ALBUMIN SYNTHESIS BY LIVER PARENCHYMAL CELLS ISOLATED FROM YOUNG, ADULT AND OLD RATS

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SUMMARY: Viable isolated parenchymal cells were incubated in a modified Waymouth medium under an oxygen tension of 30×10^3 Pa at pH 7.8. Under these conditions, hepatocytes from 3-month-old rats synthesized 5.8 µg albumin/h/10^6 cells. This value nearly equals the synthesizing capacity of intact liver tissue and is the highest activity reported so far for isolated hepatocytes. Parenchymal cells isolated from 36-month-old rats synthesized more albumin as compared to cells from 3-month-old rats. The albumin synthesizing capacity of cells isolated from 12-month-old rats was less than that of cells from 3-month-old rats.

INTRODUCTION:

Isolated liver parenchymal cells constitute a model system which is especially suitable for studying the cellular phenomena underlying the decline in liver function with age (1). Since albumin synthesis is a specific liver function performed by the parenchymal cells *in vivo*, the functional capacity of isolated cells may be judged from their competence to synthesize albumin. The first objective of this study was to develop a method for gathering baseline data on the albumin synthetic capacity of cells isolated from young rats. An important prerequisite for the method was that the capacity of the isolated cells to synthesize albumin should approach the *in vivo* synthesizing capacity of the liver, so that possible small changes due to aging could be easily detected. The second objective was to measure the amount of albumin synthesized by hepatocytes isolated from young, adult and old rats under optimal conditions.

MATERIALS AND METHODS:

Inbred female WAG/Rij rats of three age groups, young (3 months, 151 ± 9 g), adult (12 months, 199 ± 12 g) and old (36 months, 236 ± 39 g), were used. The conditions under which the rats were maintained have been previously described (1). The cells were isolated by perfusion and incubation of the liver with the enzymes collagenase and hyaluronidase as described in detail earlier (1,2).

The following method was used for determining the amount of albumin synthesized by the cells. A known number of cells in 4 ml of medium were incubated with a mixture of amino acids at 37°C in an atmosphere of 95% O_2 and 5% CO_2 for various time intervals. After incubation, the cells were destroyed by addition of Triton-X-100 in a final concentration of 1.3% to release the albumin from the cells. The samples were dialysed against distilled water for two days to remove the Triton-X-100; thereafter, the albumin was concentrated by lyophilization. The final residue was dissolved in 50 µl 0.3 M phosphate buffered saline (PBS) per 10^6 cells originally present, if the cells had been isolated from a 3-month-old rat. Since the average cell size increases with age (1), the final residue of the cells isolated from older rats was dissolved in 100 µl PBS per 10^6 cells originally present. The amount of albumin was measured by the radial immundiffusion method of Mancini (3) in a combined modification of Kalff (4) and Radl et al. (5). With this method, the albumin was precipitated by a specific antiserum to rat albumin (RARa/Alb, Nordic Diagnostics). Antisera were tested in the Ouchterlony plate. The antisera against rat serum albumin formed a single precipitation band with purified rat serum albumin and rat serum. Ten different dilutions of a reference serum (rat serum albumin, Nordic Diagnostics), and duplicate samples of the dissolved residue obtained with three different dilutions of the original cell suspension were included in each plate.

The amount of protein per 10^6 cells was determined by the method of Lowry et al. (6).

RESULTS AND DISCUSSION:

After testing various media and amino acid concentrations, incubation of the isolated cells in a modified Waymouth MB 752/1 medium appeared to result in optimum albumin synthesis. The modification of the Waymouth's medium was that 34.5 mM of $NaHCO_3$ was replaced by 25 mM HEPES, 0.56 mM L-alanine and 0.21 mM serine. The osmolality of the medium was adjusted to 308 mM by adding $NaHCO_3$. The oxygen tension of the incubation medium was maintained at at least 30×10^3 Pa (7). The influence of the pH on the amount of albumin synthesized was investigated over a pH range of 7.4 to 8.2. Maximum synthesis was found at pH 7.8. Fig. 1 shows the time course of the amount of albumin synthesized by the isolated cells. A lag period of a maximum of 30 min is observed. A comparable lag period was reported by Weigand and Otto (8). After the lag period, the

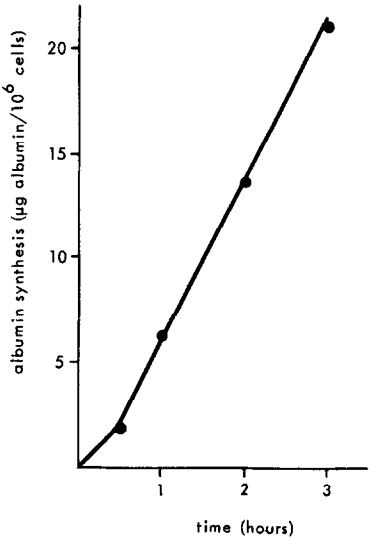

Fig. 1. Time course of albumin synthesis by hepatocytes isolated from 3-month-old rats.

curve is linear up to at least 3 h of incubation. On the basis of this result, samples were always taken after 60 min and 180 min of incubation and the capacity for albumin synthesis per time unit was calculated by subtracting the 60 min value from the 180 min value. For practical reasons, the amount of albumin present in and secreted by the cells was measured together at the different incubation times. During the incubation period of 180 min, the total number of cells did not decrease, whereas the percentage of viable cells as determined by trypan blue exclusion changed only slightly from 94 to 88%. A linear relationship between cell concentration and the amount of synthesized albumin was observed up to a cell concentration of at least 3×10^6 cells/ml medium (Fig. 2).

