Free and Membrane-Bound Polyribosomes in Normal and Rauscher-Virus-Infected Mouse Spleen Cells

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1. Free and membrane-bound polyribosomes and ribosomal monomers were isolated from normal and Rauscher-virus-infected mouse spleens by means of discontinuous sucrose density gradients. 2. The addition of ribonuclease inhibitor from rat liver was essential to protect these polyribosomes from degradation. To separate the smooth and rough membranes from ribosomal monomers an additional centrifugation step through a continuous sucrose density gradient was necessary. 3. After infection a marked increase in rRNA from both membrane-bound and free polyribosomes was observed. Treatment of the membrane-bound polyribosomes with sodium deoxycholate yielded only 80S particles even when ribonuclease inhibitor was added. 4. A striking feature of the infected spleen was the occurrence of large polyribosomes. Up to 40 monomers per polyribosome could be counted on electron micrographs.

The Rauscher virus belongs to the group of mouse RNA tumour viruses, and causes leukaemia in susceptible mice (Rauscher, 1962). The replication mechanism of these viruses is far from being fully understood; it seems to require DNA synthesis early in the infection (Temin, 1964; Bader, 1965; Duesberg & Vogt, 1969). Moreover DNA-dependent RNA synthesis is necessary to sustain virus multiplication (Duesberg & Robinson, 1967; Bases & King, 1967). This may suggest that another set of mechanisms is involved in the replication of these RNA tumour viruses as compared with the replication of RNA bacteriophages (Shapiro & August, 1966) or the polio- and mengo-viruses. The latter viruses replicate independent of DNA. The viral RNA ('plus' strand) is copied ('minus' strand) by an RNA-dependent RNA polymerase in the cytoplasm of infected cells (Baltimore, Eggers, Franklin & Tamm, 1963; Baltimore & Franklin, 1963). However, evidence has been provided that the RNA replication of tumour viruses follows the same pattern. Watson & Beaudreau (1969) reported the appearance of an RNA-dependent RNA polymerase in cells infected with avian myeloblastoma virus, whereas Biswal & Benyesh-Melnick (1969) detected a 'minus' strand in the nuclei of rat 78 A1 cells infected with mouse sarcoma-leukaemia virus. If this is true it would be interesting to know what actually happens in the period between RNA replication in the nucleus and maturation of the virus by budding from the

cell surface (Feldman & Gross, 1964; Zeigel, Tyndall, O'Connor, Teeter & Allen, 1966). The sequence of events in this period is entirely unknown. It is reasonable to assume that at a certain stage of virus multiplication, viral mRNA becomes associated with ribosomes to produce viral-specific proteins. For certain RNA viruses, such as poliovirus, specific changes in the size of the polyribosomes occur after infection of the cells (Penman, Scherrer, Becker & Darnell, 1963). The RNA and protein synthesis of this virus takes place in association with membranous structures (Caligiuri & Tamm, 1969). It has also been suggested that the endoplasmic reticulum plays a role in poliovirus multiplication (Blinzinger, Simon, Magrath & Boulger, 1969).

The aim of this study was to investigate the influence of Rauscher-virus infection on polyribosome profiles and possible differences in the occurrence of polyribosomes as free and membrane-bound structures.

MATERIALS AND METHODS

Virus and mice. The Rauscher virus was a kind gift of Dr V. Swaen (Department of Pathology, University of Amsterdam). Stocks of this virus were obtained by preparing a 19000g_{av} supernatant of a 10% (w/v) spleen homogenate in medium A (0.05M-tris-HCl, 5mM-MgCl₂, 0.07M-KCl, pH7.6). The supernatant was frozen at -76°C.

Random-bred Swiss mice were injected intraperitoneally, 3–10 days after birth, with 0.1 ml of this supernatant fraction. The mice were killed 14–20 days after infection, when their spleens weighed approx. 600 mg. Non-infected mice of the same age were used for comparison.

Liver supernatant. Rat liver supernatant as a source of the ribonuclease inhibitor was used in all experiments. Livers of 2–3-month-old Wistar rats were homogenized in 1 ml of 0.25 M-sucrose solution in medium A, to which 1 mM-dithiothreitol was added, per g of tissue. The 100 000g supernatant was prepared as described by Blobel & Potter (1966).

