Table 1

Day of irradiation	No. of females	No. of irradiated embryos Normal	Malformed	Resorbed	Control Normal	Resorbed
7	11	33	0	11	36	5
7·5	8	22	1	9	31	5
8	9	24	0	20	34	8
8·5	19	9	7	60	80	17
9	10	2	23	23	32	6
9·5	11	15	4	21	40	6
10	10	30	6	10	38	7

Seven series of female albino rats (of our own strain) between the seventh and tenth day of pregnancy were used, the difference between two series being 12 hr. of gestation. The method devised by Wilson[2] was used; the ventral abdominal wall was opened and some embryos of the right uterine horn were irradiated. The other horn and the mother were shielded by lead plates. In all, 330 implantation sites were irradiated. Only one dose of 100 r. was given at a single exposure to the unshielded embryos (85 kVp., 10 m.amp., 1·0 mm. aluminium). The shielded embryos served as control. All rats were killed on the fifteenth day of pregnancy.

From Table 1 and Fig. 1 it is readily seen that the incidence of resorption shows a peak on day 8·5.

The number of resorbed embryos irradiated on the seventh day was not significantly greater than that of controls, while on day 8·5 and 9 the difference was significant ($\chi^2 = 24$; $P < 0{\cdot}01$; $\chi^2 = 5$; $P < 0{\cdot}05$). The death-rate of irradiated embryos on day 8·5 is significantly greater than on day 7 ($\chi^2 = 9{\cdot}7$; $P < 0{\cdot}01$). In order to exclude a technical error as the cause of this high death-rate, this series was repeated and almost the same number of resorbed embryos was obtained (82·5 per cent and 75 per cent).

Russel[8], on the other hand, has shown that the radiosensitivity decreases with age of pregnancy in mice, whereas Wilson *et al.*[1] have some data similar to our own, if less clearly defined. The malformations were produced following irradiation of the stages before the ninth day of pregnancy. The control series showed no malformation at all. As to the kind of malformations, excencephalia was never observed[10], but different kinds of well-known abnormalities of the head were noted.

Whether the high incidence of resorption after irradiation on day 8·5 is caused by a relatively great number of mitoses during the onset of invagination or by differential sensitivity of cells will be the subject of future investigations.

Cytological analysis of the immediate effect of X-rays had shown that 3 hr. after irradiation there were only a few irregular mitoses, the remaining cells being normal. After 6 hr. of irradiation many pycnotic cells were already visible. Having regard to this fact we may be inclined to suppose that this dosage acts on the cells in mitosis.

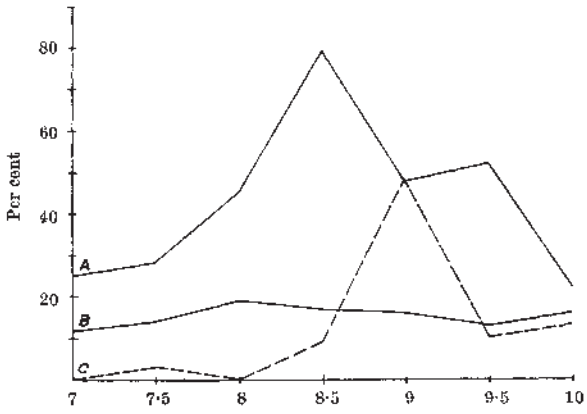

Fig. 1. *A*, Incidence of death, irradiated embryos; *B*, incidence of death, control embryos; *C*, incidence of abnormalities

Summarizing, we may state that during the early stages of the mesoderm formation in rat, there is a great increase of resorption following irradiation. Contrary to other data available on the rat embryo, there is no definite onset of incidence of malformations.

