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On the Spreading of the Presumptive Epidermis of Amphibian Embryo in Culture Medium

by

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Introduction

Spemann ('31) was the first to point out experimentally that in amphibian gastrulae spreading of the animal cap takes place by itself, neither mechanical pull nor any other influence of the marginal zone being recognized. Later Holtfreter ('33, '38, '39.) demonstrated that the presumptive ectoderm of the early gastrula showes marked spreading when it is isolated and adhered to glass plate or other inorganic substrata. Hoewver, in the normal course of development spreading of the ectoderm takes place in definite directions in different areas of an embryo. This is clearly shown by Schechtman ('32) who elucidates that the ventral epidermis shows marked stretching in the dorsal direction during neurulation, while the dorsal epidermis, after the closure of the medullary plate, stretches in the antero-posterior direction. In view of these facts, it can naturally be asked that where is involved the factor which determines the direction of spreading of ectoderm. Does it exist in the epidermis itself or comes from outside of the epidermis? To answer the question the following experiments were undertaken. The experiments were carried out by explanting pieces of the presumptive ectoderm on a glass plate or needle. As materials gastrulae and neurulae of *Triturus pyrrhogaster* were employed.

Before going further, I wish to express my sincere thanks to Prof. M. Ichikawa for his kind suggestions and encouragements throughout the course of this study. I am also deeply indebted to Prof. H. Takaya of the Konan University for his valuable criticisms.

Experiment I. Spreading of the presumptive epidermis of the early gastrula on glass plate

A square piece of the presumptive epidermis cut out from early gastrula was attached to a small piece of glass plate. Adherance of the ectoderm to the glass plate was quick and complete when it was pressed dy another piece of glass plate (Fig. 1). Cultivation of these pieces was done for about a week. During cultiva-

Table 1, Showing the extension of isolated pieces of the presumptive epidermis of early gastrula on the glass plate in area. "w_n" in mm^2 represents the area of the explants n days after the operation.

w_n / specimens	w_0	w_2	w_4
G1	1.7	1.8	—
G2	1.9	1.9	2.5
G3	1.7	2.3	2.4
G4	2.1	—	2.4

