Polyribosomes in Rat-Liver Preparations

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1. The distribution of rat-liver polyribosomes in sucrose density gradients has been investigated with regard to the effects of the preparative procedures and the physiological and pathological condition of the animal. 2. By using carefully defined conditions, three principal polyribosomal fractions have been isolated with $S_{20,w}$ values of 340, 275 and 225s in addition to the dimerized 120s and single 80s ribosomes. 3. The polyribosomes were very sensitive to treatment with ribonuclease and to mechanical stresses. 4. Incubation of dispersed hepatic cells and also cell-free preparations with puromycin in the presence of ATP and phosphoenolpyruvate caused rapid partial degradation of the polyribosomes. Treatment of the dispersed cells with actinomycin D also degraded the polyribosomes. 5. The liver polyribosomes of rats not raised under pathogen-free conditions and possibly of rats with an arthritic syndrome may be more fragile than those of healthy pathogen-free animals. 6. Treatment of pathogen-free rats with drugs stimulating liver anabolism profoundly affected the distribution of polyribosomes in sucrose density gradients.

The presence of polyribosomes, i.e. aggregates of single ribosomes held together by strands of RNA, has been demonstrated in many different cell types in various species. It is generally accepted that proteins are assembled on the constituent ribosomes of the polyribosomes although single ribosomes with attached messenger RNA may be capable of protein synthesis (Munro, Jackson & Korner, 1964; Lamfrom & Knopf, 1965). The polyribosomes are fragile structures that are extremely sensitive to minute quantities of ribonuclease and are easily broken by mechanical forces. It is uncertain therefore to what extent the polyribosomes isolated in the ultracentrifuge are fragments of larger structures degraded during cell disruption and the subsequent handling procedures. We have studied the effects of homogenizing techniques, the composition of the preparative media and the centrifugation procedures on large polyribosomal aggregates in rat-liver preparations. The influence of the condition of the animal, with regard to strain, sex, pathological state and the administration of certain drugs, on the polyribosomes has also been investigated. Finally, sucrose-density-gradient analyses have been carried out on extracts prepared from suspensions of dispersed rat-liver cells. Some of this work has been briefly described (Franklin & Godfrey, 1964).

MATERIALS AND METHODS

Animals. Two strains of rat were used, an albino strain and Norwegian hooded rats. Most of the albino and all of the Norwegian hooded rats were raised in a specific-pathogen-free animal colony (SPF rats). 'Non-SPF rats' refers to albino rats of the same strain as the SPF animals but raised in a colony not designed to exclude specific rat pathogens. All of the animals weighed 200–300 g.

Media. A stock solution was prepared containing 0.25 M-sucrose in 0.04 M-tris-HCl buffer, pH 7.7. The following media were made by adding the required amounts (final concentrations quoted) of $MgCl_2$ and KCl: medium A, 4 mM- $MgCl_2$ and 25 mM-KCl; medium B, 10 mM- $MgCl_2$ and 25 mM-KCl; medium C, 1.5 mM- $MgCl_2$ and 25 mM-KCl; medium D, 0.1 mM- $MgCl_2$ and 25 mM-KCl; medium E, 4 mM- $MgCl_2$ and 0.125 M-KCl; medium F, 4 mM- $MgCl_2$. Sucrose gradients were prepared linear over the range 10–25% (w/v) and were contained in 0.04 M-tris-HCl buffer, pH 7.7; concentrations of KCl and $MgCl_2$ corresponding to those used in the homogenizing medium were also present.

