in Figure 5. This map is so dominated by the dense substructure that the important complement structure is obscured, and, furthermore, it is doubled because the structure provides two equal Ca-Ca rotation vectors per cell. The partial Patterson, $\partial P(yz)$, of the substructure alone is shown in Figure 6. Its solution by a minimum function $\partial M_2(yz)$ of the partial Patterson is shown in Figure 7. The dots show the atom locations found by Fourier synthesis (Fig. 2).

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*A NEW THEORY OF LIMB INDUCTION**

BY B. I. BALINSKY

OSBORN ZOOLOGICAL LABORATORY, YALE UNIVERSITY, NEW HAVEN, CONNECTICUT, AND DEPARTMENT OF ZOOLOGY, UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG, SOUTH AFRICA

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In 1925 I reported that transplantation of an ear vesicle into the flank of amphibian (*Triton*) embryos may lead to the induction of a supernumerary limb.[1] The experiments were repeated and corroborated by several investigators, but very little was done to find out in what way the grafted inductor (ear vesicle or nose rudiment) causes the formation of a supernumerary limb bud. The nearest approach to this problem was made in my own experiments,[2] in which I found that the induction is accompanied by an increase in the mitotic index in the mesodermal cells participating in limb-bud formation. I interpreted this to mean that a higher level of metabolic activity brought about by the influence of the inductor allows the cells of the body wall to go through a more complex morphogenetic process (limb development), which they are not able to perform while their over-all physiological activity is low, as is normally the case in the flank area.

A method is now available to estimate the metabolic turnover of cells directly; this is the radioactive tracer method. Accordingly, I have performed experiments to test the incorporation of tagged protein and nucleic acid precursors into cells undergoing limb induction. In connection with the views proposed by Brachet,[3] I have also checked the comportment of nucleic acids in limb induction. The standard operation for limb induction—transplantation of the olfactory rudiment into the flank—was performed on *Amblystoma punctatum* embryos in the late tail-bud stage (Harrison's stage 34). At the time when the induced limb buds appeared (in stages 42–44), either the embryos were preserved for staining in sections with Azur B, or solutions of C^{14}-phenylalanine or C^{14}-adenine were injected directly into the gut. The injected larvae were preserved after 3, 6, or 24 hours, sectioned, and radioautographs were prepared, using the Ilford G-5 Nuclear Track Emulsion.

Pretreatment of sections with RNase or DNase was used to differentiate between the uptake of the C^{14}-adenine into the DNA and RNA, respectively.

The results of these experiments are as follows. The concentration of cytoplasmic RNA in mesodermal cells of the induced limb rudiments was found to be about double that in the nonlimb mesoderm cells of the body wall. The uptake of C^{14}-phenylalanine into the cells of an induced limb rudiment is not appreciably different from the uptake into mesenchyme cells of the body wall. The uptake of C^{14}-adenine into the RNA of induced limb-bud cells is about double, and the uptake of the same substance into the DNA of corresponding cells is about three times as high as the uptake into the mesenchyme cells of the body wall. As a certain increase in uptake of both protein and nucleic acid precursors would be expected, since the limb bud is a growing structure, I find that the information obtained does not justify the conclusion that the appearance of a qualitatively new formation (the supernumerary limb) can be satisfactorily accounted for as the result of a local stimulation of metabolic turnover.

Having thus failed to justify my previous expectations, I have attempted to investigate whether there are any peculiarities in the structural relationships between cells involved in limb induction (the cells of the nose rudiment, the mesenchyme, and the epidermis) which could account for their behavior. For this purpose I prepared electron micrographs of parts of the flank of operated larvae. A study of the micrographs at once showed me that there is a very marked difference in the relation to the epidermis in mesenchyme cells of the body wall and in the mesenchyme cells of induced limb rudiments. In the normal skin, at the time when the induced limb buds appear, the epidermal layer possesses on its inner surface a well-developed basement membrane consisting of several layers of fibers crossing each other at right angles, as has already been described[4] (Fig. 1). In the limb bud the basement membrane is completely absent (Fig. 2). The mesenchyme cells are partially separated from the epithelial cells by narrow intercellular spaces, which, however, do not contain an organized fibrous layer. At other points the mesenchyme cells come into direct contact with the inner surface of epithelial cells. The absence of the basement membrane in the area of limb induction may be noted in preparations stained with Mallory-Azan connective-tissue stain, though without the electron micrographs the interpretation of the latter preparations cannot be made with the necessary degree of certainty.

