Journal of Cell Science 104, 713-718 (1993)
Printed in Great Britain © The Company of Biologists Limited 1993

Lipogenic enzyme and apoprotein messenger RNAs in long-term primary culture of chicken hepatocytes

M. Douaire[1,*], B. Belloir[1], J.-C. Guillemot[1], J.-M. Fraslin[2], P. Langlois[1] and J. Mallard[2]

Laboratoire de génétique, [1]Institut National de la Recherche Agronomique and [2]Ecole Nationale Supérieure Agronomique de Rennes, 65 rue de Saint Brieuc, 35042 Rennes Cedex, France

*Author for correspondence

SUMMARY

Hepatocytes isolated from 9-week-old chickens were cultured in a serum-free, hormonally defined medium. Relative amounts of mRNAs coding for lipogenic enzymes (acetyl-CoA carboxylase, fatty acid synthase, Δ9 desaturase, malic enzyme) and apoproteins (apoprotein A1 and apoprotein B) were determined until the 12th day. β-actin and albumin mRNA, as well as albumin secretion, were also assessed.

Cellular metabolic activity appeared to be very low for the first days of culture, but increased after the 7th day. All the mRNAs studied, except for that of malic enzyme, were present from this time throughout the culture lifespan. The biological significance of the observed results and the relevance of this chicken hepatocyte culture system for long-term metabolic and genetic studies are discussed.

Key words: lipogenesis, messenger RNA, chicken, cell culture, hepatocytes

INTRODUCTION

Primary hepatocyte culture systems have successfully been used in chicken lipogenesis studies. Most of the studies have been performed using hepatocytes isolated from 13- to 19-day-old chick embryos (Goodridge et al., 1974; Liang and Grieninger, 1981; Plant et al., 1983; Back et al., 1986; Wilson et al., 1986; Cupo and Cartwright, 1989; Stapleton et al., 1990). A few studies have been performed on hepatocytes isolated from young chickens up to 15 or 20 days of age (Tarlow et al., 1977; Boehm et al., 1988). One of the major limitations of the described systems is the short cell viability (from a few hours to a week). It is known that after a few days of culture, the hepatocyte's ability to synthesize molecules, specially liver-specific mRNA, is greatly reduced (Plant et al., 1983; Jefferson et al., 1984a). Moreover, for two or three days after seeding, cell metabolism is dependent on the in vivo physiological state preceding hepatocyte collection (Tarlow et al., 1977). In primary cultures of rat hepatocytes, in vivo effects are perceptible up to 72 h (Vance and Russell, 1990). This situation has hampered some regulation studies and all long-term effect analyses.

In order to overcome these problems, a long-term system for primary culture of hepatocytes from young chickens (6- to 12-week-old) was developed (Fraslin et al., 1992), in which cells could be maintained in a serum-free, hormonally defined medium for periods of at least one month. Messenger RNA studies carried out for the first twelve days of the culture period are reported here. Three groups of mRNA were considered, according to the role of their corresponding proteins in metabolic pathways:

(1) Emphasis was laid on mRNAs coding for proteins involved in lipid metabolism: acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS), the key enzymes in fatty acid synthesis; malic enzyme (ME), the major enzyme generating NADPH required for fatty acid synthase activity, and Δ9 desaturase, which catalyses the first desaturation step of the newly synthesized, long-chain fatty acyl-CoA (C16:0, C18:0).

(2) Messenger RNAs coding for proteins taking part in the lipid secretory pathway were also considered: apoprotein B (apoB), the major apoprotein of the very low density lipoprotein (VLDL) and apoprotein A1 (apoA1), the main protein of the high density lipoprotein (HDL), also present in chicken VLDL (Hermier and Chapman, 1985).

(3) Besides these genes involved in lipid metabolism, we also analysed two other mRNAs as references: that coding for albumin, as a representative liver-specific gene, and not related to the lipid metabolism pathway, and β-actin mRNA, known as a common gene. Results demonstrated the relevance of the present hepatocyte culture system to both genetic and metabolic studies.

MATERIALS AND METHODS

Hepatocyte isolation and culture

Hepatocytes were isolated from male chickens of a high abdominal fat strain (Leclercq et al., 1980). Animals were fed ad libitum with a standard commercial diet (percentage composition by weight: protein, 20; lipids, 2.5; carbohydrates, 57.5; minerals, 6; moisture, 14) ("label" diet, Guyomarch' Cie, Saint Nolff, 56250 Elven, France), until 9 weeks of age. Hepatocytes were isolated