Liver homogenates. The animals were killed by a blow on the head and bled freely. The liver was removed into the appropriate ice-cold medium. After being weighed, the tissue was minced with scissors and disrupted in 2.5 vol. of medium with a homogenizer. Three types of homogenizer were used: (a) a stainless-steel tube with a Perspex pestle having a clearance of 1 mm. ('loose homogenizer'); (b) a Perspex tube and Teflon pestle with a clearance of 0.125 mm. ('tight homogenizer'); (c) a stainless-steel tube with a

spherical Perspex pestle having a clearance of 0.063 mm. (Dounce homogenizer). Homogenizers (a) and (b) were driven by a low-torque air motor at 1000 rev./min.; (c) was operated by hand without rotation. After homogenization (see the Results section for details), the preparation was centrifuged either at 15000g for 15 min. or at 10000g for 10 min. to remove nuclei, mitochondria and debris. The required concentration of aqueous sodium deoxycholate was added to the supernatant (see the Results section), and the mixture was layered (0.1 ml.) over the sucrose density gradient in 5 ml. tubes. The gradient was centrifuged in the SW39 rotor usually at 39000 rev./min. for 45 min. at 0–1°. Fractionation of the gradient was carried out by puncturing the tubes and collecting samples on a fraction collector. Usually 42 samples were collected and diluted fivefold before determining $E_{260m\mu}$.

Hepatic cells. The rat was killed, bled and the liver dropped into an ice-cold medium containing (per l.): NaCl, 9 g.; KCl, 0.42 g.; NaHCO_3 , 0.2 g.; sodium citrate, 7.6 g. (medium 1). The organ was perfused with medium 1 by inserting the needle of a hypodermic syringe into the cut ends of the major hepatic vessels. The liver was left for 15 min. at 0°; this appeared to increase the yield of cells. After mincing with scissors, the tissue (4 g.) was dispersed in medium 1 minus sodium citrate (25 ml., medium 2) with 10 strokes by hand in the loose homogenizer; further 4 g. portions were disrupted as required and the suspension was filtered through a stainless-steel sieve (120 mesh). The sieved suspension was centrifuged for 3 min. at 100g at 0° and the sediment resuspended gently in medium 2 with a glass rod and left for 10 min. at 0°. The hepatic cells were finally centrifuged at 50g for 3 min. and resuspended in medium A (5 ml./8 g. of original tissue). The quality of the preparation was assessed by mixing 1 drop of the cell suspension with 1 drop of 0.5% (w/v) Trypan Blue in 0.9% (w/v) NaCl. Microscopic examination of a smear of this preparation showed well-dispersed intact hepatic cells and relatively few isolated nuclei. The liver cells were disrupted by homogenizing by hand in a glass tube with a smooth Teflon pestle (clearance 0.025 mm.) at 0°. After each stroke a sample was mixed with Trypan Blue in NaCl and examined microscopically. After 8 strokes about 90% of the cells were disrupted. The homogenate was centrifuged at 15000g for 15 min. at 0° and the protein content of the supernatant determined. Sufficient aqueous sodium deoxycholate was added to give 0.43 mg. of deoxycholate/mg. of supernatant protein. Density-gradient analysis was carried out at 39000 rev./min. for 45 min.

Measurement of protein. The method of Lowry, Rosebrough, Farr & Randall (1951) was used, with crystalline bovine plasma albumin as standard.

Measurement of RNA. Ribosomal aggregates were precipitated with ice-cold 0.5N- HClO_4 and centrifuged. The precipitate was washed once and then extracted twice at 70° with 0.5N- HClO_4 . The $E_{260m\mu}$ value was determined and the RNA concentration calculated assuming an extinction coefficient of 34.2 mg./cm.

Chemicals. Sodium deoxycholate was obtained from two sources: British Drug Houses Ltd., Poole, Dorset, and T. J. Sas and Sons, Ltd., Holborn, London. Puromycin hydrochloride was purchased from Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A. Actinomycin D was kindly given by Merck, Sharp & Dohme Research Laboratories, Rahway, N.J., U.S.A. Atomid-S [ethyl 2-(p-chloro-

phenoxy)-2-methylpropionate] is a product of Imperial Chemical Industries Ltd. Cortisone acetate was obtained from Boots Pure Drug Co. Ltd., Nottingham. Crystalline pancreatic ribonuclease was obtained from British Drug Houses Ltd.

