SECRETION OF SERUM ALBUMIN BY ENZYMATICALLY ISOLATED RAT LIVER CELLS

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1. Introduction

Recently, the synthesis of albumin by enzymatically isolated rat liver cells was demonstrated [1,2]. After the treatment of the liver with collagenase and hyaluronidase various properties of the cell membrane are altered [3]. Therefore it is of interest to investigate, whether the specific function of serum protein secretion is maintained in the isolated liver cell. Most recently, Hussain and Bhargava [4] have reported that mechanically isolated liver cells cannot secrete albumin.

In contrast, this paper describes the net synthesis and secretion of rat serum albumin by enzymatically isolated rat liver cells.

2. Material and methods

The treatment of animals and the isolation of rat liver cells were described previously [1,5]. The following modifications have been made: The perfusion temperature was kept at 30°C until the enzymes were added and then increased to 37°C. Before the perfusion was stopped, the liver had been cooled by perfusion with ice-cold buffer. The shaking procedure was carried out by hand, using rotating movements for 7 min.

After isolation, the cells were washed twice and finally suspended in Krebs–Ringer–carbonate-buffer, pH 7.4, containing amino acids as described by Waymouth [6], D(-)-α-aminobenzyl-penicillin (10 μg/ml), and streptomycin (20 μg/ml). Seven identical samples of 20 ml were incubated at 37°C in a Warburg apparatus in an atmosphere of 95% O_2 plus 5% CO_2. Every 30 min one sample was removed and the incubation was stopped by rapid cooling. Subsequently, the suspension was centrifuged for 2 min at 100 *g*. The supernatant was centrifuged again for 10 min at 12 000 *g*. To concentrate the supernatant, protein was precipitated by trichloroacetic acid at a final concentration of 5%, redissolved in one-twentieth of the initial volume, and extensively dialysed against 0.01 m Tris–HCl buffer, pH 7.4. The cells were washed once, treated with sodium desoxycholate (0.8 %), and homogenised. For incorporation experiments the same buffer was used and 2–10 μCi per ml of L-[1-^{14}C]leucine (62 mCi/mmole) were added.

Protein was determined by the biuret method [7], albumin by the radial immunodiffusion of Mancini et al. [8], and protein-radioactivity by the method of Mans and Novelli [9]. Details of the methods are described elsewhere [10]. Cell counts were made in a Bürker chamber. The viability of the cells was estimated by 0.2% Trypan Blue staining.

3. Results and discussion

The initial synthesis time of a protein molecule is about 2 min [11,12]. The incorporation of radioactive leucine into total cell protein of enzymatically isolated liver cells proceeds linearly for at least 1 hr [1,5]. If newly synthesized protein passes a ruptured cell membrane, the protein radioactivity in the supernatant should increase linearly, after addition of radioactive leucine to the suspension. The appearance of radioactive protein in the supernatant after addition of L-[1-^{14}C]leucine is shown in fig. 1a. The meas-

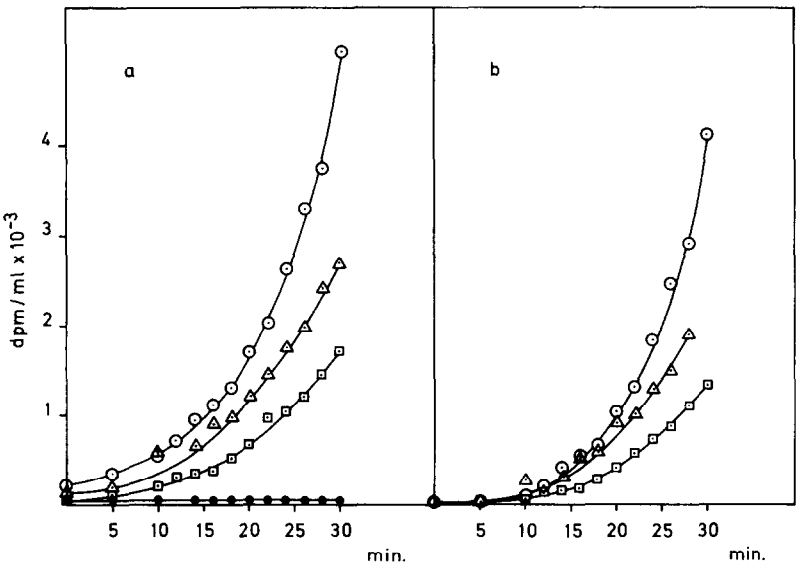

Fig. 1. Specific radioactivity of total protein in the supernatant of the cell suspension (dpm/ml) after addition of L-[1-^{14}C]leucine, in dependence of the incubation time (min). (□—□) 2 μCi/ml; (△—△) 5 μCi/ml; (○—○) 10 μCi/ml; (●—●) 2 μCi/ml plus 0.4 mg/ml puromycin. a: Measured curves. b: calculated curves, after subtraction of the linear component from the measured curves.

