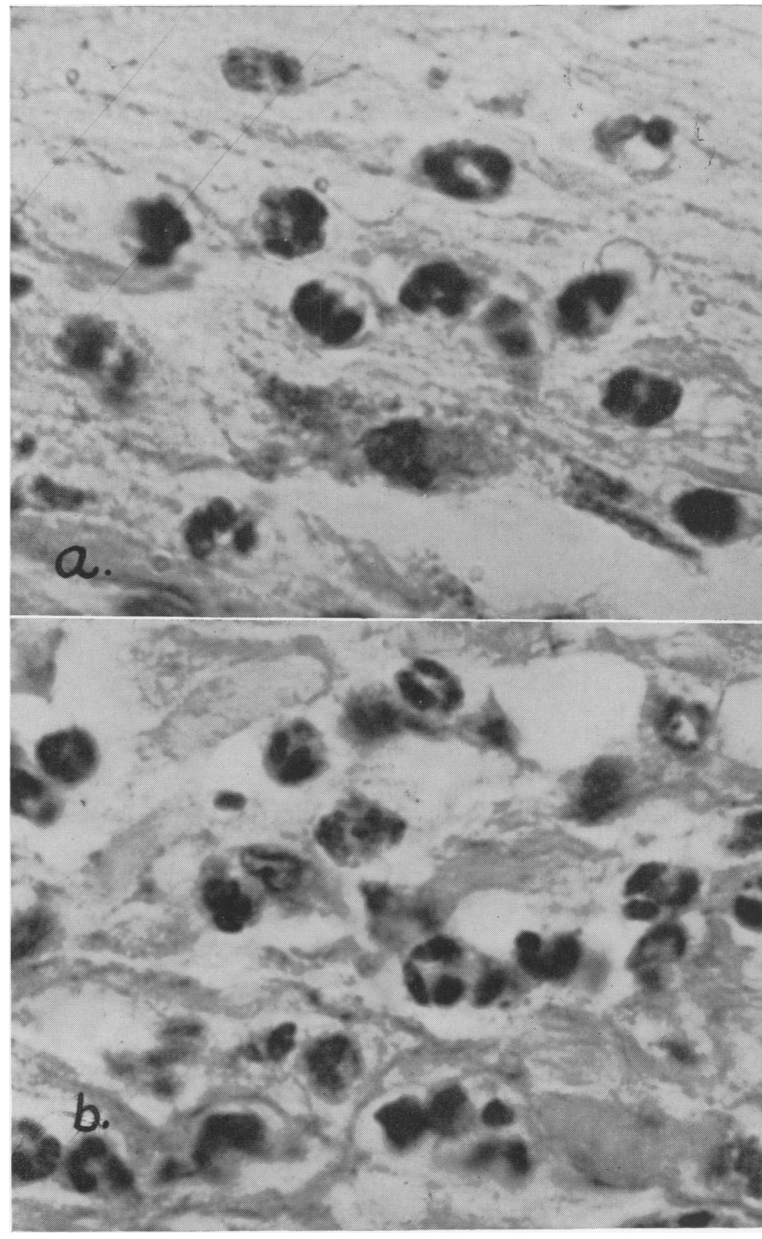

FIG. 6a, b. Photomicrographs of cellular infiltration into connective tissue layer exudate at wound margin at 6 hours after operation (H and E, × 1,400). a, unirradiated wound, and b, wound irradiated 20 minutes after operation. Polymorphonuclear leukocytes are present in large numbers in both wounds.

possible variations due to differences in behavior of anterior and posterior wounds in a single animal were ruled out. There were variations in average number of cell divisions in unirradiated wounds from animal to animal (3.69, 3.96, 4.01, 4.86, 5.19) but these values were all above average numbers for irradiated wounds (2.00, 2.81, 3.29) (Fig. 9a). Comparison of irradiated with unirradiated wounds as percentage effect in the same animal was therefore made (Fig. 8).

