THE HISTOGENESIS OF THE EPIDERMIS IN THE RAT AND MOUSE

By JEAN HANSON

Department of Zoology, Bedford College, University of London, and the Strangeways Research Laboratory, Cambridge

INTRODUCTION

Although the epidermis of the mouse and rat are much used in cancer research their normal histogenesis has not been adequately described. Moreover, the lack of information about cellular differentiation in relation to cell division and stratification during the normal development of these tissues is particularly regrettable because the subject of cellular differentiation is of fundamental importance in cancer research. In this paper I have attempted to fill in these gaps in our knowledge. I have made a comparative study of the rat and mouse because, as is well known, the epidermis of the two species differs much in its sensitivity to certain carcinogenic agents (Glucksmann, 1945). In addition, I have studied epidermis from four different skin sites because Twort & Twort (1936) have shown that, in the mouse, various sites differ in their sensitivity to carcinogenic hydrocarbons. A quantitative method for following changes in the populations of differentiating, resting (undifferentiated), dividing and degenerating cells of human carcinomata during radiation treatment has been described by Glucksmann (1941). This method was developed after previous investigations at the Strangeways Research Laboratory on the developing eyes of tadpoles and rats both normal and irradiated (Tansley, Spear & Glucksmann, 1937; Spear & Glucksmann, 1938, 1941; Glucksmann & Spear, 1939; Glucksmann, 1940). The method has recently been applied (Glucksmann, 1945) to investigations of the normal post-natal development of mouse epidermis, of regenerative hyperplasia induced in mouse epidermis by chemical depilation and by turpentine painting, and of the changes following the single and repeated application of benzpyrene to mouse and rat skin. In the work reported in the present paper a modification of Glucksmann's method has been used to supplement the qualitative investigation of normal epidermal histogenesis of rats and mice in order to express more clearly the changes in the cell population during histogenesis and, secondly, to compare the results with those of Glucksmann (1941, 1945) already referred to.

Previous work. Previous investigations of epidermal development in rats and mice are summarized in Table 1 and will be discussed in relation to my own observations.

MATERIAL AND METHODS

Mus musculus L. var. *albinus* and *Rattus norvegicus* (Erxleben) were used. The specimens examined comprise: ten embryonic mice aged 10–18 days post-coitum; nine embryonic rats aged 11–22 days post-coitum; twelve mice and six rats aged 0–100 days post-partum. Animals of both sexes were used before puberty, but after puberty males only were selected, for Bullough (1943) has shown that, in the mouse, the thickness of the epidermis and the mitotic frequency of its cells undergo cyclic changes coincident with the phases of the oestrous cycle.

Table 1. *Summary of previous work on the histogenesis of the epidermis in mouse and rat*

References		Specimens	Sites
Steiner & Hitschmann (1927)	Rats	8-, 10-, 12-, 14-, 16-, and 18-day embryos	Various
Fraser (1928)	Rats	Series from 12th day post-coitum to 2nd day post-partum; also specimen 150 days old	Mid-dorsal
Erickson (1931)	Rats	Series from birth to maturity	Tail
Maurer (1892)	Mice	Embryo 18 mm. long	Not stated
Oyama (1904)	Mice	Embryo 21 mm. long	Not stated
Reed & Alley (1939)	Mice	Embryo 10 mm. long, about 15 days old	Not stated
Gibbs (1941)	Mice	Series from 0–15 days after birth	Anterior and posterior dorsal
Glucksmann (1945)	Mice	Series from 0–100 days after birth	Anterior dorsal

Epidermis from the following four regions was used (Text-figs. 1, 2).

(1) A rectangular area extending across the back from one forelimb to the other.

(2) A rectangular area extending across the back from one hindlimb to the other.

(3) A rectangular area extending across the ventral surface from one forelimb to the other.

(4) The under surface of the forefoot between the thenar and hypothenar pads.

The same four regions were used by Twort & Twort (1936). All tissues were fixed in Zenker's fluid (with 5% acetic acid). Sections were cut usually 8μ, occasionally 10μ, thick and were stained, in duplicate sets, some with Ehrlich's haematoxylin and others by a modification of Mallory's triple staining method in which carmalum replaces acid fuchsin.

PART I. QUALITATIVE OBSERVATIONS

The lack of a generally accepted terminology for epidermal strata makes it necessary to define the names used in this paper. The abbreviation 'S.' is used for 'stratum'.

S. germinativum: a basal layer of undifferentiated cells distinguishable from cells of the S. intermedium and S. spinosum by their smaller content of cytoplasm

and by having less distinct cell outlines and less conspicuous intracellular fibrils than the cells of the S. spinosum.

S. intermedium: a transitory embryonic stratum of undifferentiated cells which eventually become spinous cells with the development of intracellular fibrils.

S. spinosum: differentiating cells with relatively more cytoplasm, more conspicuous intracellular fibrils and more clearly defined cell outlines than the cells of the S. germinativum.

S. granulosum: cells containing keratohyalin granules.

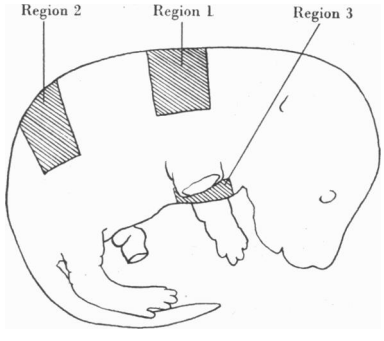
S. lucidum: cells containing eleidin.

S. corneum: fully differentiated (keratinized) cells.

S. disjunctivum: the disintegrating surface layers of the S. corneum.

Epitrichium: the keratinized layers which are shed when the first hair coat emerges.

Periderm: a transitory embryonic stratum, the most superficial in the epidermis, distinguishable from the S. germinativum and S. intermedium by its more flattened cells with denser cytoplasm.



Text-fig. 1. The position of regions 1, 2 and 3 on a 16-day mouse embryo.

It is necessary to point out that these various categories of cells, classified according to degree of cellular differentiation, are recognized by cytological criteria rather than by their location in the epidermis. In this paper the term 'differentiation' is used to refer only to the cytological changes leading to and involved in the process of keratinization.

(1) *The stages in histogenesis*

The developmental history of the epidermis can be arranged in stages according to the different strata present.

Stage A: S. germinativum only.

Stage B: S. germinativum and periderm.

Stage C: S. germinativum, S. intermedium and periderm.

Stage D: S. germinativum, S. spinosum, S. granulosum and periderm.

Stage E: S. germinativum, S. spinosum, S. granulosum, S. lucidum, S. corneum and periderm.

Stage F: S. germinativum, S. spinosum, S. granulosum, S. lucidum and S. corneum.

Stage G: S. germinativum, S. spinosum, S. granulosum, S. lucidum, S. corneum and, in region 4, S. disjunctivum.

The various stages last for different periods, their duration differs also in the mouse and in the rat, and they persist for different times in different areas.

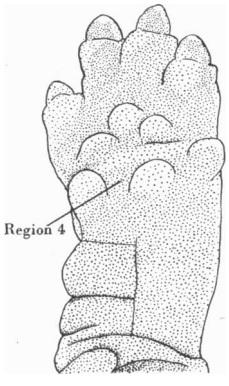
For example, the duration of stages A, B and C is not equal over the whole

of regions 1–3. In the medial parts of regions 1–3 stages A and B end on the 13th and 14th days post-coitum in the mouse, and on about the 16th and 17th days in the rat. The particulars set forth in Table 2 deal only with the lateral parts of regions 1–3. The various stages are dealt with in more detail in association with the origin and development of each stratum.

No attempt has been made to give more than an approximate estimate of the duration of any of the embryonic stages of epidermal histogenesis: litters and even embryos within one litter vary in the rate of their development (Nicholas, 1932, for the rat; Grüneberg, 1943*a*, for the mouse).

(2) *The origin and development of each stratum*

The origin and development of each stratum will be described for the mouse, and the ways in which the rat differs from the mouse will be pointed out. Changes in the number of layers in each nucleated stratum (S. germinativum, S. intermedium, S. spinosum and S. granulosum) are shown in Table 2.



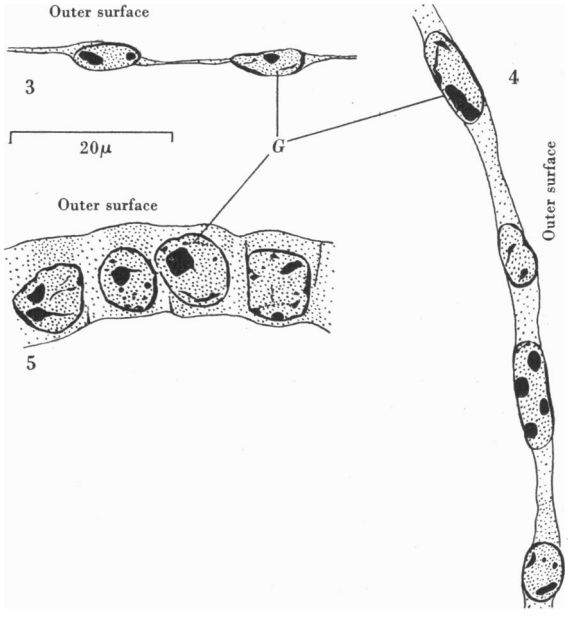
Text-fig. 2. The position of region 4 on the forefoot of a 16-day mouse embryo.

The *S. germinativum*, the deepest layer of the epidermis, is present in the youngest embryos (10-day mice and 11-day rats) and is responsible for the origin and maintenance of the other epidermal strata. Its cells are undifferentiated and in all specimens some are found dividing. In the mouse it consists of a single layer of cells which, in region 4, remains continuous at least up to 100 days after birth. In regions 1–3 it becomes incomplete by the 10th day post-partum: both spinous cells and the undifferentiated cells of the S. germinativum lie in the basal layer (Text-figs. 11, 12 and Pl. 2, figs. 10, 11), but small uninterrupted groups of undifferentiated cells remain in the immediate vicinity of the hair follicles. In the rat the S. germinativum is always a continuous layer.

