laries – presumably because, as in the skin, these lie closer to the surface of the tissue and hence suffer more injury[5,6]. The mesothelium, and the lymphatic and blood endothelium all showed very similar changes, which were naturally most pronounced adjacent to the injurious stimuli. Initially the cells appeared shrunken with dark, electron-opaque, cytoplasm and pseudopodia; after 30 min more and more cells became swollen, pale and oedematous. Some blebs were seen, together with many large vacuoles. Unlike what happens with more severe burns[15], the small vesicles seemed to increase in numbers.

The nuclei of all 3 classes of cells showed much evidence of cellular contraction (the venules in the case of histamine, and the capillaries with thermal injury). At maximum, some 30% of nuclei were affected. The contraction occurred early in all cases and passed off by 90 min. This is similar to what has been observed by intra-vital microscopy with venules[3]. The contraction of the mesothelium and of the lymphatic endothelium is important since these tissues are not subjected to the pressure of the blood. This is additional evidence that this is an active contraction and not just passive recoil[2]. The association of this contraction with the opening of the intercellular junctions, particularly in the mesothelium, indicates that it is the active contraction which opens the junctions. (Although it must be remembered that there are normally some open mesothelial junctions[16].) Of course, the poorly supported lymphatic endothelial junctions are easily opened by this contraction as well as by all the other factors normally affecting them, including the oedema[7].

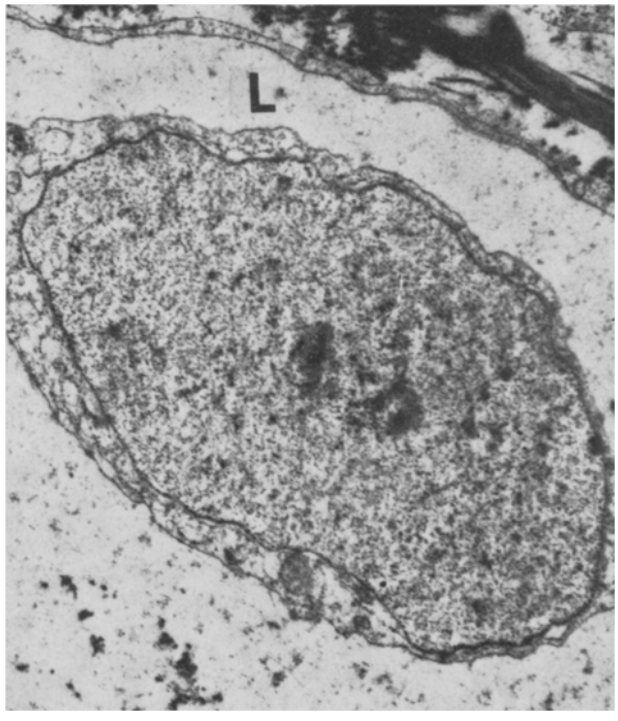

Fig. 2. A lymphatic, 30 min after burning, without Venalot treatment. The nucleus has become ovoid, with little indication of cellular contraction after this period. There is a considerable amount of protein in the tissues and in the lumen. ×8000.

The Table shows that Venalot causes some increase in the number of open blood vascular junctions as has been recorded elsewhere[11,12]. It did not appear to have any effect on the cellular contraction itself, although the slight increase in the numbers of open blood vascular junctions suggests that this may indeed be the case, but that our numbers of observations were insufficient to detect it. Any increased leakage of protein occasioned by this is more than compensated for by its action in removing protein (and hence oedema) from the tissues. This allows the lymphatics to be less dilated and to have fewer open junctions since, although some cells contract, many of them do not, and any lessening of the oedema will lessen the tension in the anchoring filaments, the dilation of the lymphatics and the separation of their endothelial cells[7].

Zusammenfassung. Hitze und Histamin vermögen eine Kontraktion der Endothelzellen feiner Venen zu erzeugen. Hitze und Histaminschädigung des Mäusezwerchfells (Endothelfensterung) führt zu Kontraktionen von Endothelzellen und Mesothelialzellen. Venalot, ein Produkt aus Cumarin und Trihydroxyethyl-Rutin, vermag das Oedem zu beseitigen.

