

here. This is consistent with the fact that the vermilion mutant is either lacking in or has a very small quantity of tryptophan pyrrolase<sup>12-14</sup> and therefore would not be expected to accumulate kynurenine anywhere in the body. We can conclude therefore that the protein yolk spheres contain at least 2 different fluorescing substances and that the more brilliant stable component is kynurenine<sup>15</sup>.

*Zusammenfassung.* Papierchromatographische und fluo-reszenzmikroskopische Untersuchungen von Ovarien der Wildtypen und der Vermilion- und Cinnabar-Mutanten von *Drosophila* zeigen, dass sich Kynurenin (der Hauptbestandteil der fluoreszierenden Komponente des Eies)

in den proteinhaltigen Dotterschollen des reifenden und des reifen Eies befindet.

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<sup>15</sup> Supported in part by NIH grant No. GM 14891 and a grant from the Research Foundation of the State University of New York.

### Differentiation of Chick Embryo Brain Cells in Culture

Previous investigations have demonstrated that neurons separated from their glial cells can be maintained in tissue culture and are able to regenerate processes. Neurons have been isolated from spinal and trigeminal ganglia<sup>1-4</sup>, from the spinal cord<sup>5-7</sup> and from the cerebral hemisphere<sup>8</sup> of chick embryos of more than 6 days of age. In all these conditions the neurons were separated when nerve fibres already existed. During the dissociation procedures all cell processes were destroyed and subsequently reappeared during the cultivation. Thus, the growth of the fibers can be considered to involve regeneration rather than differentiation.

We attempted to cultivate undifferentiated cells dissociated from cerebral hemispheres during the initial stages of differentiation when there were no nerve fibres.

*Material and methods.* Cerebral hemispheres denuded of their connective tissue covering, from 25-somite chick embryos (4.5-5 days of incubation), were passed through a nylon sieve (82  $\mu$  pore size) into chick embryo extract. One drop of the cell suspension was then placed onto a

collagen coated coverslip, supplemented with one drop of cockerel plasma and cultivated in the Maximow's double-coverslip assembly. Phase contrast microscopy was employed for observation of the cells during their subsequent growth. Some cultures were fixed and stained by the Bodian's method.

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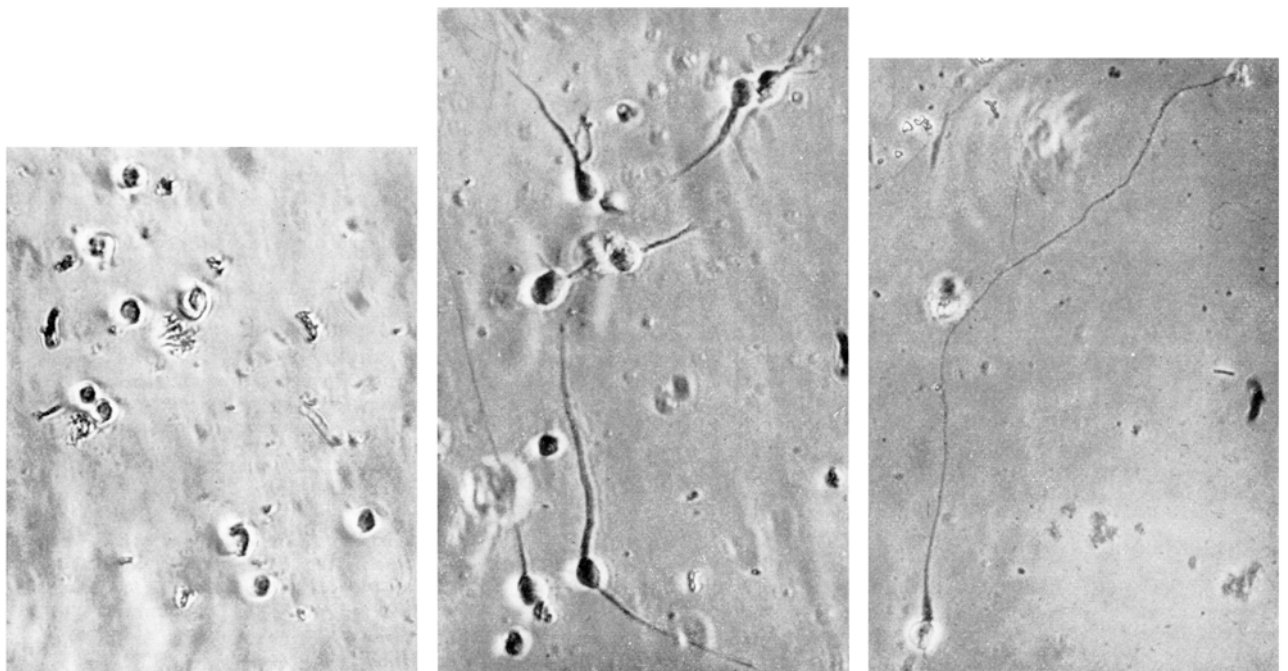


Fig. 1. Cells isolated from 5-day-old chick embryo cerebral hemispheres, photographed with phase contrast microscope.  $\times 283$ . a) Freshly isolated cells without processes. b) After 24 h in culture, cells with several processes. c) After 48 h in culture, a cell with a long process.

