Biochem. J. (1967) **103**, 280

Protein Synthesis by Microsomal Particles from Regenerating Rat Liver

By P. N. CAMPBELL, ELIZABETH LOWE and GULDBORG SERCK-HANSSEN*
Courtauld Institute of Biochemistry, The Middlesex Hospital Medical School, London, W. 1

(*Received* 17 *August* 1966)

1. Washed microsome particles from regenerating liver were shown to incorporate [^{14}C]leucine into protein more actively than similar preparations from normal liver. 2. The total incorporation in the preparations from regenerating liver increased linearly with the amount of protein incubated, whereas this was not so with preparations from normal liver. 3. The greater activity of regenerating-liver microsomes appeared to be associated with the bound polysomes. 4. The size distribution of polysomes obtained after removal of membrane with deoxycholate was the same in normal and regenerating liver. 5. In general the activity of polysome preparations from normal and regenerating liver was similar. 6. It is concluded that the greater activity of the particles in the microsome fraction from regenerating liver is to be attributed to the ribosomes bound to membrane and that their activity is controlled by factors present in the membrane.

It has previously been established in many Laboratories that the microsome fraction isolated from the livers of rats after partial hepatectomy is more active for protein synthesis than is a similar fraction from the liver of normal rats (Decken & Hultin, 1958; and see review by Bucher, 1963). The increased activity is associated with both the particles of the microsome fraction and with the soluble supernatant (cell sap), which is necessary for the protein-synthesizing activity of the isolated microsomes (Hultin & Decken, 1957, 1958; Campbell & Greengard, 1959). The continuing interest in regenerating liver is because it provides an excellent system in which to study the mechanism whereby protein synthesis is controlled. In the present paper we consider the role of the particles in the microsome fraction.

McCorquodale, Veach & Mueller (1960) claimed that the microsome fraction from regenerating-liver homogenate retained its activity for protein synthesis during incubation longer than that from normal liver. Moreover, they found that the regenerating-liver fraction was richer in ribosomes than the normal, so that when both these factors were taken into consideration the differences in activity were eliminated. Campbell, Cooper & Hicks (1964) compared the activity of the microsome fractions and the ribonucleoprotein particles obtained from the latter by treatment with deoxycholate. Since the ribonucleoprotein particles from regenerating liver no longer had the enhanced activity of the microsome fraction it was argued that the membranous component of the microsome fraction was important in controlling the rate of synthesis of protein by the ribosomes. An alternative explanation rests on the findings of Noll, Staehelin & Wettstein (1963), who showed that aggregates of ribosomes (ergosomes or polysomes) were more active for protein synthesis than were the individual ribosomes. Hence the enhanced activity of the microsome fraction from regenerating liver might be due to a higher proportion than normal of polysomes that were degraded during the removal of membrane by treatment of the microsome fraction with deoxycholate.

In the present experiments various methods for the preparation of polysomes have been examined and the activities of the products for protein synthesis compared. The hope was that it might thereby be possible to determine the relative contributions of the membrane and polysome components of the microsome fraction to the greater activity of the preparation from regenerating liver.

While this work was in progress there have been several relevant contributions to this subject. Cammarano, Giudice & Lukes (1965) found an increased complement of heavier polysomes, largely dissociated from the endoplasmic reticulum, as a conspicuous feature of the microsome fraction of regenerating liver. They found that a ribonucleoprotein-particle preparation prepared from re-

* Present address: Department of Biochemistry, University of Bergen, Norway.

generating liver by the use of deoxycholate retained some of the enhanced activity. Staehelin (1965) also reported a higher proportion of large polysomes in the regenerating-liver fraction. Tsukada & Lieberman (1965) consider that phospholipid performs an essential function in the aggregation of liver ribosomes into polysomes and that such additional polysomes are responsible for the enhanced activity of the regenerating-liver perparation.

MATERIALS AND METHODS

Chemicals. The dipotassium salt of ATP and the sodium salt of GTP were obtained from Sigma Chemical Co., St Louis, Mo., U.S.A., and pyruvate kinase was obtained from C. F. Boehringer und Soehne G.m.b.H., Mannheim, Germany. The silver–barium salt of phosphoenolpyruvic acid was prepared by the method of Clark & Kirby (1963). Solutions of free phosphoenolpyruvic acid were prepared before use and adjusted to pH 7·4 (glass electrode) with N-KOH. Tris ('specially purified') was obtained from British Drug Houses Ltd., Poole, Dorset, and polyuridylic acid from Miles Laboratories, Stoke Poges, Slough, Bucks. Sodium deoxycholate was obtained from E. Merck A.-G., Darmstadt, Germany.

