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Skin glands in the axolotl: the creation and maintenance of a spacing pattern

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SUMMARY

The morphogenesis of skin glands in the larval axolotl is described at the light microscope level. The glands are derived from the epidermis, but are eventually located in the dermis. The glands are non-randomly arranged within the skin and the spacing pattern tends towards a hexagonal array in two dimensions. Analysis of the spacing pattern in animals of different sizes reveals that a clear relationship exists between gland size and the distance between glands. Occasionally, new small glands are inserted into the pattern, suggesting that the spacing of glands in under a dynamic control throughout the growth of the axolotl. The possible mechanisms underlying the creation and maintenance of the pattern are discussed.

INTRODUCTION

Anatomical specializations of the epidermis in vertebrates are commonly arranged in defined patterns. Considerable attention has been focused on the development of feathers, scales and hairs in terms of the epithelial mesenchymal control of their formation and the tissue interactions controlling their ordered, spatial patterning (see review by Sengel, 1976). Although no definite mechanisms of spatial arrangement have yet been established, it is clear, particularly with avian feathers and mammalian hairs, that the patterns of such epidermal specializations are characterized by a general class of mechanism termed a prepattern or spacing pattern. Such mechanisms are distinct from models based on a discontinuous variable within a single field in which non-equivalent values of some variable may result in differentiation of the same or similar types of cells (Wolpert, 1971; Lewis & Wolpert, 1976). Spacing patterns or prepatterns are thought to be characterized by a variable which produces the same basic anatomical unit at equivalent concentrations. The distribution of these equivalent points in the epidermis will result from interactions in the immediate environment of the structures concerned. Apart from the amniotes, the establishment of spacing patterns has been extensively studied in insects, where the regular appearance of cuticular hairs and bristles lend themselves to analysis, and in plants where the spacing of lateral outgrowths from the stem and stomata

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on leaves have been studied (Wigglesworth, 1940; Lawrence, 1970; Claxton, 1974, 1976; Held, 1979; Sachs, 1978).

Considering the extensive use of amphibians in developmental biology, it is surprising that the analysis of spacing patterns in these animals has been limited to the one-dimensional spacing of somites in urodele and anuran embryos (see Cooke, 1981). In this study, we use the axolotl larva to examine the morphogenesis and spatial arrangement of epidermally derived glands which are situated in the dermis of the skin. The morphogenesis of the glands is described at the light microscope level and a quantitative analysis of their spacing pattern in the lateral tail skin is presented. It is established that the glands are arranged in a highly ordered array, and that a clear relationship exists between the diameter of the glands and the distance to the next gland. An important feature of the establishment of the gland spacing pattern is the relationship between the growth of the gland and the growth of the skin as a whole. The possible mechanisms underlying gland spacing are discussed in the light of previous studies of prepatterns in amniotes and insects.

MATERIALS AND METHODS

1. General

All of the axolotls used in this study were spawned in the colony at King's College. White animals were used in all cases. They were kept in standing tap water in individual plastic containers and were fed twice a week with raw liver.

2. Whole-mount analysis of gland spacing patterns

In order to visualize the gland spacing pattern easily, large pieces of skin measuring approximately 5 mm^2 were removed from the lateral tail region. The cranial margin of the skin piece was dorsal to the hindlimb and the dorsal margin was parallel to the lower edge of the dorsal fin. The skin was removed by cutting one edge with a scalpel and then gently separating the dermis from the underlying musculature with a blunt glass probe. The loosened skin was then cut off with scissors. The epidermis was separated from the dermis with fine forceps after soaking the skin piece in 100 % Holtfreter's solution for about an hour at room temperature. The treatment time was longer for the thicker skin of larger animals. The dermis was then mounted on a slide in Holtfreter's solution, coverslipped and viewed with the outer side uppermost on a Zeiss SV8 stereomicroscope with dark-field transmitted light. The glands are clearly visible using this technique (see Fig. 1). Each gland was drawn using a camera lucida so that the characteristics of the pattern could be assessed.

As a control to assess the reliability of the technique, pieces of dermis and epidermis which had been separated after treatment in Holtfreter's solution were fixed, wax embedded and sectioned to establish that all of the glands had remained in the dermis.

