Tests identical to those performed in immunoelectrophoresis were carried out in Ouchterlony immunodiffusion, in which the antigen dilution effect present in immunoelectrophoresis is not a factor. Immunodiffusion analysis has revealed the presence of two distinct antigenic components in lenses at SHUMWAY Stage 25 (weakly visible) and TAYLOR-KOLLROS Stages I and VI, corresponding to γ- and β-crystallins, and the presence of three distinct antigenic systems in lenses at TAYLOR-KOLLROS Stage XXIV, the additional component being identical with adult α-crystallins (Figure 2).

To determine the degree of tissue specificity of the absorbed antibodies, they were tested in Ouchterlony immunodiffusion against extracts of adult *R. pipiens* tissues other than lens; no reaction could be noted. This indicates that the antibodies used in this study are directed solely against components present in lens but absent in other tissues.

In conclusion, the results of this investigation indicate that the lens of *R. pipiens* contains detectable amounts of both γ- and β-crystallins by SHUMWAY Stage 25 and, together with previous immunofluorescence data[10], suggest that they appear simultaneously. Since γ-crystallins are not present in the lens epithelium at SHUMWAY Stage 25, the immunofluorescence observed there at that time using an absorbed anti-total-lens-protein antibody[10] can now be ascribed to the presence of β-crystallins. α-crystallins are the last of the lens-specific proteins to be detected during normal development of the anuran amphibian lens[19, 20].

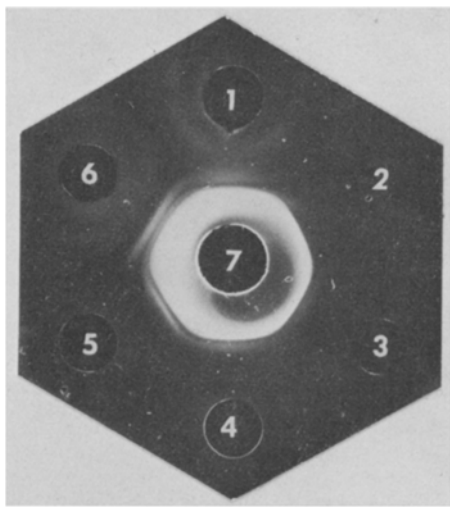

Fig. 2. Ouchterlony analysis of embryonic, larval (metamorphic), and adult *R. pipiens* lens proteins vs. absorbed antibody directed against total lens protein. Well 1, adult total-lens-proteins; Well 2, DEAE-G-100 γ crystallins; Well 3, SHUMWAY Stage 25 total lens proteins; Well 4, TAYLOR-KOLLROS Stage I total lens proteins; Well 5, TAYLOR-KOLLROS Stage VI total lens proteins; Well 6, TAYLOR-KOLLROS Stage XXIV total lens proteins; Well 7, anti-adult-total-lens protein antibody (absorbed). Incubation was for 48 h at 37 °C.

Zusammenfassung. Immunelektrophoretische und Immundiffusions-Studien zeigen, dass die Linse von *Rana pipiens* während der Entwicklung (Embryonal-Stadium 25 nach SHUMWAY) α- und β-kristalline Substanzen enthalten. Diese und frühere Ergebnisse[10] sprechen dafür, dass beide Substanzen gleichzeitig auftreten, und beweisen, dass β-kristalline Substanzen in diesem Entwicklungsstadium alleine im Linsenepithel vorkommen, während die Kristalline die am spätesten nachweisbaren linsenspezifischen Proteine (TAYLOR-KOLLROS-Metamorphosestadium VI) in der Linsenentwicklung von *R. pipiens* sind.

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Alteration of the Prospective Fate and the Inductive Power of the Definitive Streak Node in the Chick

While the young node may induce either a primitive streak or a neural structure, it is well established that the node of the definitive streak always elicits neural induction (for review, see GALLERA[1]). This change in its inductive power is correlated with some modifications of its prospective fate: at stage 2, it gives rise essentially to embryonic endoblast; later its inherent tendencies to give axial and paraxial mesoblast increase, so that no more than 50% of the node cells take part in the endoblast formation at the definitive streak stage (NICOLET[2]). Accordingly, it has been revealed that the node, whatever its age may be, does not produce a streak induction, if it differentiates partially into axial and paraxial mesoblast (GALLERA and NICOLET[3]). Hence we had suspected that this mesoblast may suppress the ability to induce a streak. Presently we see that the situation is more complex than previously thought, because, in a very peculiar environment, the definitive streak node was entirely converted into embryonic endoblast and induced a streak.

The experiments were performed on chick embryos cultured in vitro (GALLERA et NICOLET[4]) and staged after the tables of HAMBURGER and HAMILTON[5]. In the first set of experiments, 24 nodes of definitive streaks were transplanted on the posterior end of the host streak. The stages of the hosts went from stage 2 to 4. In order to recognize unequivocally the structures yielded by the grafts, 9 nodes were removed from donors, which have been previously incubated for 8 h on a medium containing 10 μC of tritiated thymidine. This time is sufficient to label all the nuclei (NICOLET[6]). In the second series, 11

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regressing nodes of stage 5 were implanted in the same way, but only on hosts of stage 2. In all experiments, implants consisted in squares, the side of which had 0.3 mm. The posterior end of the streak was previously naked from the lower layer, then received the graft, which was put with its ventral side against the ventral face of the streak. Finally its anterior edge was always oriented towards the blastoderm periphery. About 24 h later, the hosts were fixed and afterwards studied on serial sections. Those which carried a labelled node were prepared for the autoradiographic analysis according to the technique of FICQ[7].