and cultured according to the method of Fraslin et al. (1992); briefly, animals were anaesthetized (intramuscular valium injection (83 µg/kg) followed by an intravenal natrium thiopenthal (17.5 mg/kg) and heparin (1750 i.u./kg) injection). Livers were first perfused with calcium-free HEPES buffer (10 mM HEPES, 137 mM NaCl, 3 mM KCl, 3 mM Na_2HPO_4, pH 7.5) and then by the same buffer containing collagenase (0.2 mg/ml) and $CaCl_2$ (0.6 mg/ml). Livers were excised and hepatocytes collected in Leibovitz medium (Gibco, Life Technologies SARL, BP 7050, 95051 Cergy Pontoise Cedex, France). After filtering through nylon-mesh filters (60 µm and 30 µm), cells were washed in HEPES buffer to eliminate cell fragments, erythrocytes and non-parenchymal cells. Cell viability, estimated by the trypan blue exclusion test or the well-preserved refringent shape, was always greater than 80%. Hepatocytes were plated (3×10^5 cells/cm^2) into 75 cm^2 plastic flasks (Falcon^R, AES Laboratoire, BP54, 35270 Combourg, France) in William's E medium (Gibco) supplemented with chicken serum (5%), penicillin (100 i.u./ml), streptomycin (100 µg/ml), insulin (10 µg/ml) and NaCl (30 mM). When cell attachment was achieved (within 4 or 5 hours), the medium was changed for "culture medium" (William's E medium (Gibco) containing penicillin (100 i.u./ml), streptomycin (100 µg/ml), insulin (10 µg/ml), glucagon (1 µg/ml), dexamethasone (0.4 µg/ml), transferrin (100 µg/ml), NaCl (30 mM), DMSO (1%) and bovine serum albumin (1.6 mg/ml)). Medium was renewed every day. The replaced medium was kept at −20°C for further analysis. Cells were cultured at 37°C, in a 5% CO_2 atmosphere.

RNA processing

From each perfused liver, a hepatocyte sample (22.5×10^6 cells) was immediately frozen after isolation, and kept at −80°C (quoted as freshly isolated hepatocytes and used as a reference before culture for the mRNA analyses). Otherwise, cells were collected at specified days of culture: vials were washed twice with ice-cold PBS buffer (137 mM NaCl, 3 mM KCl, 4 mM Na_2HPO_4, 1 mM K_2HPO_4). Cells were scraped off with a rubber policeman, washed twice in cold PBS buffer, pelleted (800 ***g*** for 2 min at 4°C), immediately frozen and kept at −80°C.

Total RNA was isolated from frozen cells by the LiCl-urea method (Auffray and Rougeon, 1980). Electrophoresis and northern blotting were performed as follows: 10 µg total RNA from each cell pellet was denatured in 50% formamide, 17% formaldehyde, MOPS buffer (20 mM MOPS, 5 mM sodium acetate, pH 7.0, 1 mM EDTA) for 5 min at 65°C, and run on a denaturing agarose gel in MOPS buffer. Transfer onto nylon membranes (HybondN, Amersham France, 6 Avenue du Canada, BP 144, 91944 Les Ulis Cedex A, France) was achieved by capillary blotting using 10 × SSPE buffer (1 × SSPE = 150 mM NaCl, 10 mM NaH_2PO_4, 1 mM EDTA, pH 7.4). Membranes were then air-dried and irradiated with ultraviolet light (365 nm) for 3 min.

Probes

The mRNAs were hybridized with chicken DNA probes. Full length cDNA clones were used for malic enzyme (Back et al., 1986), apoA1 (Ferrari et al., 1986) and β-actin (Cleveland et al., 1980); these were kindly provided by the authors who obtained them. For fatty acid synthase, Δ9-desaturase, albumin and apoB probes, respectively, 2, 1.2, 0.7 and 1.2 kb parts of chicken cDNA, cloned in our laboratory, were used. For acetyl-CoA carboxylase mRNA, the probe was a 600-bp insert from a genomic sequence (also cloned in our laboratory), including 255-bp of coding sequence. Inserts from these different clones were purified with Geneclean^R kits (Bio 101, PO Box 2284, La Jolla, CA 92 038 2284, USA), and then radio-labelled either by nick translation or by random priming labelling kits (Boehringer Mannheim France S.A. BP 59, 38242 Meylan Cedex, France).

Hybridization and signal analysis

RNA blots were prehybridized for 2 h and hybridized for 18 h in 50% formamide with 3 × SSPE, 0.1% SDS, 0.5% zero fat milk powder and 100 µg/ml denatured herring sperm DNA. The hybridization temperature was 42°C for long-length mRNA (apoB, fatty acid synthase and acetyl-CoA carboxylase) and 50°C for the others. Membranes were then washed four times at room temperature in 2 × SSPE, 0.5% SDS, followed by three times in 0.2 × SSPE, 0.5% SDS, at 50°C for long-length messengers and 60°C for the others. Hybridization was revealed by autoradiography at −80°C, using Kodak X-Omat AR film and intensifying screens. Hybridization signals were quantified on images digitalized using a CCD camera and the Tridyn-Trimago image analysis package (IFREMER-INFO'ROP, Z.I. du Palays, 31400 Toulouse, France). Quantification was done on 256 grey levels, after correction for the film background level.