A test of homogeneity by standard chi square analysis [51] (Table 1) indicated that the difference in the fractions of cells labelled in columns A, B, C and D was far greater than could be accounted for by sampling errors ($P < < 0.001$). Similar

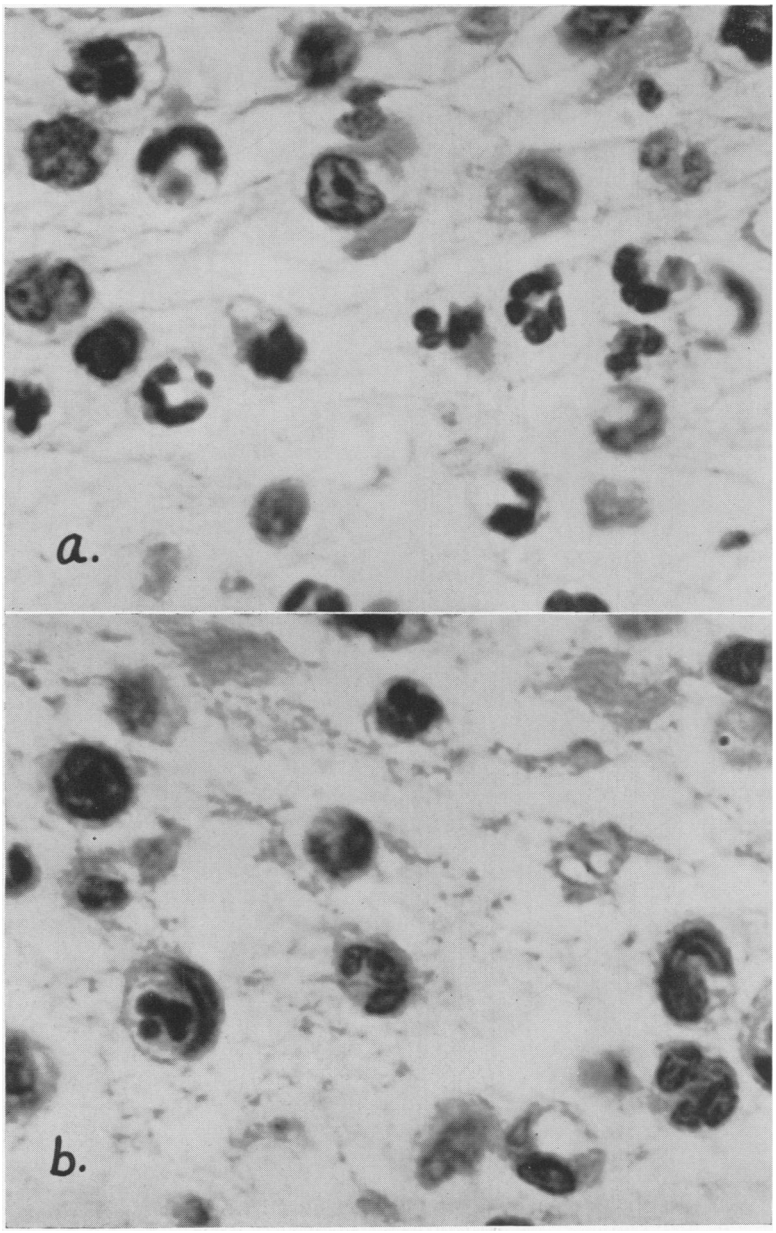

FIG. 7a, b. Cellular infiltration at 28 hours after operation. (H and E, × 1,400). Mononuclear forms are appearing. a, unirradiated wound. b, wound irradiated 20 minutes after wounding.

TABLE 1. *Number of Cells Labelled and not Labelled in Treatments*

Treatment	No Irradiation	Irradiation at Times in Relation to Wounding: −20 min.	+20 min.	+28 hrs.	Totals
Column	A	B	C	D	E
Labelled	3,313	2,052	2,189	600	8,154
Not labelled	−214	168	1,791	684	2,429
Totals	3,099	2,220	3,980	1,284	10,583
Fraction of cells labelled	1.07	0.92	0.55	0.47	0.77

Labelled cells are the total number in areas counted in all samples (see text). Number of cells not labelled is calculated as difference between control area and treated area in the same animal (or unirradiated area in Column A); this accounts for the negative value in column A due to chance circumstances. Total number of cells is the number labelled in the control in the same animal in each case. Chi square analysis is discussed in the text.

testing of the control column A against B, C and D gave P values of > 0.05, < 0.001 and < 0.001, respectively. Thus irradiation 20 minutes prior to wounding did not give a result significantly different from the control value while the other treatments did. The difference between columns C and D in the fractions of cells labelled was likewise highly significant ($P < 0.001$). In counting labelled cells not all control and treated wounds had equivalent areas surveyed. To permit statistical comparisons between equal areas the counts were standardized to the smaller area in comparing control and treated wounds in the same animal. This adjustment merely results in the computed chi squares underestimating the significance of the differences noted in Table 1.