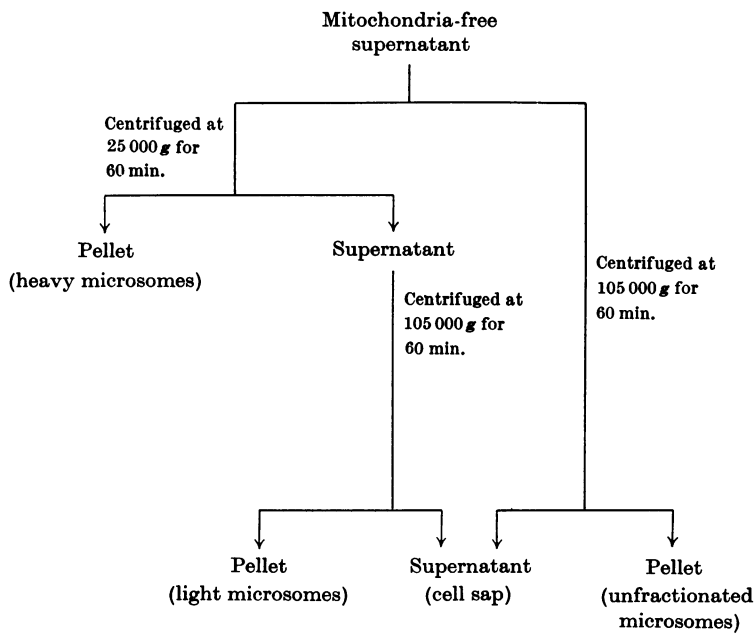
Radioactive amino acids. L-[U-^{14}C]Leucine (160mc/m-mole) and L-[U-^{14}C]phenylalanine (300mc/m-mole) were obtained from The Radiochemical Centre, Amersham, Bucks.

Animals. Adult rats (150–200g. body wt.) were Wistar albino from a closed colony bred at this Institute. The rats were starved for about 18hr. before being killed by a blow on the head followed by decapitation. Partial hepatectomy was performed by the method of Higgins & Anderson (1931).

Disruption of tissue. The liver was disrupted as follows. On removal from the animal the liver was placed in cold medium B, containing $MgCl_2$ (5mM), KCl (25mM) and tris buffer (50mM) (adjusted to pH 7·8 with HCl and checked by a glass electrode at 25°) and sucrose (0·25M) (Webb, Blobel & Potter, 1964). The tissue was placed on a filter paper to remove excess of buffer and was minced rapidly in a tissue press that had been cooled previously in ice. (The press was purchased from Climpex Ltd., London, N.W. 7.) The mince (4g.) was placed in a glass Potter-type homogenizer with 11ml. of medium B and further disrupted with a loose-fitting Teflon pestle. The clearance between the pestle (outer diam. 22mm.) and mortar was minimum 0·22mm. and maximum 0·31mm., the interior of the mortar being slightly oval in cross-section. The maximum speed of the pestle rotations was 1800 rev./min. Two complete passes were performed from top to bottom of the mortar, the complete cycle taking 10sec.

Preparation of microsomal fractions. The tissue suspension prepared as described above was centrifuged for 10min. at 12000*g* to remove cell debris, nuclei and mitochondria to give the 'mitochondria-free supernatant'. The preparation of unfractionated microsomes and heavy- and light-microsomal fractions was as described previously (Campbell, Serck-Hanssen & Lowe, 1965) except that medium A was replaced by medium B. The fractionation procedure may be summarized as shown in Scheme 1.

When washed preparations were required the pellets were gently suspended in about 4ml. of medium B, replaced in the cellulose tube of the no. 40 Spinco rotor, which was filled with medium B, and the suspension was



Scheme 1.

centrifuged at 105000*g* for 35min. in the Spinco model L ultracentrifuge.

Preparation of detergent-treated polysomes (C-ribosomes). Detergent-treated polysomes were prepared as described by Webb *et al.* (1964) by treating the mitochondria-free supernatant with 1·25% (w/v) deoxycholate and centrifuging through a double layer of sucrose (4ml. of 0·5M over 3ml. of 2·0M) in medium B at 105000*g* for 4hr. in the no. 40 rotor of the Spinco model L ultracentrifuge.

Analysis of pellet of detergent-treated polysomes. The pellet of detergent-treated polysomes was gently suspended in medium B to give an RNA concentration of 0·5–1mg./ml. Suspension was by gently stirring with a glass rod. The SW 25 rotor of the Spinco model L preparative centrifuge was used for analysis. The suspension (1ml.) was layered over a linear sucrose gradient (27ml.), which was from 10% to 40% (w/v) sucrose. The sucrose was prepared in medium B and the gradient was prepared with the apparatus described by Britten & Roberts (1960). After centrifuging for 2hr. at 63000*g* the rotor was brought to rest without the brake and the distribution of the ribosomes determined by siphoning 0·5ml. fractions from the bottom of the tube as described previously (Campbell *et al.* 1965). Each fraction was diluted with water to 1·5ml. and the extinction determined at 260mμ.