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Combined Surgical and Radiation Injury VIII: The Effect of the Gnotobiotic State on Wound Closure

Despite extensive clinical and experimental investigation, the mechanism of wound healing has not been completely elucidated. Previous studies have demonstrated a delay in wound contraction following whole body X-irradiation in the midlethal range. Retardation in wound closure was most marked when the rodent was irradiated 4 days prior to wounding[1]. The irradiation induced mortality was significantly increased by wounding. Partial bone marrow shielding[2] bone marrow transplantation[3] or the administration of radioprotective compounds[4] decreased the mortality and partially corrected the radiation induced retardation in wound contraction. A major factor underlying the augmented mortality and delayed wound healing pattern in the whole body irradiated animal may be the decreased resistance to infection characteristic of the hematologic syndrome. Although the wound healing abnormality was not corrected by the administration of antimicrobials[5], suggesting that bacterial

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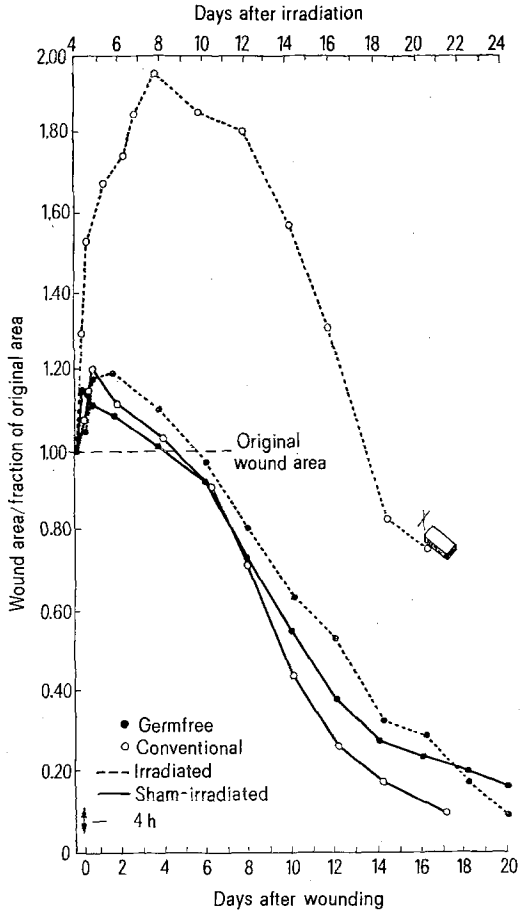
Mortality response of germfree and conventional rats to 800 R 250 KVP X-rays

Radiation response	Germfree		Conventional	
	Not Wounded	Wounded	Not Wounded	Wounded
Deaths/exposed	0/18	3/18	10/18	18/18
30-day mortality	0	21	55	100
(%; 95% C.L.)	—	(2 — 40)	(32 — 78)	—
Survival time (days)				
Mean ± S.D.	30	22 ± 3	21 ± 3	10 ± 4
Median	30	24	21	10
Range	30	12 — 26	17 — 30	7 — 16

infection did not play a predominant role, we elected to further evaluate the role of infection by studying the pattern of wound healing in irradiated and non-irradiated gnotobiotic rats.

Materials and method. 54 germfree female rats and 54 conventional rats were obtained from Charles River Breeding Farms at the age of 12 weeks. The care and handling of the germfree animal is presented in a prior publication[6]. The principles of laboratory animal care as promulgated by the National Society for Medical Research were observed. 6 weeks following shipment the experiments were initiated.

One half of the germfree group and one half of the conventional group were irradiated. Animals were exposed to 800 R delivered by means of a 250 KVP X-ray unit with 0.25 mm copper and 1 mm aluminum added filtration at a dose rate of 64 R/min. Control groups were sham irradiated. 4 days following irradiation, one half of the number of animals in each irradiated or non-irradiated group were wounded as previously described[1]. Immediately after wounding and at the time intervals indicated in the Figure, the longitudinal and lateral diameters of the wound were measured. The product of these measurements was used as an index of the initial wound size for each animal. Mortality for each major group was recorded for 30 days after irradiation.



Wound closure pattern in conventional and germfree rats. Germfree animals are indicated by the closed circles, conventional rats by the open circles. The interrupted line indicates the irradiated rats; the solid line the sham-irradiated controls. Each point represents the average of 18 rats, except as mortality intervened (see Table). There was no mortality in the non-irradiated animals. The cross in the irradiated wounded conventional group indicates the last survivor, all points from 4 h to 14 days are significantly different from all others with a p-value at least 0.05.

Results and discussion. Mortality data is presented in the Table. The expected diminished sensitivity of the germfree animal to radiation was observed. The intact germfree animals had no mortality whereas, the intact conventional animals had a 55% mortality. Both germfree and conventional animals demonstrated an increase in mortality when the non-lethal wound was superimposed on the radiation injury. The Figure presents the wound healing patterns. The patterns in the non-irradiated germfree and conventional animals were not significantly different. When irradiation preceeded wounding by 4 days, distinct changes were noted in survival as well as in wound healing in conventional but not in germfree rats. The closure patterns in the conventional rats was altered by radiation. The initial increase in size continued for 4 days, a period longer than in the unirradiated animal, until these wounds were almost twice their initial size. After the 4th day closure was underway, but the wounds were still approximately 80% of the initial size at 14 days. At 14 days wounds of the unexposed rats were less than 20% of the initial size. This retardation of contraction following radiation is compatible with our prior reports[1–5] and those of others[7,8]. In germfree rats radiation did not alter wound closure. There was no significant difference between the wound closure curve for unexposed germfree animals and those exposed to 800 R X-ray. The half-time for closure was between 8 and 9 days in both groups. Wounds of unexposed animals changed from peak size

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to half size between the 1st and 9th days post wounding. In X-irradiated animals, wounds of conventional animals fell from peak size to 1.8 on the 4th post wounding day to 0.9 on the 13th day; wounds of germfree rats from a peak of 1.2 on the 2nd post wounding day to 0.6 on the 10 th day post wounding.