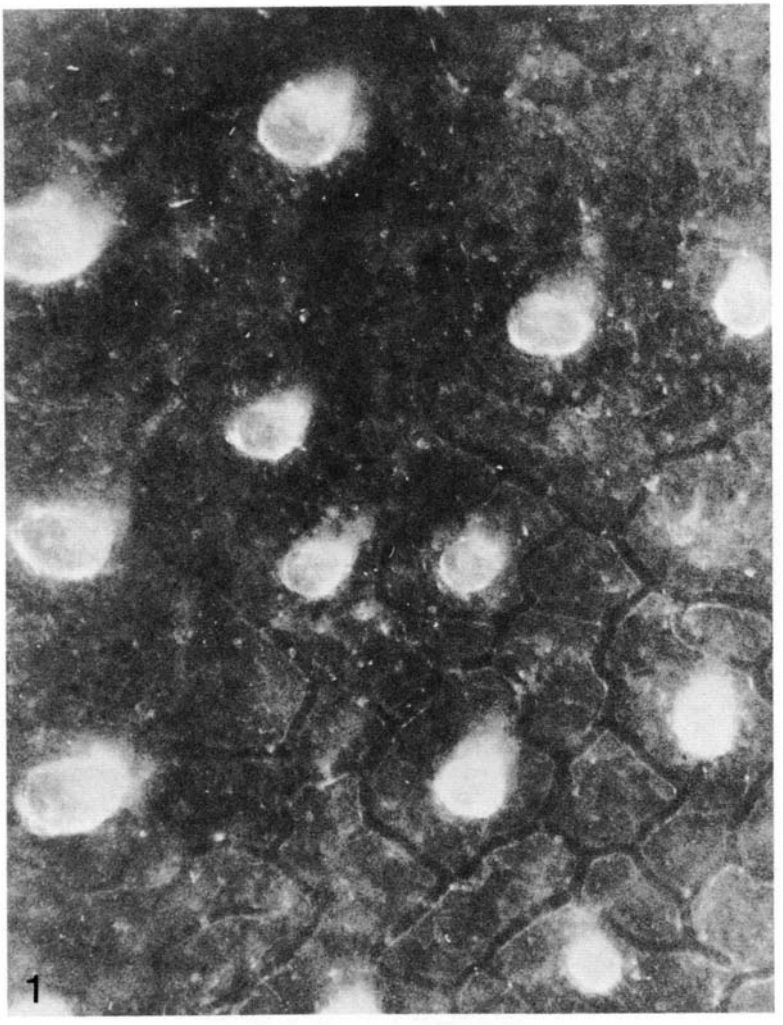

Fig. 1. Light micrograph of a region of glands in a piece of dermis from which the epidermis has been removed following treatment in Holtfreter's solution. The glands appear white in dark-field transmitted light. Blood vessels in the dermis can be seen forming rings around the glands. The preparation is unstained. Magnification ×32.

3. Histology

In order to examine the normal development and structure of the glands, whole skin pieces were removed from the lateral tail region of several animals of different sizes. These were fixed in Bouin's fluid, wax embedded and cut at 8 μm on a rotary microtome, and stained with haematoxylin and eosin or Mallory's trichrome.

4. Quantitative analysis of the spacing pattern

Several aspects of the gland spacing pattern were analysed. All measurements were taken from the camera-lucida drawings, (see results in Table 1).

1) Gland diameter was measured on a sample of glands from each animal. Between 24 and 67 gland diameters were measured for the individual animals. The mean gland diameter and frequency distribution of gland diameters were calculated for each animal.

2) Nearest neighbour analysis. The distance of each gland to its nearest neighbour was measured in a sample area of dermis for each animal. This measure, coupled with gland density (glands per mm^2), was used to establish the degree of spatial ordering of glands by the method described by Clarke & Evans (1954). This method estimates uniformity of a spatial pattern by an expression, R, which is equal to the mean of the distances of each element to its nearest neighbour divided by the reciprocal of twice the square root of the element density. R will equal 1 if the elements of the pattern are randomly distributed within a field and will approach 0 as the elements become increasingly aggregated or 2·1491 if the elements become increasingly ordered towards a perfect hexagonal array. This method has been used to describe spacing patterns in other systems, including hairs in sheep, bristles and hairs in insects, and stomata on leaves (see, for example, Claxton, 1964, 1974; Lawrence & Hayward, 1971; and Sachs, 1978).

3) As well as the nearest neighbour analysis, the distance separating glands were measured as if the pattern was a perfect hexagonal array. This series of measurements (see Fig. 2) is thought to estimate the real gland separations more realistically than a simple nearest neighbour analysis. On average this analysis produced about 200 measurements for each pattern.

Table 1.

Specimen	Animal length cm.	Mean gland diameter $\mu m \pm$ S.E.M.	Density of glands/mm^2	R (mean = 1·64)	Mean gland separations mm ± S.E.M. (brackets)
1	11·5	60 ± 1·7	8·8	1·75	0·37 ± 0·01
2	12·5	70 ± 2·5	6·21	1·6	0·43 ± 0·01
3	13	60 ± 3	6·68	1·57	0·42 ± 0·01
4	13·5	80 ± 2·4	4·81	1·68	0·46 ± 0·01
5	13·5	180 ± 5·5	3·05	1·60	0·61 ± 0·01
6	14·3	80 ± 2·5	3·21	1·66	0·57 ± 0·01
7	16·5	190 ± 10	2·41	1·62	0·64 ± 0·01
8	17·8	140 ± 10	3·20	1·74	0·58 ± 0·02
9	18	180 ± 8	3·46	1·69	0·53 ± 0·91
10	19·5	210 ± 10	1·77	1·78	0·83 ± 0·02
11	21·5	190 ± 12	2·61	1·73	0·64 ± 0·01
12	21·5	250 ± 10	1·25	1·36	0·87 ± 0·02
13	24	270 ± 10	1·56	1·57	0·82 ± 0·02

RESULTS AND DISCUSSION

1. *Morphogenesis of the glands*

Individual glands begin to form in the lower region of the epidermis close to the basement membrane. The glands first become visible as closely packed cells which form a spherical cluster (Fig. 3A). Gradually the future gland cells sink below the level of the epidermis as a unit (Fig. 3B). The basement membrane can be seen to split and form a diffuse covering around the upper part of the gland in sections stained with the Mallory technique (Fig. 3A). When glands first appear in young larvae, the dermis is very thin, consisting mainly of basement membrane and stratum compactum. As the larvae grow older, the dermis thickens considerably and its three layers become clearly visible. These are the thick basement membrane, an intermediate layer consisting of a diffuse network of extracellular fibres, scattered fibrocytes, and pigment cells, and finally a dense, continuous layer of thick extracellular fibres, the stratum compactum. Developing nests of gland cells are also seen in older larvae which have a thick dermal layer.

