DNA AND ALPHA-FETOPROTEIN SYNTHESIS IN A MONOLAYER CULTURE OF MOUSE HEPATOCYTES

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UDC 616.36-003.93-07:[616.36-008.939.633.2+616.36-008.939.6-053.13]-092.4

KEY WORDS: DNA synthesis, alpha-fetoprotein, hepatocytes, monolayer culture, dextran sulfate.

Numerous investigations of the induction of synthesis of the embryonic serum protein alpha-fetoprotein (AFP) by hepatocytes of the adult mouse liver have shown that in most cases synthesis begins in a proliferating cell population. AFP is produced by the regenerating liver after carbon tetrachloride or paracetamol poisoning or after hepatectomy [2], and AFP synthesis is observed in proliferating hepatocytes in the liver of mature mice on the 3rd-4th day of monolayer culture [3]. However, autoradiographic studies on a model of regeneration of the liver after carbon tetrachloride poisoning have shown that the processes of DNA and AFP synthesis may diverge. Only 10-15% of DNA synthesized by hepatocytes went on renewing AFP production and, conversely, from 18 to 94% of AFP-containing cells did not contain ^{3}H-thymidine [7]. On another model, mainly minimal mechanical liver damage, induction of AFP was observed in the layer of hepatocytes bordering on the incision, in the absence of regeneration and DNA synthesis [6].

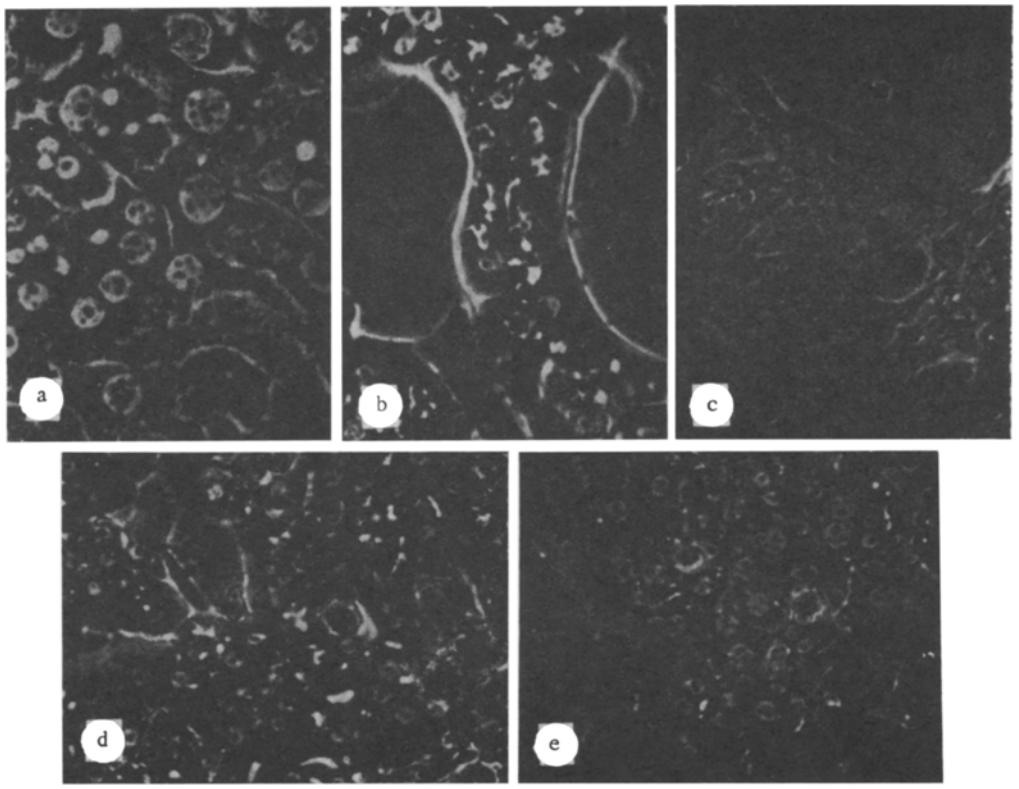

Fig. 1. Morphology of hepatocytes on 4th day of culture. a) Control culture; b-e) incubation of hepatocytes throughout period of culture (b — with DS, c — with DS + EGF, d — with DMSO + EGF). Phase contrast.

Institute of Immunology, Ministry of Health of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR, A. D. Ado.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 106, No. 9, pp. 350-353, September, 1988. Original article submitted May 12, 1987.

 0007-4888/88/0009-1328$12.50

TABLE 1. Percentage of Cells Incorporating ^{3}H-Thymidine (^{3}H) and Containing AFP on 4th Day of Culture of Mouse Hepatocytes in Control Cultures and in Cell Cultures Incubated in the Presence of DS, DMSO, DS + EGF, and DMSO + EGF

Expt. No.	Control			DS		DMSO		DS + EGF		DMSO + EGF	
	^{3}H	AFP		^{3}H	AFP	^{3}H	AFP	^{3}H	AFP	^{3}H	AFP
		total	containing ^{3}H								
1	63	25	71	0	0	10	0	30	5	35	0
2	18	58	63	0	0	3	0	10	2	20	0
3	20	5	25	0	0	7	0	15	2	35	0
$M \pm m$	33,6±15,3	11,9±6,8	53, ±15,6	0	0	6,6±2,4	0	15±6,8	3±1,0	30±5,1	0

Legend. Time of incubation of liver in collagenase solution during isolation of hepatocytes was 10 min.