Induction of rat 'arthritis'. The right hind foot pads of SPF male albino rats were injected with 0.05 ml. of liquid paraffin containing a suspension of dead tubercle bacilli (5 mg./ml.) (Newbould, 1963). The injected foot swelled considerably during the next 4 days followed by a slight regression. Ten days after the injection there was an exacerbation of the swelling accompanied by inflammation and swelling of the other feet and other collagenous areas of the body (ears, tail etc.), which persisted for several weeks. The rats were severely debilitated during the course of the syndrome with extensive weight loss in mature rats and a failure to gain weight in growing animals (Newbould, 1963).

RESULTS

Studies on preparations from whole liver

Effect of homogenizing technique on polyribosomal distribution in density gradients. Livers of SPF albino male rats were disrupted in medium A with the loose homogenizer, by using 6 strokes at 1000 rev./min. After centrifuging at 15000g for 15 min. the supernatant was made 1.1% (w/v) with respect to sodium deoxycholate and subjected to density-gradient centrifuging at 39000 rev./min. for 45 min. This procedure is referred to below as the 'standard method'. The density-gradient pattern (Fig. 1a; 'control pattern') indicates five sharply defined components having approximate $S_{20,w}$ values of 80, 120, 225, 275 and 340s, calculated by the method of Martin & Ames (1961). Examination of the samples at 320 m μ revealed a peak at about 70s, presumably due to ferritin (Munro *et al.* 1964). The 'control pattern' was extremely reproducible when the procedure described above was closely followed. The homogenization procedure appears to be critical in determining the density-gradient profile. Thus 12 strokes with the loose homogenizer, 6 strokes with the tight homogenizer (both at 1000 rev./min.) or 6 strokes with the Dounce homogenizer resulted in the disappearance of the 225s, 275s and 340s components and a more polydisperse distribution of material sedimenting faster than the 120s ribosomes (Figs. 1b, 1c and 1d). Use of the tight homogenizer increased the height of the 80s (single) ribosomes. Resolution of the 120s and 80s ribosomes was lost after using the Dounce homogenizer. Three strokes with the loose homogenizer produced a pattern similar to the control but the material sedimenting between 120 and 225s was resolved into peaks at approx. 170 and 145s (Fig. 1c).

Effect of concentration of sodium deoxycholate on polyribosomal distribution. Identical control density-gradient patterns were obtained when sodium deoxycholate from two different suppliers

Fig. 1. Effect of homogenizing technique on sucrose-density-gradient profile of liver extracts from SPF albino male rats. (a) 6 strokes at 1000 rev./min. with 'loose homogenizer'; (b) 12 strokes at 1000 rev./min. with 'loose homogenizer'; (c) 6 strokes at 1000 rev./min. with 'tight homogenizer'; (d) 6 strokes by hand with Dounce homogenizer; (e) 3 strokes at 1000 rev./min. with 'loose homogenizer'. Centrifugation procedures were as described in the text.

Fig. 2. Effect of concentrations of Mg^{2+} and K^{+} on sucrose-density-gradient profiles of liver extracts from SPF albino male rats. (a) 1.5 mM- Mg^{2+} and 25 mM- K^{+} ; (b) 10 mM- Mg^{2+} and 25 mM- K^{+} ; (c) 0.1 mM- Mg^{2+} and 25 mM- K^{+} ; (d) 4 mM- Mg^{2+} and no K^{+} ; (e) 4 mM- Mg^{2+} and 0.125 M- K^{+} .

was used to treat the mitochondrial supernatants. The profiles were similar when the mitochondrial supernatant was made 0.5%, 1.1% or 1.3% with respect to deoxycholate but at a concentration of 1.66% all five peaks of the control pattern disappeared.