Because of the experimental characteristics of blotting experiments, only a relative quantification of the hybridization signal could be obtained within a single blot for a single probe. So, in order to gather results from different blots, each mRNA quantified was expressed as a deviation from the mean value of the signals for this mRNA for each combination of blot and hybridization assay.

Albumin assay

Albumin in culture media was quantified by competitive ELISA assays (Fraslin et al., 1992): each microplaque well was coated with 450 ng of purified chicken albumin (Cappel, Organon teknica, BP 26, 94267 Fresnes Cedex, France), incubated 2 h at 37°C with 20 µl of culture medium and 37 pg of anti-chicken albumin rabbit immunoglobulin-G conjugated with peroxydase (Sigma Chimie SARL, L'Ile d'Abeau Chesnes, BP 701, 38070 St Quentin Fallavier Cedex, France). After addition of enzyme substrate, coloration was measured using a spectrophotometer (at 405 nm); this was inversely related to the albumin concentration in the sample.

RESULTS

Hepatocytes from three 9-week-old male chickens were cultured for up to 30 days keeping their typical parenchymal morphology, with characteristic polygonal shape and formation of bile canaliculi (Fig. 1). Other liver cell types were undetectable in daily observations.

Messenger RNA analyses in northern blot assays were carried out up to day 12 of the culture period. A set of blot hybridization results are shown in Fig. 2. Because of the weakness of some spots, only the β-actin, albumin and apoprotein A1 mRNA signals were quantified. Evolution according to the culture time is depicted in Fig. 3.

The β-actin mRNA content was low at the seeding time and remained at this level or below for the first four days of the culture period. From the 6th day its level began to increase, to reach on the 12th day a much higher level than the initial one (freshly isolated hepatocytes) (Figs 2, 3).

The albumin and apoprotein A1 mRNA contents displayed similar evolutions. Compared to the β-actin mRNA, the decrease during the four first days of the culture period was more marked. The increase, beginning at day 6, continued to levels rather similar to the initial ones at the end of the period studied (Figs 2, 3). Albumin secretion in culture medium was daily quantified, from day 2. Up to the tenth day, the albumin secreted remained at a low level, but