As the glands move deeper into the middle layer of the dermis, they acquire a basement membrane. They also appear to be anchored basally to the stratum

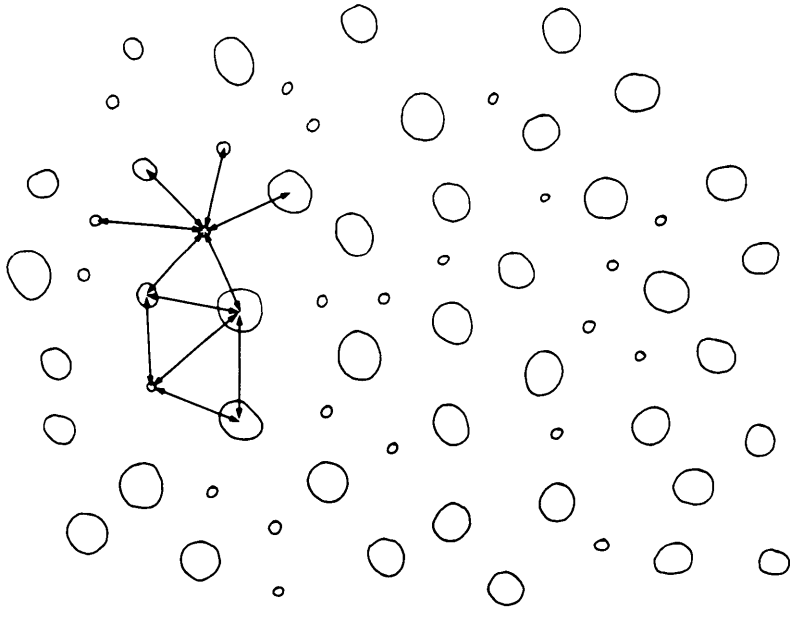

Fig. 2. Camera-lucida drawing of a field of glands examined in a separated dermis preparation. This specimen is case 11 in Table 1. The arrows represent the total gland separation measurements which were taken from every gland in the field. Also note the presence of small glands which appear in an ordered pattern with respect to the larger glands. The frequency of gland separation measurements for this specimen is shown in Fig. 7D. Magnification ×30.