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[1] Wilson, J. G., Brent, R. L., and Jordan, H. C., *Proc. Soc. Exp. Biol. and Med.*, **82**, 67 (1953).
[2] Wilson, J. G., *J. Cell. and Comp. Physiol.*, **43**, Supp. 1, 11 (1954).
[3] Russel, L. B., and Russel, W. L., *J. Cell. and Comp. Physiol.*, **43**, Supp. 1, 103 (1954).
[4] Hicks, S. P., *J. Cell. and Comp. Physiol.*, **43**, Supp. 1, 151 (1954).
[5] Auerbach, R., *Nature*, **177**, 574 (1956).
[6] Lengerova, A., *Fol. Biol.* (Praha), **3**, 321 (1957).
[7] Russel, L. B., *Proc. Soc. Exp. Biol. and Med.*, **95**, 174 (1957).
[8] Fraser, A. S., and Hall, R. J., *Austral. J. Biol. Sci.*, **11**, 425 (1958).
[9] Hicks, S. P., *Physiol. Rev.*, **38**, 337 (1958).
[10] Rugh, R., and Grupp, E., *J. Neuropath. Exp. Neurol.*, **18**, 468 (1959).
[11] Rugh, R., and Grupp, E., *Amer. J. Roentg. Rad. Therapy and Nucl.-Med.*, **84**, 125 (1960).
[12] Huber, G. C., *J. Morph.*, **26**, 1 (1915).
[13] Mulnard, J., *Arch. de Biol.*, **66**, 525 (1955).
[14] Levak, B., and Škreb, N., *Bull. Scient. Youg.*, **5**, 108 (1960).

Formation of the Scab and the Rate of Epithelization of Superficial Wounds in the Skin of the Young Domestic Pig

WHERE there is a superficial wound in the skin, new epidermis covers the denuded area by migration from the hair follicles and sweat gland ducts within the wound and from the surface epidermis at the wound edges. It has been found that epithelization is retarded by the dry scab which normally covers a superficial wound, and if the formation of the scab is prevented, the rate of epithelization is markedly increased.

These observations are derived from investigations on wound healing in the skin of pedigree Large White pigs 12–14 weeks old, fed on a standard diet without antibiotic. The experimental wounds were made with a sharp scalpel under surgically clean conditions. The wounds were 2·5 cm. square and 0·01–0·03 cm. deep. All surface epidermis and the papillary layer of the dermis were removed. Wounds were protected by a cage worn on the pig's back, so that no accidental damage was done to them.

To study the effects of keeping the wound surface moist, wounds were covered with polythene film. In all experiments control wounds were made on the same animal and were left exposed to the air.

The total area of regenerated epidermis on each wound was estimated from serial sections. Every fifth section in a series cut at 10μ was examined at × 80 magnification and the separate lengths of regenerated epidermis and the total lengths of the sections were measured to within 0·01 mm.

The results of one such experiment are presented in Table 1. This experiment suggested that moist wounds were epithelialized more rapidly than dry wounds. To confirm this suggestion, the experiment was repeated on a second pig, taking biopsy speci-

Table 1. Epithelization under Normal (Dry) and Experimental (Moist) Conditions

Time	Wound	Total length of epidermis (mm. × 10⁻²)	Total length of section examined (mm. × 10⁻²)	Percentage of epidermis
Dry Wounds				
1 day	1	1,608	71,479	2
3 days	7	26,212	77,001	34
5 days	3	55,524	77,504	72
7 days	9	77,902	78,075	100
		Also at 9 and 11 days		100
Moist wounds				
1 day	2	11,827	64,495	18
3 days	8	77,598	78,904	98
5 days	4	71,721	71,721	100
		Also at 7, 9 and 11 days		100

Fig 43. Superficial wounds, 2·5 cm.². Skin depilated with wax 3 days before wound making. 6 wounds, no dressing (dry), 6 wounds covered with polythene film (moist), spaced alternately. Serial sections at 10μ, every fifth section measured.