In region 4 of all specimens the S. germinativum consists of columnar cells (Text-fig. 5) but in regions 1–3 the cells undergo several changes in shape during development. Until the 14th day post-coitum in the mouse and the 17th day in the rat they are flattened, in vertical sections appearing elongated parallel to the skin surface (Text-figs. 3, 4, and Pl. 1, fig. 1).

They are more flattened in region 3 than in regions 1 and 2 and in the medial parts of these three regions than in their more lateral parts. Similar regional differences in the shape of the cells of the S. germinativum have been described by Steiner & Hitschmann (1927) in 10- and 14-day rat embryos, by Fraser (1928) in 12-, 13-, 14- and 15-day rat embryos and by Steiner (1929, 1930) in early human embryos.

After the S. spinosum and S. granulosum have been formed the S. germinativum over the whole of regions 1–3 consists of columnar cells (Text-fig. 8 and Pl. 1, fig. 3). Slightly later, during the development of the hair follicles, these



Text-figs. 3–5. The epidermis of an 11-day rat embryo, stage A. 3. The medial part of region 1. 4. The lateral part of region 1. 5. Region 4. *G*=S. germinativum.

Table 2. *Stages (A–G) in the histogenesis of the layers of the epidermis (S. germinativum; S. intermedium; S. spinosum; S. granulosum; S. lucidum; S. corneum; periderm); the numbers of layers in each nucleated stratum; and the duration in days, post-coitum (–), post-partum (+), of each stage in mouse and rat (M and R). P=present.*

	Strata present and number of layers in each nucleated stratum								Time of ending of stages in days	
Stage	Germ.	Int.	Spin.	Gran.	Luc.	Corn.	Per.	Region	Mouse	Rat
A	1	—	—	—	—	—	—	1–4	10 –	12–15 –
B	1	—	—	—	—	—	1	1–4	13 –	16 –
C	1	1–3	—	—	—	—	1	1–4	14 –	18 –
D	1	—	3	1	—	—	1	1–4	16 –	19 –
E	1	—	2–1	4–3M 4R	P	P	1	1–3	0+	0+
	1	—	3	4	P	P	1	4		
F	1	—	1	3M 4R	P	P	—	1–3	Emergence of 1st pelage	
	1	—	3–2	4	P	P	—	4M		
G	1	—	2M 3–2R	3M 4–3R	P	P	—	4		
	1	—	1	3–2–3	P	P	—	1–3R		
	Incomplete	—	1– Incomplete	1 or 2– Incomplete	P	P	—	1–3M		

cells become cuboidal in the stretches of epidermis between the follicles but remain columnar in the vicinity of the follicles (Text-fig. 9); this has also been noticed by Fraser (1928). No subsequent changes in cellular shape have been observed. Intracellular fibrils appear in the cytoplasm on the 14th day post-coitum in the mouse and on the 18th day in the rat.

The *periderm*, the most superficial layer of the embryonic epidermis, is formed by the S. germinativum on the 12th day of embryonic life in the rat (Text-fig. 6 and Pl. 1, fig. 2): small numbers of periderm cells are already present in the 10-day mouse embryo, the youngest that has been examined. The periderm is formed first in region 4 and in the more lateral parts of regions 1 and 2; later it appears in region 3 and in the more medial parts of regions 1 and 2. Its absence over the neural tube in young rat embryos was noticed by Fraser (1928). The periderm persists almost till birth: it has been found in the oldest rat embryo, aged 22 days, but has not been identified in the mouse after the 17th day.

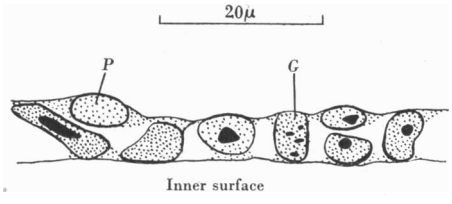


Fig. 6.

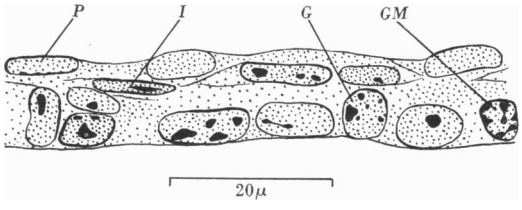


Fig. 7.

Text-fig. 6. The epidermis on the lateral part of region 1 of a 12-day rat embryo. Stage B. *P* = periderm.

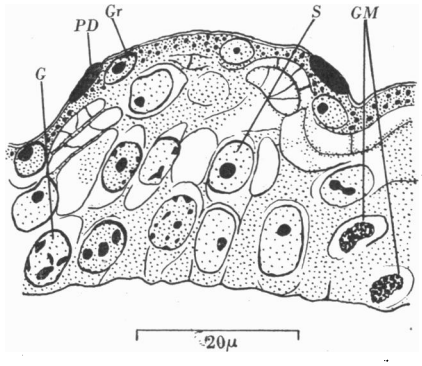
Text-fig. 7. The epidermis on the lateral part of region 1 of a 13-day mouse embryo. Stage C. *I* = S. intermedium. *GM* = dividing cell of S. germinativum.

For a short time after the periderm has been formed its cells are undifferentiated and able to divide (Pl. 1, fig. 2); cell division has ceased by the 14th day in the mouse and by the 17th day in the rat. Degenerate periderm nuclei (Text-fig. 8) are first noticed at these times and nuclei of normal appearance are no longer found after the 16th and 19th days in the mouse and rat respectively. Nuclear degeneration is accompanied by cytoplasmic changes, and the periderm soon has the appearance of a thin sheet of keratin with persistent nuclear remains (Text-fig. 9); this indicates that differentiation of the periderm probably takes place by parakeratosis as in human embryos (Pinkus, 1910). I have been unable to find, even in haematoxylin-stained sections, the basic-staining granules recorded by Fraser (1928) in the periderm of 18- and 19-day albino rat embryos.

The *S. intermedium*, situated between the S. germinativum and the periderm, is present by the 13th day of embryonic life in the mouse (Text-fig. 7) and by the 16th day in the rat. Like the periderm it is formed later in the medial than in the more lateral parts of regions 1–3; in one 13-day mouse embryo it is found in region 4 but is still absent on the trunk. Two observations indicate that the

S. intermedium is produced from the S. germinativum, viz. the cytological similarity of the cells in the two strata and the relatively small number of dividing periderm cells, probably sufficient only for increasing the surface area of the periderm and insufficient for contributing to the S. intermedium. The cells of the S. intermedium are undifferentiated and able to divide. In a short time it becomes about three cell layers thick and by the 14th day post-coitum in the mouse and the 18th day in the rat intracellular fibrils have been formed; the cells have begun to differentiate and the S. intermedium has become the S. spinosum.

The *S. spinosum*, when it is formed on the 14th day post-coitum in the mouse and on the 18th day in the rat, consists of three layers of cells. The number of layers decreases during development as shown in Table 2. On the trunk of the mouse the stratum becomes discontinuous after the 15th day post-partum, though small groups of spinous cells remain in the vicinity of the hair follicles above groups of undifferentiated cells of the S. germinativum. On the 10th day and afterwards some spinous cells are found intermingled with undifferentiated cells in the basal layer of the epidermis (Text-figs. 11, 12 and Pl. 2, figs. 10, 11). In region 4 of the mouse and in all four regions of the rat the S. spinosum and S. germinativum are always continuous strata.



Text-fig. 8. The epidermis on region 2 of an 18-day rat embryo. Stage D. *S* = S. spinosum. *Gr* = S. granulosum. *PD* = degenerating nucleus of periderm.

The S. spinosum is originally formed by the differentiation of the cells of the S. intermedium. A few dividing cells are found in the S. spinosum of region 4 until the time of birth (Pl. 1, fig. 6) but on the trunk cell division ceases earlier, by the 16th day in the mouse and by the 19th day in the rat. The cessation of cell division coincides with the time when the amount of cytoplasm is increasing and the intracellular fibrils are becoming more conspicuous. The division of spinous cells is insufficient for the maintenance of the S. spinosum, for which the S. germinativum is responsible. For a short time after the S. spinosum has been formed it is possible to distinguish between those spinous cells which have been produced by the differentiation of cells in the S. intermedium and those which have been produced by the S. germinativum; the latter, which lie deeper in the epidermis, are more polygonal in shape (Text-fig. 8).

Similarly in the early *S. granulosum* one can distinguish: (*a*) cells which are very flattened in shape, have small keratohyalin granules and have been formed by the differentiation of spinous cells of S. intermedium origin, and (*b*) cells which are less flattened, have larger granules and have never passed through a stage when they were constituents of the S. intermedium. The S. granulosum

is formed on the 14th day of embryonic life in the mouse and on the 18th day in the rat. Fraser (1928) first identified the stratum in a 20-day albino rat embryo, but it is possible that the basic-staining periderm granules which she described in younger embryos really belonged to the S. granulosum. As mentioned above, I have been unable to find such granules in the periderm; but keratohyalin granules are basic-staining and the very thin cytoplasm of the periderm is in many places difficult to distinguish in 18- and 19-day Norway rat embryos. Moreover Fraser enumerated 'six or seven' cell layers in the epidermis of an 18-day albino rat embryo and attributed only three or four of them to the 'S. intermedium' (corresponding to the S. spinosum of this paper) and S. germinativum; of the remaining three layers probably two belonged to the S. granulosum and one to the periderm.