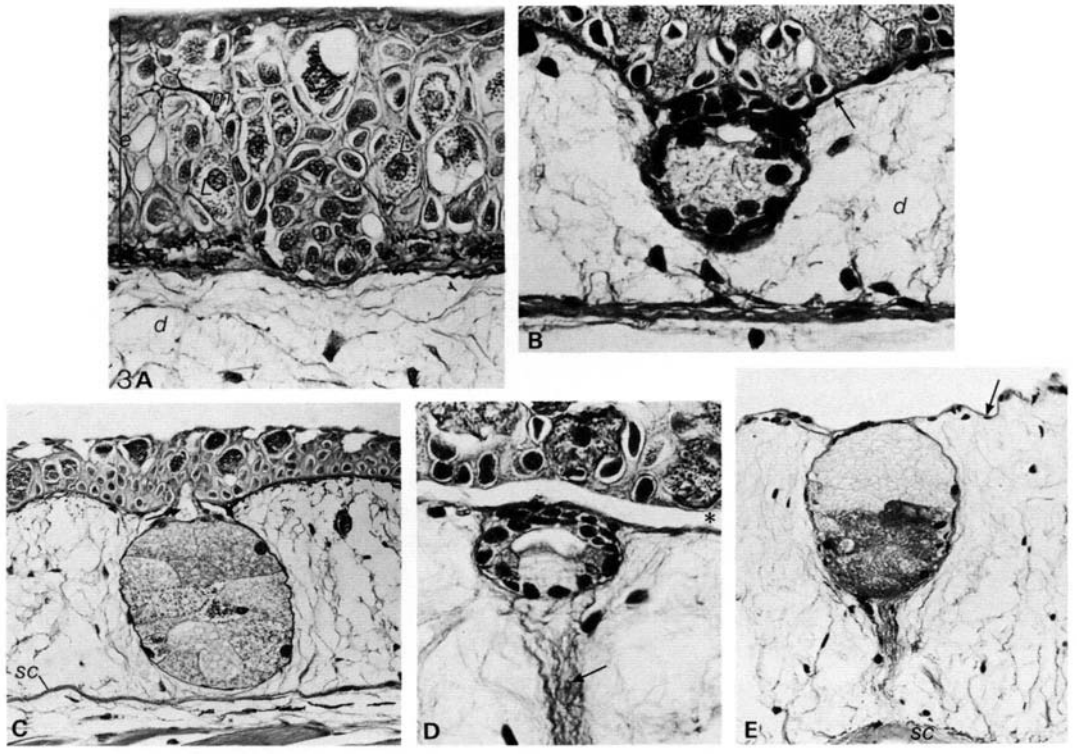

Fig. 3. Morphogenesis of the glands. (A) The earliest stage of gland formation that can clearly be recognized histologically. The gland appears as a nest of cells in the basal layers of the epidermis (e). No clear differentiation of gland cells has occured but the cells are clearly different from the epidermal Leydig cells (L). The gland cells are closely packed and the nest is deforming the basement membrane separating epidermis and dermis (d). The section is stained with Mallory's trichrome. Magnification: the nest is 74 μm in diameter. M, melanocytes. (B) Intermediate stage of gland formation, stained with Mallory's trichrome. The nest of cells has sunk into the dermis (d). The basement membrane (arrow) is split and begins to surround the perimeter of the gland. The membrane is also discontinuous at the apical region of the gland (asterisk). The gland cells are beginning to differentiate basally and the apical plug of cells is clearly visible. A small lumen is also visible near the apex of the gland. Magnification: The gland is 103 μm in diameter. m, melanocytes; sc, stratum compactum. (C) A mature gland stained with Mallory's trichrome. The gland cells are clearly differentiated and a rudimentary duct (arrow) is present. The basement membrane surrounds the gland and no muscle cells are present at the periphery. sc, stratum compactum. Magnification: The gland is 255 μm wide. (D) A small gland from a large animal (case 11, Table 1), stained with Mallory's trichrome. The immature gland is at the intermediate stage of development and has a small lumen. This specimen has been in Holtfreter's solution and shows a gap at the epidermal–dermal junction (asterisk) where separation occurs. Clear extracellular anchoring fibres can be seen (arrow) running from the base of the gland towards the stratum compactum. Magnification: The gland is 88 μm wide. (E) A mature gland in the dermis of a preparation from which the epidermis has been removed. Note the presence of the basement membrane at the epidermal–dermal junction (arrow), the normal morphology of the gland and the presence of extracellular anchoring fibres running from the base of the gland to the stratum compactum (sc). Magnification: The gland is 235 μm wide.

compactum by thick extracellular fibres which are continuous with it. The glands increase in size as the cells within the body of the gland begin to accumulate their secretory product (Fig. 3C). An apical region is distinguishable at the point of connection with the epidermis. Cells in this region appear to lag behind those in the lower part of the gland in the formation of a product and sometimes form a conspicuous cluster at the apex. In most cases, no duct is present, the gland terminating with a plug of apical cells. In a few cases, a rudimentary duct can be seen extending about a third of the way into the epidermis and ending blindly among the epidermal cells.

No evidence of muscle cells was found at the periphery of these glands, nor do they appear to be innervated (Stephens & Holder, unpublished observation). The release of the gland contents is therefore likely to be under passive control and may occur only when the skin is damaged by severe abrasion or by laceration by a predator. Such a mode of release is consistent with the absence of a duct through the epidermis.

The mature gland in axolotl larvae is composed of a spherical cluster of large deep cells and smaller apical cells. The function of the glands has not been fully ascertained as yet. The basal cells are quite hypertrophied and have vesicular and fine granular inclusions. Some of the apical cells also accumulate a granular product. The structure of these glands does not correspond in detail to glands described as mucous and poison in other urodeles and anurans (see for example, Dawson, 1920; Noble, 1954). A histochemical and electron microscopical study is currently in progress, attempting to describe the detailed morphogenesis and functioning of these glands. For the present, this histological description of their development is a sufficient background for a discussion and analysis of the pattern of spacing of the glands.

2. *Reliability of the dermis preparation technique*

In order to assess how clean the separation between epidermis and dermis was following treatment in Holtfreter's solution pieces of dermis and epidermis were fixed, wax embedded and sectioned. Several pieces of separated epidermis were examined and no glands were found. Glands at varying stages of morphogenesis were visible in separated pieces of dermis (see Fig. 3E). These included the youngest stages of glands which had begun to sink into the dermis. Sections of separated dermis and epidermis also revealed that the basement membrane remains predominantly on the dermis after treatment. The basement membrane is occasionally broken on the dermal surface and is not present over the apices of the glands.

The size of the glands present in the separated dermis was also examined in these sections in order to establish whether the smallest glands seen in whole mounts of dermis corresponded to the youngest stages of gland development. The smallest glands measured in sectioned dermis are at a range of 35 to 40 μm in width. This indicates that the smallest glands measured in whole mounts do

indeed correspond to the smallest glands visible in sectioned preparations. The smallest glands in skin from small animals and the small glands found occasionally in skin from longer animals were approximately 40 μm in diameter (see Fig. 7).

3. *General description of the spacing pattern*

The glands appear in the lateral tail region in animals of about 11–12 cm long. Before this stage in larval life the skin consists of a thin epidermis with just a few cellular layers and a dermis which has a clear stratum compactum closely opposed to the basement membrane beneath the epidermis. General examination of animals at all stages of growth after glands begin to be formed reveal their presence on the head, particularly on the dorsal surface, along the flank and on the lateral and fin regions of the tail, and on the limbs. In both the fore- and hindlimbs, glands are more numerous on the dorsal side than the ventral side, are almost completely absent in the wrist and ankle regions and occur sparsely on the digits. A more detailed analysis of the spacing and distribution of glands in limb skin is underway in order to establish whether the characteristics of the pattern are the same in different body regions.

