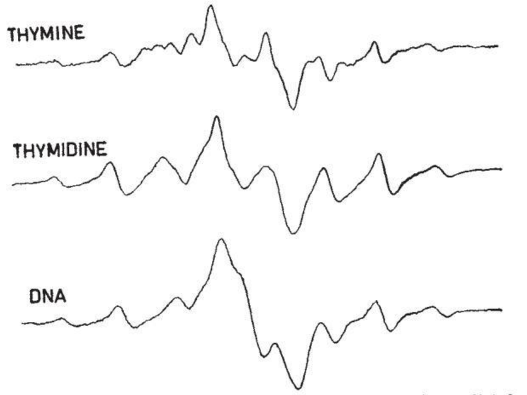

Fig. 1. Electron spin resonance spectra (first derivative) of γ-irradiated, highly purified thymus DNA and the constituents thymine and thymidine. Distance between outermost satellites is about 135 gauss. Doses about 1 Mrad

Fig. 2. Electron spin resonance (—) spectra of γ-irradiated DNA in vacuum and shortly after introduction of air (– – –)

Fig. 1 shows typical electron spin resonance spectra of DNA, thymidine and thymine. A striking similarity between these spectra can be observed. The three outer satellites on each side are present in all three spectra. Also the central regions have features in common. The thymidine spectrum agrees with that published by Gordy[2], disregarding minor details in the centre. The radicals in thymine and thymidine are quite stable, even in air, while the radicals in DNA decay already in vacuum, about 50 per cent remaining after the first 24 h. If the irradiated DNA is exposed to air, most of the radicals disappear leaving a spectrum which can be seen in Fig. 2. The latter type of spectrum is also obtained after irradiation of commercial herring sperm DNA (from L. Light and Co., Ltd.) containing 2–5 per cent protein. The decay *in vacuo* of radiation-induced radicals in the DNA, as well as their reaction with oxygen, seem to be dependent on the water content, but this dependence has not yet been investigated in detail.

In order to gain some knowledge about the radical structure, thymine was treated with heavy water, whereby the dissociable hydrogen was substituted by deuterium. The only spectral change which can be seen is a sharpening of the lines, which may be due to diminishing of the dipolar broadening and/or very weak hyperfine splitting from the exchangeable hydrogens.

The G-value (radicals per 100 eV) for DNA was found to be 0·4 in the dose range 200–600 krad. The order of magnitude agrees with results by A. Müller[10] ($G = 0{\cdot}6$) and P. Alexander *et al.*[4] ($G = 0{\cdot}2$).

Furthermore, electron spin resonance spectra have been recorded for a number of irradiated DNA and RNA constituents, that is, all the pyrimidines and purines and most of their ribose and deoxyribose derivatives, as well as most of the corresponding nucleotides. Of the constituents investigated, only thymine and thymidine (and later also thymidylic acid) gave electron spin resonance spectra similar to that of DNA. Noteworthy is that an irradiated sample of a sodium salt of highly polymeric, pure DNA (from Calbiochem, California) gave the same type of electron spin resonance spectrum as shown for DNA in Fig. 1.

In work on bacteria irradiated by ultra-violet light, Wacker *et al.*[11,12] has shown that the damage of the DNA is associated with a dimerization of thymine. Tokarskaya[13] in a recent publication "gives confirmation to the assumption that thymine is the point of injury after having studied effects of ionizing radiation on dry seeds and isolated DNA".

In view of these results, it is interesting to note that thymine is also the main centre of stabilization of radicals. This is in agreement with data given by Pullman and Pullman[14]. They conclude, after having compared mobile orders for the 4–5 bond in pyrimidines and the 7–8 bond in purines, that "the pyrimidines should thus, from that point of view, be much more reactive towards the fixation of radicals than the purines" and "the site of action of ionizing radiations and the free radicals generated by them on the purine–pyrimidine pairs of DNA is largely located in the pyrimidines".

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BIOLOGY

Effect of Air Exposure and Occlusion on Experimental Human Skin Wounds

THE benefits of special dressings versus air exposure of cutaneous wounds has long been debated. Winter and Scales[1,2] have recently added fresh insight into the problem. In the domestic pig they demonstrated that an occlusive dressing doubles the rate of wound re-epithelization when compared with wounds exposed to the air. In this communication we report parallel studies performed in man.

Our experimental subjects were healthy adult male volunteers. Each subject served as his own control. After intradermal injection of 2 per cent procaine to obtain local anæsthesia and elevate the skin, we horizontally sliced off the epidermis and upper dermis from 0·5 cm^2 marked sites on the inner surface of the arms. We utilized the inner arm, as relatively few hair follicles exist in this area. This simplifies examination of the point of origin of re-epithelization, as the epithelium spreads mainly from the periphery of the wound rather than from the transected hair follicles.