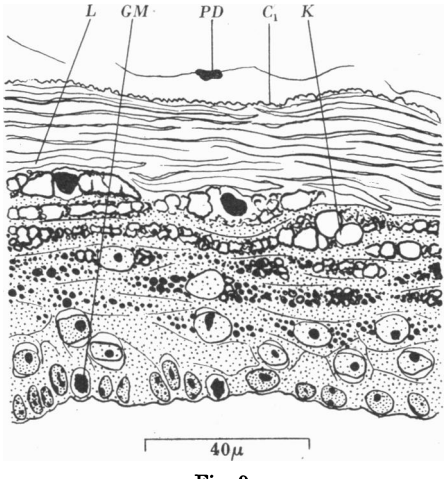


Fig. 9.

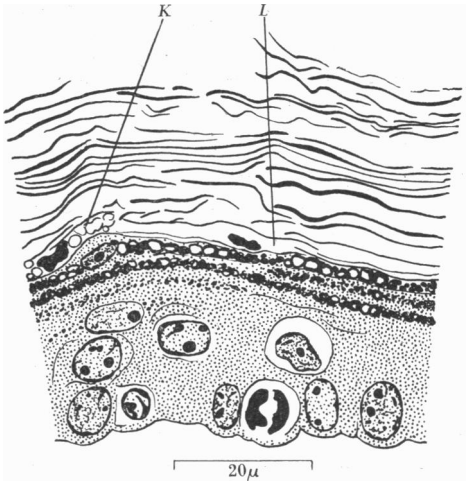


Fig. 10.

Text-fig. 9. The epidermis on region 1 of a 21-day rat embryo. Stage E. C_1 = S. corneum; K = large keratohyalin granule partly converted into eleidin; L = S. lucidum.

Text-fig. 10. The epidermis on region 1 of a mouse 3 days after birth. Stage F. Lettering as before.

Changes in the number of cell layers in the S. granulosum are shown in Table 2. On the trunk of the mouse the stratum has become discontinuous by the 20th day post-partum and is represented only by scattered cells containing small keratohyalin granules; they are found mainly in the vicinity of the hair follicles where there are also groups of undifferentiated cells and spinous cells. The stratum is never discontinuous on region 4 of the mouse or on any region of the rat. Dividing cells are never found in the S. granulosum; the nuclei in the oldest (most superficial) cells are usually degenerate.

The mode of formation of the *S. lucidum* by the differentiation of cells of the S. granulosum is indicated by the appearance presented by the most superficial cells (Text-figs. 9, 10 and Pl. 1, fig. 4) in the latter stratum. They contain a small number of very large granules probably formed by coalescence of the

smaller and more numerous keratohyalin granules of the deeper cells in the stratum. Each large granule has an outer wall of a substance giving the same staining reactions as the small keratohyalin granules; within the wall is a substance giving the same staining reactions as the cell contents of the S. lucidum, indicating that the conversion of keratohyalin to the eleidin of the S. lucidum begins in the centre of the granule. In some places in the S. lucidum it is possible to distinguish the outlines of granules which have been completely converted into eleidin and which have not yet become confluent with each other; in these places the S. lucidum cells are sometimes contiguous laterally with cells of the S. granulosum in which the granules still retain an outer covering of keratohyalin. The S. lucidum is first distinguishable on the 16th day post-coitum in the mouse and on the 19th day in the rat. It is thicker in the rat than in the mouse and, after the emergence of the first hair coat, on the lower surface of the foot than on the trunk. Its cells never divide and the few nuclei that still remain are all degenerate (Text-figs. 10, 13).

The *S. corneum* is formed on the 16th day of embryonic life in the mouse and on the 19th day in the rat. It is composed of horizontally arranged sheets of keratin representing the converted peripheral parts of the cells of the underlying strata. In the rat the outer surface of the first-formed sheet of keratin is raised into numerous small domes of about the same size as the keratohyalin granules in the outer cells of the S. granulosum (Text-fig. 9 and Pl. 1, fig. 4). This sheet of keratin can still be detected on the outer surface of the S. corneum up to the time of birth, indicating that no layers are shed from this stratum until after birth. The superficial sheet of keratin in the mouse lacks this peculiarity.

From its first appearance until the time when the first hair coat emerges the thickness of the S. corneum increases and it consists of relatively closely packed sheets of keratin in which it is difficult to distinguish the lateral cell boundaries (Pl. 1, fig. 5). On the lower surface of the foot it retains this structure for so long as its development has been studied (Text-fig. 13). On the trunk, however, the early S. corneum, or epitrichium, is shed when the first hair coat emerges.

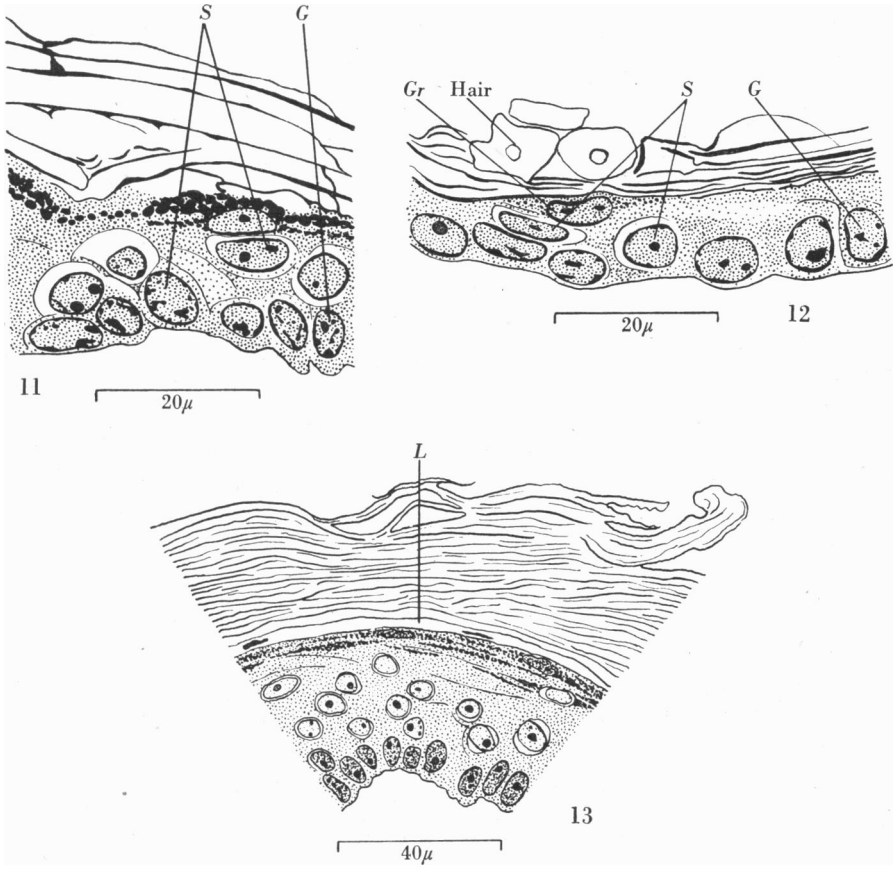
The sheets of keratin in the new S. corneum which replaces the epitrichium lie less close together and form a reticular pattern (in vertical sections) because the lateral boundaries of the cornified cells are visible (Text-fig. 11 and Pl. 2, fig. 9). These lateral boundaries usually lie vertically above each other and often above the junctions between adjacent cells in the outer layers of the S. granulosum. Fragments of the epitrichium are found outside the new S. corneum for some time after the hairs have emerged (Pl. 2, fig. 9). The new S. corneum of the mouse is considerably thinner than that of the rat.

A *S. disjunctivum* has been identified only after birth and on the lower surface of the foot; it is distinguished from the rest of the S. corneum by small differences in staining reaction and by the fact that the sheets of keratin are broken and are more widely separated from each other.

(3) *Summary of qualitative specific and regional differences*

Specific differences

(1) During embryonic life the rat enters each stage of epidermal histogenesis two to four days later than the mouse; this difference is correlated with the fact that the gestation period is about four days longer in the rat than in the mouse (Donaldson, 1924, p. 20; Grüneberg, 1943*b*, p. 6).



Text-figs. 11–13. Stage G. 11. The epidermis on region 3 of a mouse 10 days after birth. 12. The epidermis on region 3 of a mouse 100 days after birth. 13. The epidermis on region 4 of a mouse 29 days after birth. Lettering as before.

(2) At equivalent stages of development the reductions which take place in the number of cell layers in the S. spinosum and S. granulosum of region 4 and in the S. granulosum of regions 1–3 begin later in the rat than in the mouse (Table 2).

(3) The decrease in the thickness of the epidermis on the trunk after the emergence of the first hair coat proceeds further in the mouse than in the rat; and in the mouse, but not in the rat, the S. germinativum, S. spinosum,

S. granulosum and S. lucidum become disorganized and incomplete. Henceforward the S. corneum is thinner in the mouse than in the rat.

Regional differences

(1) During the early stages of epidermal histogenesis, on the medial as compared with the more lateral parts of regions 1–3 the cells of the S. germinativum are more flattened, and the periderm and the S. intermedium are formed later.

(2) The cells of the S. germinativum in region 4 are columnar in all specimens, even in the youngest embryos, whereas in regions 1–3 they are at first flattened and then cuboidal and do not become columnar until later.

(3) During the development of the first hair coat there are established other differences between the epidermis on region 4, where hairs are absent, and on the epidermis of the trunk (regions 1–3).

(*a*) The cells of the S. germinativum in region 4 remain columnar whereas in regions 1–3 they become cuboidal in the epidermis away from the vicinity of the hair follicles.

(*b*) In the spinous cells of regions 1–3 cell division ceases very soon after the formation of the S. spinosum and at the same time the amount of cytoplasm increases and the intracellular fibrils become more conspicuous. In the spinous cells of region 4 these changes do not take place until about the time of birth.

(*c*) When the first hair coat has emerged the epidermis in regions 1–3 is thinner than in region 4 (Table 2) and a different type of S. corneum has replaced the epitrichium. In region 4, however, the original type of S. corneum persists and a S. disjunctivum can be recognized.

(*d*) In the mouse the S. germinativum, S. spinosum, S. granulosum and S. lucidum remain intact in region 4 whereas they become disorganized and incomplete in regions 1–3 after the emergence of the first hair coat.