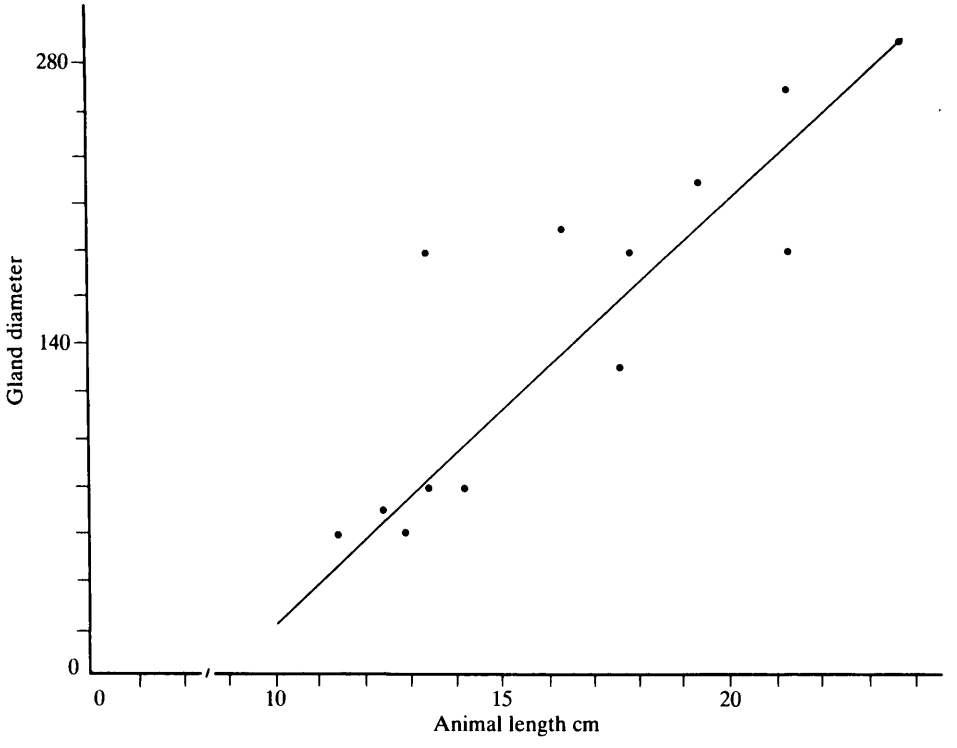

Fig. 4. Graph showing the relationship between animal length and gland diameter. The line is derived from a linear regression analysis where r = 0·95 (significant at 1 % level).

The initial appearance of the glands in different body regions appears to be roughly simultaneous. This suggests that the initial differentiation of the glands is triggered by some systemic factor. However, there is no evidence that following initiation, the local control of gland spacing is under systemic control.

4. *Quantitative analysis of the spacing patterns*

All of the data concerned with the analysis of the spacing pattern is presented in Table 1. Tail skins from 13 animals were examined. These animals ranged in length from 11·5 cm to 24 cm.

The essential features of the pattern will be assessed independently.

i) The degree of order of the spacing patterns was assessed using the R factor described above. The R values for individual cases ranged from 1·36 to 1·78 with a mean value of 1·64. It is clear at the outset of the analysis that the glands show a high degree of ordering tending towards that of a perfect hexagonal array. For reference, in other studies where this measure has been used, comparable values have been derived. Claxton (1963) obtained a mean R value of 1·67 for the pattern of developing primary skin follicles in sheep and values of about 1·4 (hairs on *Oncopeltus*: Lawrence & Hayward, 1971) and 1·37 (sternite bristles in *Drosophila*: Claxton, 1974) have been obtained for cuticular outgrowths in insects.

This method of assessment gives only an estimate of spatial order and reveals little about the mechanisms underlying the creation of the pattern (see discussion

