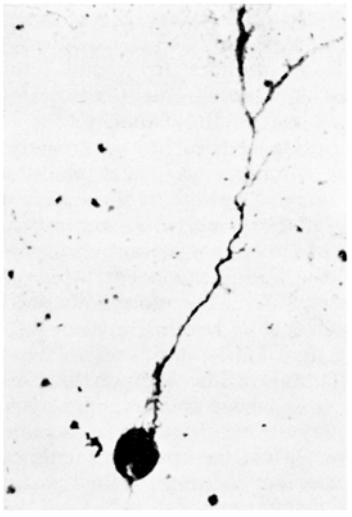
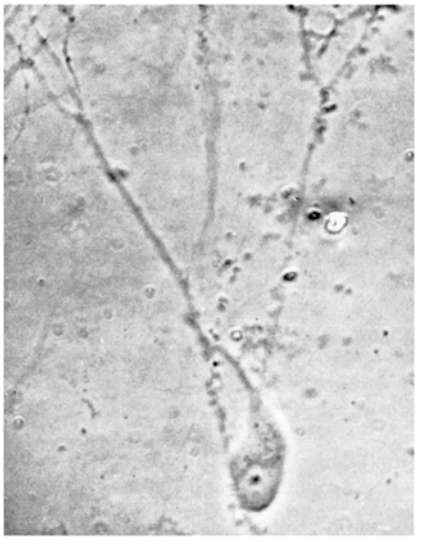


Fig. 2. Cell isolated from 5-day-old chick embryo cerebral hemispheres after 48 h in culture. Bodian's impregnation. ×400.



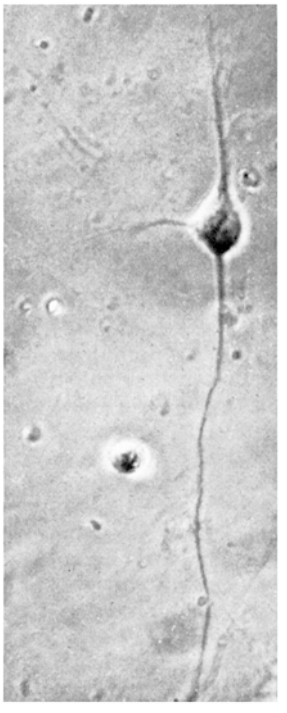


Fig. 3. Cells isolated from 5-day-old chick embryo cerebral hemispheres, photographed with phase contrast microscope. ×391. a) After 3 days in culture, a young neuroblast. b) After 6 days in culture, a pyramidal neuron.

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 ressembled 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 outgrowth of chick embryo cerebral hemispheres explants was described[9]. 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 α-amino group, α-carboxyl group and α-hydrogen of an amino acid to its transport by intestine or tumor cells has been established[1]. It has also been shown that a net charge on the side chain (R of RNH_3^+ COO^-) inhibited its transport by the intestine[1]. An apolar group enhances its transport. Oxender and Christensen[2] 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_1 mice were used. After removing the contaminating red blood cells by hemolysis, the L1210 cells were washed and suspended in 41D[3], pH 6.8; the pH of the ascitic fluid. 1 ml of the

cell suspension containing $1.0–5 \times 10^7$ cells was mixed with 0.01 ml of ^{3}H-labeled L-alanine (Ala) or one of the following: L-phenylalanine (Phe), L-tyrosine (Tyr) (1 μc, 0.2 nmoles[4]) and incubated at 4 °C for up to 20 min. At the end of incubation, 5 ml cold 0.15 *M* NaCl (NS) was added to each sample, centrifuged in the cold and the washing repeated once more. The cell sediments were resuspended in 1 ml NS and the radioactivity determined in a liquid scintillation counter and expressed as cpm per 10^7 cells. At 3000 cpm/10^7 cells, the radioactivity of the intracellular water equals that of the medium (Distribution ratio = 1). The Figure shows the uptake of the 3 amino acids for up to 20 min of incubation. No increased uptake was demonstrated when incubations were continued up to 1 h, in preliminary experiments.