PART II. QUANTITATIVE OBSERVATIONS

(1) *An analysis of the cell population*

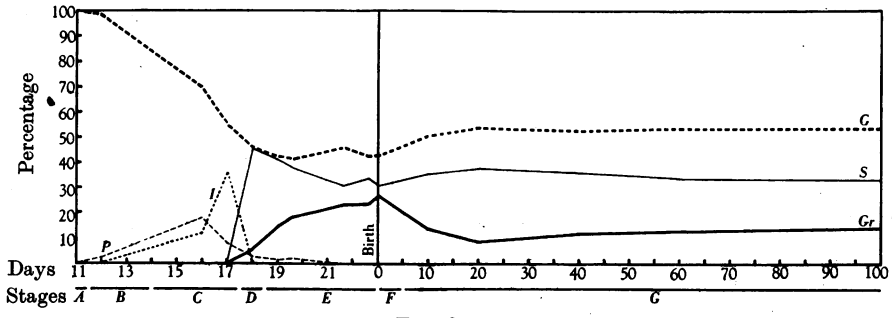
An analysis of the cell population of the developing epidermis has been made by a modification of the method used for biopsies of irradiated human carcinomata (Glucksmann, 1941) and for normal and treated mouse epidermis (Glucksmann, 1945) in which four categories of cells are counted: 'resting' (undifferentiated), degenerating, dividing, and differentiating. In the developing epidermis of the rat and mouse five categories of cells have been distinguished by the cytological criteria listed above: cells of the S. germinativum, S. intermedium, S. spinosum and S. granulosum, and cells of the periderm. In each specimen the numbers of nuclei belonging to cells in each of these five categories have been counted at all focal levels on several carefully selected rectangular areas of vertically sectioned epidermis away from the vicinity of hair follicles. The results have been expressed as percentages of the total number (usually about 500) of nuclei counted.

In Text-figs. 14–17 the percentage of each category of cells in the total population of nucleated epidermal cells is plotted against the age of the specimen. All four skin sites have been analysed separately but the results for regions 1–3 are similar and have been averaged (Text-figs. 14 and 16 for the rat and mouse respectively). The results for region 4 have been given separately (Text-figs. 15, 17). As previously mentioned the medial parts of regions 1–3 differ from the lateral parts in the youngest embryos, and in each of these cases the nuclei of all epidermal cells have been counted in each of several sections taken across the whole width of the region. The age of each embryo is usually known only to within ± 12 hr. of the approximate age, expressed in days, which has been assigned to it; moreover, as already mentioned, litters, and even embryos within one litter, vary in the rate of their development so that the embryos of the same approximate age may have reached different stages of epidermal histogenesis. For the purpose of spacing the embryos in drawing the graphs each has been assigned an age, expressed in days and fractions of days (e.g. 13·6 days), after consideration both of its approximate age and of the stage reached in epidermal histogenesis.

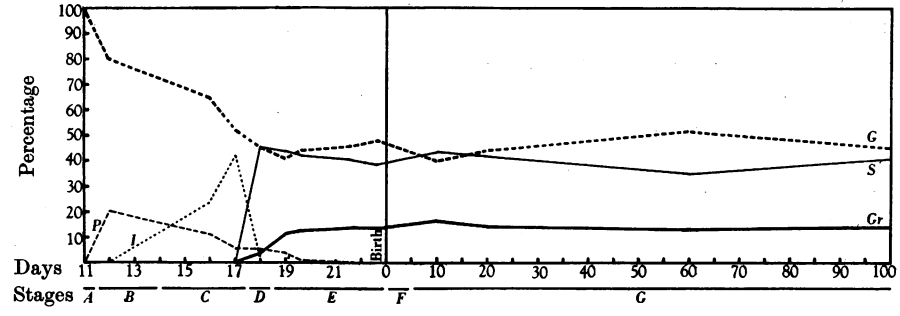
The graphical presentation of the changes in the cell population during epidermal histogenesis expresses clearly many of the features already described, such as: the time of formation of each of the nucleated strata and, in the case of the periderm and the S. intermedium, the time of its disappearance, and the combination of strata which helps to characterize the stages (A–G) of epidermal histogenesis. Moreover, the graphs demonstrate the changes in the numerical proportions of the different kinds of *cells* in the developing epidermis, whereas the qualitative observations could reveal only the developmental history of the *strata*. They show that for specimens at the same stage of epidermal development the proportion of undifferentiated cells (cells of the S. germinativum) to differentiating cells (cells of the S. spinosum and S. granulosum) is approximately the same in both the rat and the mouse. This indicates that cellular differentiation proceeds in the same manner in both species even though on the trunk of the mouse stratification of the epidermis disappears soon after birth.

During early embryonic development there is a conspicuous decrease in the percentage of undifferentiated cells and a proportionate increase in the percentage of differentiating cells. Using a similar quantitative method for analysing the cell population, Glucksmann (1945) has shown that during experimental carcinogenesis of the interscapular epidermis of the adult mouse the percentage of undifferentiated cells increases and the percentage of differentiating cells decreases, i.e. the reverse of the changes which take place during the development of the epidermis. He has also shown that when human carcinomata are successfully irradiated the percentage of undifferentiated cells decreases and the percentage of differentiating cells increases (Glucksmann, 1941).

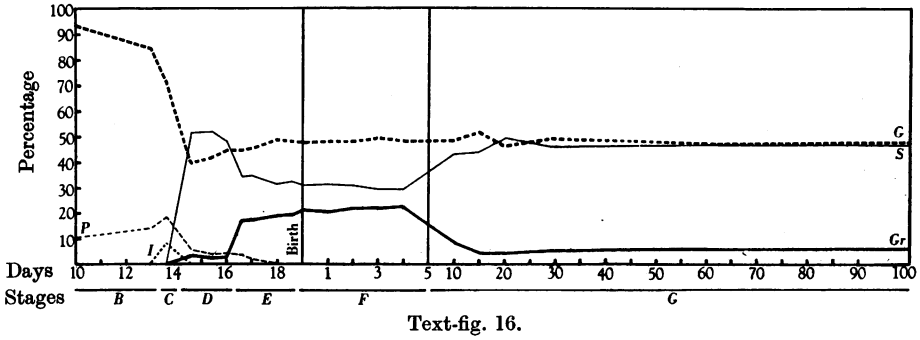
The analyses of the cell population of the developing epidermis of rats and mice lead to conclusions similar to those reached at the end of the report on



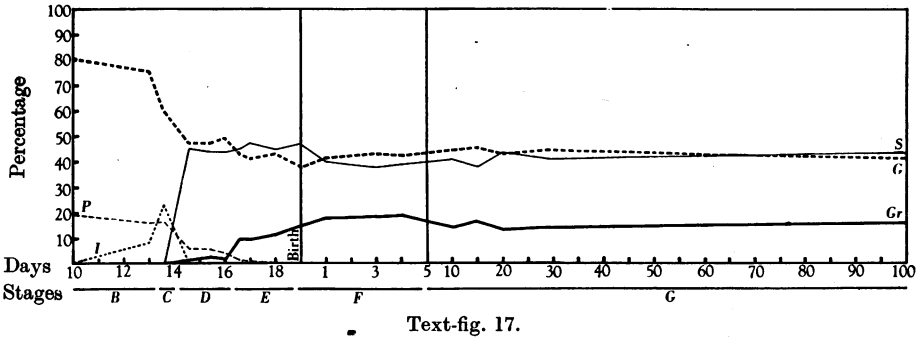
Text-fig. 14.



Text-fig. 15.



Text-fig. 16.



Text-fig. 17.

Text-figs. 14–17. The percentage numbers of five categories of cells in the total population of nucleated epidermal cells. Thick broken lines: S. germinativum; thin broken lines: periderm; dotted lines: S. intermedium; thin continuous lines: S. spinosum; thick continuous lines: S. granulosum. 14. Rat, average of regions 1–3. 15. Rat, region 4. 16. Mouse, average of regions 1–3. 17. Mouse, region 4.

qualitative observations. The most striking differences between the rat and the mouse are found on the trunk after the emergence of the first hair coat; the three trunk regions within each species closely resemble one another and differ from region 4, the differences becoming pronounced during the development of the first hair coat.

(2) *Dividing cells*

During the analysis of the cell population of the developing epidermis dividing and non-dividing cells in the S. germinativum, S. intermedium, S. spinosum and

Table 3. *The percentage numbers of four categories of dividing cells in the total population of nucleated epidermal cells. M, mouse; R, rat. The ages are given in days after copulation* (−) *or after birth* (+).