Ribonuclease inhibitor. The ribonuclease inhibitor from rat liver supernatant was a generous gift of Dr A. Gribnau (Department of Biochemistry, University of Nijmegen). It was a partly purified preparation obtained after chromatography on DEAE-Sephadex (Gribnau, Schoenmakers & Bloemendal, 1969). The concentration used was 5 mg/ml.

Homogenization and fractionation. Spleens of normal and leukaemic mice, of the same age, were homogenized in 6 ml of liver supernatant/g of tissue by five strokes of a motor-driven Teflon-glass homogenizer (2000 rev./min). The homogenate was centrifuged at 1250g_{av.} for 3 min, and subsequently at 7000g_{av.} for 10 min, in a Sorvall RC2-B preparative centrifuge.

In a separate experiment the homogenates of normal and leukaemic spleens were mixed in equal amounts but thereafter the 7000g_{av.} supernatant fraction was prepared as above.

The pellets (P1 and P2; see fractionation scheme, Scheme 1) were used for a determination of the DNA and RNA content. The supernatant fraction 2 was used for the isolation of polyribosomes. Essentially the same method was used as described by Bloemendal, Bont, de Vries & Benedetti (1967). Samples (18 ml) were layered on top of a discontinuous gradient, consisting of 10 ml of 2.0 M-sucrose and 10 ml of 1.0 M-sucrose in medium A. These gradients were centrifuged at 94 000g_{av.} for 20 h at 0°C in the SW27 swinging-bucket rotor of the Spinco L-2 preparative ultracentrifuge. Fractions (15 drops) were collected by puncturing the bottom of the tube. Of each fraction, 100 µl was diluted to 1 ml and its E_{260} and E_{280} were measured in a Zeiss spectrophotometer (PM QII).

The interface fractions (band 1, Scheme 1) were pooled and diluted to a sucrose concentration lower than 10% (w/v). Of this diluted fraction 5 ml, with or without 0.5% sodium deoxycholate, was layered on top of a sucrose density gradient, which consisted of a 3 ml 'cushion' of 68% (w/v) sucrose in medium A, with 31 ml of a linear gradient [34–10% (w/v) sucrose in medium A] on top (Talal & Dubois-Exum, 1966). This gradient was centrifuged at 94 000g_{av.} for 90 min at 0°C in a SW27 rotor and monitored automatically at 254 nm. The fractions on the 68% sucrose cushion (band 2) and a peak fraction which sedimented slower in the gradient (band 3) were pooled and their E_{260} and E_{280} measured.

Sucrose-density-gradient analysis. Pellets from the discontinuous gradients (P3) were resuspended in medium A containing ribonuclease inhibitor (5 mg/ml). The resuspended pellets were layered on top of a sucrose density gradient with a 5 ml 'cushion' of 68% sucrose in

medium A and the same linear gradient on top as described above. Centrifugation time, speed, temperature and monitoring of the gradient were also the same.

All solutions were treated with 0.1% diethyl pyrocarbonate for 60 min at 100°C to remove ribonuclease (Fedorcsák, Natarajan & Ehrenberg, 1969).

All procedures were performed at 0°C.

Electron microscopy. Material to be embedded was sedimented at 78 000g_{av.} in a Spinco 30 rotor for 11 h. Pellets were fixed for 1 h in 3% (w/v) glutaraldehyde solution in 0.1 M-sodium-potassium phosphate buffer, pH 7.4, at 0°C. Thereafter the pellets were washed twice with a solution of 0.2 M-sucrose in the same phosphate buffer, post-fixed for 30 min in a solution containing 2% (w/v) OsO₄, 0.05 M-sodium acetate and 0.05 M-sodium cacodylate, pH 7.4, and washed twice in the same acetate-cacodylate buffer, at 0°C. After dehydration in ethanol the material was embedded in Epon 812. Sections 400–600 Å thick were cut, stained with uranyl acetate and with Reynolds' (1963) lead citrate.

Fractions of a sucrose density gradient containing polyribosomes ($E_{260}^{1\text{cm}}$ between 0.2 and 1.5) were processed as follows. A small drop was placed on a grid covered with a carbon film and almost completely removed with a piece of filter paper. The grid was thoroughly washed with about 20 drops of 1% (w/v) hydrated uranyl acetate and then left to dry.

The micrographs were taken with a Philips EM300 microscope, operated at 60 kV, fitted with a cooling device.