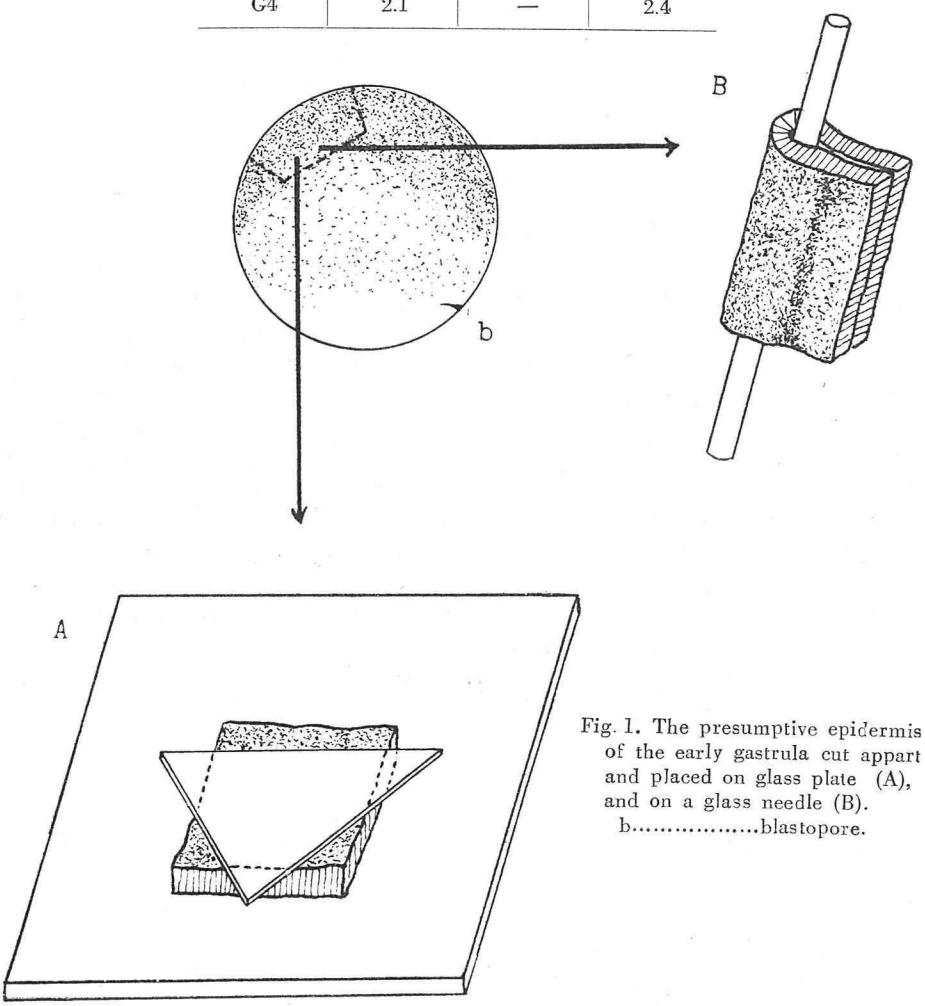

Fig. 1. The presumptive epidermis of the early gastrula cut appart and placed on glass plate (A), and on a glass needle (B).
b.....blastopore.

tion the explanted ectoderm frequently detached from the glass plate. In such case the tissue curled up immediately and produced many minute folds on its surface. It became globular in the following 2–4 days and ultimately formed a solid mass. In 4 specimens in which the ectoderm firmly adhered to the glass plate marked spreading of the tissue always occurred. In the course of spreading, there was found no minute wrincles on its surface, and it became a tissue differentiated into a thin membrane with circular contour (Fig. 2). As shown in Table 1, the spreading of the ectoderm was steady and continuous for more than 3 days. In 5 or 6 days of cultivation, disaggregation of the explanted ectoderm always followed. From the fact that the square piece of the explanted ectoderm always transformed into a circular membrane, it can be stated that the spreading was unifrom in all directions, no apparent tendency being found to spread in particular directions.

Experiment II. Spreading of the presumptive epidermis of the early gastrula on glass needle

In this operation a fine glass needle instead of a plate was used as a substratum. The needle was folded up, as is shown in Fig. 1, by a square piece of the presumptive epidermis cut out from the early gastrula. The needle used was about 0.2 mm. in diameter and 20 mm. in length. Soon after the operation the ectoderm showed marked stretching along the glass needle. In about a week after the operation a long and slender column of the tissue was produced (Fig. 2). Thus, the tissue attached to a fine needle invariably showed marked stretching only in the direction pallarel to the needle, not to other directions. The longitudinal stretching of the tissue along the needle is shown in Table 2, by measuring the daily increase of the length of the explanted tissue. Similary as in the case of spreading on the glass plate the longitudinal stretching of the presumptive

Table 2, Showing the stretching of isolated pieces of the presumptive epidermis of early gastrulae on the glass needle.
"l_n" is the length of the explants in mm. along the needle n days after the operation.

l_n / specimens	l_0	l_1	l_2	l_4	l_5	l_6	l_9
E 1	1.7	1.9	1.9	—	2.4	2.6	2.7
E 2	2.0	—	1.9	—	2.2	2.9	2.9
E 3	1.5	1.7	1.7	—	—	—	2.9
E 4	1.5	1.5	1.7	—	—	—	1.8
E 5	1.2	1.3	1.3	—	1.5	—	—
E 6	1.1	1.1	1.3	—	1.4	—	—
E 7	1.5	—	1.9	2.4	—	2.0	—
E 8	1.3	—	1.9	2.7	—	2.7	—
E 9	1.0	—	—	2.0	—	2.1	2.1
E10	1.1	—	—	1.9	—	1.9	2.2

