

Implantation of Actively Proliferating Epithelium.

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The present investigation deals with the course of events following subcutaneous implantation of actively proliferating adult epithelium. To avoid the possibility of disturbing factors being introduced by the formation of antibodies, the epithelium was implanted into the same animal or into an animal of the same species.

The effect of implantation of normal adult epithelium has, more particularly during the last two decades, been the subject of considerable study by a large number of observers, whose investigations have shown that epithelium may be successfully implanted and an epithelial cyst result, but that the implanted epithelium shows a marked tendency to die out in its new habitat.*

In the present research, instead of using normal epithelium for implantation, rapidly proliferating epithelium was employed. It was anticipated that additional information would thereby be afforded upon two important points, namely, upon the immediate influence of implantation on the activity of the epithelial cells, and (owing to the large bulk of epithelium available for implantation) upon the influence exerted by the implanted epithelium upon non-epithelial tissue elements. Both these anticipations have been fulfilled.

The use of rapidly proliferating epithelium has been rendered possible by Fischer's discovery of the action of *scharlach R* upon the epithelium of the skin of the rabbit.† The extent to which epithelial proliferation occurs may be judged by a glance at fig. 2, which shows the appearance of a section of the ear after the injection of the dye, fig. 1 showing, for comparison, a normal section drawn to the same scale.‡

* *Cp.* E. Korschelt, "Regeneration und Implantation im Tierreich," 'Verhandlungen der Gesellschaft Deutscher Naturforscher und Aerzte,' 1906, Erster Teil, S. 164.

† B. Fischer, "Die experimentelle Erzeugung atypischer Epithelwucherungen und die Entstehung bösartiger Geschwülste," 'Münch. Med. Wochenschr.,' 1906, 53 Jahrg., S. 2041.

‡ The sections represented are stained by Heidenhain's iron alum hæmatoxylin method, except in fig. 1, where methylene blue eosin is used for staining. Figs. 1 and 2 are magnified 33 diameters; the remaining figures are magnified 800 diameters.



FIG. 1.—Section of normal ear of rabbit, for comparison with the succeeding figure. In the middle the cartilage is seen. Hair follicles, containing hairs, are present; also a few sebaceous glands.
FIG. 2.—Section of rabbit's ear injected with scharlach R dissolved in olive oil. The thickness of the ear has been increased about five-fold, the increase being chiefly due to irregular hypertrophy of the Malpighian layer of the surface epithelium and of the hair follicles. The stratum corneum is also hypertrophied, and considerable increase in the nuclei of the corium is present. Fat globules, stained black, are seen: some are contained in fat cells, some have come from the oil injected. The cartilage of the ear is shown in the section, and is not markedly thickened.

A series of 31 implantations of skin from the rabbit's ear, after injection of scharlach R dissolved in olive oil, was made, the site of implantation being the skin of the back (Table I). The epithelium of the skin implanted was very actively proliferating, being in the condition shown in fig. 2. The thickness of the portion of skin implanted was about 1 mm. Before removal of skin for implantation the rabbit's ear was shaved, all loose horny epidermis removed, and the surface washed with absolute alcohol, followed in some experiments by the application of a 1 in 500 solution of mercuric chloride. The latter did not, however, present any obvious advantage, nor

Table I.—Experiments in which Portions of the Skin of the Ear of the Rabbit, previously injected with Scharlach R so as to cause Epithelial Proliferation, were implanted subcutaneously.