Labelling in vivo. Four leukaemic mice were injected intraperitoneally, 12 days after infection, with 5 µCi of ¹⁴C-labelled protein hydrolysate (52 mCi/mg-atom of C, obtained from The Radiochemical Centre, Amersham, Bucks., U.K.) and killed after 5 min. Homogenization and fractionation of the spleens was performed as described above. The resuspended pellet from the discontinuous gradient was layered on top of the continuous gradient. After centrifugation a polyribosome profile was obtained. Fractions (1 ml) were collected by puncturing the bottom of the tube and the E_{260} was measured. For determination of radioactivity, each fraction was precipitated with 5% (w/v) trichloroacetic acid after addition of 0.2 mg of bovine serum albumin (Nutritional Biochemicals Corp., Freehold, N.J., U.S.A.), kept overnight in the cold and subsequently heated for 30 min at 90°C. The precipitates were collected on 0.45 µm Millipore filters and washed several times with 5% trichloroacetic acid. The filters were dried and their radioactivities counted with 10 ml of liquid-scintillation fluid, composed of 0.3% 2,5-diphenyloxazole and 0.02% 1,4-bis-(4-methyl-5-phenyloxazol-2-yl)benzene in toluene, in a Packard Tri-Carb model 4322 liquid-scintillation spectrometer.

Determination of DNA, RNA and protein content. The 1250g_{av.} and 7000g_{av.} pellets (P1 and P2) were precipitated in ice-cold 0.5 M-HClO₄ and washed three times. The precipitate was hydrolysed twice in 0.5 M-HClO₄ at 70°C for 30 min. After hydrolysis the supernatant fraction was cleared by centrifugation (10 min, 4500 rev./min in a Christ Junior centrifuge). The 7000g_{av.} pellet (P2) was hydrolysed only once.

The DNA content of the supernatant fractions was measured by the diphenylamine reaction as described by Burton (1956).

RNA was determined by the orcinol reaction (Ceriotti, 1955).

RNA and protein contents of the polyribosomes were calculated from their extinction coefficients at 260 and 280nm (Warburg & Christian, 1941).

RESULTS AND DISCUSSION

Virus growth curve. All mice responded to the injection with Rauscher virus and developed palpable spleens within 2 weeks. Spleen enlargement was noticeable after approx. 5 days, but became striking during the second week after infection. The relation between virus quantity and spleen weight has been established previously

in Balb/c mice (Rauscher & Allen, 1964; Brodsky, Kahn, Ross, Petkov & Braverman, 1967) and in Swiss mice (Pienta & Groupé, 1967). Table 1 shows the increase in spleen weight during the first 2 weeks after infection. The aim of this experiment was to have at our disposal a criterion for infection, which in this way could easily be detected at an early stage.

Fractionation. The complete scheme of the fractionation procedure is presented in Scheme 1. Liver supernatant inhibits spleen ribonuclease (Northup, Hammond & La Via, 1967). By our method homogenization of 1g of spleen in 6ml of liver supernatant results in the maximal protection of the polyribosomes. Increase of the concentration of liver supernatant does not result in higher yields of polyribosomes. On the other hand this does not mean that the liver supernatant completely inhibits the mouse spleen ribonuclease, as demonstrated below (see under 'Large polyribosomes').

The usual way of preparing liver polyribosomes is to start with the 10000–15000g postmitochondrial supernatant fraction (Wettstein, Staehelin & Noll, 1963; Munro, Jackson & Korner, 1964; Bloemendal, Bont & Benedetti, 1964; Campbell, Serck-Hanssen & Lowe, 1965). Electron-microscopic observations, however, reveal that with spleen as starting tissue, besides mitochondria a substantial amount of ribosomes is present in the pellet (P2, Plate 1) that is obtained after centrifugation for 10min at 7000g. This is consistent with the observation that with HeLa cells most of the membrane-bound polyribosomes disappear from

Table 1. Increase in spleen weight after infection of 3-day-old Swiss mice with Rauscher virus compared with normal spleen

The values are the average spleen weights for the numbers of animals in parentheses.

Age (days)	Weight of spleens (mg)	
	Infected	Normal
4	14 (232)	14 (242)
6	30 (43)	22 (42)
8	51 (119)	29 (349)
11	186 (36)	30 (180)
15*	564 (9)	32 (136)

* In this case 1-day-old mice were infected.

Scheme 1. Fractionation scheme of a spleen homogenate.