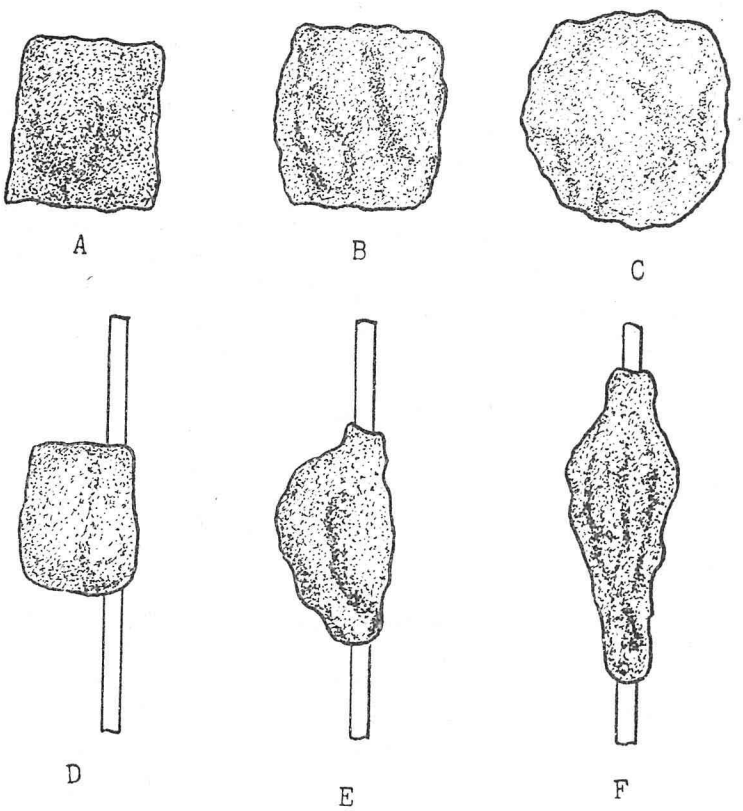

Fig. 2. above.....Spreading of the explanted presumptive epidermis of the early gastrula on glass plate (G 3 in Table 1).
A.....immediately after the operation.
B.....2 days after the operation.
C.....4 days after the operation.
below.....Stretching of the explanted presumptive epidermis of the early gastrula on the glass needle (E 8 in Table 2).
D.....immediately after the operation.
E.....2 days after the operation.
F.....6 days after the operation.

epidermis was also steady and continuous. However, the stretching always resulted in a production of a slender column of tissue in contrast to a thin membrane in the previous case.

Experiment III. Spreading of the ventral epidermis of the early neurula on glass needle

A square piece of the ventral epidermis was taken from the neurula in which the medullary folds were clearly demarcated. Soon after the isolation of the ectoderm the glass needle was attached to it in the same direction with respect to the original antero-posterior direction of the ectoderm (Fig. 3). These explants always showed marked stretching along the needle. In 2 specimens marked stretching occurred soon after the operation and continued for 7 days. In other 6 specimens stretching became marked in 1 or 2 days after the cultivation, and continued for 5 or 6 days as is represented in Table 3. As a result of the stretching, all the specimens formed a long column on the needle (Fig. 3). As compared with the case of the gastrula ectoderm, stretching of the ventral epidermis was greater, and formed more slender and longer column of tissue even within the same period of cultivation.

Stretching of the ventral epidermis occurred also along the needle, when the glass needle was placed in the dorso-ventral direction of the ectoderm. In most of the present cases, as found in the preceding explants, stretching of the tissue

Table 3. Showing the length in mm. of the isolated pieces of the ventral epidermis in which the original antero-posterior axis is kept.
"l_n" is the length along the glass needle n days after the operation.

l_n / specimens	l_0	l_1	l_2	l_3	l_4	l_5	l_6	l_7
A1	1.1	0.7	0.8	1.3	—	2.3	—	2.6
A2	1.3	1.5	2.1	3.3	—	3.4	—	—
A3	1.9	1.4	1.8	2.7	—	3.3	—	—
A4	1.4	1.3	1.3	—	1.6	—	1.7	—
A5	1.3	1.3	1.4	—	1.7	—	1.9	—
A6	1.2	1.1	1.1	—	1.3	—	1.3	—
A7	0.7	1.0	—	—	1.9	—	—	2.0
A8	0.9	1.0	—	—	1.1	—	—	1.3

Teble 4. Showing the length in mm. of the isolatd pieces of the ventral epidermis in which the original dorso-ventral axis is kept.
"l_n" is the length along the glass needle n days after the operation.

