THE FERRITIN CONTENT OF THE FREE RIBOSOME FRACTION ISOLATED FROM ADULT MALE AND FEMALE RAT LIVER

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Received 11 September 1968

1. Introduction

The absorbance at 260 mμ has usually been taken as a measure of the amount of RNA or monomeric ribosomes in sucrose gradient fractions. Some authors [1, 2], however, emphasize the importance of making a correction for ferritin, which absorbs strongly at 260 mμ, to prevent an overestimation of the number of monomeric ribosomes. Contrary to this Loeb et al. [3] found it unnecessary to make a correction as their ribosomal particles were free of significant contamination by ferritin. When preparing free ribosomes from adult rat liver using the density gradient centrifugation technique, we found that preparations from adult female rats always contained a significantly larger amount of ferritin than similar preparations from adult male rats. Since it is important to keep the ferritin level as low as possible when preparing free ribosomes and, furthermore, since this observation might explain the discrepancies in the results of the different investigators, it was considered of interest to present our results.

2. Experimental

Adult albino rats, weighing 200–220 g, of a strain bred in this Laboratory, were used. One experiment (table 2, exp. 4) was carried out with Wistar albino rats, weighing 200 g, obtained from Møllegaards Avlslaboratorium, Havdrup, Kjøbenhavn. The animals were, except when otherwise indicated, starved for about 19 h before being killed by decapitation. Two female and two male rats were used in each experiment. 10 g of the pooled livers from each sex were homogenized in Hultin's medium [4] (12.5 ml/5 g of liver) with 10 rapid strokes in a loose fitting Potter Elvehjem glass homogenizer. All steps were carried out at 0–4°. The submicrosomal fractions were isolated by a slight modification of the method described by Campbell et al. [5]. The homogenates were centrifuged for 10 min at 12000 × *g*. 20 ml of the supernatant from each homogenate was centrifuged for 45 min at 25 000 × *g* in a Spinco model L preparative centrifuge (rotor no. 65). The supernatants were centrifuged for 90 min at 105 000 × *g* to give a light microsomal fraction. The pellets thus obtained were suspended in 1.1 ml of a solution containing 1 mM $MgCl_2$, 25 mM KCl and 35 mM Tris-hydrochloric acid buffer (pH 7.8). 1 ml of the suspension was subjected to centrifugation (135 min at 22000 rev/min in the SW-25-1 rotor) in a linear gradient of 5–20% sucrose (w/v) in a solution containing 0.1 mM $MgCl_2$, 25 mM KCl and 35 mM Tris-hydrochloric acid buffer (pH 7.8) on a cushion of 50% sucrose. Fractions of 8 drops each (approx. 0.5 ml) were collected from the bottom of the gradients by puncturing the tubes with a hypodermic needle. The fractions were diluted with 2.0 ml of distilled water and the optical density at 260 mμ and 320 mμ was measured. The optical density at 260 mμ due to ferritin was calculated by means of the correction factor for ferritin suggested by Wilson et al. [1]. Protein was estimated with the Folin phenol reagent [6], using cryst. ovalbumin as standard. RNA was measured by the orcinol method (Mejbaum, 1939) [7] with purified yeast RNA as standard. The determination of ferritin iron was carried out as described by Drysdale et al. [8].