Conditions for incubation of fractions. The particulate fractions were suspended by gentle homogenization in a Potter-type homogenizer in medium A, containing $MgCl_2$ (10mM), KCl (25mM), tris buffer, pH7·8 (35mM), and sucrose (0·15M). Each incubation tube contained the particle suspension in a total volume of medium A of 0·4ml. of cell sap or pH5 fraction, ^{14}C-labelled amino acid, 2μmoles of ATP and 15μmoles of phosphoenolpyruvate in a total volume of 1·0ml. Various other additions were sometimes made as indicated for the particular experiments. Incubation was with shaking in air at 37°, for 30min. for the microsome preparations and 60min. for the polysome preparations.

Analysis of distribution of radioactive protein after incubation. When it was necessary to determine the distribution of radioactive protein among the various types of ribosomes after incubation of the microsome fraction the method previously described (Campbell *et al.* 1965) was used. The only modification was that the concentration of $MgCl_2$ in the sucrose density gradient was 5mM instead of 0·1mM.

Determination of specific radioactivity. The protein was dissolved in 98–100% formic acid (A.R.), transferred to 7cm.^{2} aluminium disks, dried under an infrared lamp and counted at infinite thinness. Radioactivity was determined in a thin-window gas-flow counter (Nuclear–Chicago Corp.). A standard planchet of 1cm.^{2} containing 1μc of ^{14}C/g. gave approx. 1800 counts/min. at infinite thickness under these conditions. The efficiency of counting ^{14}C at infinite thinness under the described conditions was 25%.

Extraction of protein for the assay of radioactivity. After incubation the protein was precipitated by the addition of trichloroacetic acid (5%, w/v), the RNA removed by heating in the same acid and the protein extracted and dried as previously described (Campbell *et al.* 1965).

Estimations. Protein was determined by the Folin–Ciocalteu method of Lowry, Rosebrough, Farr & Randall (1951), with bovine serum albumin as standard. RNA was determined by the orcinol method of Mejbaum (1939), with hydrolysed yeast RNA as standard. These methods were used for the determination of RNA-protein ratios.

RESULTS

Technique of cell disruption. Lightly chopped tissue from regenerating liver is much more easily disrupted in a Potter-type homogenizer than is normal tissue. It was thought that this could have an influence on the recovery of large polysomes from the homogenate. Accordingly the liver tissue was first passed through a metal press to produce a fine mince and was then treated in a Potter-type homogenizer with a glass mortar and a loose-fitting Teflon pestle. The pestle was turned at a controlled speed and the number of vertical movements was also controlled. The choice of Teflon rather than glass for the pestle was mainly because the wear is

Table 1. *Effect of source of cell sap on amino acid incorporation by microsome fractions from normal and regenerating liver*

Washed microsomes were incubated with phosphoenolpyruvate (15μmoles), ATP (2μmoles), pyruvate kinase (50μg.), [^{14}C]leucine (0·25μc) and cell sap (0·1ml.) in a total volume of 1ml. for 30min. The amount of protein in each tube was the same.

Source of cell sap	Source of microsomes.....	Radioactivity incorporated (counts/min./mg. of protein): Normal liver	Regenerating liver	Enhancement ratio (microsome effect)
Normal liver		364 (*a*)	942 (*b*)	2·6 (*b*/*a*)
Regenerating liver		961 (*c*)	1707 (*d*)	1·8 (*d*/*c*)
Enhancement ratio (cell-sap effect)		2·6 (*c*/*a*)	1·8 (*d*/*b*)	4·9 (*d*/*a*)

less with Teflon and so the results are more reproducible from day to day.

Variation of the speed of the rotating pestle did not consistently affect the activity of the resulting microsome pellet. An intermediate speed was therefore chosen.

Relative contribution of particles and cell sap to the enhanced activity of regenerating-liver preparations. The results in Table 1 show the effect of incubating the microsome fractions from normal and regenerating liver in the presence of homologous and heterologous cell sap. In all experiments the microsome fractions were washed with medium and resedimented in an attempt to remove as much of the soluble cytoplasm as possible from the membranous particles. The conclusion is that the particles and the cell sap contribute almost equally to the greater activity of regenerating-liver microsomes. The particle effect is greater when the incubation is in the presence of cell sap from normal rather than regenerating liver and this has been the procedure throughout the present work.