| No. of experiment. | Duration of implantation. | Condition of implanted skin at end of period of implantation. | | | |
|--------------------|---------------------------|---|------------------------------|----------------------|---------------------------|
| | | Condition of epithelium. | Mitoses in epithelial cells. | Necrotic epithelium. | Scharlach R oil globules. |
| | days. | | | | |
| 1 | 3 | In part living | Absent | Present | Present |
| 2 | 6 | None living | " | Abundant | " |
| 3 | 6 | In part living | Present | Present | " |
| 4 | 6 | None living | Absent | Abundant | " |
| 5 | 6 | " | " | " | " |
| 6 | 7 | In part living | Present | Present | " |
| 7 | 7 | " | " | " | " |
| 8 | 7 | None living | Absent | Abundant | " |
| 9 | 7 | " | " | " | " |
| 10 | 7 | In part living | Scanty | Present | " |
| 11 | 7 | " | Absent | " | " |
| 12 | 7 | " | " | " | " |
| 13 | 8 | " | Numerous | " | " |
| 14 | 14 | Very little living | Absent | " | Scanty |
| 15 | 14 | " | Scanty | " | " |
| 16 | 14 | " | " | " | Present |
| 17 | 31 | " | Absent | " | " |
| 18 | 31 | " | " | " | " |
| 19 | 31 | None living | " | " | " |
| 20 | 31 | Very little living | " | " | " |
| 21 | 71 | " | Scanty | " | " |
| 22 | 71 | None living | " | " | " |
| 23 | 71 | " | " | " | Very scanty |
| 24 | 71 | " | " | " | " |
| 25 | 80 | Very little living | Scanty | " | " |
| 26 | 80 | " | " | " | " |
| 27 | 80 | None living | " | " | " |
| 28 | 80 | " | " | " | " |
| 29 | 90 | " | " | " | " |
| 30 | 90 | " | " | " | " |
| 31 | 135 | " | " | " | " |

was it in any way inimical to the success of the implantation. The portions of skin implanted were removed at the end of from three days to four and a half months. In 17 out of the 31 implantations, living epithelium was found on removal; in the remaining 14 experiments no living epithelium was present on removal.

For comparison with the above a second series of seven implantations of normal skin from the rabbit's ear (*cp.* fig. 1) was carried out (Table II). In five of these experiments living epithelium was found on examination at the end of from seven days to four and a half months.

Table II.—Control Experiments in which Healthy Living Skin of the Rabbit's Ear was implanted subcutaneously.

| No. of experiment. | Duration of implantation. | Condition of implanted skin at end of period of implantation. | | |
|--------------------|---------------------------|---|------------------------|----------------------|
| | | Condition of epithelium. | Mitoses in epithelium. | Necrotic epithelium. |
| | days | | | |
| 1 | 7 | In part living | Very scanty | Present |
| 2 | 7 | None living | — | " |
| 3 | 31 | In part living | Very scanty | " |
| 4 | 31 | " | " | " |
| 5 | 31 | " | " | " |
| 6 | 31 | None living | — | " |
| 7 | 135 | Very little living | Absent | " |

In order to be in a position to interpret some of the appearances found in the area of implantation, a third series of five implantations was made with normal skin of the rabbit's ear, previously kept for two months in absolute alcohol. These experiments will be referred to later.

When actively proliferating epithelium was implanted it was found that, during the first fortnight of implantation, such portions of the basal prickle layer as remained living continued to divide rapidly, so that in the implanted epithelium mitoses were about as numerous as before implantation, while, in addition, loose horny epidermic scales were produced in abundance at the free surface of the epithelium. The circumstance that cell division continued for a time apparently undiminished in implanted actively proliferating epithelium was quite unexpected, for although it is known that normal epithelium preserved in a moist chamber may remain, for as much as a fortnight after removal, living and capable of being grafted on to a healthy granulating surface,* nevertheless such survival seems to be regarded, not unnaturally, as a species of suspended animation, unattended with active growth. Normal epithelium is, however, owing to its relatively slow growth and its scanty amount, unsuitable for determining the effect of implantation on cell activity, while the use of actively proliferating epithelium, on the contrary, enables this effect to be readily ascertained. The proliferating epithelium, after implantation, would appear to possess a restricted supply of nourishment, for it is not possible to recognise capillaries penetrating into the implanted skin, even at the end of the first week. The character of the

* K. Garrey, "Transplantationen in der Chirurgie," 'Verhandlungen der Gesellschaft Deutscher Naturforscher und Aerzte,' 1906, Erster Teil, S. 205.

mitoses in the implanted epithelium is the same as that noted before implantation, both somatic and reduction mitoses being seen, the former predominating, as described and figured in a previous communication.*

FIG. 4.