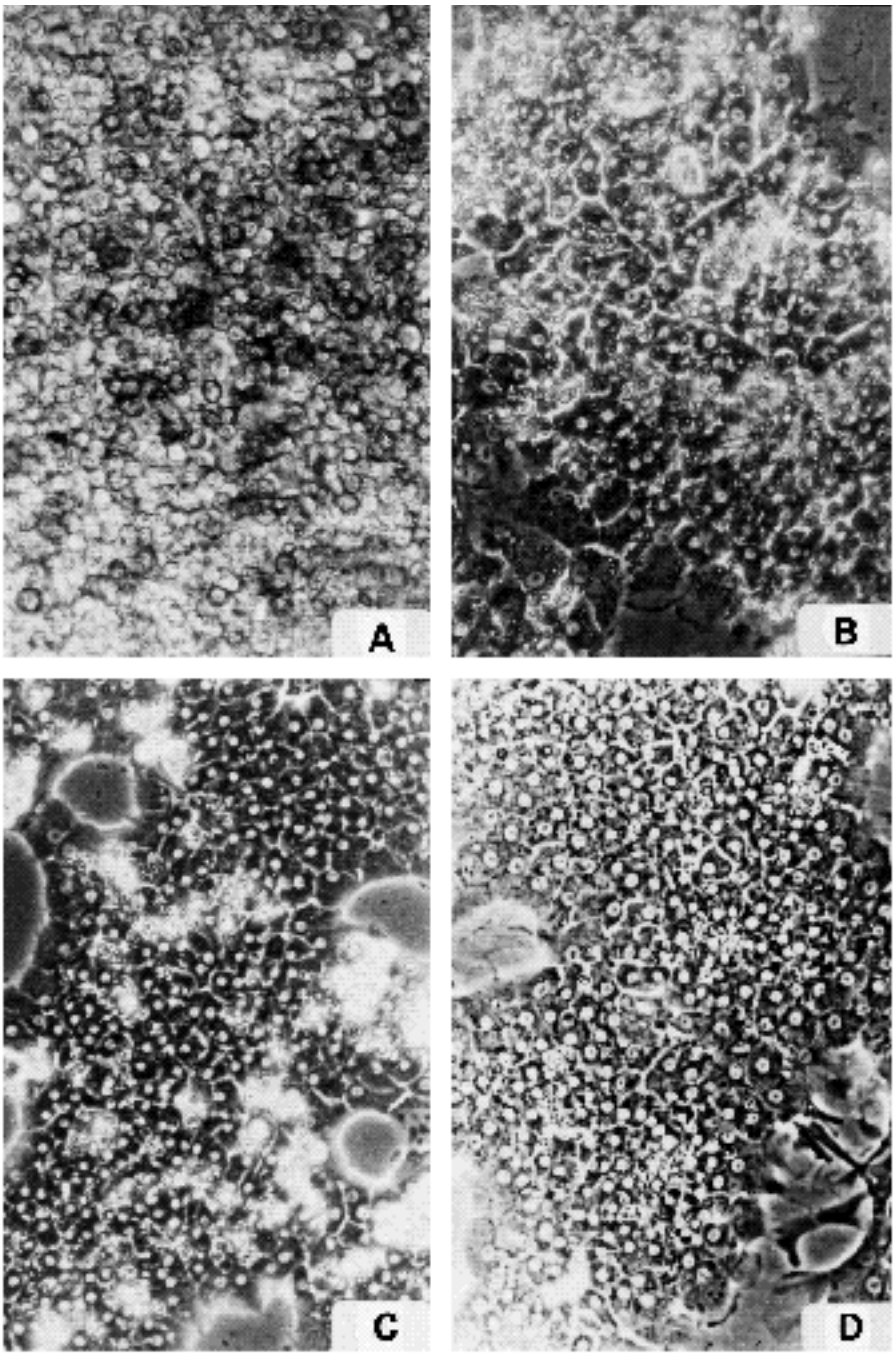

Fig. 1. Cultured chicken hepatocytes. A, seeding; B, day 4; C, day 8; D, day 27. ×200.

after the 10th day the level began to increase. The increase in secretion was also associated with a reduction of variability (Fig. 4).

Messenger RNAs coding for apoprotein B and for enzymes involved in the lipid metabolism (acetyl-CoA carboxylase, fatty acid synthase and Δ9 desaturase) were undetected in the freshly isolated hepatocytes, nor were they detected for the first days of the culture period. Only after 5 or 7 days of culture were they detected, with fatty acid synthase mRNA exhibiting a rather high level (Fig. 2).

Malic enzyme mRNA was never detected, except in RNA obtained from freshly isolated hepatocytes or after one day of culture.

DISCUSSION

In the experimental conditions described, after a 12-day culture period, hepatocytes isolated from 9-week-old chickens retained several liver-specific mRNAs, particularly those involved in the fatty acid metabolic pathway. Evolution of the mRNA levels displayed different patterns according to the genes studied. However, a general low level of expression occurred for the first days of the culture period, followed by a noticeable increase beginning at the 6th day. At the end of the period studied, some of the mRNAs were in even higher quantities than in freshly isolated cells. This phenomenon occurred for common and liver-specific genes

