POLYRIBOSOMES IN REGENERATING LIVER*

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It is well known that microsomal cell-free systems from regenerating liver (RL)are more active in incorporating labeled amino acids into proteins in vitro than similar systems from normal liver (NL). Originally it was reported that mitochondria-free supernatants from homogenates of RL are approximately 2 to 3 times as active as those from NL, depending on whether the comparison is made on the basis of equal tissue weight (Hultin and Von Der Decken, 1957) or on the basis of RNA content of the microsomes (McCorquedale et al., 1960). Microsomes from RL were found to be 1.5 times more active than control microsomes (per unit RNA) when tested with soluble components from NL (Hultin and Von Der Decken, 1958) and approximately 2.5 times more active than control microsomes (per unit protein) when tested with soluble components from RL (Hoagland, 1961).

It has been repeatedly implied, and stated at least once (Campbell et al., 1964) that ribosomes from the two sources are equally active in vitro. On the other hand, recent achievements visualizing the messenger RNA as an integral part of a polyribosome structure (Wettstein et al., 1964; Warner et al., 1962) might indicate that an enhancement of protein synthesis may be possible by attaching more ribosomes to messenger RNA (Burka and Marks, 1964).

The experiments reported here demonstrate that ribosomes from regenerating liver differ from those of normal liver not only in the amount of polysomes

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relative to monosomes, but also in the qualitative or quantitative association of the ribosomes with the endoplasmic reticulum.

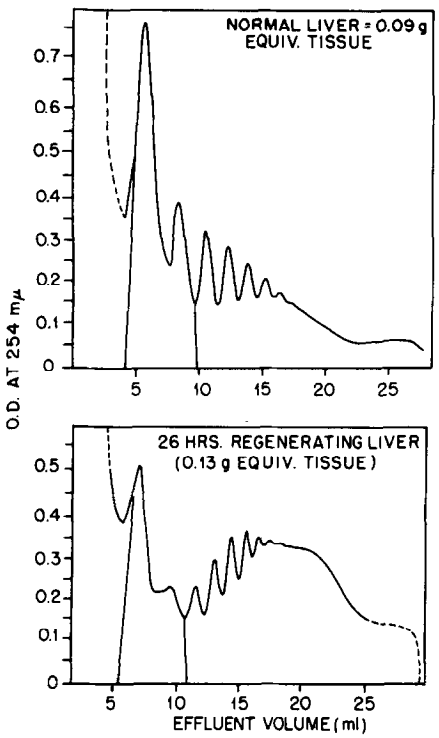

Fig. 1. Ribosome distribution patterns of normal liver (a) and regenerating livers at and 25 hours (b) after partial hepatectomy were homogenized with 2.5 volumes of Medium A (sucrose 0.25 M; TRIS-HCl pH 7.6, 0.05 M; $MgAc_2$ 0.004 M; KCl, 0.025 M). The homogenate was centrifuged at 15,000 X g for 10 minutes to remove nuclei, cell debris and mitochondria. The mitochondria-free supernatant was adjusted to 1.2% with DOC, and an amount corresponding to approximately 100-150 mg of tissue was layered as an inverted sucrose gradient (0—8.5%) on top of 28 ml of a convex exponential (10—34%) sucrose density gradient in 30 ml Spinco tubes. The gradients were centrifuged in the Sw-25 swinging-bucket rotor at 25,000 rpm (0—4°C) for 170 minutes. After centrifugation the bottom of the tube was punctured with a needle, and the optical density at 254 mμ was recorded automatically in an ISCO flow cell (1 cm light path) connected to a stripchart recorder, at a flow rate of 2.5 ml/minute. All the sucrose solutions were buffered with TKM solution (TRIS-HCl pH 7.6, 0.05 M; $MgAc_2$, 0.004 M; KCl, 0.025 M). Each gradient was derived from the pooled right lobes of the livers of four animals. The monomer peak in (a) was not seen above the height indicated by the dotted line, and its height was determined by scanning an identical preparation in a 0.2 cm light-path flow cell.

RESULTS

Typical ribosome distribution patterns of NL and RL (24 hours after surgery) are reported in Fig. 1. The number n assigned to each peak is nominal and refers to the number of ribosome units in each polysome. The first peak consists of monomers which we find to have a sedimentation constant (S°_{w20}) of 83±3 (Pfuderer et al., 1965).

The sedimentation patterns of ribosomes from NL were consistently identical to those shown in Fig. 1. In five consecutive experiments, monomers and dimers accounted for 49±1.33% of the total particle population, and aggregates of smaller than n = 8 were always represented in greater proportion than heavier aggregates.