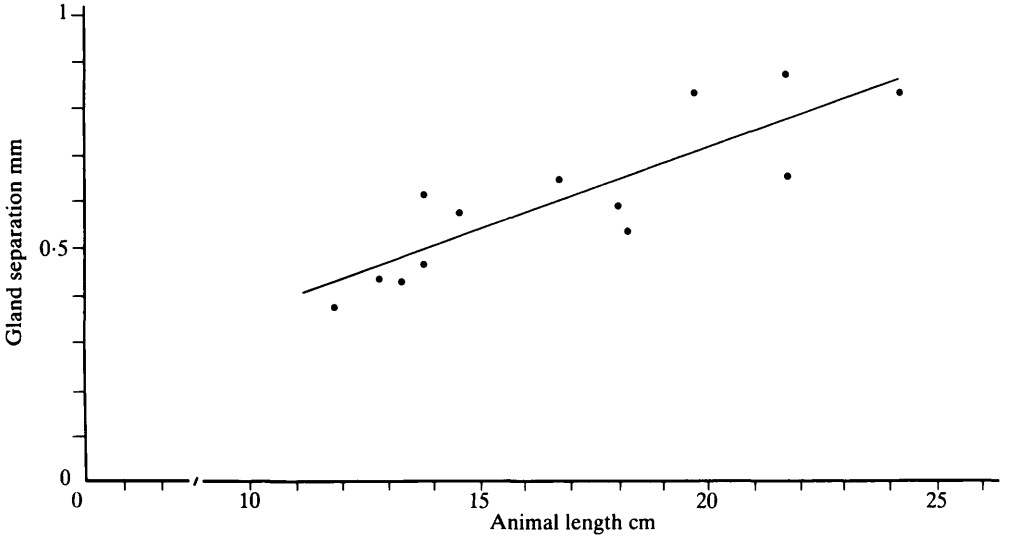

Fig. 5. Graph showing the relationship between animal length and gland separation. The line is derived from a linear regression analysis where r = 0·85 (significant at 1 % level).

by Claxton, 1964). In order to analyse further the possible mechanisms underlying the formation of the pattern, the other quantitative features must be discussed.

ii) The relationship between gland diameter, gland separation and animal size.

The animals studied ranged in size between 11·5 cm and 24 cm, thus the effects of overall growth must be considered as a central component of the analysis. From the data it can be demonstrated clearly that growth occurs both in the glands themselves and the skin in which the glands are situated. Clear linear relationships exist between increase in animal length and increase in gland diameter (Fig. 4 and Table 1) and between increase in animal length and increase in gland separation (Fig. 5 and Table 1). In addition, a clear linear relationship exists between the increase in gland diameter and the distance separating glands (Fig. 6 and Table 1). The last point will be returned to below when possible mechanisms controlling the gland spacing pattern are discussed. For the moment, however, it is used in conjunction with the relationships between these two parameters and animal length to establish that once the spacing pattern is created, gland diameter and gland separation remain in proportion as the

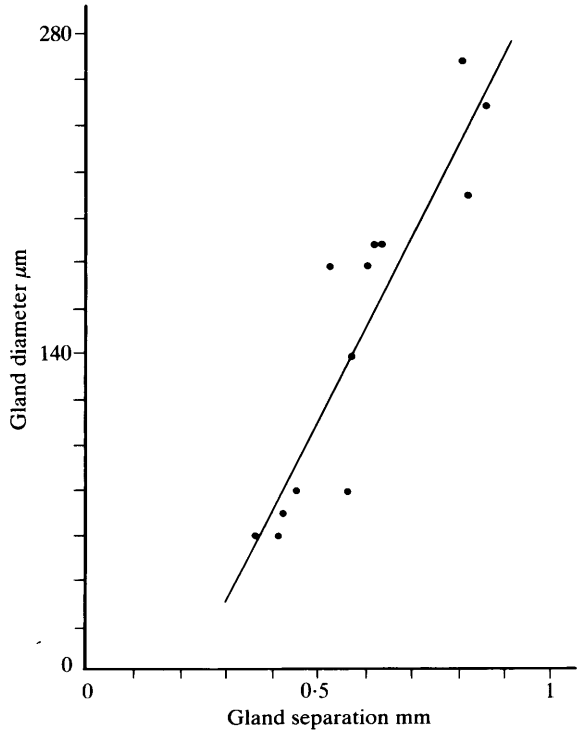

Fig. 6. Graph showing the relationship between mean gland diameter and mean gland separation for each individual case. The line is derived from a linear regression analysis where r = 0·9 (significant at 1 % level).

animals increase in size. That is, in general terms, the pattern in the smaller animals is a scaled down version of the pattern in the larger animals.

This straightforward relationship underlies the remainder of the discussion, but the picture is complicated by the detailed analysis of the overall behaviour of gland diameters in the range of animals studied. When the frequency of gland diameters in any one case is analysed, a complicating factor emerges. If gland diameter increases linearly with increase in animal length, a normal distribution of gland diameters would be expected with a gradual increase in the mean as the animals grow. Such a normal distribution is seen clearly in the small animals (see Fig. 7A) where the mean gland diameters in cases 1, 2, 3, 4 and 6 are all below 100 μm (range 60–80 μm). As the animals increase in size, so the mean gland diameters increase in size; but the frequency plots of gland diameters no longer show a range of normal distributions. In the remaining eight cases, four show a distribution which is bimodal in which the majority of gland diameters are greater than 100 μm, but a small number of glands have a diameter of less than 100 μm (see Fig. 7C); two cases have insufficient glands measured to produce a clear frequency distribution but in neither case is a gland less than 100 μm in diameter present; and in only a single case (12, see Table 1 and Fig. 7B) is a normal distribution found, the mean of which was 250 μm. In one animal (case 11, Table 1 and Fig. 7D) a bimodal distribution was seen with two clear peaks. In this case the mean of one peak was 70 μm and the mean of the other 260 μm. Thus in five cases a residual population of small glands with a diameter less than 100 μm are found (Fig. 7C). The percentage of glands which are less than 100 μm in diameter in each case are shown in Table 2.