Factors influencing the relative activity of the microsome fraction from normal and regenerating liver. The major influence on the relative activity of the microsome pellets from the two sources was the amount of microsomal protein incubated in the presence of a constant amount of cell sap and of the various metabolites.

The results in Fig. 1 show that there is a marked difference in the effect of increasing the amount of microsomal protein in the incubation medium on the total radioactivity of the synthesized protein and that this depends on the source of the microsome fraction. These results, which confirm those previously reported by Hoagland, Scornik & Pfefferkorn (1964), are consistent with the presence in the normal liver microsomes of an inhibitor that is less active in preparations from regenerating liver. Hoagland *et al.* (1964) demonstrated that GTP antagonized the inhibition so the effect of adding GTP to the incubation medium was therefore tested. GTP at a concentration of 0·25 μmole/ml. was without effect on the incorporation by microsomes of either normal or regenerating liver.

M. B. Hoagland (personal communication) has also suggested that GSSG may participate in the inhibition mediated by the membrane-associated inhibitor. The effect of adding freshly prepared GSH was tried, and also of adding reduced NADPH, which, through the transdehydrogenase present in liver extracts, would be expected to lead to the reduction of any GSSG present. The addition of GSH in the concentration range 0·3–1·2 μmoles/ml. increased the activity of microsomes of both normal and regenerating liver by about 12% at the highest concentration, but did not affect the ratio of the activities of the two preparations.

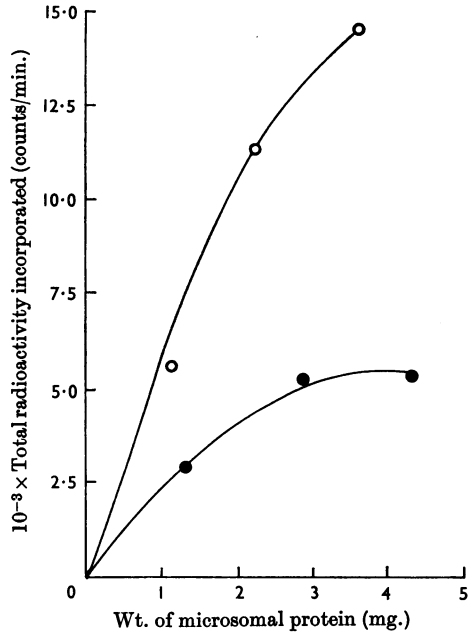


Fig. 1. Relation between amount of microsomal protein incubated and total amount of [^{14}C]leucine incorporated into protein. Washed microsomes were incubated with [^{14}C]leucine under the conditions given in Table 1 but with cell sap derived from normal liver. The amount of protein was determined by the method of Lowry *et al.* (1951) and total radioactivity was determined at infinite thinness. ○, Microsomes from regenerating liver; ●, microsomes from normal liver.

If, as was suggested by McCorquodale *et al.* (1960), the microsomal particles from regenerating liver were more stable on incubation than those from normal liver, the duration of incubation could also be important. As shown in Fig. 2 the two reach a plateau at a similar time and there is no marked difference in stability under the conditions used in the present experiments.

Experiments with submicrosomal fractions. Campbell *et al.* (1965) have described the preparation of two subfractions of liver microsomes designated 'heavy' and 'light'. The heavy fraction contains a preponderance of bound ribosomes as opposed to free ribosomes when analysed by the technique of Henshaw, Bojarski & Hiatt (1963), whereas the light fraction is richer in free ribosomes than in bound ribosomes. It is also clear that the particles in such fractions that are stimulated by polyuridylic acid are mainly the free ribosomes, which are not associated with messenger RNA. In accord with our previous experience the effect on the incorporation of [^{14}C]phenylalanine of incubating the unfractionated microsomes with

polyuridylic acid was similar irrespective of the source of the microsome fraction. This also applied when the light- and heavy-microsome fractions were used. Estimations of the RNA/protein ratios of the fractions from the two sources showed that those from regenerating liver had a slightly higher RNA/protein ratio, but otherwise the results were similar to those already published (Campbell *et al.* 1965) for normal liver. When examined by the method of Henshaw *et al.* (1963) the distribution of bound and free ribosomes between the various fractions from the two sources showed little difference.

Table 2 shows a comparison between the activity of the three microsome fractions from normal and regenerating liver. Whereas the unfractionated and heavy fractions were more active when derived from regenerating liver, the light fractions were of similar activity.

Comparison of activity of detergent-treated polysomes. Wettstein, Staehelin & Noll (1963) showed that, if the mitochondria-free supernatant from a liver homogenate was treated with deoxycholate and the resultant suspension centrifuged over a cushion of concentrated sucrose, then it was possible to obtain a pellet rich in polysomes. In particular, if the sucrose concentration was 2 M then the ribonucleoprotein particles were termed 'C-ribosomes'. This method has been used to prepare detergent-treated polysomes from normal and regenerating liver.

