

Fig. 2. Histamine activation and inhibition of brain capillary adenylate cyclase. Adenylate cyclase assay contained: 2 mM  $MgCl_2$ , 2 mM ATP (0.2  $\mu Ci$   $^{14}C$  ATP), 50  $\mu g$  bovine serum albumin, 2 mM cAMP, 1 mM tetrahydropyridine, 100  $\mu g$  protein kinase, 2 mM phosphoenolpyruvate, 50 mM Tris-HCl pH 7.4 buffer, 100  $\mu l$  brain capillary fraction (1 mg/ml protein) in a total volume of 300  $\mu l$ . The capillary fraction was homogenized with 2.0 ml 0.25 M sucrose at 0°C in a Potter apparatus. The incubation was carried out for 5 min at 32°C. The values represent the means of 3 experiments. The basal activity (control) was expressed as 100%.

Fig. 2.

to the activation of capillary adenylate cyclase. As to the origin of histamine, the physiological activator of the reticulo-endothelial system<sup>13</sup>, it is thought that, as a consequence of change of environment, histamine is released from its natural sources, that is from leukocytes and/or mast cells<sup>14</sup>.

cAMP mediation was found in the permeability-modifying effect of neurohypophyseal hormones<sup>15</sup>. On the basis of our results, the cAMP mediation in the permeability regulation of brain capillaries can be hypothesized.

**Zusammenfassung.** In den mit cAMP behandelten Kapillaren des Rattengehirns wurde mit Ferritin eine erhöhte Permeabilität gefunden. Die Adenylatzyklase-Aktivität wurde in den in Gehirnkapillaren angereicherten subzellulären Fraktionen bestimmt und mit Histamin aktiviert. Da sowohl  $H_1$ - als auch  $H_2$ -Rezeptoren in den Fraktionen vorhanden waren, wird angenommen, dass das cAMP in der Permeabilitätsregulation der Gehirnkapillaren eine Rolle als Mediator spielt.

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<sup>13</sup> M. JÁNCsó, *Nature, Lond.* 160, 227 (1947).

<sup>14</sup> H. R. BOURNE, L. M. LICHTENSTEIN, K. L. MELMON, C. S. HENNEY, Y. WEINSTEIN and G. M. SHEARER, *Science* 184, 19 (1974).

<sup>15</sup> S. K. MASER, E. HOLTZMAN, I. L. SCHWARTZ and R. WALTER, *J. Cell Biol.* 49, 582 (1971).

### At what Stage of Development does the Somitic Mesoblast Invaginate Into the Primitive Streak of Chick Embryo?

After the careful work of PASTEELS<sup>1</sup>, it was generally accepted that the presumptive somitic mesoblast was already present in the postnodal area of the fully grown primitive streak. However, WOLFF<sup>2</sup> had argued that at this stage of development, the presumptive somitic tissue is still contained in the upper layer, in two areas contiguous to the anterior part of the primitive streak. Recently, NICOLET<sup>3</sup>, on the basis of autoradiographic

study, was drawn to the same conclusion. At present, we are trying to test these 2 hypotheses.

The experiments were performed on chick blastoderms cultured in vitro (GALLERA and NICOLET<sup>4</sup>). They were distributed in 4 series (see Figure 1). Our experiments consisted either in exchanging the postnodal length (0.3 mm) of the fully grown streak with the same piece, but taken from blastoderms, labelled with tritiated thymidine (Figure 1; Exp. 1), or in explanting the postnodal area of the streak, excised with or without contiguous ectoblast, into the area opaca of the host blastoderm (Figure 1; Exp. 2, 3 and 4). The donors were always at the fully grown streak stage, the hosts were of different ages, namely: the fully grown streak stage (series 1), the long streak stage (series 2), or the head process stage (series 3 and 4). At the latter stage the ectoblast is not more competent (GALLERA and IVANOV<sup>5</sup>) so that without labelling, it was easy to distinguish the graft's structures from those of the host. The ventral side of the graft was applied against the ventral side of the host's ectoblast.

The host blastoderms were fixed about 20 h after the grafting and analyzed on serial sections. The blastoderms

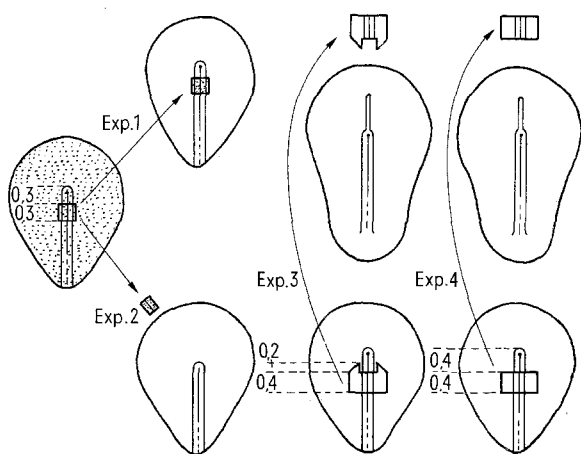


Fig. 1. Diagram showing the experimental procedures. Stippling indicates the labelling.

<sup>1</sup> J. PASTEELS, *Arch. Biol.* 48, 381 (1937).

<sup>2</sup> E. WOLFF, *Arch. Anat. Hist. Embryol.* 23, 9 (1936).

<sup>3</sup> G. NICOLET, *J. Embryol. exp. Morph.* 23, 79 (1970).

<sup>4</sup> J. GALLERA and G. NICOLET, *Experientia* 17, 134 (1961).