Effects of concentrations of Mg^{2+} and K^{+} on polyribosomal distribution. Lowering the Mg^{2+} concentration to 1.5 mM (medium C) or raising it to 10 mM (medium B) had little effect on the gradient profile (Figs. 2a and 2b). At 0.1 mM- Mg^{2+} (medium D), however, the 120s, 225s, 275s and 340s peaks were considerably reduced in height (Fig. 2c). In the absence of K^{+} (medium F) or at 0.125 M- K^{+} (medium E) the polyribosomal peaks (225s, 275s and 340s) were also much smaller (Figs. 2d and 2e).

Effects of centrifugal force on polyribosomal distribution. (a) Preliminary centrifuging. Munro *et al.* (1964) removed nuclei, debris and mitochondria from liver homogenates before density-gradient centrifuging by centrifuging at 10000g for 10 min. Staehelin, Wettstein & Noll (1963) centrifuged for

10 min. at 20000g. We have found that the conditions of this preliminary centrifugation can affect the subsequent density-gradient profile. A liver homogenate prepared by the standard method from SPF male albino rats was centrifuged at 10000g for 10 min. The five components of the control pattern were present with an extra peak at 150s, but the definition of the peaks was reduced with the 'valleys' significantly higher than in the control pattern. Considerably more material sedimented to the bottom of the gradient (Fig. 3a). The overall amount of polyribosomal material appeared to be higher than when the homogenate was centrifuged at 15000g for 15 min. A similar effect was seen when the liver was disrupted with the Dounce homogenizer and centrifuged at 10000g for 10 min. (Fig. 3b).

(b) Prolonged centrifuging. Prolonged centrifuging may lead to a loss of resolution owing to the diffusion of the sedimenting components (Brakke, 1964). Thus when a 15000g supernatant, prepared by the standard method, was centrifuged through a gradient at 27000 rev./min. for 94 min. so that the product centrifugal force $\times$ time equalled that used

Fig. 3. Effect of centrifugation procedures on sucrose-density-gradient profiles of liver extracts from SPF albino male rats. (a) Liver homogenate prepared by 'standard method' and centrifuged at 10000g for 10 min. before gradient analysis; (b) liver homogenate prepared with Dounce homogenizer and centrifuged at 10000g for 10 min.; (c) liver homogenate prepared by standard method and subjected to density-gradient centrifuging at 27000 rev./min. for 94 min. at 0°.

Fig. 4. Effect of treatment of liver extracts from SPF albino male rats with ribonuclease on sucrose-density-gradient profiles. (a) 15000g supernatant with 10 μ g. of ribonuclease/ml. at 37° for 5 min.; (b) 15000g supernatant with 5 μ g. of ribonuclease/ml. at 0° for 30 min.; (c) 340s fraction from gradient with 0.5 μ g. of ribonuclease/ml. at 0° for 30 min.; (d) 225s fraction with 0.5 μ g. of ribonuclease/ml. for 30 min. After enzyme treatment density-gradient analysis was performed at 39000 rev./min. for 45 min. The arrows in (c) and (d) indicate the $E_{260m\mu}$ of fractions 340s and 225s before treatment with ribonuclease.

to give the pattern shown in Fig. 1, the resolution of the five peaks was significantly reduced (Fig. 3c).

Properties of the polyribosomes and ribosomes of the control pattern

Protein/RNA ratios. The ribosomal and polyribosomal fractions were isolated from gradients and the protein and RNA contents determined. The protein/RNA ratios for the 80s, 120s, 225s, 275s and 340s fractions were found to be 1.00, 1.07, 1.05, 1.00 and 1.03 respectively.

Effect of ribonuclease treatment. When the 15000g supernatant was treated with pancreatic ribonuclease (10 μ g./ml.) for 5 min. at 37° before the addition of sodium deoxycholate, the 225s, 275s and 340s fractions were extensively degraded and there was a marked increase in the height of the 120s peak (Fig. 4a). After isolation from the gradient the 340s and 225s fractions were degraded to 120s and 80s ribosomes by 0.5 μ g. of ribonuclease/ml. at 0° for 30 min. (Figs. 4c and 4d). Treatment of the 15000g supernatant with 5.0 μ g. of ribonuclease/ml. at 0° (before deoxycholate treatment), however, had little effect on the density-gradient pattern subsequently obtained (Fig. 4b), except that there was more material between 170 and 140s.