The effect of the microbial flora on wound healing has been the subject of sporadic investigation and often conflicting results. ROVIN et al.[9], CARTER et al.[10] and BRODY et al.[11] were unable to demonstrate any change in healing rate, granulation tissue or rate of collagen production in germfree or conventional animals. The present study supports these findings utilizing the open wound contraction model of GRILLO[7] which eliminates problems inherent in using strength of wound as an index of healing.

In the present study irradiation did not produce a retardation in the germfree animal where there has been elimination of uncontrolled and variable bacterial infection as a complicating factor of the surgical repair mechanisms. It should be pointed out that there are other differences in the gnotobiotic animals including a diminished total lymphocyte mass and immature immunologic capacity and resultant, increased radiation resistance. Bacterial contamination which occurs following radiation injury in non-gnotobiotic animals provides a partial explanation for the lack of retardation of the wound closure pattern observed in the germfree animal. These observations suggest that wound healing abnormalities which occur following radiation in rats result, in part, from bacterial contamination and entry of more virulent organisms due to impaired immune capability secondary to hematologic radiation injury.

Zusammenfassung. Es wurde die Wirkung von Röntgenbestrahlung (800 R) am Heilungsprozess bei offenen Wunden in normalen und keimfreien Ratten untersucht. Die Bestrahlung verlangsamte die Wundheilung bei den normalen, jedoch nicht bei den keimfreien Ratten.

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The Effects of Low Concentrations of Actinomycin D upon Nucleic Acid Synthesis in Different Cell Types

An important property of actinomycin D (AMD) is to bind to the DNA molecule in mammalian cells[1]. At certain doses AMD has been found to delay initiation of DNA synthesis in Ehrlich ascites cells and mouse jejeunum in vivo[2]. This effect has also been observed in vitro at concentrations within the range 0.01–0.1 μg/ml[3,4]. Within this range of concentration, AMD appears to inhibit selectively nucleolar synthesis of RNA without having any immediate effect on either the rate of DNA synthesis or on RNA synthesis in extra-nucleolar parts of the nucleus[4–6].

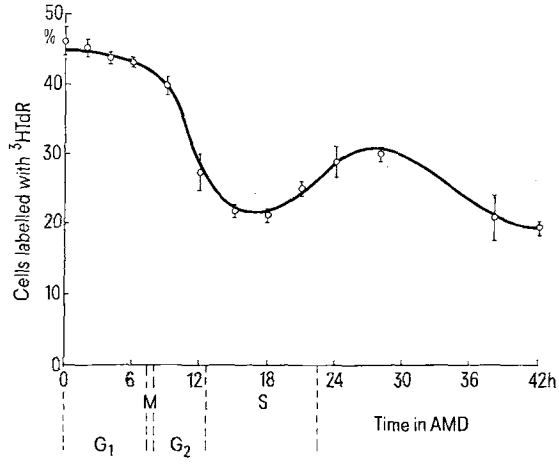

Fig. 1. Percentage of an asynchronous culture of L-cells labelled by a pulse exposure to ^{3}HTdR as a function of time in AMD. ○, 0.04 μg/ml.

RICKINSON[4] has shown that continuous incubation of an asynchronous population of L-cells with 0.04 μg/ml AMD blocks entry of cells into the DNA synthetic phase after about 6 h. The blockage is incomplete however, and some 12 h later a substantial percentage of the population succeeds in entering S phase and completes another round of DNA synthesis. In recent work on the initiation of DNA synthesis, HATFIELD et al.[7] did not observe this effect when either embryonic mouse fibrolasts (EMF) or HEp/2 cells were continuously incubated with 0.04 μg/ml AMD. The present experiments were therefore undertaken to confirm RICKINSON's result and to determine if the effect is peculiar to mouse L-cells or if it can be demonstrated for other cell types by changing the concentration of AMD.

Materials and methods. Both mouse L-cells and HEp/2 cells were used as established cell lines in these experiments. These cells were grown in Pyrex feeding bottles with medium 199 (Wellcome Ltd), and 10% foetal calf serum (Flow Laboratories), for L-cells, and Eagle's medium (Wellcome Ltd.) and 10% foetal calf serum buffered to pH 7.2 with bicarbonate for HEp/2 cells.

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