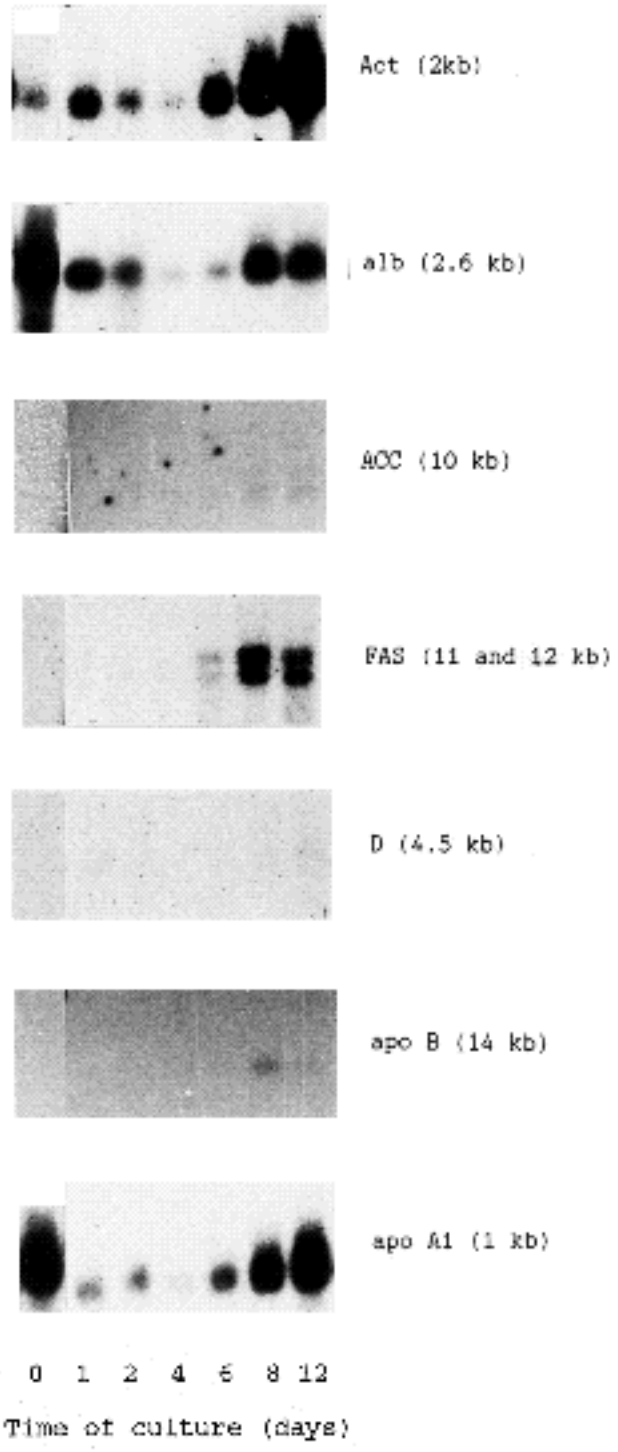

Fig. 2. RNA blot analysis from chicken hepatocytes, cultured for various times, hybridized with different probes. Act, β-actin; alb, albumin; ACC, acetyl-CoA carboxylase; FAS, fatty acid synthase; D, Δ9-desaturase; apo B, apoprotein B; apo A1, apoprotein A1. The mRNA sizes are indicated in brackets.

as well, and led us to think that transcription took place during the culture period.

In rat or mouse hepatocyte cultures it is known that mRNA levels are regulated by mechanisms rather different to those occurring in vivo (Isom et al., 1987). Moreover the

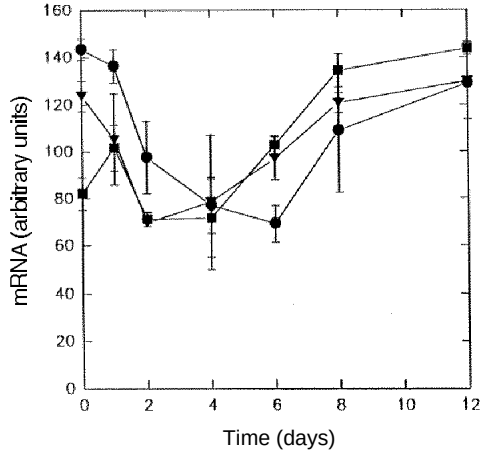

Fig. 3. Time evolution of mRNA contents (albumin ●, apoprotein A1 ▼ and β-actin ■) from chicken hepatocytes in primary culture. Each point is the mean of three measurements, each from different vials (±s.e.).

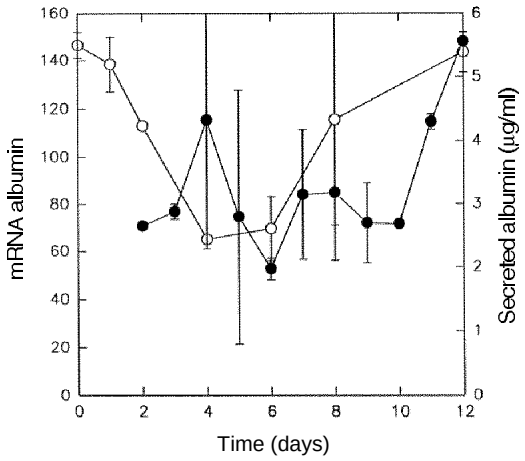

Fig. 4. Time evolution of albumin mRNA and albumin secretion by chicken hepatocytes in primary culture. Each point is the mean of two measurements, each from different vials (±s.e.).

regulation process depends on the status of the genes, according to their tissue specificity.

In vivo, transcription rates and mRNA levels for liver-specific genes are in agreement; moreover these mRNAs are less stable compared with common gene mRNAs (Derman et al., 1981). These common genes, such as actin and tubulin, have a high transcription rate but a low steady-state level (Reid et al., 1986).

In primary cultures of rat hepatocytes, the levels of common gene mRNAs increase, while those of liver-specific genes decrease throughout the culture lifespan (Clayton and Darnell, 1983). This result is achieved by an increase in the mRNA stabilization process, without change in the transcription rate for the common genes, whereas transcription is generally lower for liver-specific genes (Jefferson et al., 1984a,b; Reid et al., 1986; Isom et al., 1987).

Furthermore, the decrease which impairs the liver-specific gene transcription is achieved within a few hours of seeding (Clayton et al., 1985). The cell isolation process itself induces metabolic changes (Clayton et al., 1985),

causing the cells to leave their differentiated status and to progress to the cell cycle process (G_0 to G_1 transition) with coordinated gene regulation. Thus, immediate/early growth-associated genes are activated, whereas transcription of the differentiated-state related gene (C/EBPα) is dramatically reduced (Mischoulon et al., 1992).