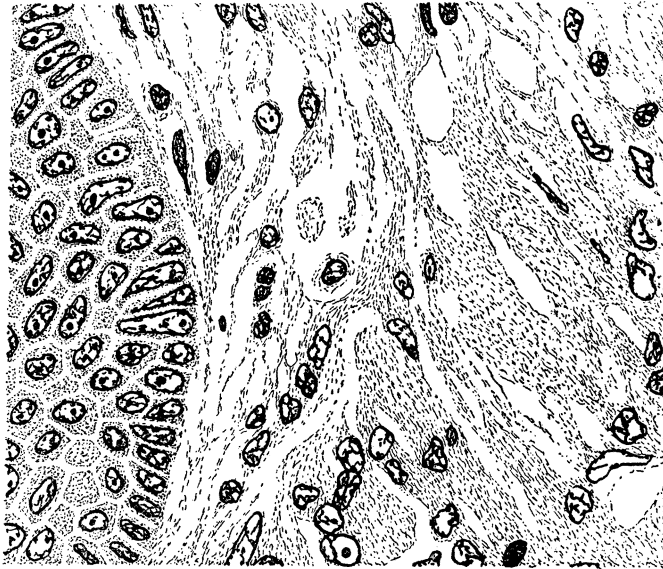


FIG. 3.

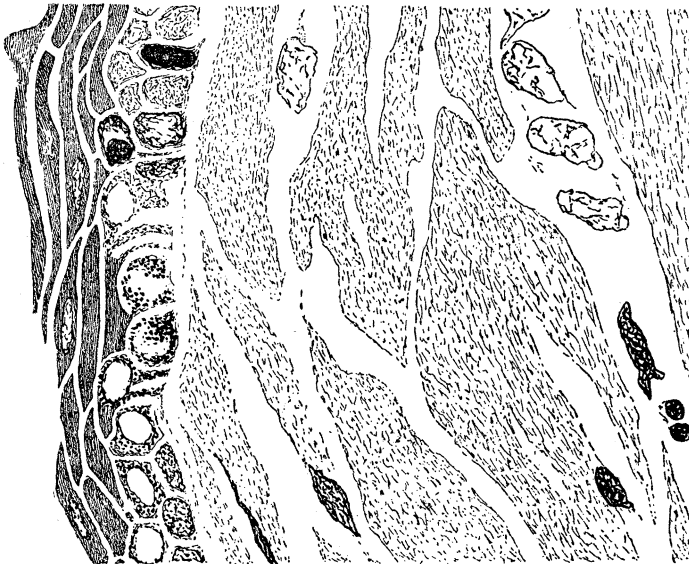


FIG. 3.—Section of normal skin of rabbit's ear, for comparison with the succeeding figure.
 FIG. 4.—Section of skin of rabbit's ear, in which a slight degree of epithelial proliferation has been produced by scharlach R. In addition, the nuclei in the corium are increased in number and stain readily; they are not so flattened as in fig. 3.

How long active proliferation continues in implanted skin cannot be definitely determined from the experiments so far made, but it was found

* J. O. Wakelin Barratt, "Mitosis in Proliferating Epithelium," 'Roy. Soc. Proc.,' B, vol. 79, 1907, p. 372.

that by the 31st day the epithelium was considerably reduced in amount, and that, as in normal skin, division figures were rare. The epithelium still surviving at this period resembled normal surface epithelium, consisting of a flattened expansion of prickle cells, covered with epidermic scales and lying upon a bed of fairly dense connective tissue. No hair follicles or sebaceous glands were seen at this period. Subsequently the epithelium surviving became very scanty in amount.

When the normal skin of the rabbit's ear was used for implantation (Table II), after the lapse of about a month, the appearance of the portions implanted was similar to that of the former series at the end of the same period of time.

In implanted skin a variable amount of the prickle layer always died. Polynuclear leucocytes appeared in large numbers about the dead portion, which could be readily recognised by the shrivelled character of its nuclei, which stained imperfectly and diffusely. Not unfrequently dead and living prickle cells lie close together, the latter showing numerous mitoses and spireme figures. Subsequently the polynuclear cells were accompanied, and later, for the most part, replaced by large mononuclear cells with abundant cytoplasm (*cp.* figs. 5 and 7), before which the prickle cells gradually disappeared, so that ultimately the place of the latter was occupied by large mononuclear cells accompanied by multinucleated giant cells, the nuclei of which lay chiefly in the centre of the cell (*cp.* figs. 6, 7, and 8). As in such cases the horny epidermic scales still persisted unaltered, acting like inert bodies, as did also the hair shafts (figs. 7 and 8), it might at first appear as if these large mononucleated cells and giant cell masses were really epithelial cells, which, owing to their changed environment, had undergone metaplasia, losing their prickles and their capacity for undergoing conversion into keratin. That this is not so, however, was shown by the experiments of the third series, in which the implantation of alcohol-hardened skin led to precisely the same appearance of large mononuclear and giant cells, before which the prickle layer disappeared (fig. 8), while the appearance of mononuclear cells was preceded by that of polynuclear leucocytes, just as in the first two series. This action of dead sterile epithelium in attracting polynuclear leucocytes shows that the presence of the latter is not necessarily due to contamination with bacteria. No evidence of the presence of bacteria in the area of implantation could be obtained by staining methods, and the appearance of the site of implantation was that of a purely aseptic process. In this connection it may be observed that, when the normal skin of the rabbit's ear was used for implantation, the same appearance of polynuclear leucocytes at first, and later of mononuclear and giant cells, occurred. Fig. 6