Thus it is clear that the pattern of growth initially suggested, where the gland diameter and gland separations remain in proportion during growth is not completely correct. These features remain sufficiently in tune to show clearly this relationship but the correspondence is not absolute. Two possible reasons have been considered for the maintenance of a low number of small diameter glands in the larger animals. The first possibility is that some glands disgorge their contents during day-to-day life and the gland diameter consequently shrinks. There are two reasons for disregarding this possibility. Firstly, should this occur, it is highly unlikely that the glands which empty should be arranged in an ordered fashion within the skin. It is much more likely, especially if the glands are emptied passively as the result of contact or damage to the skin, that large patches of empty glands would be seen. Furthermore, that small glands in large animals form in a spacially ordered manner with respect to the large glands is clear if the whole pattern is examined. A region of the dermis from case 11, which has 40 % of its glands less than 100 μm in diameter and a clear bimodal gland size distribution (Fig. 7D and Table 2) is shown in Fig. 2. The ordered appearance in space of new glands in this particular pattern can be quantitatively demonstrated by re-examining the spatial parameters after removing all glands with a diameter of less than 100 μm from the drawing of the pattern. When this is done

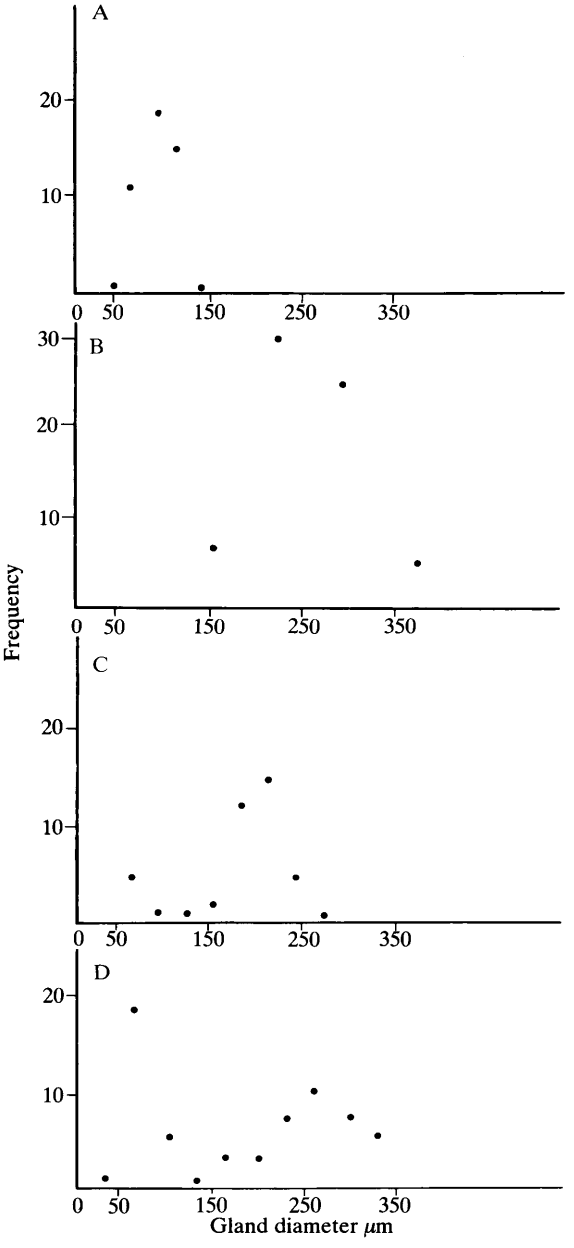

Fig. 7. The frequency of gland separation measurements for four individual cases. (A) Case 4, a 13·5 cm animal in which all of the glands are small. A unimodal normal distribution is seen with a mean gland diameter of 80 μm. (B) Case 12, a 21·5 cm animal in which a normal distribution is seen with a mean gland diameter of 250 μm. (C) Case 9, an 18 cm animal in which a residual population of small diameter glands exists. The mean gland diameter is 180 μm. (D) Case 11, a 21·5 cm animal in which a clear bimodal distribution exists. If the two peaks are separated at 150 μm, the smaller gland peak has a mean of 70 μm and the larger gland peak has a mean of 260 μm.

Table 2. *The relative number of glands with a diameter of less than 100 μm in each case*

Case	1	2	3	4	5	6	7	8	9	10	11	12	13
%	100	100	100	33	7	74	0	8	14	0	40	0	0

the gland separation mean becomes 0·83 mm with a mean gland diameter of 260 μm. Consequently, the small glands in the original pattern (those with a diameter of less than 100 μm) have a mean gland separation of 0·45 mm with a mean gland diameter of 70 μm. With reference to the relationship between gland diameter and gland separation, these two derived points fit accurately to the linear regression line (Fig. 6). Thus, the new glands in this particular pattern must be added in a spatially ordered manner.

Secondly, no discharged glands were seen histologically in larger animals. Indeed, upon histological analysis of skin from larger animals, morphologically immature glands are found which are identical in all respects to those found to make up the populations of glands in small animals (Fig. 3D). The more plausible suggestion for their appearance is that the relative growth rate of glands and the immediately surrounding skin is not always proportional. Should the skin component show an increased growth rate relative to the glands or, the glands show a decreased growth rate relative to the skin in a local region of the tail then new glands will be inserted into the pattern. This possibility will be explored further below when the likely spacing mechanisms are discussed.