The results obtained from 16 separate experiments showed that, though the activities of the polysomes from regenerating liver were consistently high for amino acid incorporation ('standard polysomes'), a proportion of the preparations from

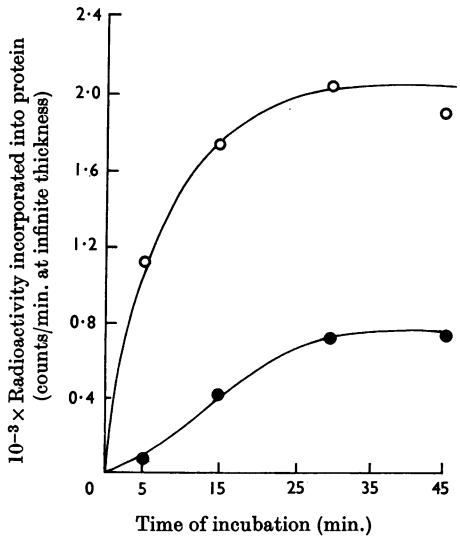


Fig. 2. Time curve for the incorporation of [^{14}C]leucine into protein by microsome fractions from normal and regenerating liver. Washed microsomes were incubated with [^{14}C]leucine under the conditions given in Table 1 but with cell sap derived from normal liver. ○, Microsomes from regenerating liver; ●, microsomes from normal liver.

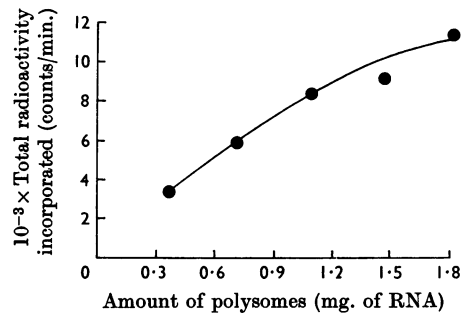


Fig. 3. Relation between amount of detergent-treated polysomes from normal liver incubated and total amount of [^{14}C]leucine incorporated into protein. Detergent-treated polysomes were incubated under the conditions given for microsome preparations in Table 1. The RNA in the preparation of polysomes was determined by the method of Mejbaum (1939) and radioactivity was determined at infinite thinness.

Table 2. *Comparison of the protein-synthesizing activities of microsome subfractions from normal and regenerating liver*

All microsome fractions were washed and incubated in the presence of [^{14}C]leucine (0·25 μc) and normal cell sap (0·2 ml.) under the conditions given in Table 1.

Fraction	Radioactivity incorporated (counts/min./mg. of protein)		
Source of microsome fraction.....	Normal liver (*A*)	Regenerating liver (*B*)	*B*/*A* ratio
Unfractionated	481	877	1·8
Heavy	510	808	1·6
Light	918	913	1·0

normal liver were defective ('defective polysomes'). The properties of the standard preparations are described first.

It was first necessary to demonstrate that under the conditions of incubation the total radioactivity of the resulting protein was proportional to the amount of ribosomal RNA in the incubation medium. As shown in Figs. 3 and 4 this was so for both preparations. Thus in making comparisons between the activity of the two preparations, not only was care taken to incubate nearly identical amounts of protein, but the amount of RNA in each incubation tube was determined from the RNA content of the polysome suspension by the orcinol technique (Mejbaum, 1939), and the specific activity/mg. of RNA calculated. In this respect the extinction coefficients of such suspensions determined at 260 and 280mμ were not an accurate estimation of the RNA present, even when a correction for ferritin was made by determining the extinction at 320mμ. The results of two experiments are shown in Table 3.

Thus for almost all the preparations of polysomes those from normal and regenerating liver were of similar activity. A typical activity curve for defective polysomes from normal liver is shown in Fig. 4. Many attempts were made to obtain reproducible conditions for the preparation of defective

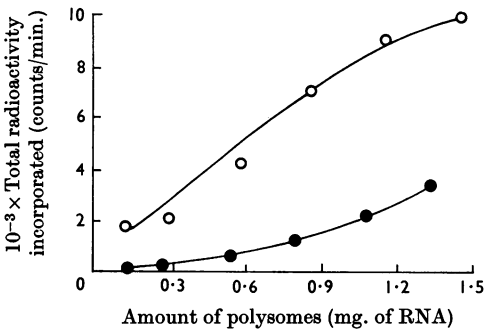


Fig. 4. Comparison between the activities of detergent-treated polysomes from regenerating liver and defective polysomes from normal liver. The polysome preparations were incubated with [^{14}C]leucine under the conditions given in Table 1. RNA and radioactivity were determined as in Fig. 3. ○, Polysomes from regenerating liver; ●, polysomes from normal liver.