Fig. 1. Result after centrifugation of supernatant fraction 2 (see Scheme 1), from 900mg of spleen tissue, in a discontinuous sucrose density gradient for 20 h at $94000g_{av}$ in a Spinco SW27 rotor. Fractions (15 drops) were collected by puncturing the bottom of the tube. The pellet (P3) was resuspended in medium A. The amount of polyribosomes was calculated based on their extinction coefficients at 260 and 280nm. $\circ$, Polyribosomes from normal spleen; $\blacksquare$, polyribosomes from leukaemic spleen. The arrow indicates the direction of sedimentation.

the supernatant after centrifugation at $8100g$ (Attardi, Cravioto & Attardi, 1969). It is obvious that a quantitative evaluation of the amount of membrane-bound polyribosomes is very difficult by using a $1250g$ or $15000g$ supernatant fraction. The former contains most of the mitochondria and cytoplasmic vesicles with ribosomes (Plate 1) whereas in the latter almost all rough membranes are lost. After considering these facts, we chose the $7000g$ supernatant as the starting material for the isolation of the spleen polyribosomes. Most mitochondria were sedimented by this procedure.

Isolation of the polyribosomes. A typical example of the result of centrifugation of the postmitochondrial supernatant fraction on a discontinuous sucrose gradient for 20 h at $94000g$ is presented in Fig. 1. The pellet (P3) contains the large polyribosomes, whereas a rather large amount of ribosomal monomers is retained in the 2.0M-sucrose layer. Electron-microscopic investigation of this layer reveals that it contains mainly ribosomes and polyribosomes up to tetramers. Heavy polyribosomes are not detected in this layer.

The interface (band 1) between the 2.0M-sucrose layer and the 1.0M-sucrose layer contains smooth and rough membranes in addition to ribosomal monomers (Plate 2a). Subsequent centrifugation through a continuous sucrose density gradient (Fig. 2 and Plates 2b and 2c) completely separates the membranous fraction (band 2) from the ribosomes (band 3).

Fig. 2. Separation of the membranous fraction from free ribosomes. The interface fraction (band 1) from the discontinuous gradient was diluted and applied to a continuous gradient of 10–34% (w/v) sucrose with a 3ml 'cushion' of 68% (w/v) sucrose. Centrifugation was for $1\frac{1}{2}$ h at $94000g_{av}$ in a Spinco SW27 rotor. The gradient was automatically monitored at 254nm. Band 2 contained the smooth and rough membranes; band 3 consisted mainly of monomers. —, Polyribosomes from normal spleen; $\cdots$, polyribosomes from leukaemic spleen. The arrow indicates the direction of sedimentation.

Membrane-bound and free polyribosomes from rat liver can be separated by methods described previously (Bloemendal *et al.* 1964; Webb, Blobel & Potter, 1964). A survey of the functional differences between these two classes of ribosomes has been given by Campbell (1970). For spleen polyribosomes Talal & Dubois-Exum (1966) applied a different procedure. However, all these methods do not result in a complete separation when leukaemic spleen is the source of the polyribosomes. When the method of Bloemendal *et al.* (1964) or Webb *et al.* (1964) is applied there is a substantial contamination with 80S particles in the membrane-bound fraction, whereas in the Talal & Dubois-Exum (1966) method there is a distribution of smooth and rough membranes throughout the whole gradient, as can be demonstrated by electron microscopy. Further, in the last method the membrane fraction, which is located on the 68% sucrose 'cushion', is contaminated with large free polyribosomes. For this reason the modified procedure as described in the Materials and Methods section and summarized in Scheme 1, has been

EXPLANATION OF PLATE I

Ultrathin section of pellet P2 (Scheme 1), showing large vesicles containing ribosomes, besides mitochondria and membrane-bound ribosomal material.

J. TH. M. BURGHOUTS, A. L. H. STOLS AND H. BLOEMENDAL

Table 2. RNA and protein contents of the subcellular fractions from normal and leukaemic spleens