Later on during the time course of culture, besides the general processes already mentioned, the actual situation may vary according to the culture system. Different factors, such as extra-cellular matrix (Fujita et al., 1987) or cell-cell interaction (Fraslin et al., 1985), are known to influence gene expression. The composition of the culture medium is another important factor influencing the regulation of gene expression (Plant et al., 1983; Wilson et al., 1986; Isom et al., 1987). Thus, mRNA stability increases in cultured rat hepatocytes, whatever the culture medium is, for the common genes, but only after hormone addition for the liver-specific genes (Jefferson et al., 1984a, 1985). Moreover, a defined component may have various effects according to the remaining medium and the precise gene studied. Insulin, for instance, has a negative effect on albumin mRNA level in rat hepatocytes cultured in a hormonally defined medium (Jefferson et al., 1984b), whereas in chicken hepatocytes it prevents the decline of this messenger, and this is achieved within 24 h without insulin (Plant et al., 1983). The effects of insulin on other defined mRNAs are totally different (Plant et al., 1983; Jefferson et al., 1984a; Isom et al., 1987).

The chicken hepatocyte culture medium used here is characterized by the presence of both DMSO and a high level of hormones, compared to other media that have been used for chicken hepatocyte culture (Tarlow et al., 1977; Wilson et al., 1986; Plant et al., 1983; Stapleton et al., 1990).

In rat hepatocytes cultured in a DMSO-supplemented, hormonally defined medium, a low level of mRNA synthesis occurs throughout the culture lifespan (after the 6th day), with an increased stabilization process (Isom et al., 1987). In chicken hepatocytes cultured under similar conditions as those described here, for a much longer period, albumin secretion reached a steady-state level after about 10 days of culture and throughout the remaining culture lifespan (Fraslin et al., 1992). The results reported here deal with the beginning of the culture period, but they seem to be in accordance with the evolution described later in the culture lifespan. A well differentiated metabolic state could be achieved within about ten days of culture.

As for the hormone medium composition, insulin and glucagon are known for their antagonistic effects on lipogenesis. In chick embryo hepatocyte culture, insulin added with triiodothyronine increases the rate of fatty acid synthase and malic enzyme gene transcription, whereas added alone it has no effect. These hormones have also an effect on the mRNA stabilization process (Back et al., 1986; Stapleton et al., 1990). In both cases, glucagon greatly reduces the mRNA persistency. In the present experimental conditions, the insulin and glucagon amounts are much higher. This might explain why the malic enzyme mRNA, which is very susceptible to glucagon concentration, was not detectable after the second day of culture. As for the other mRNAs, the high insulin level might have a positive effect on both transcription and mRNA stabilization processes.

Therefore, in chicken hepatocytes under our experimental conditions, the use of a hormone-rich medium seems to have favoured a stabilization process. However, stabilization alone does not explain the level of the liver-specific mRNA observed in the last days of the experimental period: the estimated half-lives for various mRNAs in cultured hepatocytes from different species are in the range of hours, and up to one day (Plant et al., 1983; Goodridge and Adelman, 1986; Back et al., 1986; Pullinger et al., 1989). So, after a few days, it is thought that the observed mRNA levels are due to de novo synthesis.

Other signs reinforce this assessment. These mRNAs appear to have been particularly sensitive to the stress of cell isolation: they were undetectable either in freshly isolated hepatocytes or after 1 or 2 days of culture, although they are easily detected in vivo (Douaire et al., 1992). So it appears that the mRNAs present in vivo have been dramatically impaired by cell isolation process. A similar situation has been already described for C/EBPα mRNA in rat cultured hepatocytes (Mischoulon et al., 1992). Thus, it could be considered that the mRNAs observed after day 6 of the culture result, at least in part, from transcription processes occurring during the culture period. The evolution of albumin secretion, following that of the mRNA, also suggests that regulation does not occur at a post-transcriptional level. However we cannot rule out that mRNA stabilization also occurs and has a role in determining the actual mRNA levels.

The results presented here show that chicken hepatocytes cultured in a suitable medium are able to recover differentiated status after a few days, as illustrated by albumin secretion and the appearance of several specific mRNAs. It seems that this biological pattern occurs every time hepatocytes are cultured in a medium that allows their maintenance for a period of several weeks (Isom et al., 1987; Mann et al., 1989). For a few days following the culture establishment, hepatocytes are degenerating or recovering from the effects of cell isolation before reaching a differentiated biological steady state. The time necessary to overcome what we can call the culture stress is not to be considered as a loss of time. Indeed it allows the cells "to forget" their in vivo history. It is usually admitted that the complex signal network which regulates gene expression and protein synthesis disappears within 2 or 3 days after stimulation removal (Tarlow et al., 1977; Guillouzo, 1986). So when the cultured hepatocytes have recovered their differentiated status they are biologically free to respond to any new biological situation provided by the culture conditions.

The culture of chicken embryo hepatocytes is commonly used in metabolic and genetic studies. The present results were obtained on cultured hepatocytes isolated from growing chickens. This system provides two advantages: (1) processing the liver of one 9-week-old chicken provides 5×10^8 to 10^9 cells (Fraslin et al., 1992), allowing the comparison of various experimental conditions in a single experiment; (2) the collected hepatocytes have been fully developed in vivo. All regulatory systems have been established and just need to be initiated in culture conditions. So it may be assumed that their ability to respond to various experimental situations does not suffer from the particular situa-

tion which might occur in embryo or chick cells (Takai et al., 1988). Such a system would be particularly useful in the study of genetic or metabolic regulations that occur in the chicken. Moreover, human and chicken lipid metabolism is very similar: liver plays a greater role than adipose tissue for fatty acid synthesis, whereas the situation is reverse in rodents (Griminger, 1976). Thus this chicken hepatocyte culture system could be suitable for human metabolism studies as well.