A standard analysis of variance [51] comparing the variance of the numbers of labelled cells in microscopic fields with the variance of the number of labelled cells in the entire area enumerated in the slides was completed to evaluate the degree of uniformity, or lack thereof, in the healing process. The variance ratios indicated in four of the eight wounds studied a variation in labelled cell counts greater than explained by random sampling. (P values

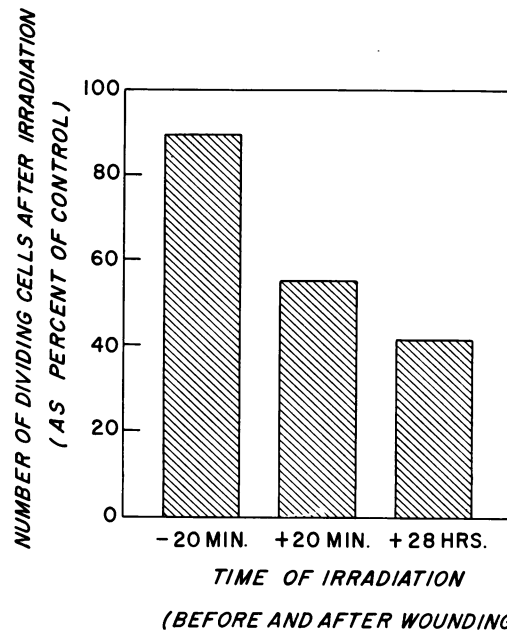

FIG. 8. Chart of the average number of tritium labelled cells per high power field present in the area of proliferation 5 days after irradiation at varying times before and after operation (20 minutes prior, and 20 minutes and 28 hours after wounding) expressed as percentage of the number present at 5 days in unirradiated wounds. The graph represents data from counting of 12 zones. Similar data were obtained from counting of 10 zones, and from plotting of cells per zone to eliminate a possible factor of variation due to edema. Cells within the epithelial, dermal, or muscular layers were not counted.

for F ratios: > 0.25, > 0.2, > 0.05, > 0.01, > 0.001, < 0.001, < 0.001, < 0.001.) This heterogeneity demonstrated the importance of counting large numbers of cells in many different areas to obtain an unbiased impression of activity in the whole wound, confirming the presence of wide variations in precise stage and activity of proliferation in a single healing wound due to yet undefined factors.

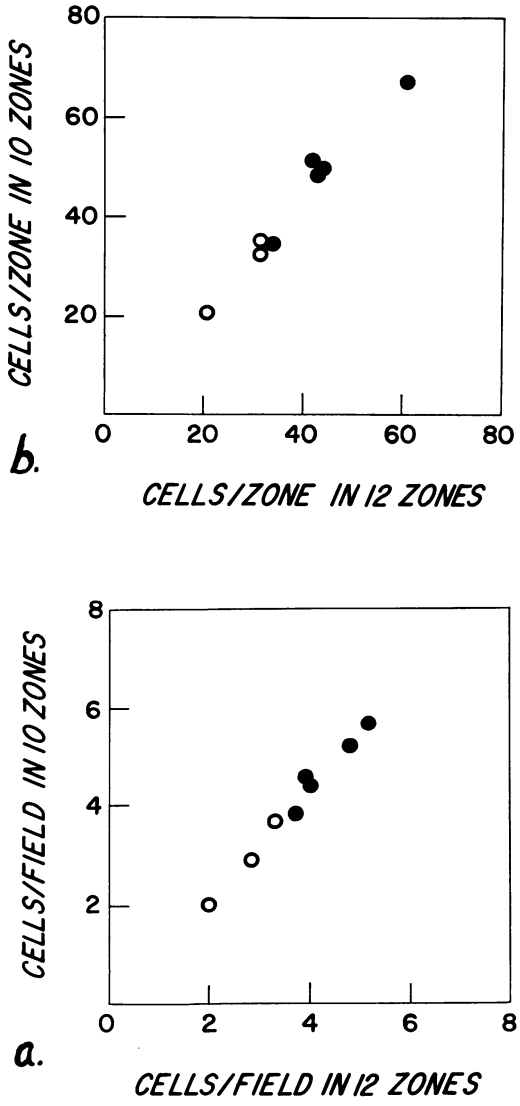

FIG. 9a, b. Correlation diagrams. In Figure 9a the average number of labelled cells per microscopic field when fields in 12 zones were counted is compared with a similar number obtained from counting 10 zones, "shifted" as described in the text. Figure 9b demonstrates correlation between average number of labelled cells in a zone when 12 zones were counted and when 10 zones were counted. See text. Correlations between fields and zones were also close.