Recentrifuging of polyribosomal fractions. The 340s, 275s and 225s fractions were isolated from

gradients and diluted to reduce the sucrose concentration with medium A minus sucrose. Portions (0.2 ml.) were layered over fresh gradients and centrifuged at 39000 rev./min. for 45 min. at 0–1°. Fractionation of the gradients showed that the 340s peak had split into a mixture of 340s, 275s, 225s, 150s, 120s and 80s fractions, the 275s peak into 275s, 225s, 150s, 120s and 80s fractions and the 225s peak into 225s, 150s, 120s and 80s fractions (Figs. 5a, 5b and 5c).

Effect of incubation of 15000g supernatant with puromycin. Williamson & Schweet (1965) found that incubation of reticulocyte polyribosome preparations with puromycin degraded the polyribosomes and increased the number of single and dimerized ribosomes. This effect was dependent on the addition of a source of 'high-energy' phosphate esters. The 15000g supernatant from the liver of SPF rats was incubated for 10 min. with puromycin (0.4 mM) in the presence and absence of ATP (1 mM) and phosphoenolpyruvate (5 mM) before the addition of deoxycholate. The mixture was then chilled and made 1.1% (w/v) with respect to sodium deoxycholate and centrifuged through a density gradient at 39000 rev./min. for 45 min. In the presence of ATP and phosphoenolpyruvate, puromycin partially degraded the 340s, 275s and 225s poly-

Fig. 5. Sucrose-density-gradient profiles obtained after recentrifuging of polyribosomal fractions isolated from gradient. (a) 340s fraction; (b) 275s fraction; (c) 225s fraction.

ribosomes, and markedly increased the 120–80s ribosomes and the material sedimenting at about 170–150s. Incubation with puromycin alone or with ATP and phosphoenolpyruvate in the absence of drug had little effect on the sedimentation profile.

Effect of sex and strain of rat on polyribosomes. Liver extracts were prepared by the standard method from female albino SPF rats. The density-gradient profile was very similar to that obtained from male rats. There was, however, a small peak sedimenting at about 370s that has not been observed in the liver preparations from male rats and considerably more material between 160 and 140s. Standard-method preparations were also obtained from the livers of SPF males of the Norwegian hooded strain. The polyribosomal distribution was very similar to that of the albino rats, although the 340s and 225s peaks were smaller and there was a marked peak at about 150s.

Liver sedimentation profiles from rats not raised under specific pathogen-free conditions. Liver extracts were made from non-SPF rats by the standard method. In the sedimentation profile the 80s and 120s ribosomes were evident but there was a broad distribution of material sedimenting faster than the single and dimerized ribosomes with no discrete polyribosomal peaks. However, when the livers from non-SPF rats were disrupted by 3 strokes with the loose homogenizer at 1000 rev./min., the 340s, 275s and 225s peaks appeared together with a component at about 150s.

Effect of development of arthritis syndrome. Within 24hr. of the injection of the suspension of

Fig. 6. Changes in sucrose-density-gradient profiles of liver extracts prepared by the 'standard method' from SPF albino male rats (a) 24hr., (b) 48hr. and (c) 10 days after injection of a suspension of dead tubercle bacilli in liquid paraffin into the right hind foot pad.

dead tubercle bacilli in liquid paraffin into the foot pad there were striking changes in the sedimentation profiles of liver extracts prepared by the standard method. The polyribosomal components were less well defined and there was a small increase in the height of the 80s fraction (Fig. 6a). After 48hr. the polyribosomal peaks disappeared and there was an increase in the 120s and 80s ribosomes (Fig. 6b). During the next 6 days there was a further decrease in the amount of polyribosomal material (Fig. 6c), and although the rats were kept for 32 days after the injection the sedimentation profiles did not return to the control pattern.