The sedimentation patterns of ribosomes from RL were consistently similar to those reported in Fig. 1b and were characterized by a marked shift toward aggregates of heavier size (n = 8) with concomitant reduction of monomers and smaller oligomers. In five consecutive experiments, within 4 and 80 hours after partial hepatectomy, monomers and dimers accounted for 23 to 26% of the total particle population. Results to be presented will demonstrate that there is a trend toward accumulation of aggregates of heavier size during the first 48 hours after surgery and an opposite trend toward reappearance of smaller polysomes (but not of monomers) between 48 and 80 hours after surgery (Cammarano and Novelli, in preparation).

The data reported in Table 1 extend and substantiate the above findings. When ribosomes from NL and from RL at 26 hours after surgery were tested in the presence of a supernatant obtained from the same homogenate from which the particles were derived, the ribosomes from the experimental livers incorporated approximately twice as much C^{14}-leucine as those from control livers. The greater activity of RL ribosomes was considerably reduced when the ribosomes were tested with a supernatant fraction from NL. In contrast, the supernatant fraction of RL failed to stimulate the ribosomes of NL up to the level of those from RL. Essentially similar phenomena occur at the microsomal level when the specific activities are expressed on an RNA basis (Cammarano and Novelli, in preparation.)

In a recent report, Webb et al. (1964) have demonstrated that in NL the bulk of the polyribosomes are intimately bound to the endoplasmic reticulum (ER), whereas monomers and perhaps dimers are essentially free in the cytoplasm. In some of the minimal deviation Hepatomas, which still preserve the ER (Novikoff, 1962), the

TABLE 1

Amino acid incorporating activity of ribosomes from normal liver (Nr) and regenerating liver (Rr) at 25 hours after surgery tested with 105,000 X g supernatants from normal liver (Ns) and regenerating liver (Rs).

Source of ribosomes and cell sap	SA (cpm/mg ribosomal RNA)
Nr + Ns	20,000
	24,000
Rr + Rs	47,000
	50,000
Nr + Rs	29,000
	32,000
Rr + Ns	32,000
	30,000

For preparation of ribosomes, mitochondria-free homogenates from NL and RL were supplemented with DOC (1.2%) and centrifuged for 4 hours at 105,000 X g through a discontinuous gradient of sucrose (4 ml of 0.5 M over 3.0 ml of 1.0 M) in 12 ml Spinco tubes. Supernatants were obtained by centrifuging portions of the untreated mitochondria-free homogenate for 3 hours at 105,000 X g and were dialized for 3 hours vs 3000 volumes of medium A (see legend for Fig. 1).

The incubation mixtures for amino acid incorporation in vitro included (per ml): TRIS-HCl pH 7.6, 25 μmoles; $MgAc_2$, 4 μmoles; KCl, 50 μmoles; 2-mercaptoethanol, 20 μmoles; phosphocreatine, 12.5 μmoles; phosphocreatine-kinase, 20 μgrams; ATP (K salt), 0.5 μmoles; GTP (Na salt), 0.5 μmoles; L-C-14-leucine (SA 246 μC/μmole) 0.5 μC; ribosomes, 300 μgrams RNA; 105,000 X g, supernatant, 700 μgrams protein.

Radioactivities were measured by the method of Bollum (1959) as modified by Mans and Novelli (1961) using a Packard TRI-carb scintillation spectrometer (approximately 50% counting efficiency). Incubations were carried out for 45 minutes.

majority of the polyribosomes are either free or less tightly attached to lipoprotein membranes (Webb et al., 1964). For comparative purposes it was of interest to determine whether a similar phenomenon occurs in the tissue of origin undergoing a rapid but controlled rate of growth.

The technique was essentially that described by Webb et al. (1964). A portion of the mitochondria-free supernatant from homogenates of either NL or RL was made 1.2% with respect to sodium deoxycholate (DOC) and layered over

a discontinuous gradient of sucrose whose bottom layer was less dense than the ribosomes but denser than the microsomes (4.0 ml of 0.5 M over 3 ml of 2.0 M in a 12 ml Spinco tube). Equal portions of the same supernatant were layered on similar gradients but without prior detergent treatment. Both portions were centrifuged simultaneously at 105,000 X g for 4 hours. The resulting ribosome pellets were redissolved in the homogenizing medium (see legend for Fig. 1) and the ribosomes [C ribosomes (Wettstein et al., 1964)] were immediately separated by size in a sucrose gradient.