Discussion

The marked inhibition of later fibroblastic division in wounds which resulted from a single local application of x-irradiation at 28 hours after wounding indicated that a significant number of fibroblasts or their progenitor cells were present in the area of the wound at 28 hours. Since many polymorphonuclear leukocytes and mononuclear cells had infiltrated the wound zone prior to 28 hours (Fig. 7) some of these cells could have been precursor cells of a later fibroblast population. Irradiation was therefore applied 20 minutes after wounding—a time before there was any infiltration by cells of vascular origin (Fig. 5). Again, marked inhibition of later fibroblastic division resulted. Since no apparent inhibition of initial cellular infiltration from the blood stream followed irradiation 20 minutes after wounding (Fig. 6), it was concluded that the effect on fibroblast proliferation resulted from influences applied to cells resident in the tissues of the wound area at the time of wounding. Certainly the direct effect could not have been upon cells yet to arrive in the wound area from distant sites. Further, the cells of hematogenous origin which enter the wound area in the early hours are chiefly neutrophils, as demonstrated in these experiments and by others.[32, 46, 50] Even enthusiastic proponents of vascular origins of fibroblasts have not proposed that neutrophils are pluripotential cells. Hence a large proportion, if not the major portion of fibroblasts would seem to originate from cell precursors present in resting local mesenchymal tissues.

The local irradiation dose used was moderate. At this dose histologic recovery oc-

curred in time in all wounds and cell necrosis was not seen.[22] Cells in an irradiated field may be variably affected. It was not to be expected that total inhibition of cell division would result, especially when measured four to five days after irradiation. The difference in inhibition observed between irradiation applied shortly after wounding and 28 hours after wounding may have arisen not so much from an added day of recovery time available in the first instance, as from the differential sensitivity of cells in various phases of their proliferative cycle. Whatever may be the stimulus to division which injury supplies, steps preceding visible division are obviously much further along at 28 hours than at 20 minutes following stimulation. It is accepted that cells become highly sensitive to irradiation just prior to visible division.[17, 45, 52] Earlier experiments presented indirect evidence of rapidly increasing tissue sensitivity to irradiation after the stimulus of wounding, with a peak effect following irradiation given at 36 hours.[22] Cells not initially affected by irradiation, either due to statistical chances of irradiation or due to their being in a relatively insensitive phase, might account for a large population in four or five days. It cannot, however, be excluded that some of the difference measured in inhibitory effect between wounds irradiated at 20 minutes and at 28 hours after wounding might have resulted from augmentation of a principally local source of fibroblasts by cells of vascular origin arriving during this interval.

The small late effect noted when irradiation was applied just prior to wounding was not statistically significant and may not be a constant phenomenon. The small size of the effect may reflect relative insensitivity of resting, unstimulated connective tissue cells to irradiation, especially at this dose level. A generally low rate of division has been observed for normal connective tissue cells and hence only a very few cells would be in an optimally sensitive premitotic phase.[29, 34]

Inhibition of later proliferation caused by a primary irradiation effect on extracellular components of the wound seemed unlikely since so few of these components were present at 20 minutes. It is, however, impossible to state categorically that radiation changes in the extracellular molecular constituents of the local connective tissue or products resulting from irradiation injury exerted no influence on the cellular processes shortly to occur. Mechanical blockade was similarly not a convincing possibility since no secondary accumulation of infiltrating cells was seen early or late to suggest such a barrier. Indeed, a relatively normal infiltration from the vascular system was observed early after irradiation. It has been proposed that one effect of irradiation is to inhibit capillary proliferation which then secondarily limits fibroblast movement but not fibrogenesis, resulting in erection of a fibrous barrier to further proliferation.[59] In the present experiment no excessive proliferative attempts by fibroblasts were observed adjacent to nonproliferating capillaries. Thus, while capillary inhibition did occur, and it is probable that in time fibrous precipitation might result locally from the activity of arrested fibroblasts, there was no evidence found to suggest a limitation of recruitment from the vascular system in the area as a result of irradiation effect on capillaries.