ured curves can be explained as the result of the addition of a straight line and a steeply rising curve. Subtracting the former curve from the latter, typical secretion curves of rat serum proteins are obtained, with a secretion time of about 10 min (fig. 1b). For rat liver in vivo a secretion time of 14 min was reported [10,13]. The initial cell suspension contains about 20% Trypan Blue stained cells (fig. 2). The membrane of the stained cells are defective and may be permeable for proteins. In addition, a number of cells may be destroyed during the centrifugation procedure. These facts could explain the linear component of the measured radioactive protein curves in the supernatant. It remains unexplained why the secretion time is 4 min shorter for serum proteins of the isolated cells than the secretion time in vivo.

Albumin was determined separately, in the cells and in the supernatant (fig. 3). Because of the limited sensitivity of the immunological method, serum albumin cannot be detected in the supernatant before 60 min after the incubation has been started. Thereafter, the secretion of albumin is linear for 120 min at a rate of 0.63 $\mu g/10^6$ cells/hour. This is based on the calculation for a total of $2.39 \pm 0.17 \times 10^6$ cells per ml. Assuming that the stained cells do not synthesize albumin, a secretion rate of 1.29 $\mu g/10^6$ cells/hour can be calculated. If one gram of liver wet weight

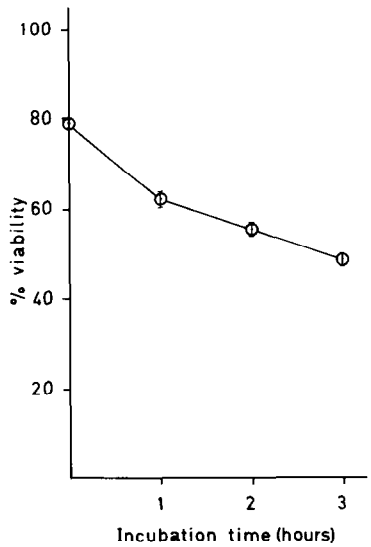

Fig. 2. Percentage of unstained cells in the suspensions during the incubation period of 3 hr (mean ± SD, n = 5).

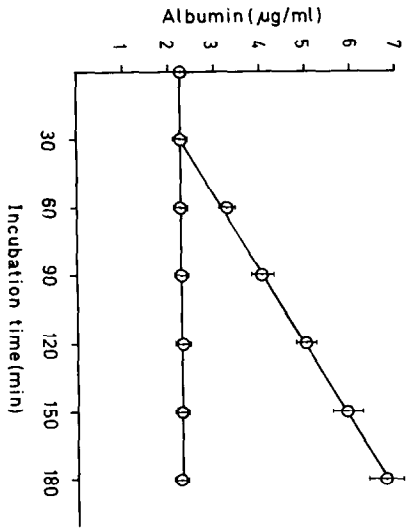

Fig. 3. Immunologically determined albumin (µg/ml) in the cells (lower curve) and in the supernatant (upper curve), in dependence of the incubation time (min). One ml of the suspensions contained (2.39 ± 0.17) × 10^6 cells. Each point represents the mean ± SD of 5 experiments.

contains 1.7 × 10^8 cells (14), albumin is secreted at a rate of 45 µg–92 µg/g/hour. In comparison, 470 µg were reported for fasted liver in vivo [13], 470 µg for isolated perfused liver [15], and 161 µg for liver slices [16].

At the beginning of the incubation the albumin content of one cell was found to be 0.96 pg. As the total cell protein is 2.7 ng [5], cell albumin accounts for about 0.04% of the total cell protein. After addition of 0.4 mg/ml puromycin, no protein radioactivity (fig. 1a) or albumin in the supernatant was found. The basic albumin content of the cell was not released after addition of puromycin. Therefore, it is very likely that this albumin pool does not participate in the continual pathway of synthesis and secretion. It is tempting to propose that this albumin is identical with the nuclear and mitochondrial fraction of the liver albumin, reported by Peters Jr. [13] to be 82 µg per g liver wet weight, corresponding to 0.48 pg per cell.

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