Effect of intraperitoneal injection of cortisone acetate. There have been many reports describing the effects of cortisone on protein and RNA synthesis in the liver and there is general agreement that the hormone induces the new synthesis of hepatic enzymes (Feigelson & Greengard, 1962). It was therefore decided to investigate the effects of the administration of cortisone to the rat on the polyribosomal sedimentation profile of the liver. Cortisone acetate was given by intraperitoneal injection to SPF male albino rats in three doses of 5mg. each; the second injection was given 4hr. after the first and the third 16hr. after the second injection. The animals were killed 2hr., 24hr., 48hr. and 7 days after the last injection and liver extracts prepared by the standard method. The sedimentation profiles obtained after 2hr. showed striking changes from the control pattern; the 340s, 275s and 225s peaks disappeared and the height of the

80s fraction increased (Fig. 7a). After 24 hr., however, the polyribosomal peaks began to reappear and this trend was maintained at 48 hr. with a marked increase in the amount of material sedimenting between 180 and 150s (Figs. 7b and 7c). The control pattern had not returned 7 days after the injections (Fig. 7d).

Effect of administration of Atromid-S. The administration of Atromid-S to rats lowers the concentrations of total lipid and cholesterol in the blood and liver (Thorpe & Waring, 1962). An anabolic effect on the liver, which occurs within 3 days of daily administration of Atromid-S (0.25%, w/w, in powdered rat-food pellets) is due mainly to the deposition of protein (Dr D. S. Platt, unpublished work). Liver extracts were prepared by the standard method from SPF male albino rats after 5 days of administration of Atromid-S in the diet (0.25%, w/w). The sedimentation profile (Fig. 8) revealed a marked increase in the amount of material sedimenting between 180 and 130s and a greater proportion of material sedimented faster than 340s.

Sucrose-density-gradient profiles of extracts from dispersed hepatic cells. Suspensions of dispersed hepatic cells from SPF male albino rats were carefully homogenized in medium A as described in the Materials and Methods section. The 15000g supernatant was treated with sodium deoxycholate and subjected to density-gradient analysis. The pattern obtained was almost identical with the control pattern from whole liver (Fig. 9a).

Effect of incubation of hepatic cells with puromycin. Dispersed cells were incubated in medium A (8g. of

original liver/5ml.) with 10 μ g. and 50 μ g. of puromycin/ml. for 30 min. The drug caused extensive degradation of the polyribosomes and a considerable increase in the 120s and 80s ribosomes (Figs. 9b and 9c). Incubation of the cells alone had no effect on the density-gradient pattern.

Effect of incubation of hepatic cells with actinomycin D. Dispersed cells were incubated in medium A with 10 μ g. and 50 μ g. of actinomycin D/ml. Incubation was continued for 1 hr. in 10 μ g. of actinomycin D/ml. and polyribosomes were progressively degraded with accompanying increase in the 120s and 80s ribosomes and material sedimenting between 170 and 140s (Figs. 10a and 10b). The higher concentration of drug completely degraded

Fig. 8. Sucrose-density-gradient profile of liver extracts prepared by the 'standard method' from SPF albino male rats after 5 days of administration of Atromid-S in the diet (0.25%, w/w).

Fig. 7. Changes in sucrose-density-gradient profiles of liver extracts prepared by the 'standard method' from SPF albino male rats (a) 2 hr., (b) 24 hr., (c) 48 hr. and (d) 7 days after intraperitoneal administration of cortisone acetate.

Fig. 9. Sucrose-density-gradient profiles of extracts from dispersed liver cells from SPF albino male rats. (a) Control preparation; (b) and (c) cells incubated with 10 μ g. and 50 μ g. of puromycin/ml. respectively for 30 min. at 37° before disruption and density-gradient analysis.