Synthesis of DNA occurs prior to the earliest events of mitosis and probably independently of mitosis.[7, 25, 56] The precise radiosensitivity of nucleic acids and nucleoprotein is not known[17, 24, 43] nor has the mechanism by which irradiation causes reversible and irreversible mitotic arrest yet been finally clarified.[45] Since observations were made four to five days after irradiation, no conclusions could be made about primary effects of irradiation on

DNA synthesis and mitosis, but this interval probably assured the validity of using thymidine incorporation into DNA as an indicator of pre-mitotic synthetic activity preceding actual division.

Additional qualitative observations of epithelium, dermis and capillaries in these studies by the technic of DNA labelling have confirmed a number of points about the behavior of these elements in wound repair. The epithelium has again been seen to show proliferative activity chiefly proximally from the tip of epithelium advancing over the wound, denoting the migratory aspect of epithelial coverage,[19, 32] and proliferation has been seen to occur almost wholly in the basal layer of epithelium. Intradermal *fibroblasts* have been shown to be inactive and the dermis to play a passive role. Recognition of dermal inertness in wound healing is becoming generally accepted.[19] Cellular proliferative activity was also identified in the new capillaries of the granulation tissue, supplying additional evidence of growth by this means[12] a not yet fully accepted mechanism;[32, 58] the budding tips, however, were not specifically identified in this study.

After a century of experimentation and discussion, the origin of fibroblasts in wound repair has remained unclear. The concept that fibroblast proliferation after injury originates chiefly from locally present fibroblasts or other mesenchymal progenitors permanently resident in the adjacent tissues, is widely accepted. However, evidence continues to be presented suggesting that precursor cells arrive in the wound from the vascular space following injury. The controversy has been complicated by variable nomenclature and the difficulties of positive morphologic identification of single cells. Another difficulty has been unilateral consideration on the one hand of the potential of a cell or cells derived from the blood, and, on the other hand, the possibly different problem, quantitatively, of predominant origin of fibrogenetic cells in a wound.

The controversy began when assertions of the histogenous origin of fibroblasts by Marchand[37] and Aschoff[5] were sharply challenged by Maximow[38-40] and Bloom, who described conversion of lymphocytes into fibroblasts.[6] Arey[4] reviewed the conflicting theories in 1936 and since then Allgöwer[1] has done so in a monograph reporting his own earlier experiments on this question. No attempt is made here to review in detail the extensive literature on this subject. Static observations of the evolution of cells[19, 26, 28] have continued to present difficulties in morphologic identification even with electron microscopy.[46] With direct observations of the continuing close morphologic relationship between proliferating fibroblasts and capillaries in repair[12, 53] it becomes difficult to accept diapedesis as the explanation of the perivascular concentration of fibroblasts, a finding often presented as argument in favor of a vascular origin of these cells. Observation of early fibroblast proliferation[47] makes it unnecessary to invoke conversion of vascular infiltrates to explain the development of large fibroblast populations by the fourth and fifth days after wounding. Results obtained by modification of the vascular response by total body irradiation and its influence on repair[1] are difficult to interpret because of profound abnormalities resulting from such irradiation.[52] Except for some support from Carrel and Ebeling,[9, 10] the tissue culture experiments of Bloom were not successfully repeated[14, 15, 23, 42] until Allgöwer and Hulliger demonstrated that cells obtained by intracardiac puncture were capable of producing hydroxyproline-containing material in culture.[2, 30, 31] Shelton and Rice applied chamber technics of *in vivo* culture[48, 49] in investigation of this problem, and cellular evolution has been studied in man recently by sequential smear technics[50] and by use of implanted

diffusion chambers containing white cells.[44] The exact potential of the lymphocyte remains unsettled.[20, 33, 57, 62] MacDonald recently studied wounds at different time intervals by labelling dividing cells with tritiated thymidine,[36] and concluded from observations of the morphology of the labelled cells, their distribution, and their further patterns of division, that fibroblasts originated locally, apparently from a system of cells surrounding blood vessel, hair follicles and muscle bundles.

Thus, although identification of conversion of lymphocyte or monocyte into fibroblast is not conclusive, a hydroxyproline-containing material, presumably collagen, has been obtained from cells cultured from the blood stream. If contamination could be fully eliminated as the source, such cells, whatever their origin, even if fibroblasts washed from the marrow or other sites, could certainly supply a portion of the collagen producing cell population of a wound. Much less certain would be the number of fibroblasts derived from this source.