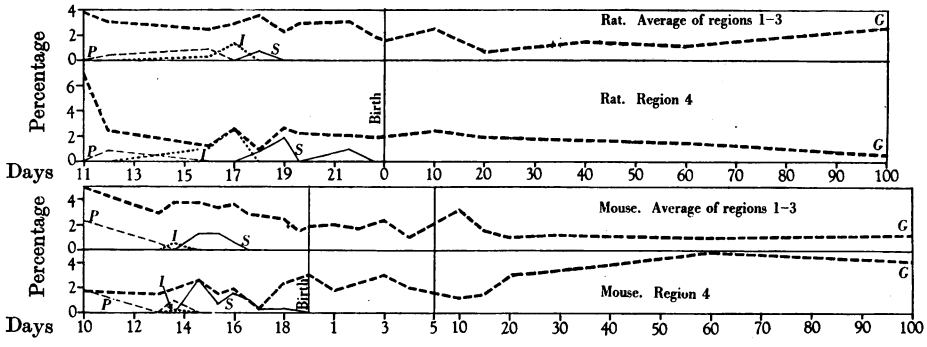
	S. germinativum				S. intermedium				S. spinosum				Periderm			
	Average regions 1–3		Region 4		Average regions 1–3		Region 4		Average regions 1–3		Region 4		Average regions 1–3		Region 4	
Age (days)	M	R	M	R	M	R	M	R	M	R	M	R	M	R	M	R
10−	4·96	—	1·74	—	0	—	0	—	0	—	0	—	2·27	—	1·74	—
11−	—	3·81	—	7·05	—	0	—	0	—	0	—	0	—	0	—	0
12−	—	3·12	—	2·50	—	0	—	0	—	0	—	0	—	0·18	—	0·83
13−	2·99	—	1·53	—	0	—	0	—	0	—	0	—	0·46	—	0	—
13−	3·79	—	1·95	—	0·53	—	0·19	—	0	—	0	—	0	—	0·97	—
14−	3·82	—	2·64	—	0	—	0	—	1·29	—	2·64	—	0	—	0	—
15−	3·44	—	1·53	—	0	—	0	—	1·31	—	0·77	—	0	—	0	—
16−	3·71	2·53	1·96	1·30	0	0·32	0	1·11	0·56	0	1·57	0	0	0·84	0	0
16−	2·87	—	1·06	—	0	—	0	—	0	—	0·89	—	0	—	0	—
17−	2·81	2·95	0·38	2·61	0	1·38	0	2·61	0	0	0·38	0	0	0	0	0
18−	2·57	3·61	2·41	0·99	0	0	0	0	0	0·70	0·37	0·79	0	0	0	0
18−	1·58	—	—	—	0	—	—	—	0	—	—	—	0	—	—	—
19−	—	2·35	—	2·72	—	0	—	0	—	0	—	1·88	—	0	—	0
19−	—	2·97	—	2·33	—	0	—	0	—	0	—	0	—	0	—	0
21−	—	3·18	—	2·12	—	0	—	0	—	0	—	0·96	—	0	—	0
22−	—	1·96	—	1·98	—	0	—	0	—	0	—	0	—	0	—	0
0+	1·91	1·61	3·09	—												
1+	2·12	—	1·86	—												
2+	1·75	—	—	—												
3+	2·45	—	3·08	—												
4+	1·16	—	2·10	—												
10+	3·26	2·58	1·23	2·53												
15+	1·62	—	1·57	—												
20+	1·10	0·76	3·13	1·97												
29+	1·29	—	3·42	—												
40+	—	1·52	—	—												
60+	1·01	1·19	4·86	1·55												
100+	1·32	2·60	4·15	0·56												

periderm were recorded separately. The results of the observations on dividing cells are given in Table 3 and Text-fig. 18. The mitotic period is defined as extending from the earliest recognizable prophase to the latest recognizable telophase.

As might be expected the aggregate percentages of the various categories of dividing cells in the total population of nucleated epidermal cells is, on the whole, higher during embryonic life than after birth except in region 4 of the mouse. After the 15th day post-partum the percentage of dividing cells in the mouse is considerably higher in region 4 than on the trunk and also exceeds the percentage of dividing cells in any of the four skin regions of the rat. The

difference between the average mitotic indices of region 4 and region 1–3 of the mouse after the 15th day post-partum is statistically significant. The difference between the average mitotic indices of region 4 of the mouse and region 4 of the rat after the 15th day post-partum is statistically significant. The production of more epidermal cells might be expected on the lower surface of the foot, where greater friction is sustained by the skin surface (Thuringer, 1939) than on the trunk where the surface is protected by the hair coat; it is not clear, however, why more new cells are formed on the foot of the mouse than of the rat.

The difference between the average mitotic indices in the epidermis of the trunk of the mouse and the trunk of the rat after the 15th day post-partum is not statistically significant. For the adult animal, the surface area of which is



Text-fig. 18. The percentage numbers of four categories of dividing cells in the total population of nucleated epidermal cells. The ages are given in days after copulation or after birth. Thick broken lines: S. germinativum; thin broken lines: periderm; dotted lines: S. intermedium; thin continuous lines: S. spinosum.

almost constant, the duration of keratinization can be calculated from the mitotic index and the duration of mitosis (Glucksmann, 1945). Since in growing rats and mice the mitotic indices are not significantly different and the duration of mitosis is presumably the same, it might be assumed that keratinization proceeded at the same rate in both species. This would be true, provided the surface areas increased at the same rate, but in fact this is not so. During the first 100 days after birth the surface area of the rat increases more rapidly than that of the mouse (Lee, 1929; Grüneberg, 1943*b*). Some of the dividing cells in both species are used for lateral growth of the epidermis but more are used in the rat than in the mouse. It may therefore be concluded that keratinization takes longer in the rat than in the mouse. Glucksmann (1945) has shown that the time taken for keratinization in the adult mouse is approximately the same as the duration of the hair cycle (3–4 weeks). The hair cycle in the rat is longer, about 35 days (Butcher, 1934) and it is interesting that the duration of keratinization, also, is probably greater in the rat than in the mouse.

Cell division in the epidermis of the rat has been studied by Loeb & Haven (1929*a*), Carleton (1934, 1939) and Blumenfeld (1939) and in the epidermis of

the mouse by Picón (1933), Carleton (1934), Cooper & Franklin (1940), Blumenfeld (1943), Bullough (1943), and Cowdry & Thompson (1944). Picón has described a considerable decrease in mitotic frequency during the first month after birth, a slow rise to the 6th month and a slow fall to the 12th month after which the frequency remained constant. His results cannot be discussed in detail because the frequency has been expressed as the number of mitoses per unit surface area of epidermis and no allowance has been made for the decrease in epidermal height which occurs after birth. It is also unprofitable to discuss Carleton's results because she counted the dividing cells both in the epidermis and in the hair follicles, and the observations reported in the later paper were made after the injection of colchicine which arrests cell division at metaphase. Cooper & Franklin have investigated the diurnal mitotic rhythm in the epidermis of the ears of mice 15 days post-partum. The percentage of dividing nuclei in the total number of epidermal nuclei varied from 0 to 0·393. These values are considerably lower than the average (1·611) of my results for all four skin regions of a 15-day mouse; this disparity is presumably due to the difference in the sites that have been examined. Cowdry & Thompson found a mitotic frequency of 2·525 % in the epidermis of the hindfoot pads of mice 10 days after birth; in the region between the thenar and hypothenar pads, which is probably subjected to less friction than the pads themselves, I have found a frequency of 1·23 % in a mouse of the same age.

The determinations of mitotic frequency made by Blumenfeld (1939) on 28-day rats and by Blumenfeld (1943) and Bullough (1943) on adult mice cannot be discussed because they have been based on counts of dividing nuclei per unit volume (Blumenfeld) or unit area (Bullough) of epidermis; in addition Bullough injected colchicine before the tissues were fixed. Loeb & Haven (1929*a*) used rats of unstated age, presumably adult. The average percentages of dividing cells in the total epidermal cell population of the ears and chest were found to be 0·306 and 0·327 respectively. These values are lower than the average value (2·092) obtained for the four skin regions, trunk and foot, of a 100-day rat; the value for the chest epidermis is also lower than the value (2·13) for the anterior ventral region of this 100-day rat. Differences in age and site of epidermis may account for these divergences.

(3) *The height of the nucleated layers*

The height of the nucleated layers of the epidermis, i.e. the distance from the basement membrane to the outer limit of the S. granulosum has been measured at all stages of development; usually about ten measurements have been made at carefully selected positions in each specimen. The periderm has been excluded from the measurements. During part of embryonic development the outer layers of the epidermis are more folded than the inner layers, so that at the summits of the folds the epidermis is thicker than it is in the valleys of the folds (Pl. 1, fig. 3). In agreement with the observations of Fraser (1928) on the rat and Grüneberg (1943*a*) on the mouse these folds have been found to be most

conspicuous during the 19th day in the rat and the 17th day in the mouse. On the epidermis of these embryos equal numbers of height measurements at summits and valleys have been made and average values for epidermal thickness obtained. The epidermis of the three trunk regions of the youngest embryos is thinner medially than laterally; in these specimens the average height of the epidermis over the whole region has been found.

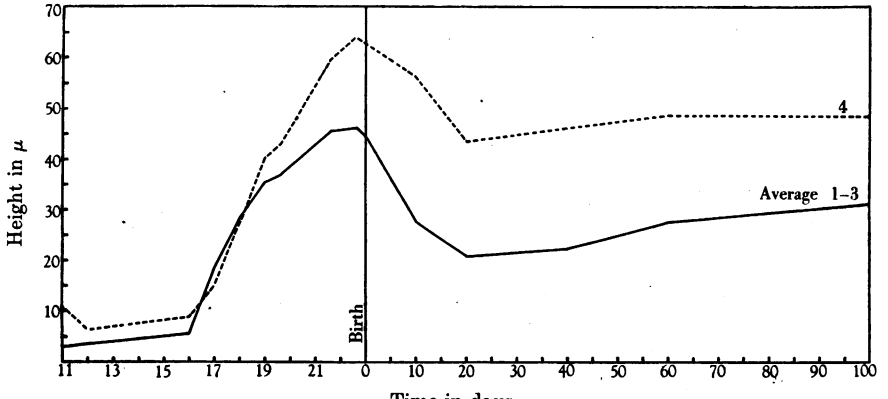
In Text-figs. 19 and 20 the height of the nucleated layers of the epidermis is plotted against the age of the specimen. The results for regions 1–3 of each animal are similar and have been averaged; the results for region 4 are given separately.

The changes in epidermal height are due almost entirely to changes in the number of strata and in the number of cell layers in each stratum (Table 2) but apparently are sometimes the result of changes in the height of individual cells.