represents a section of a portion of the ear of a healthy rabbit implanted *sub cute* for 31 days (Experiment 3, Table II). It must therefore be concluded that the dead prickly layer exerts a chemiotactic action, which is at

FIG. 6.

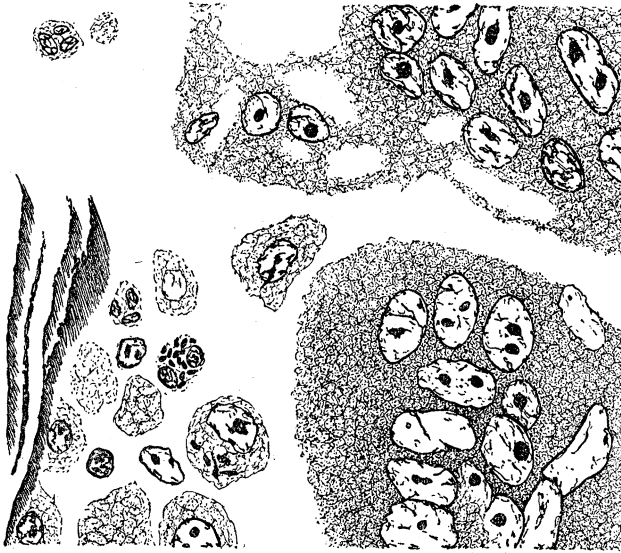


FIG. 5.



FIG. 5.—Section of skin of rabbit's ear, implanted for seven days under the skin of the back of the rabbit. Previous to implantation, epithelial proliferation had been brought about by means of scharlach R. The prickly cells are still living, and are normal in aspect, one division figure being seen. The nuclei in the corium are surrounded by abundant cytoplasm; well-defined collagen bundles are not present. In contact with the epithelium in the upper left-hand corner necrotic material is seen, consisting of ill-defined spherical masses, among which darkly staining bodies, apparently degenerated nuclei, are found.

FIG. 6.—Section of healthy skin of rabbit's ear, implanted for 31 days under the skin of the back of the rabbit. Above is the stratum corneum unaltered. Below this are seen large mononucleated cells, together with two polynuclear cells, one of which shows basophile granules. Still lower are portions of two large giant cells.

first acute, leading to the attraction of polynuclear leucocytes, and, later, becoming less intense, affects mononuclear cells. The source of the latter cells need not be considered here, since the experiments made do not throw any new light upon their mode of origin, beyond the fact that mitoses of the

somatic type are frequent among these cells, showing that they multiply *in situ*. The giant cells, which are a striking feature of sections of the area of implantation, do not resemble the giant cells of tubercular lesions, but are

FIG. 8.

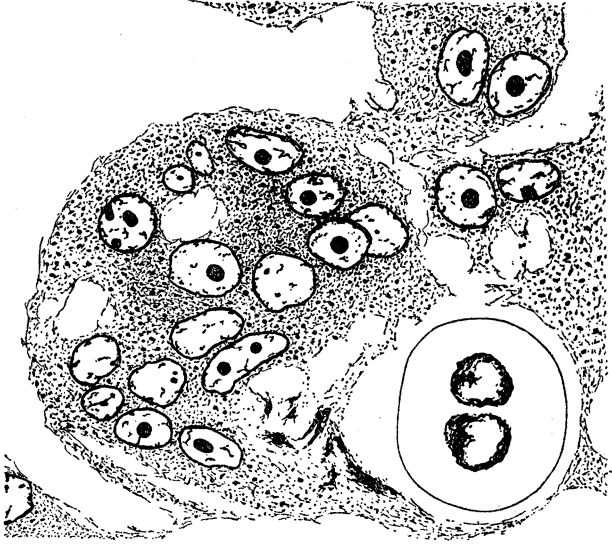


FIG. 7.