The present experiments did not deny the possibility that some cells arriving from the vascular system either had or could develop the ability to function as fibroblasts, and might contribute to fibroplasia after injury, but rather indicated that the major source of fibroblasts in repair was from pre-existent, local, previously resting cells. It can not be finally stated whether these cells are differentiated but resting fibroblasts, or whether this represents an example of *modulation* of resident mesenchymal cells as proposed by Weiss.[61] We could not separate the intimate partnership of the fibroblast–capillary system which springs into action from dormant connective tissue in response to injury. The repair response seems to parallel the regenerative response in amphibia in its local character as demonstrated by Butler and O'Brien in the urodele,[8] quantitatively supported by Chalkley[11] and confirmed in the recent experiments of Hay.[27]

Summary and Conclusions

1. Local x-irradiation of experimental wounds 28 hours after injury resulted in over 50 per cent reduction in subsequent fibroblast and capillary endothelial cell proliferation, measured five days after wounding by incorporation of tritiated thymidine into DNA. When irradiation was applied prior to the onset of any cellular infiltration from the blood, 20 minutes after wounding, marked reduction in later cellular proliferation also resulted.

2. These findings indicated that fibroblasts of wound repair arose predominantly by proliferation of locally resident connective tissue cells, rather than from precursor cells recruited via the vascular system.

Acknowledgments

The generous help of Dr. Majic S. Potsaid, Department of Radiology, Massachusetts General Hospital, is gratefully acknowledged. The invaluable technical assistance of Mrs. Hilliard Macomber is similarly noted. Dr. Benjamin A. Barnes, Department of Surgery, Massachusetts General Hospital, was most helpful in statistical analyses.

References

1. Allgöwer, M.: The Cellular Basis of Wound Repair. Springfield, C. C Thomas, 1956.
2. Allgöwer, M. and L. Hulliger: Origin of Fibroblasts from Mononuclear Blood Cells: A Study on *in vitro* Formation of the Collagen Precursor, Hydroxyproline, in Buffy Coat Cultures. Surgery, **47**:603, 1960.
3. Amano, M., B. Messier and C. P. Leblond: Specificity of Labelled Thymidine as a Deoxyribonucleic Acid Precursor in Radioautography. J. Histochem. Cytochem., **7**:153, 1959.
4. Arey, L. B.: Wound Healing. Physiol. Rev., **16**:327, 1936.
5. Aschoff, L.: Das Reticulo-endotheliale System. Ergeb. d. inn. Med. u. Kinderheilk., Bd. 26, S. 1, 1924.
6. Bloom, W.: Mammalian Lymph in Tissue Culture. From Lymphocyte to Fibroblast. Arch. Exper. Zellforsch., **5**:269, 1928.