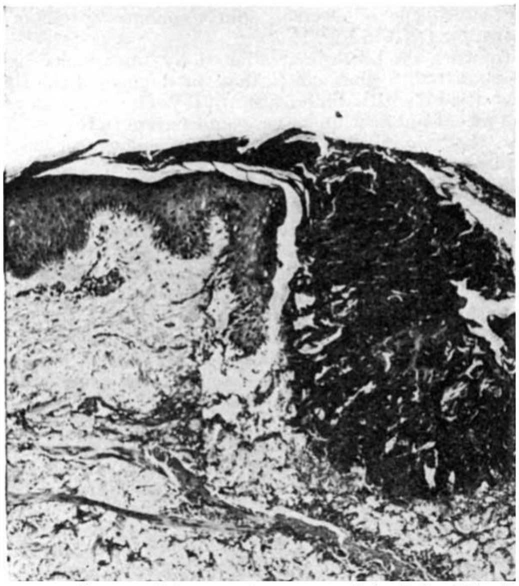

Fig. 1. Air exposed human cutaneous wound (control). Note the angle at which epithelium has grown beneath the eschar

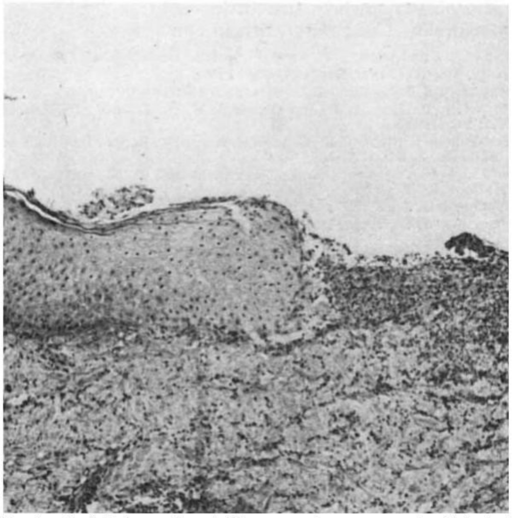

Fig. 2. Occluded experimental human cutaneous wound. Note lack of eschar and straight epithelial growth

After obtaining hæmostasis, we applied 0·1 c.c. of 0·5 per cent neomycin in saline to prevent infection. The control wound was left exposed to the air and the experimental wound occluded with sterile polyethylene film. We excised the wounds with an 8 mm cutaneous punch at three-, five-, seven-, and nine-day intervals respectively. After formalin fixation, the specimens were bisected, paraffin embedded, serially sectioned, and stained with hæmatoxylin and eosin. We examined the slides at 60 times magnification.

With some experience it was possible to determine histologically the site of the original wound as well as the area of re-epithelization. We measured the length of the original wound and that of the new epithelium in every fifth section and totalled for each biopsy. Only growth from the edges was recorded, and that from hair follicles and eccrine sweat gland ducts disregarded.

Table 1. Epithelization

	Subject	Wound No.		Total length section examined (mm × 10^{-2})	Total new epidermis (mm × 10^{-2})	Per cent new growth
3 days	*A*	51	E*	17,599	2,005	11·4
		52	O*	5,693	1,257	22·0
	B	53	E	22,656	2,456	10·8
		54	O	12,411	5,857	47·4
	C	116	E	8,902	1,424	16·0
		117	O	6,639	1,898	28·5
	D	120	E	7,960	1,183	15·4
		121	O	1,942	1,238	63·9
5 days	*E*		E	3,509	1,014	28·8
			O	3,746	1,606	42·8
	C	118	E	5,167	668	21·1
		119	O	2,551	1,978	73·9
	D		E	2,691	989	36·5
			O	2,730	1,365	50·0
7 days	*F*	105	E			100·0
		106	O			100·0
	G	107	E			100·0
		108	O			100·0
9 days	*F*	103	E			100·0
		104	O			100·0
		109	E			100·0
	G	110	O			100·0

*E, control, air-exposed. *O, occluded.

The results are summarized in Table 1. Most occluded specimens showed so much more epithelization than the air-exposed wounds that the difference was obvious without benefit of measurement. No difference was observed by the seventh day, as both wounds were 100 per cent epithelized at this time. No wound infection occurred.

Histologically, in the air-exposed wounds we noted the epithelium had to grow at right angles to the surface in order to find a plane of cleavage to proliferate under the eschar (Fig. 1). Occluded wounds allowed for no eschar formation, so that the epithelium spread directly across the wound surface (Fig. 2). This alone may explain the difference in rates of epithelization.

We do not know whether these observations will fall in the realm of biological curiosity, or if they will have practical importance in the treatment of cutaneous wounds and burns in man. In the past, infection precluded the practical use of such occlusion, as these dressings provided the moisture necessary for the multiplication of pathogenic organisms. With the introduction of potent anti-bacterial agents for the skin, it may now be practical to take advantage of such an occlusive dressing as was used in this study. Clinical investigation is needed to determine its usefulness in burn and wound therapy.