Centrifugal fraction	Content							
	Per g of spleen				Per 100mg of DNA*			
	Normal		Leukaemic		Normal		Leukaemic	
	mg of RNA	mg of protein	mg of RNA	mg of protein	mg of RNA	mg of protein	mg of RNA	mg of protein
Pellet P1	5.42		7.49		17.24		37.82	
Pellet P2	0.51		0.77		1.62		3.89	
Supernatant fraction 2	5.20	19.38	6.58	24.97	16.54	61.63	33.23	126.60
(a) Free polyribosomes	4.64	9.24	5.56	12.23	14.76	29.38	28.07	62.01
Pellet P3†	0.39	0.48	0.89	1.79	1.24	1.53	4.49	9.08
2.0M-Sucrose layer†	2.83	4.68	3.47	5.93	9.00	14.88	17.52	30.06
Ribosomes, band 3†	1.42	4.08	1.20	4.51	4.52	12.97	6.06	22.87
(b) Membrane-bound polyribosomes, band 2	0.56	10.14	1.02	12.74	1.78	32.25	5.15	64.59
Total RNA	11.13		14.84		35.39		74.94	

* mg of DNA/g of spleen: normal, 31.40; leukaemic, 19.74.

† Constituents of free polyribosome fraction.

used. It has the advantage of a more consistent separation between membrane-bound polyribosomes and contaminating monomers.

Composition of the centrifugal fractions. To verify whether a difference does exist between the two tissues, a comparison has been made between RNA and protein content of the various fractions from normal and Rauscher-virus-infected spleen cells. To get a better insight into the distribution per cell, the values based on DNA content have also been included (Table 2). Calculations of the RNA content of the membrane-bound fraction based on the extinctions at 260nm and 280nm is not entirely correct since the smooth membranes also contribute to the extinction besides light-scattering due to membrane components (Attardi *et al.* 1969). The orcinol reaction, after removal of sucrose, yields lower RNA contents, which vary from 37–57% in different experiments. The RNA content of free polyribosomes as measured by the orcinol reaction

yields values between 80 and 100% of the amount of RNA calculated after measurement of the extinction at 260 and 280nm.

It may be concluded from Table 2 that there is a marked increase in both free and membrane-bound polyribosomes after infection with the Rauscher virus. This increase is especially pronounced when the different fractions are related to the DNA content. The majority of the polyribosomes from the leukaemic spleen is apparently not bound to membranes. This is consistent with the conception that most polyribosomes in rapidly multiplying cells are free (Penman, Greenberg & Willems, 1969). The same phenomenon has been observed in cells treated with chemical carcinogens (Benedetti & Emmelot, 1966).

Membrane-bound polyribosomes. When the gradient fraction containing the membrane-bound polyribosomes, from both normal and leukaemic spleen, is treated with 0.5% sodium deoxycholate and applied to the continuous sucrose density gradient as described in the Materials and Methods section, only an increase in 80S particles is noticed. Extra addition of the ribonuclease inhibitor (5mg/ml) does not alter this feature. Apparently no polyribosomes can be released as such. This may mean that either only monomers are attached to membranes or the polyribosomes are degraded by nuclease released during solubilization of the membranes by sodium deoxycholate.

Large polyribosomes. Pilot experiments revealed that 5 days after infection, concomitantly with a significant increase in spleen weight, a shift towards larger polyribosomal aggregates occurred. This observation was confirmed by electron microscopy.

EXPLANATION OF PLATE 2

(a) Ultrathin section of the pelleted band 1, consisting of membranous as well as ribosomal material. (b) Ultrathin section of the pelleted band 2, consisting mostly of smooth membranes and of membrane-bound polyribosomes. (c) Ultrathin section of the pelleted band 3, consisting of ribosomes (and possibly small polyribosomes) only. (d) Polyribosomes, from the peak fraction located on the 68% sucrose 'cushion' of the linear sucrose density gradient (Fig. 3), spread on carbon film and positively stained with 1% hydrated uranyl acetate. Careful examination of the micrographs reveals the presence of a few clusters containing up to 40 ribosomes per strand. Magnification is the same in all four preparations.

Fig. 3. Polyribosome profiles obtained from the pellets (P3) of the discontinuous gradients. The pellets were resuspended in medium A with ribonuclease inhibitor (5 mg/ml) and were layered on a continuous gradient of 10–34% (w/v) sucrose with a 5 ml 'cushion' of 68% (w/v) sucrose. Centrifugation was for 1½ h at 94 000 g_{av} in a Spinco SW 27 rotor. The gradients were automatically monitored at 254 nm. ----, Polyribosomes from 1320 mg of normal spleen; ····, polyribosomes from 1320 mg of leukaemic spleen; —, polyribosomes obtained after mixing the homogenates from 660 mg of normal and 660 mg of leukaemic spleen. The arrow indicates the direction of sedimentation.