7. Bucher, N. L. R. and D. Mazia: Deoxyribonucleic Acid Synthesis in Relation to Duplication of Centers in Dividing Eggs of the Sea Urchin, *Strongylocentrotus Purpuratus*. J. Biophys. Biochem. Cytol., 7:651, 1960.
8. Butler, E. G. and J. P. O'Brien: Effects of Localized X-radiation on Regeneration of the Urodele Limb. Anat. Rec., **84**:407, 1942.
9. Carrel, A. and A. H. Ebeling: Pure Cultures of the Large Mononuclear Leucocytes. J. Exp. Med., **36**:365, 1922.
10. Carrel, A. and A. H. Ebeling: The Transformation of Monocytes into Fibroblasts through the Action of Rous Virus. J. Exp. Med., **43**: 461, 1926.
11. Chalkley, D. T.: The Cellular Basis of Limb Regeneration. Chapter III, pp. 34–58, in Regeneration in Vertebrates, Ed. C. S. Thornton, Chicago, U. Chicago Press, 1959.
12. Clark, E. R. and E. L. Clark: Microscopic Observations on Growth of Blood Capillaries in Living Mammal. Am. J. Anat., **64**:251, 1939.
13. Doniach, I. and S. R. Pelc: Autoradiograph Technique. Br. J. Radiol., 23:184, 1950.
14. Ebert, R. H. and H. W. Florey: The Extravascular Development of the Monocyte Observed *in vivo*. Br. J. Exp. Path., **20**:342, 1939.
15. Ebert, R. H., A. G. Sanders and H. W. Florey: Observations on Lymphocytes in Chambers in the Rabbit's Ear. Br. J. Exp. Path., 21:212, 1940.
16. Edwards, L. C. and J. E. Dunphy: Wound Healing. I. Injury and Normal Repair. N. E. J. M., **259**:224, 1958.
17. Errera, M.: Effects of Radiation on Cells, pp. 695–740, in The Cell, Biochemistry, Physiology, Morphology. Ed. J. Brachet, A. E. Mirsky. New York, Academic Press, 1959.
18. Fitzgerald, P. J.: Autoradiography in Cytology, in Analytical Cytology, Methods for Studying Cellular Form and Function. ed. R. C. Mellors. New York, McGraw-Hill, 1959.
19. Gillman, T. and J. Penn: Studies on the Repair of Cutaneous Wounds. Med. Proceedings, 2:121, 1956.
20. Gowans, J. L.: The Effect of the Continuous Re-infusion of Lymph and Lymphocytes on the Output of Lymphocytes from the Thoracic Duct of Unanesthetized Rats. Br. J. Exp. Path., **38**:67, 1957.
21. Grillo, H. C., C. Levene and J. Gross: Unpublished observations.
22. Grillo, H. C. and M. S. Potsaid: Studies in Wound Healing. IV. Retardation of Contraction by Local X-irradiation, and Observations Relating to the Origin of Fibroblasts in Repair. Ann. Surg., **154**:741, 1961.
23. Hall, J. W. and F. Furth: Cultural Studies on the Relationship of Lymphocytes to Monocytes and Fibroblasts. Arch. Path., **25**:46, 1938.
24. Harbers, E.: Wirkungen ionisierender Strahlen auf die Nukleinsäuren. Strahlentherapie, **112**: 333, 1960.
25. Harrington, H.: Effect of Irradiation on Cell Division and Nucleic Acid Synthesis in Strain U-12 Fibroblasts. Biochim. Biophys. Acta, **41**:461, 1960.
26. Hartwell, S. W.: The Mechanisms of Healing in Human Wounds. C. C Thomas, Springfield, Ill., 1955.
27. Hay, E. D. and D. A. Fischman: Origin of the Blastema in Regenerating Limbs of the Newt *Triturus Viridescens*. Develop. Biol., 3:26, 1961.
28. Howes, E. L. and S. C. Harvey: The Clinical Significance of Experimental Studies in Wound Healing. Ann. Surg., **102**:941, 1935.
29. Hughes, W. L., V. P. Bond, G. Brecher, E. P. Cronkite, R. B. Painter, H. Quastler and F. G. Sherman: Cellular Proliferation in the Mouse as Revealed by Autoradiography with Tritiated Thymidine. Proc. Nat'l. Acad. Sci., **44**:476, 1958.
30. Hulliger, L.: Über die unterschiedlichen Entwicklungs-fähigkeiten der Zellen des Blutes und der Lymphe in vitro. Arch. Pathol. Anat. u. Physiol. Virchow's, **329**:289, 1956.
31. Hulliger, L. and M. Allgöwer: Proliferation und Differenzierung monocytarer Zellen des peripheren Blutes. Schweiz. Med. Wochenschrift, **91**:1201, 1961.
32. Johnson, F. R. and R. M. H. McMinn: The Cytology of Wound Healing of Body Surfaces in Mammals. Biol. Rev. (Cambr. Phil. Soc.), **35**:364, 1960.
33. Kelsall, M. A. and E. D. Crabb: Lymphocytes and Plasmacytes in Nucleoprotein Metabolism. Ann. N. Y. Acad. Sci., 72:295, 1958.
34. Leblond, C. P., B. Messier and B. Kopriwa: Thymidine-H^3 as a Tool for the Investigation of the Renewal of Cell Populations. Lab. Investig., **8**:296, 1959.
35. Leblond, C. P. and B. E. Walker: Renewal of Cell Populations. Physiol. Rev., **36**:255, 1956.
36. MacDonald, R. A.: Origin of Fibroblasts in Experimental Healing Wounds: Autoradio-

graphic Studies Using Tritiated Thymidine. Surgery, **46**:376, 1959.

