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.

Table II. Cell growth. Uptake and incorporation of ^{3}H-L-leucine, ^{14}C-L-serine, ^{3}H-uridine into the control (C) and treated (T) cultures

	4.5 h				9 h				18 h			
	C		T		C		T		C		T	
Cell counts [a]	28.6 ±	3.1	24.6 ±	2	25.6 ±	2	22.8 ±	2.8	22.3 ±	1.2	25.3 ±	6.3
^{3}H-L-leucine [b]												
Acid soluble	9,293 ±	577	9,895 ±	1,037	11,700 ±	1,142	11,313 ±	465	24,848 ±	2,124	19,129 ±	1,218
Acid insoluble	32,682 ±	745	27,632 ±	1,907	50,384 ±	4,217	35,860 ±	1,226	91,474 ±	1,959	83,504 ±	16,040
A. sol./A. insol.	0.28		0.36		0.23		0.31		0.27		0.23	
'Secreted' proteins [c]					56,423 ±	2,904	35,868 ±	4,100				
^{14}C-L-Serine [b]												
Acid soluble	4,660 ±	179	4,180 ±	189	5,928 ±	997	6,075 ±	353	12,496 ±	1,982	12,821 ±	2,876
Acid insoluble	9,105 ±	324	7,613 ±	450	13,372 ±	1,296	11,843 ±	600	20,643 ±	908	19,834 ±	8,614
A. sol./A. insol.	0.51		0.55		0.44		0.51		0.60		0.64	
^{3}H-Uridine [b]												
Acid soluble	5,584 ±	376	5,364 ±	908	14,815 ±	313	6,260 ±	452	34,032 ±	4,293	19,933 ±	8,614
Acid insoluble	16,543 ±	2,521	16,267 ±	1,794	46,519 ±	2,377	21,362 ±	2,874	192,306 ±	18,570	97,758 ±	29,950
A. sol./A. insol.	0.34		0.33		0.32		0.30		0.18		0.20	

Each value is the mean of 3 independent experiments, each in duplicate + SD. [a] Number of cells 10^{6}/culture. [b] cpm/culture. [c] cpm/ml nutritional media.

with the nutrient containing 0.5 μCi/ml of ^{3}H-uridine (Radiochemical Centre, Amersham; specific activity 41 Ci/m*M*). The cultures were continued for 4.5, 9 and 18 h. In a third experiment, media were replaced after 18 h of progesterone stimulation and cultures incubated for other 48 h. At the end of incubation, cells were detached by treatment with 0.02% EDTA (37 °C, 10 min), washed with EDTA and recovered by centrifugation (50 *g*, 10 min). Cell counts were carried out by means of a Bürker chamber. Cells were subsequently homogenized at 0–4 °C in 1 ml of 10% TCA using a Potter homogenizer with a teflon pestle. The disrupted cell suspension was centrifuged (1000 *g*, 10 min) and acid-soluble and acid-insoluble material separately recovered and processed. 20 μl of acid-soluble material were placed on a Whatman glass fibres disc GF/A (W. and R. Balstone Ltd., Maistone, England), transferred into a vial, added with 10 ml of a Spectrafluor PPO-POPOP scintillation fluid (Radiochemical Centre, Amersham) and radioactivity measured. The TCA precipitate was washed twice with 5% TCA, methanol-ethyl ether (1/1 v/v), ether and then dissolved in 1 ml Soluene (Packard Instrument Co. Inc.) and added to 10 ml of Spectrafluor PPO-POPOP scintillation fluid. Culture media recovered by centrifugation of disrupted cell suspension were precipitated with 10% TCA, repeatedly washed with 5% TCA and used for newly synthesized secreted proteins and phosphorous determinations. Radioactivity was recorded for 10 min with a Packard scintillation counter 2425. Protein phosphorous was determined according to BARLETT's method[6], after alkaline hydrolysis considered specific for phosvitin[7].

Results and discussion. The results of our experiments are reported in Tables I and II. Progesterone does not affect the survival and multiplication of the liver cells in vitro, as demonstrated by the cell counts. Moreover, hormone is unable to induce phosvitin synthesis during both the 18 h stimulation period and the subsequent 48 h, when estradiol-17-β is able to do so. (Table I) On the other hand, protein and RNA synthesis are affected by hormonal treatment. At 9 and 18 h, ^{3}H-uridine incorporation into RNA (TCA-precipitate) is depressed into the treated cultures in comparison with the controls (9 h: 46%, 18 h: 51%). However, this decline is dependent upon a parallel reduction of labelled nucleotide uptake by the liver cells. Indeed, the ratios acid-soluble/acid-insoluble radioactivity are in close agreement between the treated and control cultures. Hormone therefore acts on cellular permeability. This effect is not present at 4.5 h. Also ^{3}H-L-leucine and ^{14}C-L-serine incorporation into proteins (TCA precipitate) is decreased at 4.5 h (84% as measured both as labelled leucine and labelled serine incorporation) and at 9 h (71% and 88% respectively). Since there is no difference between control and treated cultures in labelled amino-acid uptake, the protein synthesis diminution is real. Correlatively a decrease in 'secreted' protein synthesis at 9 h is detected in progesterone treated cultures (61% in comparison to the controls).

In conclusion, progesterone interfers with the liver cells protein synthesis. Since this effect is independent of phosvitin production stimulation, we suggest that the phosvitin synthesis and that of 'secreted' proteins are not mutually related, and that the last effect is not specifically estradiol-17-β mediated.

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