The above information makes it possible for me to propose an entirely new theory of limb induction. It may be taken as being firmly established that limb development can proceed only when there is a harmonious interaction between the mesoderm and epithelium of a limb rudiment.[5, 6] In this interaction the mesoderm not only causes the epithelium to react but is itself controlled by an influence exerted by the epidermis. Stimuli of an inductive nature thus have to pass both ways between the mesoderm and the epithelium. The transmission of an inducing stimulus is in some cases at least (neural-plate induction, lens induction) dependent on a direct contact between the acting and the reacting components. If the same were true in the case of a limb rudiment, one would expect that the intervention of a well-developed fibrous basement membrane would effectively preclude the possibility of limb development even if the components (mesenchyme and epidermis) were capable of participating in such a process.

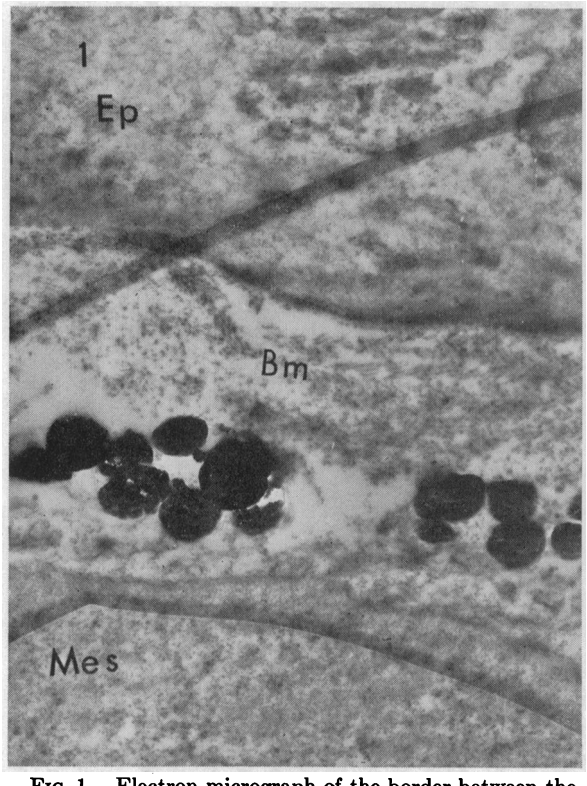

FIG. 1.—Electron micrograph of the border between the epidermis and mesenchyme in normal skin of *Amblystoma* at the stage when limb inductions appear. *Ep*, epidermis; *Mes*, mesenchyme; *Bm*, basement membrane of epidermis.

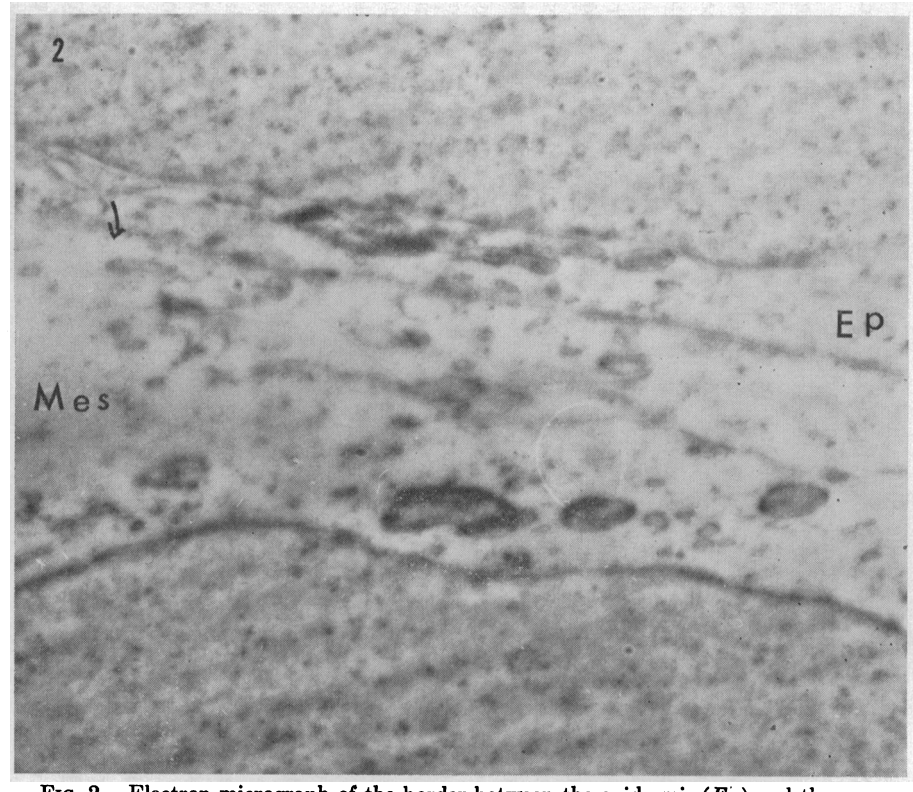

FIG. 2.—Electron micrograph of the border between the epidermis (*Ep*) and the mesenchyme (*Mes*) in an induced limb rudiment. The arrow shows a section in which the cytoplasm of the mesenchyme cell is in direct contact with the cytoplasm of the epidermal cell.