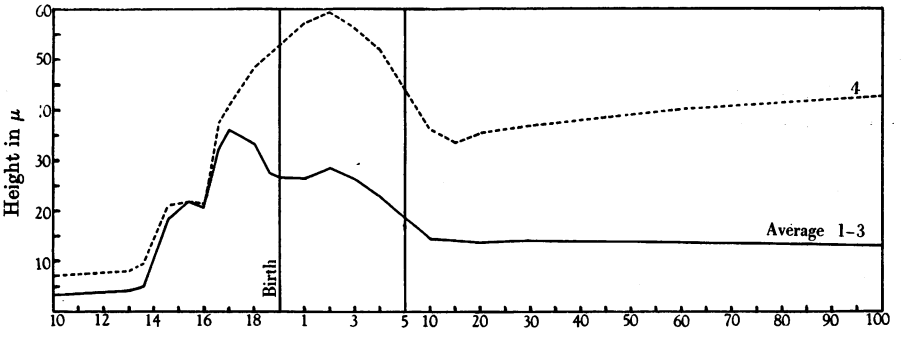
The decrease taking place in the height of the epidermis of the mouse just after birth is accompanied by a disorganization of the strata of nucleated cells indicating that the thinning of the epidermis may be due, at least in part, to lateral cell displacement during stretching; this suggestion has also been made by Gibbs (1941). She has found that from the 4th to the 13th days, correlated with the development of the tertiary and quaternary hair follicles, the epidermis on the anterior and posterior dorsal regions of the mouse 'decreased in actual thickness by a suppression of the cell layers of the strata granulosum and intermedium' (her 'stratum intermedium' corresponds to the S. spinosum of this paper). From the 13th to the 15th days the 'decrease in thickness of epidermis ceased, and a comparatively constant condition was maintained'. The graphs given by Gibbs for the anterior and posterior dorsal regions differ in two respects from Text-fig. 20: in the first place the epidermis was approximately 5μ thicker both before and after the decrease; and in the second place the main decrease did not begin until after the 4th day, whereas in Text-fig. 20 it is seen to begin after the 3rd day. Gibbs has found that the posterior dorsal epidermis is consistently thicker than the anterior dorsal epidermis between birth and the 15th day. A statistical treatment of my results and an examination of many more specimens would be necessary before reaching any conclusions about differences in epidermal height between the three trunk regions that have been studied; but, except in the 2-day animal, the epidermis from the posterior dorsal region of the mouse is apparently slightly thicker than that from the anterior dorsal region after birth.

The decrease in thickness of the post-natal epidermis of the mouse was noticed by David (1934) who found that it coincided with the completion of growth of the first pelage. The epidermis normally remained thin for the rest of life but could be made to regain its original thickness by various means: under the stimulus of grafting; after treatment with benzyl mercaptan; and after the application of collodion dressings. Hammet (1931) described the structure of the epidermis from the interscapular region of the adult mouse and its reaction to benzyl mercaptan but, as David pointed out, he did not realize

that the hyperplastic epidermis showed a close resemblance to the early postnatal epidermis. Picón (1933), who studied the frequency of mitosis in the epidermis of the mouse from birth to the age of 33 months, published a series of photographs of sections through the skin on the dorsum showing a striking decrease in epidermal thickness between the first and 7th days after birth, and a further decrease between the 2nd and 4th weeks. A decrease taking place



Text-fig. 19.



Text-fig. 20.

Text-figs. 19, 20. The height of the nucleated layers of the epidermis, excluding the periderm. Continuous lines: average of regions 1–3; broken lines: region 4. Fig. 19. Rat. 20. Mouse.

just after birth in the number of nucleated cells, both undifferentiated and differentiating, per unit surface area of interscapular mouse epidermis has been described by Glucksmann (1945). Fraser (1928) measured the height of each stratum of the dorsal epidermis during embryonic development in the rat; the aggregate heights of the S. germinativum (corresponding to the S. germinativum and S. spinosum of this paper) and the S. granulosum agree closely with the measurements that I have made.

DISCUSSION

(1) *Specific and regional differences*

Qualitative and quantitative studies on epidermal development have demonstrated differences between the mouse and the rat and between the lower surface of the foot (region 4) and the trunk, three parts of which have been examined (regions 1–3). These differences develop during the growth of the first pelage and reach their full expression at the time of its emergence on the surface. The epidermis on the trunk of the rat is then thicker than that of the mouse and all the strata are uninterrupted, whereas in the mouse the S. germinativum, S. spinosum and S. granulosum are incomplete. In both species the epidermis is thicker in region 4 than in regions 1–3 and in the mouse its strata are not disorganized.

These specific and regional differences can be correlated with the type of hair coat or with the absence of hair. It is probable that the closer and finer fur of the mouse gives a better protection to the skin surface than the coarser and less dense fur of the rat. On the lower surface of the foot hair follicles are absent and the friction sustained by the skin surface is greater than on the trunk. In other places where hair is absent in the mouse, e.g. around the anus and nipples and on the tail (Fekete, 1941) the epidermis is thick and stratified.

There are indications that these specific and regional differences in epidermal structure and thickness are related to differences in relative sensitivity to certain carcinogenic agents. It is well known that the epidermis of the mouse is much more sensitive than that of the rat (Glucksmann, 1945), and Twort & Twort (1936) have shown that the epidermis on the trunk of the mouse is more sensitive than the epidermis on the lower surface of the foot. Similarly the epidermis of genetically hairless rats has more cellular layers and a thicker S. corneum than that of their haired litter mates and is less susceptible to carcinogenesis induced by ultra-violet radiation (Hueper, 1941). The significance of the correlation between type of hair coat, epidermal structure and relative sensitivity to carcinogenic agents has been discussed by Glucksmann (1945) who concluded, however, that 'a specific sensitivity of the mouse epidermis to the growth-stimulating effect of the carcinogenic hydrocarbons must be assumed'.

Glucksmann (1945) has pointed out that there is no justification for the assertion, frequently encountered in the literature (David, 1934; Hammett, 1931), that the non-stratified epidermis of the adult mouse is 'undifferentiated' in comparison with the stratified 'differentiated' epidermis of the new-born mouse (David) or of the adult animal during wound repair or after treatment with chemical irritants (David; Hammett). He has shown that both undifferentiated (S. germinativum) cells and differentiating (S. spinosum and S. granulosum) cells can be identified in the thin nucleated part of the epidermis lying beneath the S. corneum on the trunk of the adult mouse; the observations

reported in this paper agree with his description. These observations indicate that cellular differentiation proceeds in a similar manner in both non-stratified and stratified epidermis. Further evidence is provided by the results of the analysis of the cell population (Text-figs. 14, 16), which show that the proportion of undifferentiated to differentiating cells is approximately the same in both species. Therefore, although stratification may be interpreted as an indication of *histological* differentiation, the modification or absence of stratification does not necessarily imply the modification or absence of *cellular* differentiation. Stratification is simply the result of the aggregation of cells into different layers according to the stages they have reached in differentiation.

(2) *Cell division and differentiation*

It has been found, with one exception, that in the rat and mouse the differentiating (keratinizing) cells of the epidermis do not divide and cell division is a function of the undifferentiated cells, viz. the cells of the S. germinativum and S. intermedium and those of the periderm before they begin to differentiate. There is one exception to this statement: for a short time after the formation of the S. spinosum during embryonic life its cells are able to divide. Division of spinous cells soon ceases on the three trunk regions but continues for a longer time on the lower surface of the foot. The cessation of division of spinous cells coincides with the time when the cytoplasm increases in amount and the intracellular fibrils become better developed. It may be suggested that the loss of viability is a result of the cytoplasmic changes involved in differentiation, such as the development of intracellular fibrils; or, as suggested by Dawson (1940), a single factor may both impair viability and initiate differentiation.

Although it has been widely accepted that cell division and cell differentiation are mutually exclusive (Lewis, 1939; Dawson, 1940) the division of cells located in the S. spinosum has been recorded several times. In the epidermis of the human scalp and prepuce Thuringer (1924, 1928) found not only that the cells of the S. spinosum were able to divide, but also that more than half of the total number of dividing cells in the whole epidermis were located in this stratum. In the epidermis of the pads on the feet of cats he recorded (1939) more dividing cells in the basal part of the S. spinosum than in either the basal layer of the epidermis or the more superficial parts of the S. spinosum. Cowdry & Thompson (1944) obtained similar results for the hind-foot pads of 10-day-old mice.

[They found that in normal animals the site of maximal mitotic frequency was the proximal third of the suprabasal part of the epidermis whereas after treatment with colchicine the site of maximal frequency moved to the middle third. They suggested that the level of maximal mitotic frequency 'is not fixed but subject to change in different physiological and pathological conditions'. In my opinion the results are to be interpreted as an expression of the normal upward migration of differentiating cells in the epidermis. Cells in which division has been arrested at metaphase by colchicine proceed to differentiate

and to migrate upwards through the epidermis thus increasing the apparent mitotic frequency of the more superficial parts of the epidermis.]

Cowdry, van Dyke & Geren (1946) have recently found that the site of maximal mitotic frequency is in the basal layer of the hyperplastic epidermis on the backs of young mice treated with methylcholanthrene. They attribute to the carcinogen the difference between this result and that of Cowdry & Thompson (1944).

As shown in the previous discussion cytological criteria are essential for judging the stage of differentiation reached by a cell, and the histological criterion of location in a particular stratum is of little significance without verification by cytological examination. Thuringer (1924, 1928, 1939) and Cowdry & Thompson (1944) and Cowdry *et al.* (1946) have identified cells by their position in the epidermis, a procedure which is apt to give misleading results when oblique sections, particularly in the neighbourhood of papillae or ridges, are examined. In such sections which, to judge from his photographs, were apparently used by Thuringer (1924), cells of the basal layers may appear to be situated more superficially. Moreover, Thuringer (1924) has recorded an average of 4 % of nuclei in the prophase of mitosis amongst all the dividing cells that he counted; but it is known from direct observations on dividing cells (Lewis, 1939) that prophase lasts for more than half the duration of mitosis. One must conclude that many of the dividing cells in early prophase were not recognized by Thuringer. In most epidermal cells, however, the early part of mitosis probably takes place while the cell is in the basal layer of the epidermis although some dividing cells may leave the basal layer before division is completed. The exclusion from his counts of these cells in prophase further invalidates his deduction that most of the dividing cells in the epidermis are spinous cells.

