Acta Medica Okayama

Volume 42, *Issue* 1 1988 *Article* 7

FEBRUARY 1988

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Abstract

Upon addition of epidermal growth factor (EGF, 0.1 microgram/ml) and insulin (0.1 microM), adult rat hepatocytes proliferated and increased 120-134% in number in serum-free primary culture. However, in the absence of the growth factors, hepatocytes decreased in number with time. The average albumin secretion per cell was much lower in the proliferating cultures than in the non-proliferating cultures. The results suggest that albumin production in hepatocytes decreases during cell proliferation.

KEYWORDS: serum-free primary culture, epidermal growth factor, insulin, hepatocyte proliferation, albumin secretion

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*PMID: 3284281 [PubMed - indexed for MEDLINE]

Acta Med Okayama 42 (1) 41-43 (1988)

— Brief Note—

Decreased Albumin Secretion in Serum-Free Primary Cultures of Adult Rat Hepatocytes during Proliferation Induced by Epidermal Growth Factor and Insulin

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Upon addition of epidermal growth factor (EGF, 0.1 μg/ml) and insulin (0.1 μM), adult rat hepatocytes proliferated and increased 120-134% in number in serum-free primary culture. However, in the absence of the growth factors, hepatocytes decreased in number with time. The average albumin secretion per cell was much lower in the proliferating cultures than in the non-proliferating cultures. The results suggest that albumin production in hepatocytes decreases during cell proliferation.

Key words : serum-free primary culture, epidermal growth factor, insulin, hepatocyte proliferation, albumin secretion

Adult rat hepatocytes in primary culture retain various liver-specific functions at *in vivo* levels (1, 2). Moreover, it has become possible to induce proliferation of primary cultured hepatocytes by addition of growth factors, such as epidermal growth factor (EGF) and insulin (3-6). Therefore, primary culture of adult rat hepatocytes is a suitable model for studying the interaction between cell proliferation and expression of liver-specific functions. In the present study, we investigated what relation exists between hepatocyte proliferation and albumin secretion, a typical liver-specific function.

As reported previously (2), hepatocytes, having an initial viability of 85% to 90% as measured by trypan blue exclusion, were isolated from normal Donryu male rats (3-month-old) by liver perfusion with type I collagenase (Sigma Chemical Co., St. Louis, Mo., USA). The isolated hepatocytes were suspended in DM-160 medium (Kyokuto Pharmaceutical Industrial Co., Ltd., Tokyo, Japan) or Williams' medium E (WE, Flow Laboratories, Irvine, UK), inoculated at a density of 8×10^5 cells per 4-ml of medium per 60-mm Falcon plastic dish, and cultured in a humidified atmosphere of 5% CO_2 and 95% air at 37℃. To enhance the attachment efficiency of inoculated hepatocytes, dexamethasone-21-disodium phosphate (10 μM, Nippon Merck Banyu Co., Tokyo, Japan) and insulin (1.6 μM, Sigma Chemical Co.) were added to the cultures during the first 20 h (1). Twenty hours after inoculation, the culture medium was replaced with DM-160 or WE, with or without EGF (0.1 μg/ml, Sigma Chemical Co.) and insulin (0.1 μM). The culture medium was renewed 10 h and 24 h after the first renewal. The contents

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of albumin in the spent culture media were determined by a radioimmunoassay as reported previously (7). At the indicated times, the cultured hepatocytes were detached from dishes by treatment with 0.1% trypsin (1 : 250, Difco, Detroit, Mich., USA) in Ca^{2+}-and Mg^{2+}-free phosphate-buffered saline containing 0.02% ethylenediaminetetraacetic acid (Sigma Chemical Co.) as reported previously (2), and the numbers of viable hepatocytes were determined by trypan blue exclusion in a hemocytometer.