In the first series, the results were as follows: 19 nodes were implanted prior to stage 3+ and all induced a streak which was growing up in opposite direction to that of the host (Figure 1). The autoradiographic examination shows that all the cells coming from the node were incorporated into the endoblastic layer, especially all around the node and in front of it (Figure 3). The size of the streak was stage dependent. It became smaller as the host was older. As for the 5 nodes transplanted at stage 4, they differentiated according to their prospective fate (Figure 4) and have provoked a neural induction only in one case. In the second series, the prospective fate of the 11 nodes did not change (Figure 2), but only 2 succeeded in inducing a small neural structure.

All these results require some comments. In previous studies (GALLERA et NICOLET[8]), it has been mentioned that the capacity to form a streak quickly disappears after stage 3. Here again, no streak induction was obtained after this stage. Hence the ectoblast lost this competence in this region in the same time as in other blastoderm areas. Furthermore, they confirm earlier conclusions (GALLERA[8]) according to which the posterior half of the area pellucida reacts very weakly to the neural inductive stimulus, since, amongst 16 grafts which gave rise to axial and paraxial mesoblast, only 3 have elicited a reduced neural response. On the posterior end of the young streak, the node of stage 4 behaved as a younger node. It was converted into embryonic endoblast and induced a streak. In other words, its mesoblastic tendencies were lost at the same time as it recovered the faculty of inducing a streak. In fact several authors, e.g. ABERCROMBIE[9], have already stressed that the prospective fate of the node may be altered up to the long primitive streak stage. The main interest of the present results is to have shown conspicuously that such alteration is still possible at stage 4, if the node is subjected to the influences of a very particular environment. Although the mechanisms of this change await further clarification, the high endoblastic content of the node of stage 4 may be held responsible for the maintenance of its streak inducing capacity, as it does not contain endoblast at stage 5 (NICOLET[2]). On the other hand, it must not be overlooked that, at stage 4, the prospective fate of the node is far from being so well stabilized as at stage 5 (GRABOWSKI[10], GALLERA[11])[12].

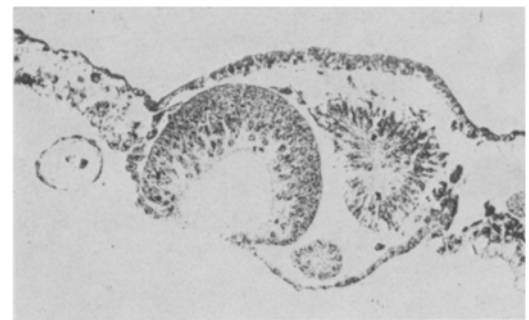
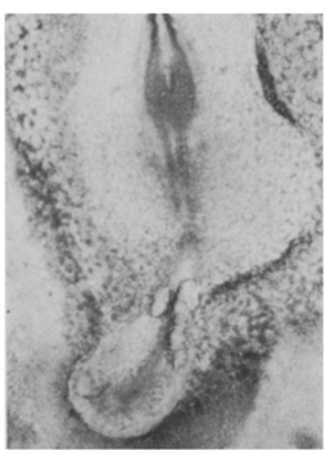


Fig. 4. Implanted at stage 4, nodes of the definitive streak differentiated into embryonic endoblast, axial and paraxial mesoblast. In this case, it has moreover built somites and neural tissue. The overlying ectoblast did not react to the neural inductive stimulus, but was clearly thicker than normal. × 280.



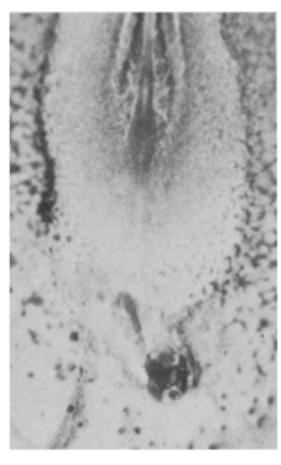


Fig. 1. Behind the embryonic body of the host, the node of stage 4 has induced a small area pellucida, in which a streak was growing up. × 20.

Fig. 2. The node of stage 5 was always included in the area vasculosa and gave rise to a notochordal rod and several somites. × 20.

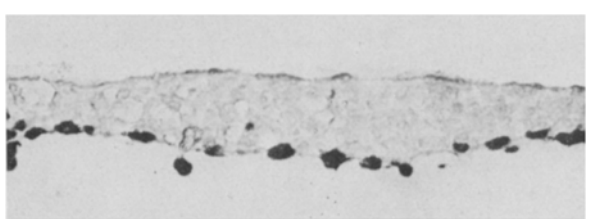


Fig. 3. Whenever a streak was induced, all the cells of the labelled node were incorporated into the lower layer, especially in front of the induced streak as shown here. × 250.

Résumé. L'implantation du noeud de Hensen de la ligne primitive achevée sur l'extrémité postérieure de la jeune ligne primitive modifie sa destinée présomptive. Au lieu de se différencier en partie en mésoblaste axial et paraxial, il se transforme entièrement en endoblaste embryonnaire et provoque l'induction d'une autre ligne primitive.

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