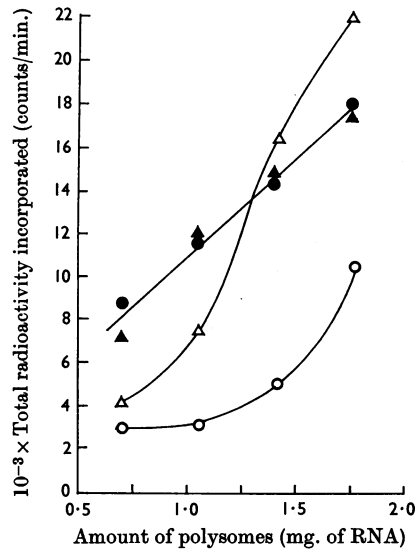


Fig. 5. Effect of addition of GTP on the activity of defective polysomes from normal liver. The preparations of detergent-treated polysomes were incubated with [^{14}C]leucine under the conditions given in Table 1, except for the addition of GTP where indicated. RNA and radioactivity were determined as in Fig. 3. The experiments with standard and defective polysomes respectively were performed on different occasions so that the absolute activities of the two preparations are not strictly comparable. ●, Standard polysomes; ▲, standard polysomes plus 0·25μmole of GTP/ml.; ○, defective polysomes; △, defective polysomes plus 0·25μmole of GTP/ml.

Table 3. *Comparison of incorporation of* [^{14}C]*leucine by standard detergent-treated polysomes from normal and regenerating liver*

The pellets of detergent-treated polysomes were suspended in medium B and incubated in the presence of [^{14}C]leucine (0·25μc) and cell sap (0·1ml.) under the conditions given in Table 1.

	Source of polysomes	Total radioactivity incorporated (counts/min.)	Amount of RNA (mg.)	Radioactivity incorporated (counts/min./mg. of RNA)
Expt. 1	Normal liver	2500	1·2	2083
	Regenerating liver	3084	1·5	2055
Expt. 2	Normal liver	6653	1·3	5280
	Regenerating liver	10187	1·7	5888

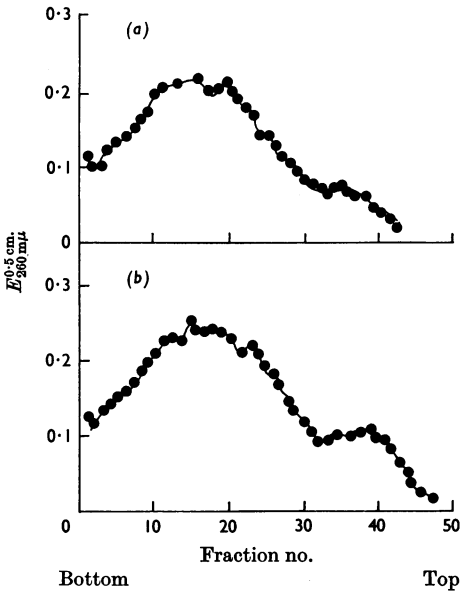


Fig. 6. Analysis of size distribution of detergent-treated polysomes from normal and regenerating liver. The method of analysis is described in the Materials and Methods Section. 'Top' indicates the top of the sucrose density gradient: sedimentation is from right to left. The 0·5ml. fractions were removed by suction after centrifugation in the SW25/1 rotor of the Spinco model L preparative centrifuge. Single ribosomes present in the preparation would sediment to about tube 35. (*a*) Polysomes from regenerating liver; (*b*) polysomes from normal liver.

ribosomes from normal liver without success. We have, however, found that the addition of GTP to the incubation medium will restore the activity of defective ribosomes to some extent. An experiment illustrating this is shown in Fig. 5. The addition of GTP to standard polysomes has virtually no effect. With defective polysomes the effect of GTP is very small at low concentrations of ribosomes, but is quite marked as the amount of polysomes in the medium increases.

Analytical comparison of detergent-treated polysomes. As shown in Fig. 6 no striking difference could be found in the sedimentation profiles of the two preparations. This indicates that there is no marked difference between the size distribution of the polysomes in the two preparations. This is contrary to the report of Cammarano *et al.* (1965), who found that there was a preponderance of larger polysomes in the preparation from regenerating liver.

DISCUSSION

Protein synthesis can be controlled at two different levels, called 'coarse control' and 'fine control' by Kornberg (1965). Coarse control is exerted on the genes and determines the range of proteins that may be synthesized by the particular cell; fine control determines the amount of each protein to be synthesized within a limited range. Fine control can be further classified depending on whether the control is over all protein (general) or whether the synthetic rate of only certain proteins is changed (specific).