Fig. 10. Sucrose-density-gradient profiles of dispersed liver cells incubated at 37° with actinomycin: (a) 10 $\mu\text{g./ml.}$ for 15 min.; (b) 10 $\mu\text{g./ml.}$ for 1 hr.; (c) 50 $\mu\text{g./ml.}$ for 15 min.

the polyribosomal and ribosomal components within 15 min. (Fig. 10c).

DISCUSSION

There is no evidence as to whether the discrete polyribosomal aggregates described in this paper with $S_{20,w}$ values of 225, 275 and 340s (and possibly 150s, which may be an aggregate of 120s and 80s ribosomes mediated by Mg^{2+} ; Peterman, 1960) exist in liver cells of the living rat. Because of their great sensitivity to ribonuclease and mechanical stress the study of polyribosomes by homogenization and centrifuging is fraught with possible artifacts. The sedimentation properties of large macromolecules in centrifugal fields impose other difficulties. The experiments described here clearly demonstrate the way in which the $E_{260m\mu}$ sedimentation profiles of liver-mitochondrial supernatants depend on the technical procedures used and on the physiological or pathological condition of the animal.

The 'control' pattern we obtained for the distribution of polyribosomes in sucrose gradients differs considerably from patterns reported by other workers for liver preparations (Staehelin *et al.* 1963; Munro *et al.* 1964). The sharply defined components at 225, 275 and 340s have not been described elsewhere. The appearance of these components was dependent on the severity of the procedure used to disrupt the whole liver, suggesting considerable fragility in their structure. It is unlikely that these discrete components are

stabilized by fragments of endoplasmic reticulum since they were stable over a range of concentrations of deoxycholate (0.5–1.33%). A higher concentration of deoxycholate (1.66%) degraded the 120s and 80s ribosomes as well as the polyribosomes.

The polyribosomes were stable in a wide range of Mg^{2+} concentrations, suggesting they are not aggregates caused by non-specific magnesium binding. The Mg^{2+} ion appears to be important in stabilizing the polyribosomal structure since the peaks disappeared in 0.1 mM- Mg^{2+} . Munro *et al.* (1964) have also reported that rat-liver polyribosomes are unstable in 0.1 mM- Mg^{2+} . Though the concentration of K^+ was also important in maintaining the integrity of the polyribosomes the effects of this ion were not studied in detail, so that its role remains unclear.

Strong evidence that the 225s, 275s and 340s fractions are polyribosomal aggregates linked together by a labile RNA molecule was provided by the effects of ribonuclease. The polyribosomes were much more sensitive to ribonuclease after isolation from the sucrose gradient. A high concentration of ribonuclease (10 $\mu\text{g./ml.}$) at 37° was required to degrade the polyribosomes before treatment of the mitochondrial supernatant with deoxycholate. It is likely therefore that the endoplasmic reticulum in some way protects the polyribosomal structure against ribonuclease attack. Alternatively, the resistance of polyribosomes in the mitochondrial supernatant to ribonuclease could be due to the presence of some inhibitor of the enzyme such as that described by Shortman (1961).

A significant proportion of polyribosomal material may be sedimented during the preliminary centrifuging of the homogenate. Centrifuging at 10000g for 10 min. reduced the resolution of the 225s, 275s and 340s peaks as well as increasing the overall amount of polyribosomes in the gradient. In this context Brakke & Daly (1965) have discussed the loss in resolution of components in density-gradient centrifuging when the concentration of the sedimenting components is increased. A further fall in the resolution of the polyribosomal peaks was evident in our experiments when the period of density-gradient centrifuging was extended and the centrifugal speed reduced. A similar effect has been reported by Brakke (1964) in a study of the effect of centrifugation conditions on the sedimentation of virus particles in sucrose-density gradients. To obtain high resolution it is important to use a low macromolecular concentration and maximal centrifugal force with the minimum period necessary for an adequate movement of the components along the gradient.