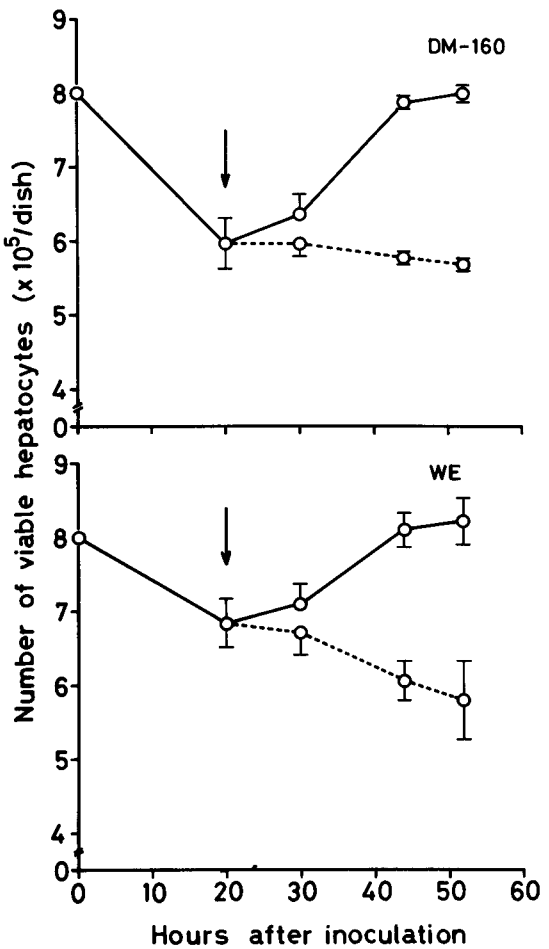

Fig. 1 Changes in the number of adult rat hepatocytes in serum-free primary culture. Freshly isolated hepatocytes were inoculated at an initial density of 8×10^5 cells per 60-mm dish. Twenty hours after inoculation (arrows), 0.1 μg/ml of epidermal growth factor and 0.1 μM of insulin were added to the cultures. The solid and dotted lines indicate the presence and absence of the growth factors, respectively. The hepatocyte numbers are expressed as the mean of three dishes. The vertical lines show the SD.

Since DM-160 and WE allowed hepatocytes to attach to plastic dishes at the highest efficiency among ten synthetic media tested, these two media were used in the present study (data not shown). Freshly isolated hepatocytes in DM-160 and WE attached firmly to plastic dishes, at attachment efficiencies of 75% and 86%, respectively, 20 h after inoculation (Fig. 1). At this time, EGF and insulin were added to the cultures. After addition of the growth factors, hepatocytes in DM-160 and WE increased up to 134% and 120% in number, respectively (Fig. 1). Growth rates in both DM-160 and WE were the highest from 10 h to 24 h after addition of the growth factors. On the other hand, in the absence of the growth factors, hepatocytes decreased gradually in number in both DM-160 and WE. The average albumin secretion per cell was much lower in the proliferating cultures than in the non-proliferating cultures (Table 1).

Table 1 Albumin secretion by proliferating and non-proliferating hepatocytes in serum-free primary culture

Hours after cell inoculation[b]	Albumin secretion (ng/10^5 cells/h)[a]			
	DM-160 medium		Williams' medium E	
	NP[c]	P[d]	NP[c]	P[d]
0-20	326±36	—	513±7	—
20-30	384±19	315±39	651±28	557±37
30-44	707±73	322±30	882±52	609±150
44-52	574±35	419±17	701±67	689±66

a: Albumin contents in the spent culture media were measured by a radioimmunoassay. Results are expressed as the mean±SD of three dishes.

b: Isolated hepatocytes were inoculated at an initial density of 8×10^5 cells per 60-mm dish. Twenty hours after inoculation, 0.1 μg/ml of epidermal growth factor (EGF) and 0.1 μM of insulin were added to the cultures.

c: Non-proliferating cultures without EGF and insulin.

d: Proliferating cultures with EGF and insulin.

The most remarkable difference was observed from 10 h to 24 h after addition of the growth factors, when the cell growth rates were the highest (Fig. 1 and Table 1). The results suggest that albumin production in hepatocytes decreases during cell proliferation.

Shreiber *et al.* (8) have reported that the albumin concentration in the serum of partially hepatectomized rats decreased dramatically to 59% of that of normal rats 4 days after the operation. Tuczek *et al.* (9) have observed immunohistochemically and autoradiographically that the albumin content is much lower in dividing hepatocytes than in non-dividing ones in regenerating livers of mice having undergone partial hepatectomy. Furthermore, a prominent reduction of albumin mRNA has been observed 50 h after partial hepatectomy, compared to untreated and sham-operated rats (10). The results of the present study are consistent with these *in vivo* findings. It has also been reported that other hepatocyte-specific characters, such as the induction of tyrosine aminotransferase and serine dehydratase, were reduced by the stimulation of the growth of hepatocytes in primary culture (11). Cell division and expression of specific, differentiated functions seem to have a mutually exclusive relationship.

The serum-free primary culture of adult rat hepatocytes described in the present study seems to be a suitable model for further investigation of the mechanism through which hepatocyte proliferation and expression of specific, differentiated functions are regulated.

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Received July 14, 1987; accepted October 27, 1987