37. Marchand, F.: Der Prozess der Wundheilung. Deutsche Chirurgie, herausg. von v. Bergmann und v. Bruns, Lief. 16, Stuttgart, 1901.
38. Maximow, A.: Experimentelle Untersuchungen über entzündliche Neubildung von Bindegewebe. Ziegler's Beitr. z. path. Anat. u. allg. Path, Suppl. 5, 1902.
39. Maximow, A.: Development of Non-granular Leucocytes (Lymphocytes and Monocytes) into Polyblasts (Macrophages) and Fibroblasts in vitro. Proc. Soc. Exp. Biol. and Med., **24**:570, 1926–7.
40. Maximow, A.: Cultures of Blood Leukocytes. From Lymphocytes and Monocytes to Connective Tissue. Arch. exper. Zellforsch, **5**: 169, 1928.
41. Mazia, D. and N. L. R. Bucher: A Method for Avoidance of Electrostatic Flashing in Preparing Autoradiographs with Stripping Film. Experientia, **16**:215, 1960.
42. Medawar, J.: Observations on Lymphocytes in Tissue Culture. Br. J. Exp. Path., **21**:205, 1940.
43. Painter, R. B., V. W. R. McAlpine and M. Germanis: The Relationship of the Metabolic State of Deoxyribonucleic Acid During X-irradiation to HeLa S3 Giant Cell Formation. Rad. Resch., **14**:653, 1961.
44. Petrakis, N. L., M. Davis and S. P. Lucia: The *in vivo* Differentiation of Human Leukocytes into Histocytes, Fibroblasts and Fat Cells in Subcutaneous Diffusion Chambers. Blood, **17**:109, 1961.
45. Puck, T. T.: Cellular Aspects of Irradiation and Aging in Mammals. Fed. Proc., **20**:31, 1961.
46. Ross, R. and E. P. Benditt: Wound Healing and Collagen Formation. I. Sequential Changes in Components of Guinea Pig Skin Wounds Observed in the Electron Microscope. J. Biophys. Biochem. Cytol., **11**:677, 1961.
47. Schilling, J. A., B. V. Favata and M. Radakovich: Studies of Fibroplasia in Wound Healing. Surg., Gynec. & Obst., **96**:143, 1953.
48. Shelton, E.: Differences in the Potentialities of Normal and Malignant Lymphocytes Grown in Diffusion Chambers. Pathologie-Biologie, **9**:542, 1961.
49. Shelton, E. and M. E. Rice: Growth of Normal Peritoneal Cells in Diffusion Chambers: A Study in Cell Modulation. Am. J. Anat., **105**:281, 1959.
50. Sieracki, J. C. and J. W. Rebuck: Role of the Lymphocyte in Inflammation, pp. 71–81, in The Lymphocyte and Lymphocytic Tissue, ed. J. W. Rebuck, Paul B. Hoeber, Inc., N. Y., 1960.
51. Snedecor, G. W.: Statistical Methods. 5th Edition, Iowa State Univ. Press, Iowa, 1956.
52. Spear, F. G.: Some Biological Aspects of Experimental Radiology, Int. Rev. Cytol., **7**:1, 1958.
53. Stearns, M. L.: Studies on Development of Connective Tissue in Transparent Chambers in Rabbit's Ear. Am. J. Anat., **66**:133, 1940.
54. Taylor, J. H.: Autoradiography at the Cellular Level, in Physical Techniques in Biological Research, ed. G. Oster and A. W. Pollister, Acad. Press. N. Y., 1956.
55. Taylor, J. H., P. S. Woods and W. L. Hughes: The Organization and Duplication of Chromosomes as Revealed by Autoradiographic Studies Using Tritium Labelled Thymidine. Proc. Nat'l. Acad. Sci., **43**:122, 1957.
56. Till, J. E.: Radiation Effects on the Division Cycle of Mammalian Cells *in vitro*. Ann. N. Y. Acad. Sci., **95**:911, 1961.
57. Trowell, O. A.: The Lymphocyte. Int. Rev. Cytol., **7**:236, 1958.
58. van den Brenk, H. A. S.: Studies in Restorative Growth Processes in Mammalian Wound Healing. Brit. J. Surg., **40**:525, 1956.
59. van den Brenk, H. A. S.: Effects of Ionizing Radiations on the Growth of the Mammalian Repair Blastema (*in vivo*). Studies in Sandison-Clark Ear Chambers. Progress in Radiobiol., **4**:386, 1956.
60. Watts, G. T., H. C. Grillo and J. Gross: Studies in Wound Healing. II. The Role of Granulation Tissue in Contraction. Ann. Surg., **148**:153, 1958.
61. Weiss, P.: Perspectives in the Field of Morphogenesis. Quart. Rev. Biol., **25**:1777, 1950.
62. Yoffey, J. M. and F. C. Courtice: Lymphatics, Lymph and Lymphoid Tissue. Cambridge, Harvard University Press, 1956, p. 414.