Other workers have found that nearly all the dividing cells in the epidermis of the mouse (Hammett, 1931; Picón, 1933) and of the rat and guinea pig (Loeb & Haven, 1929*a*, *b*) are situated in the basal layer. Two histological methods (Broders, 1920; Glucksmann, 1941) for judging the malignancy of human carcinomata are based largely on the belief, which has not been disproved, that a differentiating cell has lost the ability to divide and is no longer a dangerous source of new malignant cells.

GENERAL SUMMARY

1. A comparative account has been given of the histogenesis of the epidermis on the trunk and foot of albino mice and Norway rats aged 10 days post-coitum to 100 days post-partum.

2. Observations on cell division have led to the conclusion that in the epidermis of the mouse and rat differentiating cells do not normally divide.

3. Differences in epidermal development have been found between the rat and mouse and between the trunk and the lower surface of the foot. These differences can be correlated with the density of the hair coat or with its

absence. The type of epidermis which is relatively resistant to chemical carcinogenic agents (the epidermis of the rat and that of the mouse's foot) has been shown to differ from the type which is relatively sensitive (the epidermis of the trunk of the mouse).

This investigation was aided by emoluments from the Beilby Research Scholarship of Bedford College and the New Zealand Junior Research Scholarship of the British Federation of University Women, and by grants from the British Empire Cancer Campaign and the Sir Halley Stewart Trust. I am much indebted to Dr H. B. Fell for extending to me the facilities of the Strangeways Research Laboratory where the greater part of this investigation was made, and to Prof. H. Munro Fox, F.R.S., in whose Department at Bedford College it was completed. I wish to record my gratitude to Dr A. Glucksmann who suggested the work and who throughout its course gave his valued advice and encouragement.

REFERENCES

BLUMENFELD, C. M. (1939). Periodic mitotic activity in the epidermis of the albino rat. *Science*, **90**, 446–447.

BLUMENFELD, C. M. (1943). Studies of normal and abnormal mitotic activity. II. The rate and the periodicity of the mitotic activity of experimental epidermoid carcinoma in mice. *Arch. Path. Lab. Med.* **35**, 667–673.

BRODERS, A. C. (1920). Squamous-cell epithelioma of the lip. A study of five hundred and thirty-seven cases. *J. Amer. med. Ass.* **74**, 656–664.

BULLOUGH, H. F. (1943). Cyclical changes in the skin of the mouse during the oestrous cycle. *J. Endocrinol.* **3**, 280–287.

BUTCHER, E. O. (1934). The hair cycles in the albino rat. *Anat. Rec.* **61**, 5–20.

CARLETON, A. (1934). A rhythmical periodicity in the mitotic division of animal cells. *J. Anat., Lond.*, **68**, 251–263.

CARLETON, A. (1939). A note on the effect of colchicine on the skin of young rats. *J. Anat., Lond.* **73**, 416–418.

COOPER, Z. K. & FRANKLIN, H. C. (1940). Mitotic rhythm in the epidermis of the mouse. *Anat. Rec.* **78**, 1–8.

COWDRY, E. V. & THOMPSON, H. C. (1944). Localisation of maximum cell division in epidermis. *Anat. Rec.* **88**, 403–409.

COWDRY, E. V., van DYKE, J. H. & GEREN, B. B. (1946). Localisation of stratum of maximum mitotic frequency in epidermal methylcholanthrene carcinogenesis in mice. *Cancer Res.* 620–624.

DAVID, L. T. (1934). Studies on the expression of genetic hairlessness in the house mouse (*Mus musculus*). I. Skin folds. II. Precocious hair regeneration. III. Reactions to benzyl mercaptan. IV. Skin grafts and parabiosis. *J. exp. Zool.* **68**, 501–518.

DAWSON, A. B. (1940). Cell division in relation to differentiation. *Growth*. Supplement: Second symposium of development and growth, pp. 91–106.

DONALDSON, H. H. (1924). *The Rat. Data and Reference Tables for the Albino Rat* (Mus norvegicus albinus) *and the Norway Rat* (Mus norvegicus). *Mem. Wistar Inst.* no. 6, 2nd ed. Philadelphia.

ERICKSON, T. C. (1931). The post-natal development of the caudal integument in the rat. *Amer. J. Anat.* **47**, 173–194.

FEKETE, E. (1941). Histology. In *Biology of the Laboratory Mouse*, ed. G. D. Snell. Philadelphia.

FRASER, D. A. (1928). The development of the skin of the back of the albino rat until the eruption of the first hairs. *Anat. Rec.* **38**, 203-224.

GIBBS, H. F. (1941). A study of the post-natal development of the skin and hair of the mouse. *Anat. Rec.* **80**, 61–82

GLUCKSMANN, A. (1940). Development and differentiation of the tadpole eye. *Brit. J. Ophthal.* pp. 153–178.

GLUCKSMANN, A. (1941). Preliminary observations on the quantitative examination of human biopsy material taken from irradiated carcinomata. *Brit. J. Radiol.* (N.S.), **14**, 187–198.

GLUCKSMANN, A. (1945). The histogenesis of benzpyrene-induced epidermal tumours in the mouse. *Cancer Res.* **5**, 385–400.

GLUCKSMANN, A. & SPEAR, F. G. (1939). The effect of gamma radiation on cells *in vivo*. Pt. II. 1. Single exposures of the fasting tadpole at room temperature. 2. Single exposures of the normal animal at low temperature. *Brit. J. Radiol.* (N.S.), **12**, 486–498.

GRÜNEBERG, H. (1943*a*). The development of some external features in mouse embryos. *J. Hered.* **34**, 88–92.

GRÜNEBERG, H. (1943*b*). *The Genetics of the Mouse.* Cambridge: University Press.

HAMMETT, F. S. (1931). The proliferative reaction of the skin to sulfhydryl and its biologica significance. *Protoplasm*, **13**, 331–347.

HUEPER, W. C. (1941). Cutaneous neoplastic responses elicited by ultra-violet rays in hairless rats and their haired litter mates. *Cancer Res.* **1**, 402–406.

LEE, M. O. (1929). Determination of the surface area of the white rat with its application to the expression of metabolic results. *Amer. J. Physiol.* **89**, 24–33.

LEWIS, W. H. (1939). Some contributions of tissue culture to development and growth. *Growth.* Supplement: First symposium on development and growth. pp. 1–14.

LOEB, L. & HAVEN, F. L. (1929*a*). Quantitative studies on the growth of the epidermis. *Anat. Rec.* **42**, 217–241.

LOEB, L. & HAVEN, F. L. (1929*b*). The relation between functional states of the sex organs in the female guinea pig and the cell proliferation in the epidermis. *Anat. Rec.* **43**, 1–26.

MAURER, F. (1892). Haut-Sinnesorgane, Feder- und Haaranlagen, und deren gegenseitige Beziehungen, ein Beitrag zur Phylogenie der Saugethierhaare. *Morph. Jb.* **18**, 717–804.

NICHOLAS, J. S. (1932). Variability in rat embryo development. *Anat. Rec.* **52** (Supplement), 71.

OYAMA, R. (1904). Entwickelungsgeschichte des Deckhaares der weissen Maus (*Mus musculus*, varietas *alba*). *Arb. anat. Inst., Wiesbaden*, **23**, 585–608.

PICÓN, J. M. O. (1933). Über Zellteilungsfrequenz und Zellteilungsrhythmus in der Epidermis der Maus. (Ein Beitrag zur Kenntnis der Zellteilungsfaktoren.) *Z. Zellforsch.* **19**, 488–509.

PINKUS, F. (1910). The development of the integument. In *Manual of human embryology*, ed. F. Keibel and F. P. Mall, **1**. Philadelphia. Lippincott.

REED, S. C. & ALLEY, A. (1939). The production of pigment in grafts of mouse skin grown on the chorio-allantois of White Leghorn chicks. *Anat. Rec.* **73**, 257–268.

SPEAR, F. G. & GLUCKSMANN, A. (1938). The effect of gamma radiation on cells *in vivo*. Single exposures of the normal tadpole at room temperature. *Brit. J. Radiol.* (N.S.), **11**, 533–553.

SPEAR, F. G. & GLUCKSMANN, A. (1941). The effect of gamma radiation on cells *in vivo*. Pt III Spaced radiation. *Brit. J. Radiol.* (N.S.), **14**, 65–76.

STEINER, K. (1929). Über die Entwicklung und Differenzierungsweise der menschlichen Haut. 1. Über die frühembryonale Entwicklung der menschlichen Haut. *Z. Zellforsch.* **8**, 691–720.

STEINER, K. (1930). Über die Entwicklung und Differenzierungsweise der menschlichen Haut. II. Die embryonale Entwicklung der Hautgebiete mit frühzeitiger Mehrschichtigkeit des Epithels. *Z. ges. Anat.* 1. *Z. Anat. EntwGesch.* **93**, 750–764.

