Table II. Infection of mouse cells and clones of mouse-human cell hybrids with adenovirus type 12[a]

Cell clone	Human chromosomes present	Frequency of T-antigen positive cells[d] (%)	Ad12 DNA synthesis[e]		Frequency of V-antigen positive cells[f] (%)
			Input DNA (cpm)	Fraction in hybrid (%)	
C1-1 D	None	65–82	88,141	< 0.05	< 0.1
Hybrid C1 8[b]	B5, C6, C7, C11, C12, D14, E17, F19, F20	67–80	13,869	< 0.1	< 0.1
Hybrid C1 84[c]	A3, B5, C7, C11, C12, E17, F19, F20	65–80	78,826	< 0.05	< 0.1
Hybrid C1 82	A3, B5, C7, C11, C12, D14, E17, F19, F20, G22	84–92	12,377	9.37	30
Hybrid C1 15 BUDR	A3, B5, C7, C11, C12, D14, F19	70–84	26,829	< 0.1	< 0.1
Hybrid C1 75	A3, B5, C7, C11, C12, D14, D15, E17, F19, F20	61–72	14,280	10.97	15–20
Hybrid C1 21	B5, C7, D14, E17, F19, G21	65–85	10,857	< 0.1	< 0.1
Hybrid C1 16	A3, C7, C11, C12, E17, F19, F20	65–80	23,566	< 0.1	< 0.1
Hybrid C1 91	C7, D15, E17	65–80	15,600	< 0.1	< 0.1

[a] Yield of infectious virus above backrround was not detected in any clone so far tested. [b] Clones C1 8, C1 15, C1 16, C1 21, C1 75, and C1 91 are hybrids of LN-SV human cells with C1-1 D mouse cells. [c] Clones C1 82 and C1 84 are hybrids of KOP-2 normal human fibroblasts with C1-1D mouse cells. [d] The cells were stained for adenovirus type 12 specific T-antigen at 32 h after infection as previously described[2]. The ranges correspond to results obtained in 3 experiments. [e] The cells were labeled from 18 to 22 h after infection with either ^{3}H-thymidine (10 μCi/ml) or ^{32}P-phosphate (500 μCi/ml). The DNA was isolated from infected and control cells, denatured, sonicated, and hybridized to purified Ad12 DNA immobilized on nitrocellulose filters under previously described conditions[10]. Radioactivity bound to filters not containing Ad12 DNA was subtracted as background. Each hybridization was performed with a minimum of 2 filters containing 5 μg of Ad12 DNA. [f] The cells were stained for V antigen at 42 and 48 h after infection by the previously described indirect immunofluorescent method[3]. Results in 2 independent experiments are presented.

hypoxanthine-aminopterin-thymidine (HAT) medium or in Eagle's medium containing bromodeoxyuridine[8]. At least 25 metaphases of each clone population were analyzed as previously described[9]. Only low passage clones were used for experiments.

The mouse cells, the human cells (WI-38), and the mouse-human cell hybrids were infected with adenovirus type 12 at input multiplicity of infection of 100 plaque-forming cells (PFU) per cell. At 32 h after infection, the cells were stained for adenovirus-specific T-antigen that was detected in 60–80% of mouse cells, in 90–95% of human cells, and in 61–92% of different hybrid cells. At 42, 48 and 52 h after infection, the cells were stained for adenovirus capsid proteins. No virus capsid proteins were detected in mouse cells; positive staining was detected in 95% of human cells. The presence of virus-specific capsid proteins was observed in 2 clones of hybrid cells, C1 82 and C1 75, but not in 6 other hybrids tested (Table II).