TABLE 2. Percentage of Cells Incorporating ^{3}H-Thymidine (^{3}H) and Containg AFP on 4th Day of Culture of Mouse Hepatocytes in Control Cultures and in Cell Cultures Incubated with DS

Expt. No.	Control			DS		
	^{3}H	AFP		^{3}H	AFP	
		total	containing ^{3}H		total	containing ^{3}H
4	20	14	10	4,5	1,8	30
5	72	30	70	33	5	33
6	40	17	40	4	1	0
7	27	20	20	14	4	10
8	38	30	30	14	6	15
$M \pm m$	39,4±3,88	22,2±3,04	34±11,4	13,9±5,5	3,56±0,95	17,6±6,3

Legend. Time of incubation of liver in collagenase solution during isolation of hepatocytes was 20-30 min.

The relationship between proliferation and AFP synthesis by hepatocytes in monolayer culture is not yet clear. To shed light on this problem it was decided to study the effect of various substances on hepatocytes. Dextran sulfate (DS) which, as the writer showed previously [4], inhibits AFP synthesis without affecting the spectrum of the other serum proteins, dimethyl sulfoxide (DMSO) – an agent inducing differentiation in different cell systems [9], and also epidermal growth factor (EGF), which stimulates hepatocyte proliferation.

The simultaneous influence of DS on migration, proliferation, secretory activity, and intercellular interaction was first demonstrated for lymphocytes [5], and later for other cells also [8, 10]. However, it is not yet clear how all these phenomena are linked together. A proliferating hepatocyte culture, synthesizing AFP, is a convenient system for such an investigation.

EXPERIMENTAL METHOD

A suspension of hepatocytes was obtained from the liver of adult female C57BL/6 mice and cultured by the method described in [3]. After perfusion of the liver, it was additionally incubated in a 0.02% solution of collagenase for 10 or 20-30 min. The cells were cultured in Petri dishes 40 mm in diameter, previously covered with 1% gelatin solution. Into each dish 400,000-500,000 cells were introduced. On the first day of culture, after a change of medium, a series of agents was added to the culture: DS ("Pharmacia," Sweden), in a concentration of 100 µg/ml, DMSO (2%), and EGF ("Sigma," USA), in a concentration of 50 ng/ml throughout the period of culture.

The localization of AFP in the hepatocyte cultures was studied by the indirect immunoperioxidase method described in [3], on the 4th day of hepatocyte culture. ^{3}H-thymidine was added to the dishes on the 3rd day of culture for 18-20 h in a concentration of 2 mCi/ml. In some experiments ^{3}H-thymidine was added on the 1st day of culture for 70 h, knowingly before the beginning of cell proliferation. After fixation, AFP was detected immunohisto-

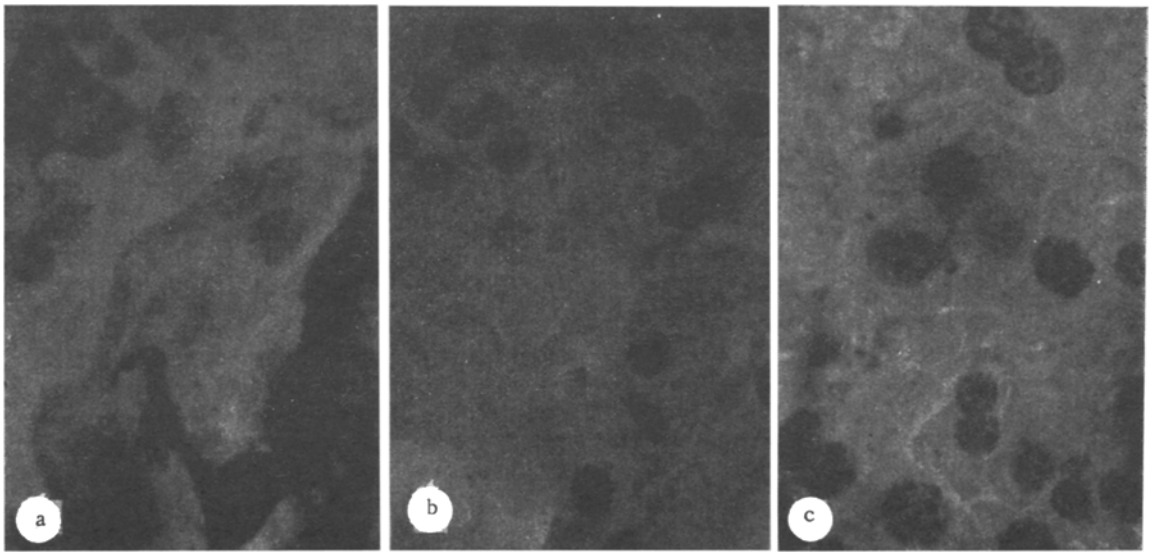

Fig. 2. Combined detection of incorporation of ^{3}H-thymidine by hepatocytes and AFP synthesis. a) Control culture; b) culture of cells incubated with DS + EGF; c) culture of cells incubated with DMSO + EGF. Culture of hepatocytes on 4th day. Arrows indicate nuclei incorporating ^{3}H-thymidine, double arrow — AFP-containing cells. Immunoperoxide staining.

chemically, and the dishes were then coated with emulsion. The autoradiographs were developed after 4-5 days in the usual way. The preparations were then stained with hematoxylin and the labeled nuclei counted among no fewer than 600 nuclei in total.