A possible spacing mechanism

A number of different mechanisms have been put forward to account for regular spacing of equivalent structures. These vary from mechanisms based on measuring distance by counting cell number (see Held, 1979), to complex interactions between equivalent structures and their immediate environment involving diffusible products of either or both. Wigglesworth (1940) proposed that bristles on the abdomen of *Rhodnius* became evenly spaced following the breakdown of a substance produced continuously by the epidermal cells from which the bristles developed. Such a model suggests that an initially random pattern of bristles within a field will gradually become ordered as development proceeds (see Lawrence & Hayward, 1971). Such a system may be at work in the axolotl but this seems unlikely because the first patterns which are visible in the smallest animals with patterns are as ordered as the established patterns in larger animals. However, the technique used in this study may not allow the true starting conditions to be visualized if very small glands come away with the epidermis when it is stripped from the dermis after treatment with Holtfreter's solution. Similarly, the initial stages of gland morphogenesis may not be visible

in the dermis of small animals which may show an initially random gland pattern. We feel that the dermal preparations do allow visualization of the smallest stages of gland morphogenesis because glands as small as 40 μm in diameter are seen in the dermis of the smallest animals. These correspond to the size of the earliest stages of gland morphogenesis seen in sections when the cells are merely aggregates of epidermal cells lying in the epidermal–dermal junction (Fig. 3A). Thus, although we cannot rule out Wigglesworth's suggested mechanism, the non-random gland patterns in the small animals make it unlikely. It is also possible that local alterations of gland position relative to other glands brought about by cell movement or localized epidermal expansion due to growth may occur as a fine tuning mechanism which establishes the highly ordered array very soon after the initiation of gland differentiation. Such fine tuning events have been suggested in the formation of bristle patterns in *Drosophila* (Held & Bryant, 1983). Should a mechanism such as that described by Wigglesworth, with the epidermal cells acting as a source and the glands as a sink of some substance, be at work, then the picture will be further complicated by the fact that the epidermis of the larval axolotl becomes thicker with age, with more cell layers being added. A further complication is that the epidermis of the axolotl maybe continuously migrating over the surface of the animal. One advantage to the Wigglesworth competition model is that the random starting conditions deny the possibility of glands being in constant anatomical positions in different individuals (see Maynard-Smith & Sondhi, 1961). Although this facet of the problem has not been quantitatively examined it seems unlikely to be the case as so many glands are present in the lateral tail region. This feature of the pattern needs to be examined in more detail.

At this stage of the analysis, the more likely mechanism would involve the production of an inhibitor of gland formation by the glands themselves. A simple interpretation of the data in this study would be that a gland produces an inhibitory substance in an amount that is related to its own size. Thus, as the glands increase in size the distance to the next gland will increase in size as is observed (see Fig. 6). A similar relationship has been observed between bristle length and interval spacing in the third sternite of *Drosophila* (Claxton, 1974). The linear relationship between gland diameter and gland separation is not absolutely straightforward however, because small glands do appear in specific positions relative to the larger glands in some regions of skin in some of the larger animals (see Table 2 and Fig. 7C–D). If an increase in gland diameter and gland separation (skin growth) remained exactly in register throughout the growth of an animal no new glands should be added to the pattern. We suggest, therefore, that the occurrence of these small glands is due to localized increases in skin growth rate as compared to increase in gland diameter which causes the inhibitory influences of gland formation to fall sufficiently to allow new glands to form. It is difficult to explain why such local aberrations in epidermal growth rate may occur. However, this may be related to the fact that the axolotl is a constantly

growing animal and its rate of growth in the laboratory is very much dependent on the frequency of feeding and the size of the container in which the animal is maintained. These conditions may vary for any one animal during one or two years in the laboratory. Any variation in conditions that lead to gland growth lagging behind the general growth rate of the surrounding skin will result in inhibitory fields moving sufficiently far apart to allow the specification of new glands. Variations in animal growth rate leading to local alterations in skin growth rate would therefore influence the pattern but growth rate per se is not a component of the actual spatial control mechanism. The relationship between gland diameter and gland separation also allows the estimation of the diameter of an inhibitory field. When glands are 60 μm in diameter the inhibitory field is about 400 μm (see Fig. 6). When the gland diameter increases to 250 μm the inhibitory field is 850–900 μm.

Such a simple lateral inhibition model would suggest that gland spacing will rapidly become ordered after the systemic trigger for gland differentiation has initiated the pattern. The most popular type of mechanism would involve the reaction–diffusion kinetics of two substances with long-range inhibition and short-range activation (Turing, 1952; Geirer & Meinhardt, 1972). The specific features of such a gradient of lateral inhibition may be taylor made to fit the exact characteristics of gland spacing. One explanation for the insertion of new glands into a pattern which shows the general characteristic of proportional growth between glands and epidermis may be that an absolute threshold for new growth initiation does not exist. Rather, the likelihood for gland initiation is a probabalistic event with the possibility of gland initiation increasing as the lateral inhibition gradient falls with greater distance from the gland centres. The overall fit of such variations in a lateral inhibition model to the data presented here will be discussed elsewhere. Similarly, the predictions of such a model can be tested by examining the effects of established glands on the features of a new gland region forming in a regenerating region of tail skin. Such experiments are now underway.

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