The increase in the rate of protein synthesis that takes place in that part of the liver which remains after partial hepatectomy is an example of fine control. At present it is not known which specific proteins are concerned in the increase in protein synthesis. As stated in the introduction, various possible sites for the operation of the fine-control mechanism have been considered previously, but no firm conclusions have been made. In the present paper, which is solely concerned with the particulate components of the microsome fractions, four possible reasons for the higher activity of the microsome fraction from regenerating liver must be discussed. These are, first, that the control is exerted by some factor present in the membrane of the rough endoplasmic reticulum, secondly, that the morphology of the ribosomes in the fractions differ, thirdly, that the polysomes in the regenerating-liver fraction are larger than those from the normal liver, and, finally, that there is some factor tightly bound to the polysomes that affects their activity.

It was first necessary to examine the conditions under which a particulate fraction from regenerating liver is more active for protein synthesis than a similar fraction from normal liver. This is necessary because it has long been realized that the soluble fraction from regenerating liver is more active than that from normal liver. As shown in Table 1 the contributions of the particulate and soluble fractions to the greater activity of the microsome fraction from regenerating liver are approximately equal. For this reason washed microsomes have been used with the object of limiting the effect of the higher activity of the cell sap.

The results shown in Fig. 1 indicate that the washed particulate fraction from regenerating liver is more active than that from normal liver when both are incubated in the presence of normal cell sap. Moreover, the enhancement factor varies according to the amount of the fraction that is incubated. In contrast with regenerating liver, with normal liver an increase in the amount of particulate protein in the incubation medium does not lead to a proportional increase in the total radioactivity of the resulting protein. Several possible reasons for this may be mentioned. First, the particles either contain some free leucine or

contain proteolytic enzymes that release leucine during the incubation. This endogenous leucine would lower the specific radioactivity of the precursor leucine in the incubation medium, which would result in a plateau of activity, as seen in Fig. 1. Though this is possible it does not account for the difference between normal and regenerating liver. Another explanation would be that an inhibitor is present in the normal fraction that is virtually absent from the regenerating-liver preparation. Hoagland *et al.* (1964) first obtained evidence of such an inhibitor and their results suggested that GTP was involved. No evidence was found in the present experiments that GTP or glutathione is implicated. The ineffectiveness of glutathione is in accord with previous experiments (Campbell & Greengard, 1959; Rendi & Campbell, 1959). The inhibitor appears to be associated with the membrane components of the microsomes, since the relationship between the amount of RNA and total activity of the standard polysome preparations shows no indication of the presence of inhibitor (Figs. 3 and 4). The inhibitor seems to exert its effect early in the incubation of microsomes, since the enhancement ratio is high at 5min. (22), lower at 15min. (4·1) and lowest at 45min. (2·6) (see Fig. 2). The nature of the inhibitor remains unknown.

The estimation of the RNA/protein ratio of the microsome preparations showed that, though that from regenerating liver was higher in RNA, the difference was not sufficient to account for the greater activity on this basis. The time–activity curve reached a plateau at much the same time, so that at 30min. this would not be a factor. It therefore seems unlikely that the explanation of McCorquodale *et al.* (1960) is satisfactory.

Since it has been shown previously that the free ribosomes are comparatively inactive in protein synthesis in the absence of added synthetic polynucleotides (Henshaw *et al.* 1963; Campbell *et al.* 1964, 1965), a difference in the relative proportions of the different kinds of ribosomes could affect the activity of the microsome fraction based on RNA content. We have previously shown that the effect of polyuridylic acid on the incorporation of phenylalanine is similar for the microsome fraction from normal and regenerating liver (Campbell *et al.* 1964), suggesting that the proportion of free ribosomes is similar in the two preparations. The presence of polysomes not attached to membrane (free polysomes) was demonstrated in the microsome fraction from normal liver by Campbell *et al.* (1965), and so the proportion of bound to free polysomes might also differ. In this connexion the results in Table 3 are pertinent. The light fraction will contain a high proportion of free ribosomes and free polysomes and shows no difference between normal and regenerating liver. However, the fractions rich in bound polysomes are more active from regenerating liver. It seems likely that the major contribution to the higher activity of regenerating-liver preparations is to be attributed to the bound polysomes.

As indicated in Fig. 6, we have not been able to detect any significant difference in the size distribution of the polysomes isolated after removal of the membrane with deoxycholate. The results of Blobel & Potter (1966) and of Lawford, Langford & Schachter (1966) have cast doubt on the value of such estimations, since these authors have shown that disruption of the membrane by deoxycholate activates a nuclease that breaks up the polysomes, and that this nuclease is inhibited in part by a factor present in the cell sap. Since Shortman (1962) has shown that the activity of this inhibitor rises after partial hepatectomy, a variation in the size of the polysomes isolated after removal of the membrane could merely reflect the activity of the nuclease and its inhibitor.