l_n / specimens	l_0	l_1	l_2	l_3	l_4	l_5	l_6	l_7
L1	1.7	1.3	0.6	1.4	—	3.2	—	3.9
L2	2.1	1.7	1.7	1.3	—	1.1	—	—
L3	2.0	1.9	1.3	1.5	—	2.3	—	2.3
L4	1.7	1.7	1.5	—	1.6	—	1.9	—
L5	1.4	1.7	2.0	—	3.1	—	3.9	—
L6	0.9	0.9	—	—	1.5	—	—	2.0
L7	0.9	0.9	—	—	1.1	—	—	2.2
L8	1.0	1.0	—	—	1.0	—	—	1.7

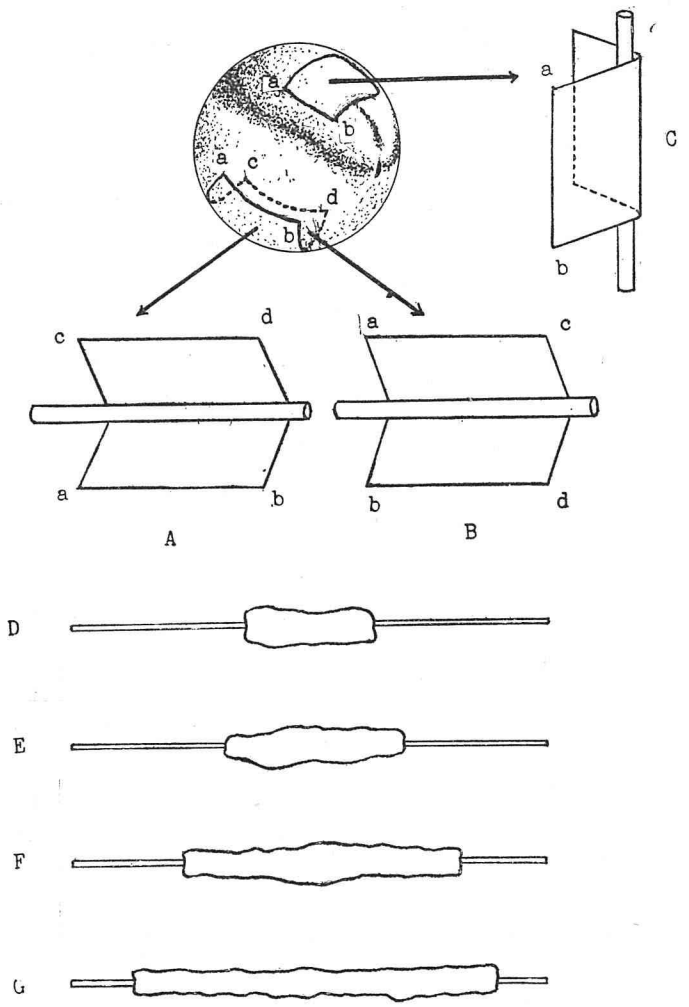

Fig. 3 above.....The ventral epidermis (A and B) and the medullary plate (C) of early neurulae cut appart and attached to glass needles.

A.....Glass needle is applied in the same direction with the original antero-posterior direction of the ectoderm.

B.....Glass needle is placed in the same direction with the original dorso-ventral direction of the ectoderm.

C.....Glass needle is placed in the same direction with the antero-posterior axis of the medullary plate.

below.....Stretching of the explanted piece of the ventral epidermis on the glass needle. (L 5 in Table 4)

D.....immediately after the operation

E.....2 days after the operation.

F.....4 days after the operation.

G.....6 days after the operation.

became marked in 1 or 2 days after the operation and continued for about a week, as is shown in Table 4. As a consequence, a long and slender column of epidermal tissue was formed along the needle in this case, too.

From the above results of experiments it can be stated that the isolated piece of the ventral epidermis of the early neurula always has a marked stretching tendency on the glass needle, and that the direction of the stretching is always along the needle, regardless of the original polarity of the explanted epidermis.