The authors thank D. Chauvel and A. Leduc for their skilful technical assistance. They are grateful to Drs D. Cleveland, A. Goodridge and S. Ferrari for their gifts of plasmids. They acknowledge Dr R. S. Powell for his thoughtful criticism of the manuscript. This investigation was supported by the Institut National de la Recherche Agronomique, and research grants from the Ministry of Research and Technology and the Regional Council of Brittany.

REFERENCES

Auffray, C. and Rougeon, F. (1980). Purification of mouse immunoglobulin heavy-chain messenger RNAs from total myeloma tumor RNA. *Eur. J. Biochem.* **107**, 303-314.

Back, D. W., Wilson, S. B., Morris, S. M. Jr and Goodridge, A. G. (1986). Hormonal regulation of lipogenic enzymes in chick embryo hepatocytes in culture. *J. Biol. Chem.* **261**, 12555-12561.

Boehm, K. D., Hood, R. L. and Ilan, J. (1988). Induction of Vitellogenin in primary monolayer cultures of cockerel hepatocytes. *Proc. Nat. Acad. Sci. USA* **85**, 3450-3454.

Clayton, D. F. and Darnell, J. E. Jr. (1983). Changes in liver-specific compared to common gene transcription during primary culture of mouse hepatocytes. *Mol. Cell Biol.* **3**, 1552-1561.

Clayton, D. F., Harrelson, A. L. and Darnell, J. E. Jr. (1985). Dependence of liver-specific transcription on tissue organization. *Mol. Cell Biol.* **5,** 2623-2632.

Cleveland, D. W., Lopata, M. A., MacDonald, R. J., Cowan, N. J., Rutter, W. J. and Kirschner, M. W. (1980). Number and evolutionary conservation of α- and β-tubulin and cytoplasmic β- and γ-actin genes using specific cloned cDNA probes. *Cell* **20**, 95-105.

Cupo, M. A. and Cartwright, A. L. (1989). Lipid synthesis and lipoprotein secretion by chick liver cells in culture; influence of growth hormone and insulin-like growth factor-I. *Comp. Biochem. Physiol.* **94B**, 355-360.

Derman, E., Krauter, K., Walling, L., Weinberger, C., Ray, M. and Darnell, J. E. Jr. (1981). Transcriptional control in the production of liver-specific mRNAS. *Cell* **23**, 731-739.

Douaire, M., Le Fur, N., El Khadir-Mounier, C., Langlois, P., Flamant, F. and Mallard, J. (1992). Identifying genes involved in fatness genetic variability in the growing chicken. *Poultry Sci.* **71**, 1911-1920.

Ferrari, S., Drusiani, E., Calandra, S. and Tarugi, P. (1986). Isolation of a cDNA clone for chick intestinal apolipoprotein AI (Apo-AI) and its use for detecting apo-AI mRNA expression in several chick tissues. *Gene* **42**, 209-214.

Fraslin J.-M., Kneip, B., Vaulont, S., Glaise, D., Munnich, A. and Guguen-Guillouzo, C. (1985). Dependence of hepatocyte-specific gene expression on cell-cell interactions in primary culture. *EMBO J.* **4**, 2487-2491.

Fraslin, J.-M., Touquette, L., Douaire, M., Menezo, Y.,Guillemot, J.-C. and Mallard, J. (1992). Isolation and long term maintenance of differentiated adult chicken hepatocytes in primary culture. *In Vitro Cell. Dev. Biol.* **28A**, 615-620.

Fujita, M., Spray, D. C., Choi, H., Saez, J. C., Watanabe, T., Rosenberg, L. C., Hertzberg, E. L. and Reid, L. M. (1987). Glycosaminoglycans and proteoglycans induce gap junction expression and restore transcription of tissue-specific mRNAs in primary liver cultures. *Hepatology* **7**, 1S-9S.

Goodridge, A. G. and Adelman, T. G. (1976). Regulation of malic enzyme synthesis by insulin, triiodothyronine and glucagon in liver cells in culture. *J. Biol. Chem.* **251**, 3027-3032.

Goodridge, A. G., Garay, A. and Silpananta, P. (1974). Regulation of lipogenesis and the total activities of lipogenic enzymes in a primary culture of hepatocytes from prenatal and early postnatal chicks. *J. Biol. Chem.* **249**, 1469-1475.

Griminger, P. (1976). Lipid metabolism. In *Avian Physiology* (ed. Sturkie, P. D.) pp 252-262. Springer-Verlag: New York.