STEINER, K. & HITSCHMANN, O. (1927). Über die Entwicklung der Basalmembran des Hautepithels. I. Die Entwicklung der Basalmembran bei *Mus decumanus albus*. *Z. Zellforsch.* **5**, 150–173

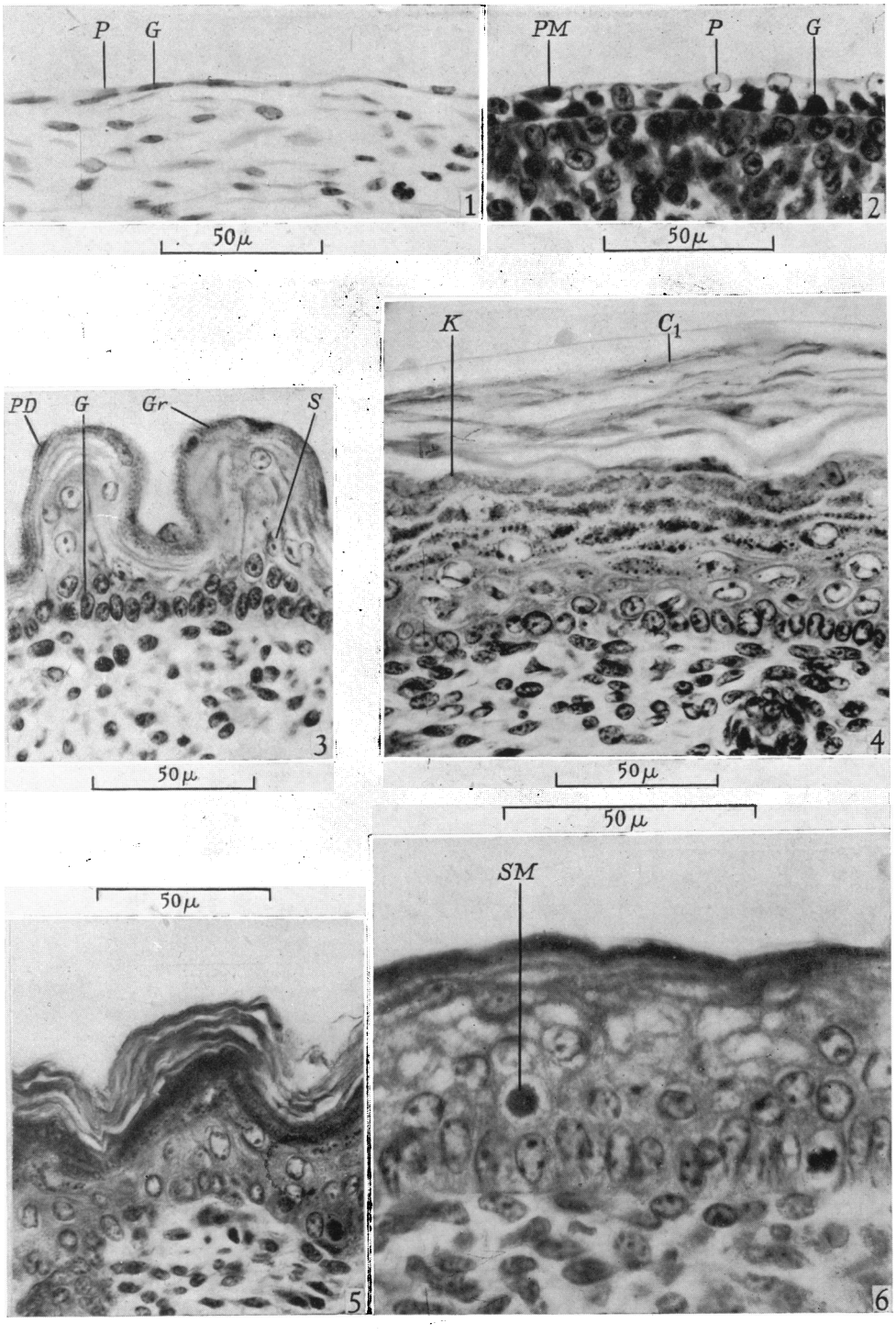
TANSLEY, K., SPEAR, F. G. & GLUCKSMANN, A. (1937). The effect of gamma rays on cell division in the developing rat retina. *Brit. J. Ophthal.* pp. 273–298.

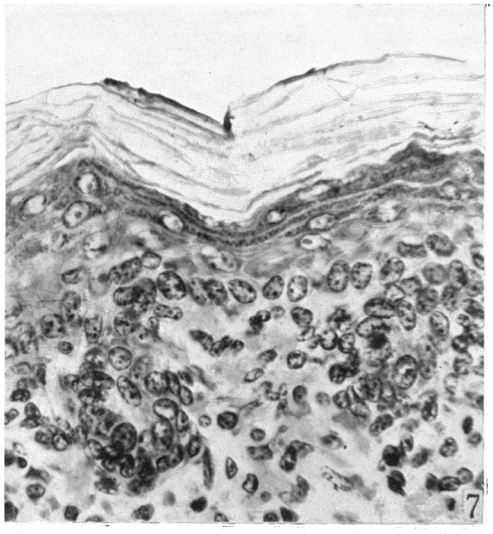
THURINGER, J. M. (1924). Regeneration of stratified squamous epithelium. *Anat. Rec.* **28**, 31–44.

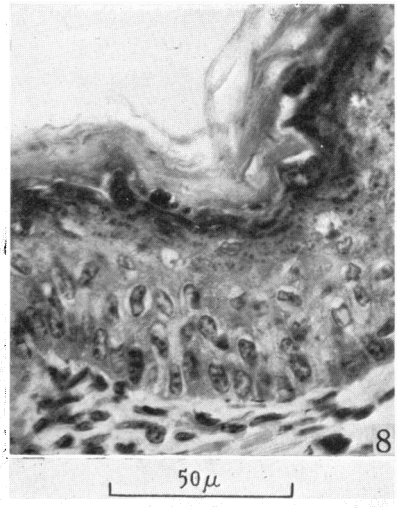
THURINGER, J. M. (1928). Studies on cell division in the human epidermis. II. A. Rate of cell division in the prepuce. B. Influence of various factors on cell division. *Anat. Rec.* **40**, 1–13.

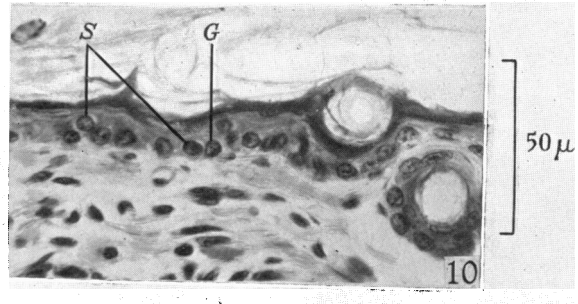
THURINGER, J. M. (1939). Mitotic index of palmar and plantar epidermis in response to stimulation. *J. Invest. Derm.* **2**, 313–326.

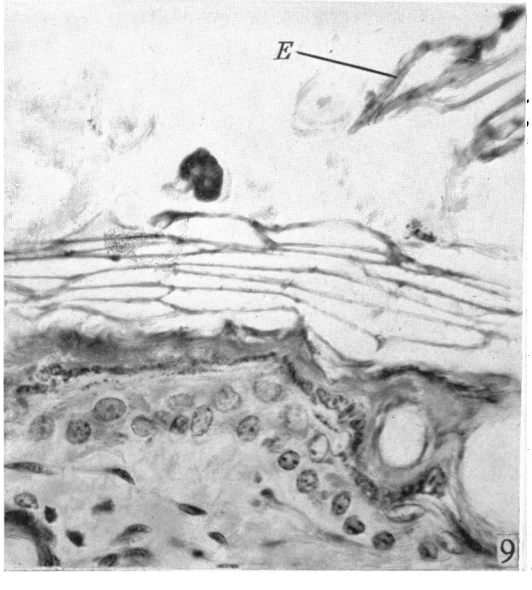
TWORT, J. M. & TWORT, C. C. (1936). The variable sensitivity of different sites of the skin of mice to carcinogenic agents. *J. Path. Bact.* **42**, 303–316.

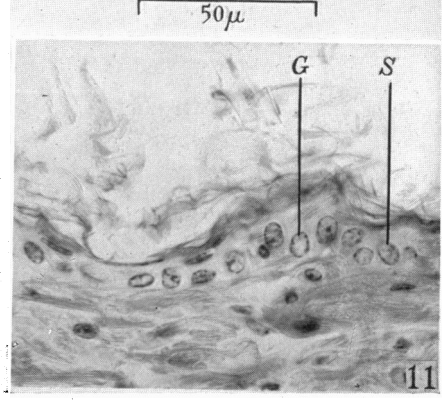

P
G
1
50μ
PM
P
G
2
50μ
K
C_1
4
50μ
PD
G
Gr
S
3
50μ
50μ
SM
5
6


7
50μ


8
50μ


S
G
10
50μ
50μ


E
9


50μ
G
S
11

EXPLANATION OF PLATES

C_1, first-formed sheet of keratin; *E*, epitrichium; *G*, non-dividing cell of S. germinativum; *GM*, dividing cell of S. germinativum; *Gr*, S. granulosum; *I*, non-dividing cell of S. intermedium; *K*, large keratohyalin granule partly converted into eleidin; *L*, S. lucidum; *P*, non-dividing cell of periderm; *PD*, degenerating nucleus of periderm; *PM*, dividing cell of periderm; *S*, non-dividing cell of S. spinosum; *SM*, dividing cell of S. spinosum.

Fig. 1. The epidermis on region 1 of a 13-day mouse embryo. 8μ. Modified Mallory.
Fig. 2. The epidermis on region 4 of a 12-day rat embryo. 10μ. Modified Mallory.
Fig. 3. The epidermis on region 3 of a 19-day rat embryo. 8μ. Ehrlich's haematoxylin.
Fig. 4. The epidermis on region 2 of a 21-day rat embryo. 8μ. Ehrlich's haematoxylin.
Fig. 5. The epidermis on region 1 of an 18-day mouse embryo. 8μ. Modified Mallory.
Fig. 6. The epidermis on region 4 of a 16-day mouse embryo. 8μ. Modified Mallory.
Fig. 7. The epidermis on region 2 of a mouse one day after birth. 8μ. Ehrlich's haematoxylin.
Fig. 8. The epidermis on region 4 of a mouse 4 days after birth. 8μ. Modified Mallory.
Fig. 9. The epidermis on region 2 of a rat 10 days after birth. 8μ. Ehrlich's haematoxylin.
Fig. 10. The epidermis on region 1 of a mouse 10 days after birth. 8μ. Ehrlich's haematoxylin.
Fig. 11. The epidermis on region 2 of a mouse 60 days after birth. 10μ. Ehrlich's haematoxylin.