The induction of a heterotopic limb may then well be a direct result of the absence of the epidermal basement membrane at a particular spot, correctly timed, so that the competent mesodermal cells are able to establish direct contact with the epidermis. The grafts serving as limb inductors appear to be capable of influencing adversely the development of the basement membrane in adjoining sections of the skin. In the case of the nose rudiment, a zone denuded of the basement membrane is found around the opening (the nostril) by which the grafted olfactory sac communicates with the exterior. The basement membrane is also greatly reduced (even to complete disappearance) in the epidermis overlying a grafted ear vesicle. If induced limb rudiments are present, these invariably occupy the position where the deficiencies of the basement membrane are the greatest, but areas with a reduced basement membrane often reach beyond the induced limb rudiments. According to the theory proposed here, the potential limb cells of the mesoderm establish a contact with the epidermis in the denuded areas, are trapped there, and thus form the rudiment of an induced limb. It is possible that once a limb rudiment has been started, it retards further the development of the basement membrane. This suggestion may perhaps account for the development of the normal forelimbs in urodeles, while in the case of the hind limbs there may prove to be some local factors preventing the formation of the basement membrane until the hind-limb development gets under way.

It is to be hoped that some means may be found to destroy the basement membrane of the epidermis selectively. Performed at an appropriate stage and place, this should lead to supernumerary limb development and thus furnish a crucial test for the new theory. In the meantime I should like to indicate that some known phenomena appear to fall in line with the new interpretation.

1. Holtfreter[7] has reported a very peculiar transformation of fin folds into limbs. One can understand this transformation if one takes into account that the fin folds in question were situated in the area of the lateral plate mesoderm, which is presumably the source of the mesodermal limb cells, and, furthermore, that the basement membrane of the epidermis, as I have seen in my preparations, is extremely attenuated in the dorsal fin fold and disappears toward its tip. It is very likely, then, that lateral mesoderm cells may, under the circumstances, migrate into the fin fold and, finding contact with the epidermis, settle down to form a limb rudiment.

2. It has been repeatedly asserted that if the amputation surface of a limb is covered by skin (whether by natural overgrowth from the sides or deliberately, by the experimenter), regeneration is suppressed. The dermis of the skin has usually been held responsible for this effect. In the light of the present experiments it would seem that the important factor may well be the basement membrane of the epidermis covering the wound. The success of the methods proposed to renew or extend the regeneration capacity in tadpoles and adult frogs[8, 9] may depend on the effective prevention or retardation of basement-membrane development.

3. In Nassonov's experiments[10] on additional limb development after ligaturing of limbs or after implantation of pieces of cartilage under the skin of a limb, etc., the mechanism of the morphogenetic stimulus may well have been a local destruction of the epidermal basement membrane. The limb mesoderm cells coming into contact with the denuded area may then have formed a new blastema, from which stage onward the development would have followed the pathway of normal regeneration.

A study of the basement membrane of the epidermis in all similar cases is desirable and will easily show whether my suggestions are correct.

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ABNORMAL BRAIN SIZE AND FUNCTIONAL REGULATION IN FUNDULUS

BY JANE M. OPPENHEIMER AND NAOMI VASSADY

DEPARTMENT OF BIOLOGY, BRYN MAWR COLLEGE

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Introduction.—The development of the behavior pattern has been described[1] for a group of *Fundulus heteroclitus* embryos with central nervous systems abnormal as a result of transplantation experiments that had been performed during gastrula stages. The results of that investigation showed a surprising degree of functional regulation in embryos in which supernumerary brain parts had developed in confluence with the primary brain. It was therefore concluded that the developing central nervous system can under some conditions integrate considerably more than the usual amount of material into an orderly functioning whole.

Not all the operated embryos, however, regulated completely. Since the deficits in behavior of the nonregulating embryos could not always be correlated with specific visible morphological disturbances of the brain, it has seemed desirable to attempt a quantitative study of the abnormal brains in order to ascertain whether brain size may have been a factor affecting the degree of regulation which took place in the operated embryos.

Procedure.—Paper reconstructions were made of the brains of forty-five of the fifty embryos that were discussed in detail in the earlier communication. With the aid of the camera lucida, alternate sections of the brains studied were drawn on heavy bond paper at a magnification of 130×. The reconstructions constituted by the alternate drawn sections were weighed and their weights doubled to give the total relative weights indicated for each separate embryo by the vertical light gray and dark gray bars in Figure 1. The protocol numbers of the embryos, shown along the abscissae of the graph, correspond to those in Tables 1–5 of the original communication. The asterisks designate the brains illustrated by photomicrographs in the plates of that article.