Experiment V. Spreading of the isolated medullary plate on glass needle

From neurulae of the same stage as used in the preceding experiment, a rectangular piece of the medullary plate was cut out. The piece to be explanted was taken out from a middle third of the plate (Fig 3). The piece of the medullary plate was carefully removed from its mesodermal components and attached to the glass needle. The needle was always placed in the same direction with the antero-posterior axis of the isolated piece. As a rule, the explanted tissue did not show marked stretching in any direction during the whole course of cultivation for about a week, except for one in which stretching of the tissue was found along the needle. Even in this case stretching was encountered only in a small restricted portion, and the remaining portion formed a solid globular mass. The portion stretched produced a slender protrusion that quite resembled, in the external appearance, to the column-like structure formed by the ventral epidermis attached to the glass needle. It was noteworthy that the ciliary beating was clearly shown in this portion of the tissue. Since previous workers (Woerdemann '25, Twitty '28, and Holtfreter '31.) do not recognize the ciliary beating in the developing neural tissue, it is questionable whether the stretching portion of this explant was derived actually from the neural tissue.

In the remaining explants of the experiments 3 cases failed to attach to the needle. Such tissue did not show stretching in any direction, and always formed a solid globular mass, 4 specimens attached to the needle successfully, but showed no marked stretching along the needle (Table 5). They resulted in the production of spherical masses owing to slight shrinkage of the tissue during cultivation.

Table 5 Showing the length in mm. of the isolated pieces of the medullary plate in which the original antero-posterior axis is kept. "l_n" is the length along the glass needle n days after the operation.

l_n / specimens	l_0	l_1	l_2	l_4	l_6	l_7
M1	1.3	1.3	—	1.1	—	1.1
M2	1 5	1.1	—	0.9	—	0.9
M3	1.1	—	1.1	—	1.1	—
M4	1.5	—	1.5	—	1.2	—
M5	1.5	—	2.1	—	—	1.9

Discussion

In the present experiments it has been shown that the marked stretching is always a rule in the presumptive epidermis of the gastrula, even when they are cut appart and placed on glass plate. These stretched pieces always result in the production of thin membranous tissues of epithelial appearance. Therefore, it is apparent that the presumptive epidermis has a marked tendency to spread by itself. However, this tendency becomes manifest only when the tissue is placed on an appropriate substratum. In the absence of substratum, the tissue always proudces a solid mass without forming membranous epithelial tissue. This result clearly indicate the importance of the substratum for the developing epidermal tissue to undergo marked stretching.

Similar stretching is also shown in the ventral epidermis of the neurula when it is attcahed to the glass needle. As is demonstrated by Schechtman ('32) by means of vital staining, the ventral epidermis of the neurula normally undergoes, in its intact position, the most pronounced stretching in the linear direction, i.e. in the dorso-ventral direction. Stretching in the other direction is scarcely found. In connection with this fact, our experiments further disclose that this linear stretching of the tissue is not necessarily concerned to the intrinsic polarity of the tissue. Because, the tissue stretches always in the direction along the needle used as substratum, without apparent connection with the polarity of the tissue. Therefore, it can be stated that it is not the internal polarity of the epidermis, but external influences that determine the direction of stretching of the tissue. The fact that the same tissue of gastrula epidermis behaves quite differently according as it is placed on a glass plate or on a needle, provides another evidence which demonstrates influences of the substratum upon the direction of stretching of the epidermis.

In view of the facts, evidenced by the experiments, that the substratum is indispensable for the stretching of the presumpiive epidermis, the mesodermal substratum in intact embryo may be considered to have most important rôle.

Differing from the presumtive epidermis, a piece of the medullary plate ectoderm does not undergo stretching even when it is placed on the glass needle. According to Nakamura ('42) and others, the posterior half of the medullary plate shows marked stretching in the antero-posterior direction. Nevertheless, a piece of the ectoderm derived from this area of the plate does not show any stretching on the glass substratum. Therefore, as far as the stretching of the tissue is concerned, the medullary plate ectoderm seems to differ greatly from the presumptive epidermis. It is problematical whether the difference merely comes from the intrinsic properties of the two different tissues or not.

Summary

1) The presumptive epidermis cut out from the gastrula and the neurula shows marked stretching when placed on the glass plate or needle.
2) It has an inherent tendency of stretching. This tendency becomes manifest only when the tissue is placed on an appropriate substratum.
3) The direction of the stretching is not inherently determined in the tissue. It seems to be markedly influenced by substratum.
4) The tissue in the medullary plate does not stretch when cut appart and placed on a glass needle.

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