Pronounced quantitative differences between normal and infected spleen polyribosomes exist after 2 weeks. This is illustrated in Fig. 3 when the pellets (P3) from the initial discontinuous gradients are resuspended and examined on continuous gradients. The large polyribosomes accumulate on the sucrose 'cushion'. These polyribosomes are barely observed in the corresponding fractions of the normal spleen. As it cannot be excluded that the absence of large aggregates in the polyribosomes of the normal spleen is due to endogenous ribonuclease, we carried out an experiment in which equal amounts of homogenates of normal and infected spleen were mixed. This does not result in a complete disappearance of the large polyribosomes, whereas an obvious increase of 80S particles occurs (Fig. 3). From this experiment and the results mentioned above under 'Fractionation' it can be concluded that neither liver supernatant nor purified ribonuclease inhibitor completely inhibits the nucleases present in mouse spleen. Northup *et al.* (1967) claim to have achieved a complete inhibition of endonuclease action on polyribosomes from rat spleen cells with crude liver-cell extracts. If this is true, in mouse spleen a ribonuclease is present that is insensitive to the ribonuclease inhibitor. An acid ribonuclease and an alkaline ribonuclease have been described for rat liver (Roth, 1954; Rahmann, 1966). However, only the latter is susceptible to the action of the ribonuclease inhibitor (Roth, 1956; Hunter & Korner, 1968). Notwithstanding the fact that all our solu-

tions are kept at pH 7.6, we still may encounter a residual effect of the acid ribonuclease. Such a ribonuclease, assayed at pH 5.8, has been described for normal and leukaemic mouse spleens (Chakrabarty, Friedman & Ceglowski, 1969).

The conclusion seems to be justified that this residual ribonuclease activity is not responsible for the absence of large polyribosomes in the normal spleen, as these large polyribosomes from the leukaemic spleen do not disappear after mixing the homogenates of normal and infected tissues.

The role of polyribosomes in virus infection is obvious. After infection with a leukaemia virus a complex process takes place in the spleen. Besides virus replication (Rauscher, 1962), a process of cellular involvement must exist. Both require polyribosomes to produce specific proteins. The viral RNA has a sedimentation coefficient of approx. 70S (Galibert, Bernard, Chenaille & Boiron, 1965; Duesberg & Robinson, 1966). More recent investigations indicate that this RNA molecule is composed of subunits of 37S (Blair & Duesberg, 1968; Duesberg & Cardiff, 1968). The viral RNA ('plus' strand) hybridizes with specific RNA from the nuclei of infected cells. This complementary virus-specific RNA ('minus' strand) has a sedimentation coefficient of 31–36S and 18–22S respectively (Biswal & Benyesh-Melnick, 1969). If this RNA, either as 'plus' or as 'minus' strand, acts as mRNA one would expect to find large polyribosomes on investigation with the electron microscope. This phenomenon has been demonstrated in HeLa cells

Fig. 4. Polyribosome profile from leukaemic spleen after pulse-labelling *in vivo* for 5 min with $5\mu\text{Ci}$ of ^{14}C -labelled protein hydrolysate. Centrifugation was the same as described in the legend of Fig. 3. Fractions (1 ml) were collected by puncturing the bottom of the tube and the E_{260} was measured. Determination of radioactivity was as described in the Materials and Methods section. ■, $E_{260}^{1\text{cm}}$; ●, radioactivity (c.p.m.); ▲, specific radioactivity (c.p.m./ E_{260} unit).

infected with poliovirus. The 35S poliovirus RNA serves as messenger in the large polyribosomes, which contain 35 ribosomes per viral mRNA molecule (Summers, Maizel & Darnell, 1967).

In our experiments, samples taken from the fractions on the sucrose 'cushion' (Fig. 3) and investigated in the electron microscope revealed the presence of a few large polyribosomes of up to 40 monomers (Plate 2d).

Labelling experiments *in vivo* indicate that the largest polyribosomes (Fig. 4, fractions 3 and 4) show the highest specific radioactivity, i.e. incorporate more ^{14}C -labelled amino acids per unit of RNA than the slower-sedimenting fractions on the 'cushion'.

Note added in proof. Meanwhile we have observed large polyribosomes of approximately the same size (as described for leukaemic spleen) in cultured spleen-thymus cells infected with Rauscher virus. These large polyribosomes were absent from uninfected control cells.

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