<sup>5</sup> J. GALLERA and I. IVANOV, *J. Embryol. exp. Morph.* 72, 693 (1964).

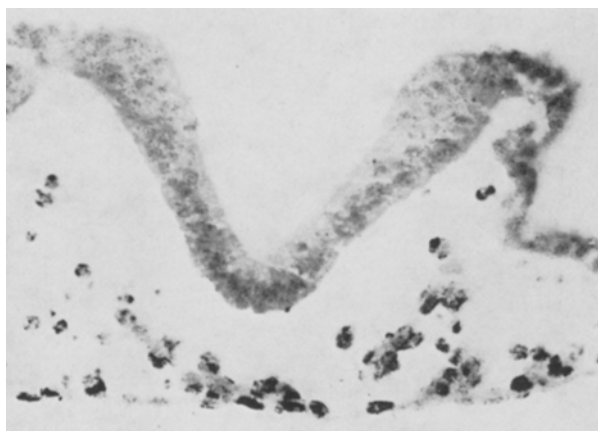


Fig. 2. Transverse section through the graft's derivatives and the induced neural groove. The labelled cells are localized in the embryonic endoblast and in the head mesoblast.  $\times 320$ .

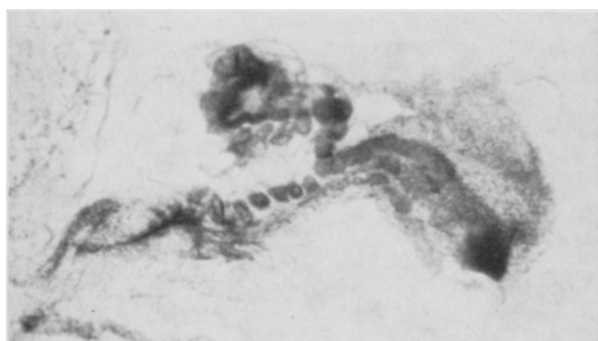


Fig. 3. In toto view of a graft of the series 3. It gave rise to 15 somites.  $\times 60$ .

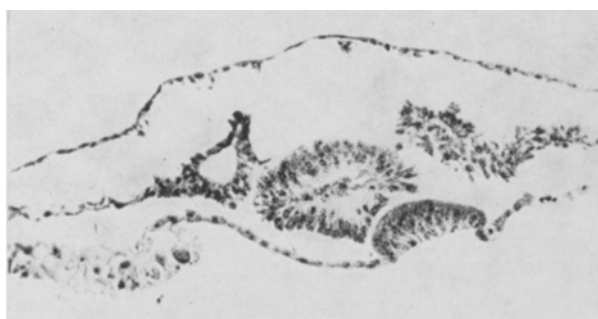


Fig. 4. Transverse section passing through the other graft of series 3. Neural plate, somites and lateral plates derived from the self differentiation of the graft.  $\times 170$ .

Series	No. of experiments	Derivatives of the grafts			
		Somites	Lateral plate	Head mesoblast	Embryonic endoblast
1	5	0	0	5	5
2	5	0	0	5	5
3	7	5	7	7	7
4	8	1	8	8	?

carrying the labelled grafts were prepared for the autoradiographic analysis, according to the method of Ficq<sup>6</sup>.

Series 1 (5 experiments). Only the blastoderms of which the development was perfectly normal were taken in account. In all cases, the labelled cells were found in the dorsal wall of the foregut, except in its most rostral part, and in the head mesoblast. Finally, in one case we have found some labelled cells scattered in the first somites.

Series 2 (5 experiments). The grafts induced neural structures in the host ectoblast and they gave rise to head mesoblast and to embryonic endoblast (Figure 2).

Series 3 (7 experiments). The grafts were taken from the postnodal area of the fully grown streak with some contiguous ectoblast. The latter was prolonged by 2 triangles of more anterior ectoblast. The most anterior part of these triangles reaching the level of the primitive pit (Figure 1; Exp. 3). 5 grafts have formed several somites (Figures 3 and 4), arranged in an irregular way. 6 gave rise to a small neural plate, a few mesenchyme cells and some quantity of very thin embryonic endoblast.

Series 4 (8 experiments). The grafts of these series differ from those of the preceding one, indeed they were not prolonged forward by the two ectoblastic triangles (Figure 1; Exp. 4). However, its derivatives were very different. Indeed only 1 graft was able to form few somites. On the other hand, all the grafts gave rise to a short lateral plate and to some mesenchyme cells.

Although the number of our experiments was limited, their results were coherent and significant, as seen in Table.

As can be seen, the explants which included exclusively the postnodal length of the primitive streak have never formed somites. They gave rise only to embryonic endoblast and to head mesoblast. Whereas the majority of the grafts, which included besides the postnodal fragment of the primitive streak two areas of ectoblast situated close to the more anterior part of the streak (series 3), formed typical somites. It is thus obvious that at the fully grown streak stage the presumptive somitic tissue is still localized in the upper layer just behind the level of Hensen's node. Indeed, our grafts in which the ectoblast were more posterior, (series 4) gave rise mainly to lateral plate mesoblast<sup>7</sup>.

*Résumé.* Les expériences microchirurgicales et l'analyse autoradiographique ont montré qu'au stade de la ligne primitive achevée le mésoblaste somitique présumé est encore contenu dans le feuillet externe des deux côtes de la région directement postnodale de la ligne primitive.

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<sup>6</sup> A. FICQ, in *The Cell* (Academic Press, New York 1959), p. 67.

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