The partial degradation of the polyribosomal fractions into smaller fragments that occurs during isolation and recentrifuging suggests that rupture

may occur at specific weak points in the polyribosomal structure. Rifkind, Luzzato & Marks (1964) have described large polyribosomes containing up to 20 single ribosomes in intact reticulocytes. These structures were characterized by exposed lengths of messenger RNA appearing between clusters of ribosomes. They suggested that the preponderance of much smaller polyribosomes in sucrose-density gradients of reticulocyte lysates may be due to the rupture of larger aggregates at the sites of exposed messenger RNA. It is possible that similar intervals between ribosomal clusters may occur along the length of the liver polyribosomes, which could account for the peculiar manner in which these structures fragment during isolation from the gradient and recentrifugation.

The interpretation of the partial degradation of the polyribosomes to 120s and 80s ribosomes during incubation of the mitochondrial supernatant with puromycin in the presence of ATP and phosphoenolpyruvate is uncertain. Williamson & Schweet (1965) found that incubation of reticulocyte polyribosomes with puromycin caused a similar degradation. In contrast with their findings with reticulocyte preparations, however, we observed only very minor degradation of the liver polyribosomes in the absence of puromycin. Williamson & Schweet (1965) found an extensive conversion of reticulocyte polyribosomes into 80s ribosomes under these conditions, which was only slightly accelerated by puromycin. The pronounced acceleration by puromycin of the degradation process in the rat-liver preparations might be attributed to the stripping away of the nascent polypeptides by the antibiotic. It is possible that the nascent polypeptides in some way stabilize liver polyribosomes, although Williamson & Schweet (1965) have discounted this possibility in reticulocyte polyribosomes.

Not surprisingly, there were minor differences in the sedimentation profiles between liver preparations from rats of different sexes and strains. The failure to obtain the discrete polyribosomes by the standard method from non-SPF animals is puzzling. Since they were demonstrable when the severity of homogenization was reduced, the liver polyribosomes of the non-SPF rats may be more fragile than those of SPF rats. Alternatively, some property of the liver of SPF rats might protect the polyribosomes during disrupting of the cells. Similar considerations could apply to the changes in the sedimentation profile that occur during the development of the rat arthritis syndrome, during which the animals are severely debilitated. It is not clear whether there is a fall in the polyribosomal content of the liver cells or whether there is an increased sensitivity to homogenization.

Under conditions of increased protein synthesis

in the liver, induced by the administration of cortisone or Atromid-S, there were marked changes in the sedimentation profiles. There was no obvious reduction in the total amount of polyribosomes, but rather a redistribution of material in the gradient. Again the significance of these results is not clear, but in some way they may reflect the changes in protein and RNA synthesis in the liver induced by drug administration.

The experiments with dispersed hepatic cells underline the fragility of the discrete polyribosomal aggregates. Before reproducible sedimentation profiles could be obtained it was necessary to control carefully the procedure for the disruption of the cells. More vigorous homogenization or ultrasonic disruption of the cells degraded the polyribosomes and greatly increased the height of the 120s and 80s peaks. The rapid degradation of the polyribosomes and 120s and 80s ribosomes produced by incubation of the cells with a high concentration of actinomycin D (50 $\mu\text{g./ml.}$) suggests, in accordance with Revel & Hiatt (1964), that the drug may induce polyribosomal fragility with consequent disintegration during homogenization. This effect is thought to be in addition to the inhibition of RNA synthesis by actinomycin D. The degradation of the polyribosomes induced by incubation of the cells with puromycin resembles the effect of this drug observed by Williamson & Schweet (1964) on intact reticulocytes.

It is not yet clear whether the polyribosomes described in this paper, or indeed in other published reports of polyribosomes in disrupted cell preparations, are a true indication of the size and nature of polyribosomes in the intact cell. Preliminary experiments suggest that there is not necessarily any obvious correlation between the type of sedimentation profile and the rate of protein synthesis *in vivo* or *in vitro*. The functional significance of the polyribosomes described here is therefore uncertain. However, our results emphasize the difficulties in the interpretation of polyribosomal sedimentation patterns.

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