In summary, experimental split thickness wounds in human volunteers were either air-exposed or occluded with a polyethylene film. Re-epithelization was more rapid in the occluded than in the air-exposed control. This has been verified for wounds of up to duration of five days.

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Hinman and Maibach have shown that in human skin wounds, as in pig's skin, epidermal regeneration is faster when the wound surface is moist than when exposed to the air and dry. There is little doubt that this is due to the way the scab is formed on an exposed wound surface.

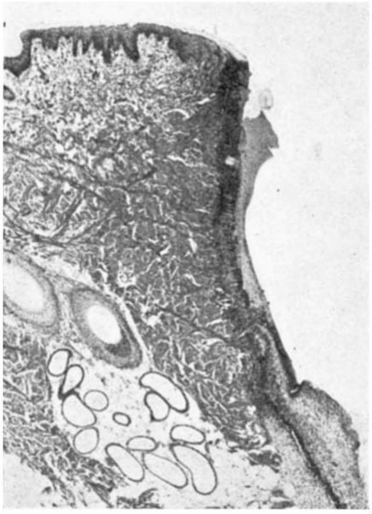

Fig. 1. Edge of deep wound in pig's skin after two days. The exposed dermal edge invaded by leucocytes was dry, and epidermis was migrating through the dermis behind the dehydrated layer

Migrating epidermis passes through the dermis and the fibrous tissue impedes the movement of the epidermal cells. The only other similar investigation known to me was by Braun and Magazanik[1], who compared wounds in rabbits' ears exposed to the air with similar wounds covered with paraffin wax. They observed that the covered wounds epithelized faster. They also noted that on the exposed wounds a superficial part of the dermis was incorporated into the scab.

Several reports have referred to the presence of collagen in the scabs of healing wounds. James[2] found hydroxyproline in scabs formed over cutaneous wounds in rats. However, no fibres were seen in the scabs and it was tentatively suggested that the hydroxyproline was present in a pre-collagenous form. Zahir[3] repeated the examination of scabs from wounds in guinea pigs and rats and concluded that the hydroxyproline found in scabs was a constituent of formed collagen. Collagen fibres were identified histologically, particularly in the margins of the scab.

Fig. 2. Edge of deep wound in pig's skin after six days. The original edge of the wound has been separated from the remainder of the dermis by the migrating epidermis and pushed into a horizontal position by the exudate

Devenyi and Holczinger[4] found that the scab on deep wounds in rat skin included "the necrotic and demarcated parts of the wound-edge".

Hadfield[5] recently concluded that the essential component of the scab which covered the floor and sides of deep wounds in rat skin was collagen fibre.

Collagen fibres were identified in the scab of an eight-day-old wound in the skin of the human thigh which was allowed to heal without a dressing[6].

Forage[7] reported on two similar burns, one of which was left to heal with the epidermal roof of the blister intact, and the other without the protection of the dead epidermis. The protected wound healed faster. Where the blister was opened, the scab was formed of dermis which died as a result of exposure to the air.

Further studies of wounds in pig's skin now in progress have shown that at the edge of deep wounds exactly the same phenomena occurred as at the surface of superficial wounds. The exposed dermal tissue was dehydrated, leucocytes accumulated beneath the surface, and the new epidermis migrated through the fibrous tissue behind the dry layer (Fig. 1). The dehydrated dermal tissue at the side of the wound was separated off by the migrating epidermis and carried up by the exudate which gradually filled the wound cavity. It eventually came to lie horizontally on the surface of the skin at the edge of the wound (Fig. 2). The manner of formation of the scab confirms Zahir's observations and explains why the hydroxyproline content of scabs on deep wounds was greatest at the margins.

The biological interest in these observations is that in dry-skinned animals generally it is probable that epidermal regeneration is through the dermis, not through a blood clot as has been thought[8].

Indeed, the blood clot appears to play a minor part in wound healing aside from its initial function of stemming the loss of blood. Tissue fluid which oozes on to the injured surface has more significance in the formation of the scab and the protection this later affords to the migrating epidermis.

With regard to the treatment of wounds, a number of dressings in common use are more or less occlusive and have the same effect as polythene film in keeping the exudate moist and permitting faster epithelization. Gauze impregnated with soft paraffin is one such dressing.

Biopsy specimens of superficial wounds in pig's skin, healing under Tulle Gras dressings, showed that the new epidermis moved through a moist exudate over the original wound surface.

In these cases the dressing performs the protective function of the normal dry scab and premature removal of the dressing will cause the death of the new epidermis from dehydration.

Apart from effects on epithelization recent work on full thickness wounds in pig's skin has shown that occluded wounds contract more rapidly and the scars are smaller than on wounds exposed to the air.

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