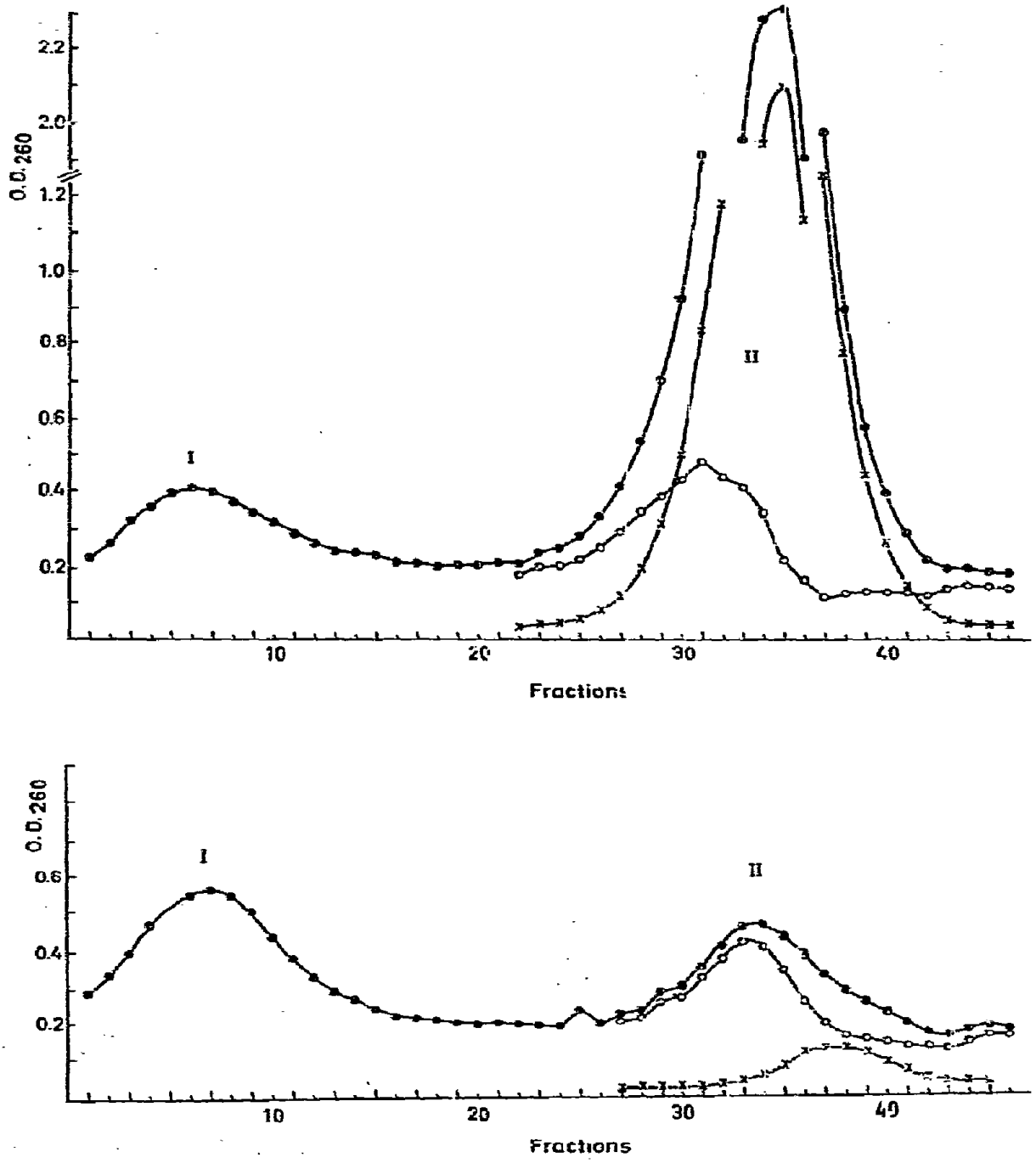

Fig. 1. Density gradient centrifugation of the light microsomal fraction from female (a) and male (b) rat liver.
●—●—● $O.D._{260}$ measured
x—x—x— $O.D._{260}$ due to ferritin
o—o—o— $O.D._{260}$ after correction for ferritin

Table 1

The protein, RNA and iron content of peak II (free ribosomes + ferritin) isolated from female and male rat liver by density gradient centrifugation of the light microsomal fraction.

Sex	Protein (mg)	RNA (mg)	Iron (mg)
Male rat liver	1.2	0.4	0.2
Female rat liver	2.8	0.6	0.47

The light microsomal fraction was isolated from 10 g of liver as described in the Experimental section.

3. Results and discussion

A comparison of the results obtained from the gradient centrifugation of the light microsomal fraction isolated from female and male rat livers respectively, is shown in fig. 1. Peak I contains the membrane-bound polysomes and peak II contains the free ribosomes and the ferritin. It is seen from fig. 1 that the $O.D._{260}$ due to ferritin in the free ribosome preparation from females is very large compared to that from males. The amount of free ribosomes (corrected $O.D._{260}$) is about the same in the two liver preparations.

The protein, RNA and iron content of the fractions (26–44) in fig. 1(a) and the fractions (29–44) in fig. 1(b) are given in table 1. It appears that the iron content of peak II from the female liver is 24 times that of peak II from the male liver, whereas the protein content of the former is approximately twice the value of the latter.

If we assume an RNA/protein ratio of 0.5 for the free ribosomes [5], the results indicate that the free ribosome preparation from the female livers contains a higher amount of ferritin protein and that the apoferritin from the female liver contains more iron compared to similar preparations from the male liver.

The results from six experiments, which include non-starved rats and Wistar albino rats, are listed in table 2. In all experiments the Fe/RNA ratio of peak II is considerably higher for female than for male rat livers.

This finding might explain why Loeb et al. [3] who used male rats found it unnecessary to make a correction for ferritin, while Wilson et al. [1] using female rats and Munro et al. [2] using female and male rats, reported a significant contamination by ferritin.

Table 2

A comparison of the Fe/RNA ratio of peak II (free ribosomes + ferritin) obtained from female and male rat liver by density gradient centrifugation of the light microsomal fraction.

Experiment	Fe/RNA Male	Fe/RNA Female
1	0.05	0.78
2 *	0.12	0.39
3	0.06	0.40
4 [a]	0.08	0.38
5 [b]	0.19	0.49
6 [b] *	0.20	0.44

a = Wistar albino rats
b = non-starved rats
* These experiments were carried out by Mr. Eirik Bjørklid.

Hence in order to keep the ferritin at a low level when preparing free ribosomes from rat liver, male rats should be used.

Acknowledgement

It is a pleasure to thank Prof. S. Laland for helpful advice. Financial support from The Norwegian Research Council for Science and the Humanities is gratefully acknowledged.

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