The distribution patterns of ribosomes obtained with DOC (+DOC) and without DOC (-DOC) from mitochondria-free supernatants of NL and RL are recorded in Fig. 2a and in Fig. 2b respectively. Figure 2a demonstrates that in the absence of DOC the majority of the polyribosomes from NL do not sediment

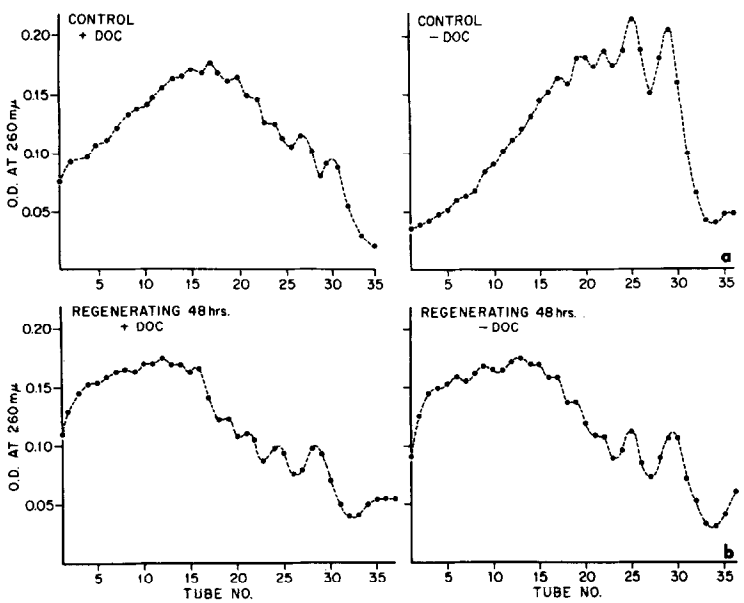

Fig. 2. Sucrose density gradient analysis of C ribosomes obtained by the standard procedure (+DOC) and by the modified procedure (-DOC) from normal liver (a) and from regenerating liver (b). The patterns with DOC and without DOC were obtained from equal volumes of the same homogenate and are therefore strictly comparable. The C ribosomes were layered on top of 28 ml of a linear (10-34%) sucrose gradient. The gradients were centrifuged 2 hours at 25,000 rpm in the Sw-25 swinging-bucket rotor at 0—4°C. After centrifugation the tubes were punctured with an hypodermic (19-gauge needle) and fractions of 11 drops each were collected, diluted with water, and their optical density at 260 mμ determined in the Beckman DU spectrophotometer. In order to allow a comparison, the optical densities were normalized to the same input.

through the 2.0 M sucrose, but remain at the 2.0 M to 0.5 M interface in association with the membrane fraction. Figure 2b demonstrates that in RL the ribosome distribution patterns obtained with and without DOC are identical.

In NL the recovery of ribosomes by the modified procedure (-DOC) was approximately 50% of that obtained by the standard procedure (+DOC) from an equal volume of the same homogenate. In RL the recovery of ribosomes was the same whether or not DOC was used during the preparative procedures. Electron microscopy studies of regenerating liver do not indicate that these phenomena may be due to a diminution of the endoplasmic reticulum (Cammarano and Novelli, in preparation).

DISCUSSION

An increased complement of heavier polyribosomes, largely dissociated from ER, is a conspicuous (although not exclusive) feature of the microsomal fraction of RL and might explain, in part, its greater activity in vitro.

The fact that the greater potential of RL ribosomes for amino acid incorporation is restrained by a soluble fraction from normal liver indicates that changes in the amount and in the composition of ribosomal population in vivo are paralleled by simultaneous change(s) in the activity of cytoplasmic component(s).

A similar increase in liver polyribosomes, with concomitant reduction of monomers and smaller oligomers, has been recently reported by Sidransky et al. (1964) for rats force fed a threonine-deficient diet. Aside from the possibility of a minor fragility of RL ribosomes which has been excluded (Cammarano and Novelli, in preparation), our results imply conclusions similar to those postulated by Sidransky et al. (1964): Either there is an increased output of longer mRNAs or there are more ribosomes than normal accommodated on the same length of mRNA; the latter change might be the result of an "uncoupling" between the speed at which the ribosomes move along the mRNA and the rate at which they attach to mRNA. As an alternate hypothesis, an increase in polyribosomes, with relative decrease of smaller aggregates, might indicate a marked increase in the stability of a fraction of mRNA. The basis for a similar hypothesis has been recently discussed by Noll et al. (1963).

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