To determine whether Ad12 DNA was synthesized in infected cells, the mouse and hybrid cells were pulse-labeled with ^{3}H-thymidine or ^{32}P-phosphate from 18 to 22 h p.i., the DNA was isolated, purified, and hybridized to adenovirus type 12 DNA. Newly synthesized virus-specific DNA was detected only in clones C1 82 and C1 75, i.e., those which also contained viral capsid antigens. Both clones contained the human chromosomes A3, B5, C7, C11, C12, D14, E17, F19 and F20, derived from either normal human fibroblasts or SV40-transformed Lesch-Nyhan cells. The remaining hybrids were not able to support replication of viral DNA or synthesis of viral capsid proteins (Table II). No increase of infectious virus above background was detected in any of the hybrids.

The detailed study of molecular events during abortive infection of mouse cells and different mouse-human cell hybrids with Ad12 is in progress[11].

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Effects of Dibutyryl Cyclic AMP on Cultured Brain Cells from Chick Embryos of Different Ages

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Summary. The effect of dibutyryl cyclic AMP on cultivated astroblasts from 8-, 12- and 15-day-old chick embryo brain has been studied. The results show that these cells must have reached some degree of maturation in ovo to become morphologically differentiated by the cyclic nucleotide.

It has been shown that cultures from 17–21-day-old rat embryos and from newborn rats are composed predominantly of undifferentiated glial cells (astroblasts). Several authors have reported that the addition of monobutyryl[3] or dibutyryl cyclic AMP[4,5] induces morphological alterations, converting these cells towards a more differentiated state. Electron microscopic studies of this material have demonstrated the presence of an abundant cytoplasmic fibrillar network which is a characteristic of differentiated astrocytes[6]. To study the influence of

cell maturation on the response to N^6, O^2-dibutyryl adenosine 3′:5′monophosphate (dBcAMP), we investigated the effect of this cyclic nucleotide on cultures of astroblasts from chick embryos of different ages.

Materials and methods. Cerebral hemispheres from 8-, 12- and 15-day-old chick embryos were chemically dissociated by using a 48 µm pore size nylon sieve as previously described[7]. The dissociated cells were grown in Falcon plastic Petri dishes in Eagle's medium (BME, Institut Pasteur, Paris) supplemented by 20% fetal calf

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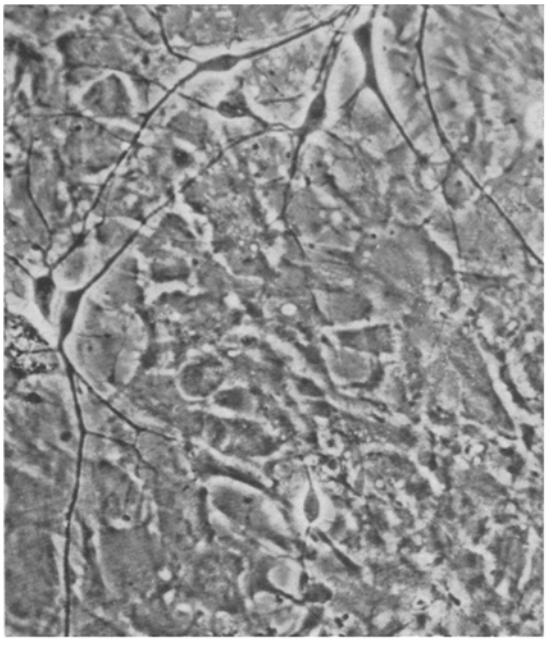