Table 2. Epithelization under Normal (Dry) and Experimental (Moist) Conditions

Time	Wound	Total length of epidermis (mm. × 10⁻²)	Total length of section examined (mm. × 10⁻²)	Percentage of epidermis
Dry wounds				
3 days	3	59,481	102,865	58
	5	32,940	81,635	40
	6	23,224	70,050	33
	8	24,892	66,119	38
	9	23,232	65,183	36
	12	30,254	90,756	33
Total (6 wounds)		194,023	476,608	41 (40·7)
Moist wounds				
3 days	1	83,804	84,410	99
	2	87,120	87,120	100
	4	106,804	106,902	100
	7	101,362	101,574	100
	10	78,045	79,089	99
	11	71,557	76,434	94
Total (6 wounds)		528,692	535,529	99 (98·7)

Fig 47. Superficial wounds, 2·5 cm.². Hair was clipped short immediately before wound making. 6 wounds, no dressing (dry), 6 wounds covered with polythene film (moist), distributed at random. Serial sections at 10μ, every fifth section measured.

Table 3. 't' Test of Significance, Difference between Dry and Moist Wounds, Data as in Table 2

		n	$\bar{x}$	s	t	p
Total length of epidermis	dry	6	32·6	14·1	6·970	< 0·001
	moist	6	88·1	13·6		

mens from all the wounds at three days (Table 2). There was only about half as much new epidermis under the normal dry scab as under the polythene film. The difference is statistically highly significant (Table 3).

The conclusion from these experiments is that when superficial wounds in the skin of young domestic pigs are kept moist under cover of a relatively inert and impermeable film, epithelization of the denuded surface is about twice as rapid as on wounds exposed to the air.

The explanation for this large difference in the rate of epithelization can be deduced from the histology of the wounds. Normally, when no dressing is used, and the wound is freely exposed to the air, the wound is covered by a dry, serous scab within 24 hr. Within the dermis under the wound, extravasated polymorphonuclear leucocytes migrate upwards and accumulate within the fibrous tissue immediately below the wound surface. Migration of epidermis from hair follicles and the wound edges has just begun at 24 hr. The moving sheet of epidermal cells passes through the fibrous tissue below the leucocytic layer. Therefore a superficial layer of the fibrous tissue of the dermis is included in the scab, and the original wound surface lies within the scab above the new epidermis. On the other hand, when the wound is kept moist under a polythene film, epidermis migrates through the serous exudate on the wound surface above the fibrous tissue of the dermis. A normal scab including fibrous tissue is not formed, and leucocytes migrate out of the dermis into the exudate. In contrast to the normal dry wound, the original wound surface is below the new epidermis.

From the experiments described, it appears that where there is a superficial wound in the skin a part of the dermis is dehydrated by exposure. The regenerating epidermis migrates along the outermost level, which is sufficiently moist for the life of the cells, below the dehydrated layer of the dermis. Leucocytes are trapped at the surface of the dermis because they are unable to move through the dry tissue. The normal scab prevents the ingress of dirt and microorganisms and protects the delicate cells of the new epidermis from dehydration. It is formed, however, at the expense of some dermal tissue, and the rate of epidermal migration is less than the potential maximum. Migration is impeded by the bundles of collagen in the path of the epidermal cells.

It is interesting to inquire how the epidermis is able to pass through the fibrous bundles. There is no histological evidence that a layer of separation develops between the leucocytic layer and the underlying tissue. The fibres remain apparently intact between the dermis and the leucocytic layer up to the very edge of the advancing sheet of epidermis. Possibly the epidermal cells push through the collagen, the fibres being weakened by altered physical conditions at this level, or the collagen is dissolved locally by an enzyme secreted by the epidermal cells themselves, or by the leucocytes.

The demonstration that a simple change in physical conditions at the wound surface can have such a marked effect on the rate of epithelization has an important bearing on experimental methods in wound healing. It would be unwise to draw conclusions about the specific effects of various substances on the rate of wound healing, where the results may be complicated by occlusion of the wound, for example, by dressings or greasy bases. There is histological evidence, too, that the effects of keeping the wound surface moist extend beyond the phase of epithelization. The new connective tissue under the regenerated epidermis appears earlier than normal, suggesting that the stimulus for its production is linked with the presence of epidermis. It is hoped to report more fully on these observations in due course.

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A Pair of Compatible Strains of *Absidia glauca* which has become Heterogamous in Culture

A pair of compatible ('plus' and 'minus') strains of the heterothallic mould *Absidia glauca* which had already been maintained for some years in the culture collection of the Department of Botany of the Imperial