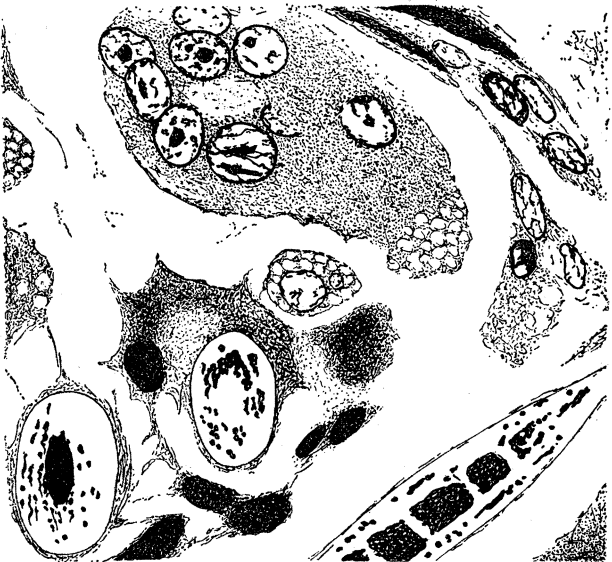


FIG. 7.—Section of nodule resulting from implantation, for 31 days, of skin of rabbit's ear, in which epithelial proliferation had been brought about by injecting scharlach R. No prickles cells are present. Three pigmented hair shafts are seen, two in transverse, one in oblique, section; the latter seems to exhibit slight superficial erosion, the others show no erosion. Mononucleated cells with abundant cytoplasm are seen, and also a giant cell.

FIG. 8.—Section of nodule resulting from implantation of normal skin of the ear of the rabbit for 14 days under the skin of the back of the rabbit. Previous to implantation, the piece of skin employed had been kept in absolute alcohol for two months. An unaltered hair follicle is seen with a giant cell beside and in part surrounding it, together with several large mononucleated cells possessing abundant cytoplasm. This section shows that the cell masses seen in figs. 6 and 7, beneath unaltered stratum corneum or around hair-shafts, cannot be considered as arising by a metaplasia of the implanted epithelial cells.

like those of myeloid sarcoma, the nuclei being placed centrally. Apart from the *débris* which they exhibit (figs. 5 to 8), the sections recall very vividly the aspect of sarcoma tissue of the round celled variety. Both the giant cells and the single large mononucleated cells exhibit granular cytoplasm, which

often contains numerous pigment granules (fig. 8), and sometimes presents a vacuolated character (fig. 7).

It may here be observed that, when epithelial proliferation is produced in the rabbit's ear by the injection of scharlach R (fig. 4), the nuclei of the corium are increased and mononuclear cells rich in cytoplasm become observable. That this is not due to the action of scharlach R, is shown by the fact that repeated attempts to obtain such cell infiltration by injection of this dye, dissolved in olive oil, into the deeper layers of the subcutaneous tissue and also into the testis of the rabbit failed. It follows, therefore, that, as the result of the more active metabolism of the prickle cells under the influence of scharlach R, a chemiotactic action is set up, leading to the appearance of these cells.

The implanted normal corium in the second and third series of experiments did not excite any marked chemiotactic response, no invasion of new cells into it being noted. The cartilage survived in most implantations; when it underwent necrosis, however, it did not become invaded by new cells, its hyaline substance remaining as an inert body, and its nuclei becoming shrivelled and staining indistinctly.

Summary.

1. During the first 7 to 14 days after implantation of actively proliferating epithelium of the rabbit's ear, mitosis proceeds apparently undiminished in amount in such portions of the prickle layer as escape necrosis.

2. In contrast to living prickle cells, dead prickle cells, when implanted under the skin, exert a powerful chemiotactic action, determining at first the appearance of polynuclear cells and later of large mononuclear and giant cells; before the latter the dead prickle layer slowly disappears. Even living prickle cells, when actively proliferating under the influence of scharlach R prior to implantation, induce chemiotactically, through their increased metabolism, the appearance of numerous cells, mostly mononucleated, in the subjacent corium; this is, however, much slighter in degree than that induced by dead proliferated epithelium.

3. No metaplasia of actively proliferating epithelium occurs as a result of implantation.

4. Horny epidermic scales and hair shafts remain intact, behaving as inert bodies.