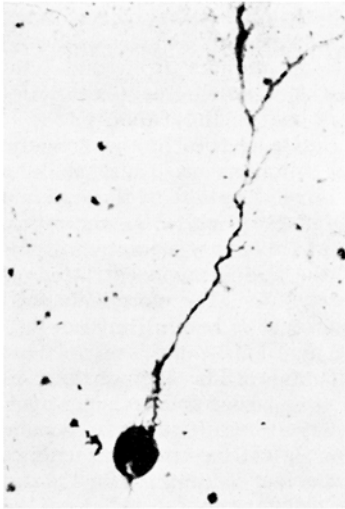


Fig. 2. Cell isolated from 5-day-old chick embryo cerebral hemispheres after 48 h in culture. Bodian's impregnation.  $\times 400$ .

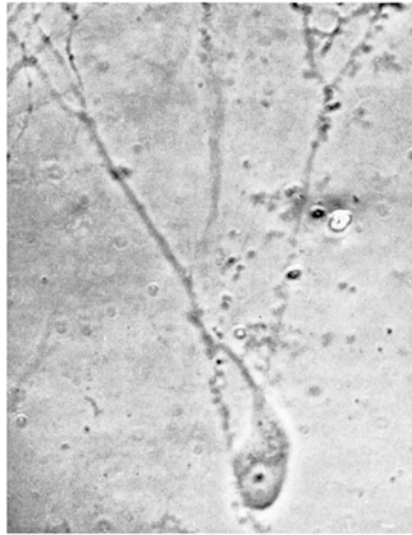
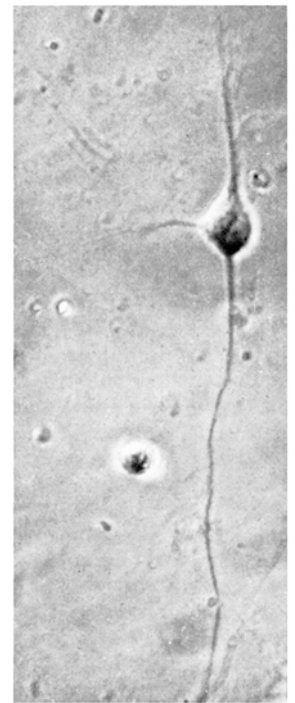


Fig. 3. Cells isolated from 5-day-old chick embryo cerebral hemispheres, photographed with phase contrast microscope.  $\times 391$ . a) After 3 days in culture, a young neuroblast. b) After 6 days in culture, a pyramidal neuron.



**Results.** Immediately following isolation, the cells were of a round-shaped morphology and identical to each other (Figure 1a). After 15 h of cultivation, many cells were observed to have formed one, two or more short processes. These processes developed rapidly (Figure 1b) and after 48 h were seen to be quite extended from their cell bodies (Figure 1c). These processes, as well as their soma, displayed positive reaction to Bodian's impregnation (Figure 2). After 24 h, approximately 45% of the cells had formed processes, and by 48 h between 80 and 90% of cells possessed fibres.

Nucleoli could sometimes be distinguished in the cell body, at which time the cells resembled young neuroblasts (Figure 3a). After 3 to 4 days in culture, most of the cells maintained a spherical morphology and were unipolar or bipolar. Some cells, however, evolved to the multipolar type and after 5 days pyramidal neurons were observed to have differentiated (Figure 3b).

The results of this study revealed undifferentiated cells, isolated from cerebral hemispheres, were able to develop processes. They were further able to differentiate into bipolar, unipolar and multipolar neuroblasts without contact either between each other or with glia cells.

In a previous study in this laboratory, a stimulatory effect by embryonic brain extract upon the fibre out-

growth of chick embryo cerebral hemispheres explants was described<sup>9</sup>. Investigations as to the influence of this extract upon the differentiation of isolated neuroblasts in long-term culture are now in progress.

**Résumé.** Des cellules, encore à un stade indifférencié, dissociées d'hémisphères cérébraux d'embryon de Poulet ont formé, en culture, des fibres nerveuses. La plupart des cellules se différencient en neuroblastes unipolaire et bipolaire; certaines évoluent vers le type multipolaire sans contact direct avec des cellules gliales.

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### Uptake of Alanine, Phenylalanine and Tyrosine by L1210 Cells at 4°C: Possible Effect of Lipid Solubility

The importance of the  $\alpha$ -amino group,  $\alpha$ -carboxyl group and  $\alpha$ -hydrogen of an amino acid to its transport by intestine or tumor cells has been established<sup>1</sup>. It has also been shown that a net charge on the side chain (R of  $\text{RNH}_3^+ \text{COO}^-$ ) inhibited its transport by the intestine<sup>1</sup>. An apolar group enhances its transport. OXENDER and CHRISTENSEN<sup>2</sup> showed the effect of structural changes of the side chain on the transport of neutral

amino acids by Ehrlich cells. This paper presents the findings in the effects of the introduction of a phenyl group and the hydroxylation of the phenyl group on the uptake of L-alanine by L1210 cells at 4°C.

L1210 cells from ascitic fluid of BDF<sub>1</sub> mice were used. After removing the contaminating red blood cells by hemolysis, the L1210 cells were washed and suspended in 41D<sup>3</sup>, pH 6.8; the pH of the ascitic fluid. 1 ml of the