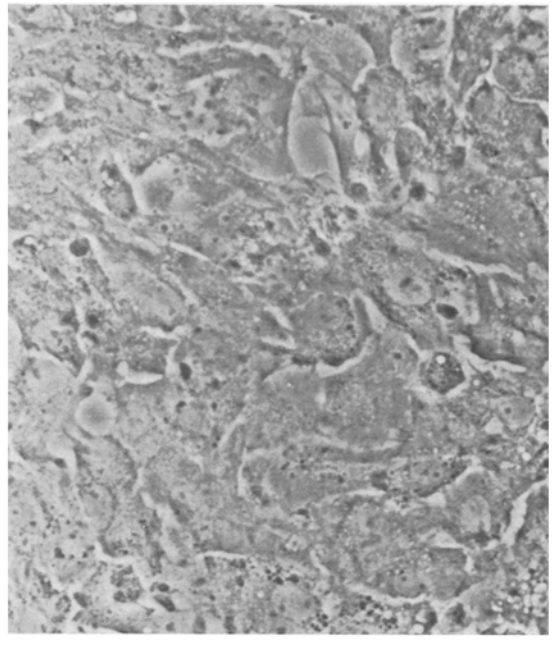

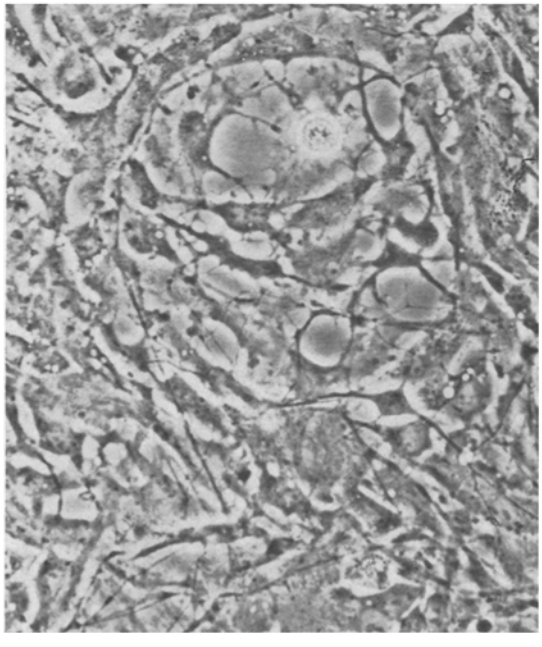

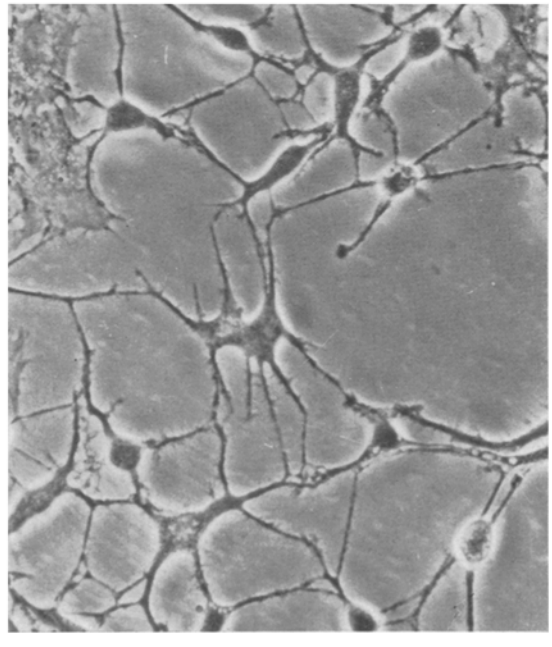

Figs. 1–4. Phase contrast micrographs of dissociated cerebral hemisphere cells from chick embryos cultivated during 2 weeks. × 250.
1. 8-day-old embryos: Mature neurons with long fibre processes are lying on a monolayer of polygonal-shaped flat cells.
2. 15-day-old embryos: only undifferentiated glial elements can be observed in these cultures.
3. 8-day-old embryos: culture treated 4 h with DBcAMP in which few cells assume a star-like appearance.
4. 15-day-old embryos: culture treated 4 h with DBcAMP: most cells have multiple branched processes and resemble mature astrocytes.

serum (GIBCO). The cultures were incubated at 37 °C in a humidified 5% CO_2/95% air atmosphere. The medium was changed every 2 days.

After 2 weeks of cultivation, confluency was reached. At this point in time, the normal nutrient medium was replaced by a serum-free Eagle's medium supplemented with 1 m*M* cBcAMP (Sigma Chemical Co.). The cultures were observed with an inverted phase-contrast microscope.