EXPERIMENTAL RESULTS

As was shown previously [3], 18-20 h after adhesion the isolated hepatocytes spread out to form a monolayer of polygonal cells. The cells preserved their regular shape for 2 days, after which an irreversible evolution of the culture began: the cells became elongated and fibroblast-like in shape, with numerous outgrowths; some cells formed a network above the layer of hepatocytes (Fig. 1a). Addition of DS retarded the spreading of the cells and on the first day led to the formation of monolayer trabecular structures composed of polygonal cells with pursed, thickened edges (Fig. 1b). The structures were preserved throughout the period of culture. On addition of both DS and EGF to the culture, the hepatocytes in the trabeculae were well spread out, and the shape of the cells changed from polygonal to elongated (Fig. 1c).

Incubation of the liver in a solution of collagenase for 20-30 min during isolation of the hepatocytes abolished the effect of DS on preservation of the regular polygonal shape of the cells. The formation of trabecular bands under the influence of DS was significantly modified. Bands were formed later — after 48 h of culture; they consisted of elongated cells and a surface network of hepatocytes. In the presence of DMSO, 40-48 h after its addition to the culture the cell monolayer was divided by lacunae, and monolayer bands or sheets of regularly packed polygonal cells were formed (Fig. 1b). The formation of characteristic bile capillaries was observed. On the addition of DMSO together with EGF, the shape of the cells was unchanged, the cell population was definitely increased, and a dense monolayer separated by small lacunae was formed (Fig. 1e).

In the control cultures labeled nuclei were found on the 3rd day, coinciding with the beginning of AFP production by the cells and a change in cell morphology (Fig. 2a). The percentage of labeled nuclei varied in different experiments from 20 to 72, and the percentage of AFC-containing cells in these same experiments varied from 5 to 30 (Tables 1 and 2). Independently of the time of incubation in collagenase solution, moderately strong correlation was observed ($r = 0.69$) between the intensity of proliferation and the number of AFP-containing cells (Tables 1 and 2). Of the AFP-containing cells 10-70% incorporated ^{3}H-thymidine, whereas the rest synthesized AFP without entering on the S phase of the cycle. Cell proliferation is thus not an essential condition for AFP synthesis, as previous investigations also have shown [3]. Cells forming a network above the layer of hepatocytes and having no contact with the substrate in most cases synthesized AFP.

Addition of DS to a culture of cells incubated during isolation in a solution of collagenase for 10 min caused complete suppression of AFP and DNA synthesis. Addition of both DS and EGF led to resumption of DNA synthesis, but AFP synthesis was resumed only in a small percentage of cells (Table 1). Total inhibition of AFP synthesis by the cells also took place on the addition of DMSO to the cultures. Under these circumstances there was also a marked increase in incorporation of ^{3}H-thymidine (Table 1). After addition of both DMSO and EGF to the cultures DNA synthesis was restored almost to its initial level, but induction of AFP was not observed.

During incubation of the liver in collagenase solution for 20-30 min DS did not inhibit AFP and DNA synthesis by the cells completely, but only to a certain and different degree. Reduction of the percentage of AFP-containing cells (Table 2) corresponded to the reduction in the percentage of cells incorporating ^{3}H-thymidine ($r = 0.56$).

Thus the present investigation confirmed the presence of correlation between DNA and AFP synthesis in a monolayer culture of hepatocytes [3]: coincidence between time of induction of synthesis, moderatly strong correlation between the parameters in the control cultures and when depressed by DS, and simultaneous inhibition under the influence of DS or DMSO.

However, under the influence of DMSO and EGF restoration of proliferation to the control level was not accompanied by induction of AFP. This is evidence that processes of DNA and AFP synthesis in a monolayer hepatocyte culture may be uncoupled. Inhibition of AFP synthesis by DS, DMSO, or DMSO together with EGF corresponds to maintenance of the regular polygonal shape of the hepatocytes and the formation of a trabecular structural organization. This rule is perhaps in agreement with the hypothesis put forward to explain regulation of AFP synthesis by contact interactions between hepatocytes [1].

It is an interesting fact that long incubation of the liver in collagenase solution abolishes the effect of DS on hepatocyte morphology and on DNA and AFP synthesis. This may be evidence that the participation of certain membrane components of the cell, which are lost on proteolytic degradation, is essential in the mechanism of action of DS.

The author is grateful to R. M. Khaitov, D. I. Abelev, and R. I. Ataullakhanov for guidance during the work and to A. S. Gleiberman for constant advice and practical help with the research.

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