With due observance of the experimental conditions mentioned above, the quantity of albumin synthesized by hepatocytes isolated from 3-month-old rats amounted to 5.8 µg albumin/h/10^6 cells, which represents about 70% of the amount synthesized in an *in vivo* situation (7,9). In the study of East *et al.* (10), the albumin synthesis appeared to be 0.85 µg albumin/h/10^6

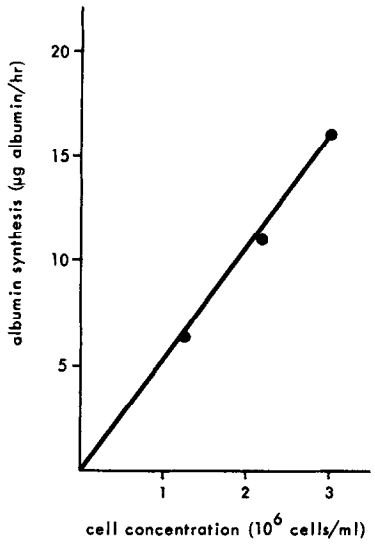

Fig. 2. Effect of cell concentration in the incubation medium on the albumin synthesis by parenchymal cells isolated from 3-month-old rats.

cells. Approximately the same value has been claimed by some other authors (8, 11). These values are only 10% of that observed *in vivo* (7,9). A much higher albumin synthesis was measured by Jeejeebhoy *et al.* (7) who, also using Waymouth's medium, found the amounts of albumin synthesized by isolated hepatocytes to be up to about 40% of the amount synthesized in the *in vivo* situation. The method used in this report results in a higher value than the values reported for albumin synthesis in the literature up to now. The fact that the synthesis of albumin nearly equals the value reported for the *in vivo* situation indicates that the isolation procedure did not negatively influence the functional capacity of the isolated cells. The high quality of the cells was also confirmed by the finding that the isolated hepatocytes are capable of performing all of the various steps of the bromsulfophthalein (BSP) clearance mechanism (2).

The second objective of the study was to compare the capacity for albumin synthesis of parenchymal cells isolated from adult and old rats with the capacity of cells from 3-month-old rats. Table 1 shows that a

Table 1. Albumin synthesis by hepatocytes isolated from young, adult and old rats*

Age (months)	Protein content ($mg/10^6$ cells)	Albumin synthesis** (µg albumin/h/10^6 cells)	Albumin synthesis** (µg albumin/h/mg protein)
3	1.65 ± 0.12 (6)	5.8 ± 0.8 (12)	3.5 ± 0.5
12	1.78 ± 0.25 (3)	3.7 ± 0.3 (8)	2.1 ± 0.4
36	2.01 ± 0.11 (5)	9.8 ± 1.7 (7)	4.9 ± 0.9

*Mean ± S.E.M.; number of different cell preparations between parentheses

**The values observed for the various age groups differ significantly ($p \leq 0.05$) from each other, except for the 3- to 36-month value, when expressed on a protein basis

significant increase was measured for the amount of albumin synthesized by cells isolated from 36-month-old rats. When expressed on a protein basis, the albumin synthesis did not significantly increase with age, due to an increase in the protein content of the cells with age. An intermediate age group (12 months) was studied to determine whether or not these observed differences are related to the maturation of the rat or are really age-related phenomena. Hepatocytes isolated from 12-month-old rats synthesized less albumin, expressed per 10^6 cells or per mg protein, than cells from 3- and 36-month-old rats (Table 1).

A decrease in albumin synthesis during the maturation of the rat has also been observed *in vivo* (12,13). This decrease would be attributable to the fact that the turnover rate of albumin decreased during the maturation of the rat (12). The observed increase in albumin synthesis after 12 months of age is in good agreement with *in vivo* results obtained by many investigators (14-17). Thus, the age-related changes obtained with the isolated liver parenchymal cells completely parallels the *in vivo* situation. It can be concluded that the age-related changes at the organ level can be at

least partly explained by changes in the functional capacities of the individual cells.

An interesting phenomenon was observed by Ove et al. (16) with in vivo experiments and by Chen et al. (18) with isolated microsomes. In agreement with our data, young rats synthesized less albumin than old rats. However, when the albumin concentration in the blood was decreased by bleeding the rats, the capacity for albumin synthesis of young rats increased with respect to non-bled young rats, whereas bleeding did not influence the capacity for albumin synthesis in old rats.

In analogy to these phenomena, the high values for the albumin synthesis of the cells isolated from old rats might be explained by the fact that these cells may function at their maximal capacity. Hepatocytes isolated from young and adult rats may not have fully utilized their functional reserve capacity.

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