Results and discussion. After 2 weeks of cultivation in normal nutrient medium, cultures from 8- and 12-day-old chick embryos consist of a monolayer of polygonal flat cells (mainly astroblasts and few mesenchymal cells) upon which differentiated neurons are present[7] (Figure 1). In contrast, in cultures from 15-day-old chick embryos, very few neurons are present and almost all the cells are flat and polygonal (Figure 2).

No morphological alteration of the flat cells could be observed after removing serum from the culture medium. After addition of dBcAMP to this serum-free medium, the polygonal flat cells (astroblasts) assume a star-like appearance (astrocytic-like cells). This change can be observed as early as after 2 h of incubation. However, the relative amount of morphologically changed cells differed widely according to the age of the embryo.

In 8- and 12-day-old chick embryo cultures, most cells retain an undifferentiated morphology, while some others undergo an astrocytic-like transformation after 2 or 24 h incubation in the presence of dBcAMP (Figure 3). A few more cells are altered, however, in 12-day-old than in 8-day-old chick embryo cultures. In contrast, in cultures from 15-day-old embryos, many astroblasts are transformed into multipolar cells with many processes, as shown in Figure 4.

Previous studies have shown that cyclic nucleotides affect the morphology of cultured astroblasts from 17–21-day-old rat embryos and from newborn rats[3–5]. In the present experiments, the same morphological alteration could be seen in chick embryo cultured astroblasts after the addition of dBcAMP. It is of interest that the response to dBcAMP is quantitatively much more important in cultures from 15-day-old than in cultures from 8- or 12-day-old chick embryos. These results appear to indicate that astroblasts must have reached some degree of maturity in ovo to become morphologically changed by cyclic nucleotides. We think that this phenomenon further agrees with a specific and differentiating action of cyclic AMP.

Influence of Progesterone on Protein and RNA Synthesis in Cultured Chick Embryo Liver Cells[1]

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Summary. Chick embryo liver primary cultures, when added with progesterone, exhibit, in comparison with the controls, a normal growth, a decline of both ^{3}H-uridine uptake and incorporation into total RNA, a decline of ^{3}H-leucine and ^{14}C serine incorporation into the proteins. Progesterone is not able to stimulate phosvitin synthesis induction.

Analysis of in vitro effect on target tissues and cells of steroid hormones represents an important tool for studying the mechanisms of their action. In previous research, we have demonstrated that estradiol-17-β induces in chick embryo liver primary cultures the de novo synthesis of the yolk protein phosvitin[2] and a declined production of the 'secreted' proteins[3]. In addition, a decreased incorporation of ^{3}H-uridine incorporation into the total RNA has been detected[4]. Inhibition of protein synthesis and labelled uridine incorporation can be observed in vitro also in isolated bone cells after hydrocortisone administration[5]. We have therefore investigated whether similar effects could be demonstrated in liver cells following progesterone treatment.

Material and methods. 14-day chick embryo liver cells were cultured as previously described[2]; 24 h after plating, test cultures were supplied with nutrient containing progesterone (Merck, 500 μg/culture in 10 μl of propylene glycol) and controls with nutrient added with propylene glycol alone. Two independent sets of experiments with labelled precursors were carried out: in the first, both control and treated cultures were supplied with the nutrient containing 2 μCi/ml of ^{3}H-L-leucine (Radiochemical Centre, Amersham; specific activity 58 Ci/m*M*) and 0.2 μCi/ml of ^{14}C-L-serine (Radiochemical Centre, Amersham; specific activity 174 mCi/m*M*); in the second,

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Table I. Alkali-labile protein phosphorous

	Culture media (μg/ml)[a]
Control cultures (18 h in propylene glycol containing medium)	0
Test cultures (18 h in progesterone containing medium)	0
Test cultures (18 h in progesterone containing medium and 48 h in normal medium)	0
Test cultures (18 h in estradiol containing medium and 48 h in normal medium)	3.7[8]

[a] Mean of 4 independent experiments.