Guillouzo, A. (1986). Plasma protein production by cultured adult hepatocytes. In *Isolated and Cultured Hepatocytes* (ed. A. Guillouzo and Ch. Guguen-Guillouzo), pp. 155-170. John Libbey Eurotext Ltd., London.

Hermier, D. and Chapman, M. J. (1985). Lipoprotéines plasmatiques et engraissement: Description d'un modèle chez le poulet domestique, *Gallus domesticus.Reprod. Nutr. Dev.* **25(1B)**, 235-241.

Isom, H., Georgoff, I., Salditt-Georgieff, M. and Darnell, J. E. Jr. (1987). Persistence of liver-specific messenger RNA in cultured hepatocytes: different regulatory events for different genes. *J. Cell Biol.* **105**, 2877-2885.

Jefferson, D. M., Clayton, D. F., Darnell, J. E. Jr and Reid, L. M. (1984a). Post-transcriptional modulation of gene expression in cultured rat hepatocytes. *Mol. Cell Biol.* **4**, 1929-1934.

Jefferson, D. M., Liverpool, C. and Reid, L. M. (1984b). Hormonal modulation of steady state levels of specific mRNAs in primary cultures of adult rat hepatocytes. *J. Cell Biol.* **99,** 201a.

Jefferson, D. M., Reid, L. M., Giambrone, M.-A., Shafritz, D. A. and Zern, M. A. (1985). Effects of dexamethasone on albumin and collagen expression in primary cultures of adult rat hepatocytes. *Hepatology* **5**, 14-20.

Leclercq, B., Blum, J. C. and Boyer, J. P. (1980). Selecting broilers for low or high abdominal fat: Initial observations. *Bri. Poult. Sci.* **21**, 107-113.

Liang, T. J. and Grieninger, G. (1981). Direct effect of insulin on the synthesis of specific plasma proteins: Biphasic response of hepatocytes cultured in serum- and hormone-free medium. *Proc. Nat. Acad. Sci. USA* **78,** 6972-6976.

Mann, D., Bartley, W., Strain, A. J. and Bailey, E. (1989). Regulation of malic enzyme expression in hepatocytes in culture. *Biochem. Soc. Trans.* **18**, 652.

Mischoulon, D., Rana, B., Bucher, N. L. R. and Farmer, S. R. (1992). Growth-dependent inhibition of CCAAT enhancer-binding protein (C/EBPα) gene expression during hepatocyte proliferation in the regenerating liver and in culture. *Mol. Cell Biol.* **12**, 2553-2560.

Plant, P. W., Deeley, R. G. and Grieninger, G. (1983). Selective block of albumin gene expression in chick embryo hepatocytes cultured without hormones and its partial reversal by insulin. *J. Biol. Chem.* **258**, 15355-15360.

Pullinger, C. R., North, J. D., Teng, B., Rifici, V. A., Ronhild de Brito, A. E. and Scott, J. (1989). The apolipoprotein B gene is constitutively expressed in HepG2 cells: Regulation of secretion by oleic acid, albumin, and insulin, and measurement of the mRNA half-life. *J. Lipid Res.* **30**, 1065-1077.

Reid, L. M., Narita, M., Fujita, M., Murray, Z., Liverpool, C. and Rosenberg, L. (1986). Matrix and hormonal regulation of differentiation in liver cultures. In *Isolated and Cultured Hepatocytes* (ed. A. Guillouzo and Ch. Guguen-Guillouzo), pp. 225-258. John Libbey Eurotext Ltd., London.

Stapleton, S. R., Mitchell, D. A., Salati, L. M. and Goodridge, A. G. (1990). Triiodothyronine stimulates transcription of the fatty acid synthase gene in chick embryo hepatocytes in culture. *J. Biol. Chem.* **265**, 18442-18446.

Takai, T., Saito, Y., Yamamoto, K. and Tanabe, T. (1988). Developmental changes of the content of acetyl-CoA carboxylase mRNA in chicken liver. *Arch. Biochem. Biophys.* **266**, 313-318.

Tarlow, D. M., Watkins, P. A., Reed, R. E., Miller, R. S., Zwergel, E. E. and Lane, M. D. (1977). Lipogenesis and the synthesis and secretion of Very Low Density Lipoprotein by avian liver cells in nonproliferating monolayer culture. *J. Cell Biol.* **73**, 332-353.

Vance, J. E. and Russell, J. C. (1990). Hypersecretion of VLDL, but not HDL, by hepatocytes from the JCR:LA-corpulent rat. *J. Lipid Res.* **31**, 1491-1501.

Wilson, S. B., Back, D. W., Morris, S. M., Jr., Swierczynski, J. and Goodridge, A. G. (1986). Hormonal regulation of lipogenic enzymes in chick embryo hepatocytes in culture. *J. Biol. Chem.* **261**, 15179-15182.

(Received 22 April 1992 - Accepted, in revised form, 30 November 1992)