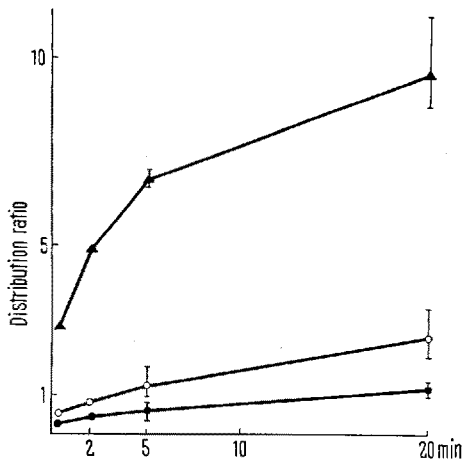
As shown in the Figure alanine is taken up by L1210 cells sluggishly at 4 °C. The intracellular concentration reaches that of the medium in 20 min. Phenylalanine is taken up extremely rapidly while tyrosine, much less so.

Since the cell membrane is rich in phospholipids, one possible explanation of these striking differences is that the phenyl group enhances the lipid solubility of alanine while hydroxylation of the phenyl group reduces its lipid solubility. The pk_3 of the OH group is 10.07[5]. It is, therefore, not dissociated at pH 6.8 and hence no net charge is introduced by the OH group. To estimate the lipid solubilities of these amino acids, 1 ml of NS containing 1.0 μc of labeled amino acid was thoroughly mixed with 2 ml of a phospholipid mixture consisting of chloroform:methanol:n-heptane (6:3:1 by volume) containing lecithin 600 mg/100 ml and cholesterol 200 mg/100 ml. After centrifugation, aliquots from the lipid solvent layers were taken for measurement of radioactivities. The results are shown in the Table.

Lipid solubilities of Ala, Tyr and Phe

Phospholipid solution (cpm/0.1 ml)	Ala	Tyr	Phe
Range	46–59	242–278	711–1066
Average	51	262	920

Aliquots of each labeled amino acid was extracted with a phospholipids solution (see text) and the radioactivities measured. 3 experiments.



Uptake of alanine, tyrosine and phenylalanine at 4 °C by L1210 cells. No further uptake is noted when incubation is continued to 60 min.

The differences of the radioactivities of the 3 amino acids in the lipid solvent fractions are quite striking and do correlate with the rates of uptake of these amino acids by lipid cells. However, even with Phe, the radioactivity in the chloroform phase is only a fraction of the aqueous phase, whereas, the cellular concentration of Phe is 10 times that of the medium. Therefore, enhanced lipid solubility of an amino acid is helpful but not sufficient for optimal uptake by L1210 cells.

How L1210 cells can accumulate Phe 3 times the concentration in the medium after 20 sec and 10 times after 20 min at 4 °C is not known. It is difficult to reconcile with the concept that concentrative uptake is energy dependent. There is some evidence (unpublished data) that Phe taken up by the cells are concentrated on the membrane rather than distributed evenly in the intracellular water. Cell membrane consists of proteins, lipids and carbohydrates such as neuraminic acid derivatives. At the molecular level, the membrane probably is composed of myriads of hydrophobic and hydrophilic groups and side chains of amino acids, sialic acids, etc., which serve as receptors or barriers to outside substances. Possibly the high uptake of Phe is due to the hydrophobic affinity of the phenyl moiety and a remarkable fit to the receptors on the cell surface. Conceivably, there may be a practical application for this property. For instance, the introduction of a Phe group to a chemotherapeutic agent may enhance its cellular permeability and efficacy.

As pointed out by PARDEE[6], the first step in cellular transport is adsorption (uptake) of the substance to be transported to the surface of the cell. Working at 4 °C slows down this first step so that some of the characteristics can be studied. The present work describes for the first time the effect of a phenyl group and the hydroxylation thereof on the uptake of alanine by L1210 cells[7].

Résumé. L'introduction d'un groupe phényl facilite grandement le transfert de L-alanine tandis que l'hydroxylation du groupe phényl diminue la facilité de transfert.

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[4] Purchased from New England Nuclear Corp.
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