The general conclusion from the results on the activity of the polysome preparations must be that those from regenerating liver have the same activity as those from normal liver. We must, however, add a rider that sometimes the polysomes from normal liver are peculiarly inactive and the curve relating the amount of ribosomes to total amount of radioactive amino acid incorporated is not linear. It appears that sometimes the polysomes from normal liver are isolated with an inhibitor tightly bound. That this inhibitor may have guanosine-triphosphatase activity is suggested by the enhanced effect of GTP on the activity of defective polysomes (see Fig. 5). This does not, however, seem to be the full explanation, because of the small effect of GTP at low ribosome concentrations. This accounts for the fact that the curve of activity for defective ribosomes in the presence of GTP is unexpectedly steep. That factors can be very tightly bound to ribosomes has been shown by Ibuki, Gaşior & Moldave (1966), who find that their transferase II is thus bound to liver ribosomes.

In conclusion, therefore, it appears that the greater activity of the particles in the microsome fractions from regenerating liver is to be attributed to the ribosomes bound to membrane. The activity of such ribosomes seems to be controlled by a factor associated with the membranes, but the nature of the factor is at present unknown.

This work was made possible by a grant to the Medical School from the British Empire Cancer Campaign. The receipt of a Wellcome Research Fellowship by G.S.-H. is gratefully acknowledged, as is a personal grant to P.N.C. from the Medical Research Council. We are also grateful to Mr Richard Parkin for performing the partial hepatectomies.

REFERENCES

Blobel, G. & Potter, V. R. (1966). *Proc. nat. Acad. Sci., Wash.*, **55**, 1283.

Britten, R. J. & Roberts, R. B. (1960). *Science*, **131**, 32.

Bucher, N. L. R. (1963). *Int. Rev. Cytol.* **15**, 245.

Cammarano, P., Giudice, G. & Lukes, B. (1965). *Biochem. biophys. Res. Commun.* **19**, 487.

Campbell, P. N., Cooper, C. & Hicks, M. (1964). *Biochem. J.* **92**, 225.

Campbell, P. N. & Greengard, O. (1959). *Biochem. J.* **71**, 148.

Campbell, P. N., Serck-Hanssen, G. & Lowe, E. (1965). *Biochem. J.* **97**, 422.

Clark, V. M. & Kirby, A. J. (1963). *Biochim. biophys. Acta*, **78**, 732.

Decken, A. von der & Hultin, T. (1958). *Exp. Cell Res.* **14**, 88.

Henshaw, E. C., Bojarski, T. B. & Hiatt, H. H. (1963). *J. molec. Biol.* **7**, 122.

Higgins, G. M. & Anderson, R. M. (1931). *Arch. Path.* **12**, 186.

Hoagland, M. B., Scornik, O. A. & Pfefferkorn, L. C. (1964). *Proc. nat. Acad. Sci., Wash.*, **51**, 1184.

Hultin, T. & Decken, A. von der (1957). *Exp. Cell Res.* **13**, 83.

Hultin, T. & Decken, A. von der (1958). *Exp. Cell Res.* **15**, 581.

Ibuki, F., Gaşior, E. & Moldave, K. (1966). *J. biol. Chem.* **241**, 2188.

Kornberg, H. L. (1965). *Symp. Soc. gen. Microbiol.* **15**, 8.

Lawford, G. R., Langford, P. & Schachter, H. (1966). *J. biol. Chem.* **241**, 1835.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). *J. biol. Chem.* **193**, 265.

McCorquodale, D. J., Veach, E. G. & Mueller, G. C. (1960). *Biochim. biophys. Acta*, **46**, 335.

Mejbaum, W. (1939). *Hoppe-Seyl. Z.* **258**, 117.

Noll, H., Staehelin, T. & Wettstein, F. O. (1963). *Nature, Lond.*, **198**, 632.

Rendi, R. & Campbell, P. N. (1959). *Biochem. J.* **72**, 435.

Shortman, K. (1962). *Biochim. biophys. Acta*, **61**, 50.

Staehelin, M. (1965). *Biochem. Z.* **342**, 459.

Tsukada, K. & Lieberman, I. (1965). *Biochem. biophys. Res. Commun.* **19**, 702.

Webb, T. E., Blobel, G. & Potter, V. R. (1964). *Cancer Res.* **24**, 1229.

Wettstein, F. O., Staehelin, T. & Noll, H. (1963). *Nature, Lond.*, **197**, 430.