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A Comparative Study *in vivo* and *in vitro* of the Ability of Ribosomes from *Xenopus* Liver and Ovary to Incorporate L-[U-^{14}C]Leucine

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1. A system for the incorporation *in vitro* of amino acids into protein is described for the South African clawed toad (*Xenopus laevis laevis* Daudin). 2. The incorporation of L-[U-^{14}C]leucine by *Xenopus*-liver microsomes is very much greater per mg. of microsomal RNA than the incorporation by ovary microsomes. 3. The incorporation by *Xenopus*-liver and -ovary polysomes is approximately the same when expressed per mg. of polysomal RNA. 4. It was predicted from the above results that ovary microsomes should contain a ribosomal fraction inactive in protein synthesis. This was shown to be the case by a labelling experiment *in vivo* with L-[U-^{14}C]leucine. 5. The labelling experiment *in vivo* also showed that the active polysomal fraction in ovary is associated with membranes and is liberated by treatment with deoxycholate; this is also true of liver microsomes *in vivo*. 6. The results are discussed in relation to previous work on the synthesis of proteins by amphibian ovarian tissue, and on the role of bound and free ribosomes in protein synthesis.

Cytoplasmic protein synthesis is associated with ribosomes bound into polymeric structures called polysomes (Tissières, Schlessinger & Gros, 1960; Warner, Rich & Hall, 1962; Marks, Burka & Schlessinger, 1962; Gierer, 1963; Wettstein, Staehelin & Noll, 1963; Warner, Knopf & Rich, 1963). Electron microscopy of tissues actively synthesizing protein reveals that most ribosomes are bound on to membranes, but there are some free (non-bound) ribosomes and polysomes (Porter, 1954; Palade, 1955; Palade & Siekevitz, 1956*a*,*b*; Slautterback & Fawcett, 1959; Prescott, 1960; Siekevitz & Palade, 1960; Birbeck & Mercer, 1961). In reticulocytes, where no secretory function is known, it has been observed that polysomes are free in the cytoplasm (Marks, Burka, Rifkind & Danon, 1963). In adult rat liver, where both free and bound ribosomes occur, the activity of the free ribosomes in protein synthesis *in vitro* is low compared with the bound fraction unless synthetic polynucleotide is added (Henshaw, Bojarski & Hiatt, 1963; Campbell, Cooper & Hicks, 1964). However, Campbell, Serck-Hanssen & Lowe (1965) conclude that the free polysomes in both young and adult rat liver are active in protein synthesis *in vitro* to the same extent as bound polysomes, whereas the free monomer ribosomes are inactive, unless synthetic messenger is added.

It has been shown that it is possible to isolate ribosomes from ovary, eggs and embryos of *Rana pipiens* (Kohne, 1965) and of *Xenopus* (Brown & Littna, 1964). Labelled amino acids are incorporated into the cytoplasm and nucleus (germinal vesicle) of frog oocytes (Ficq, 1964; Kemp, 1955; Merriam, 1966). The experiments reported below constitute a comparative study of the capacity of ovarian and liver ribosomes to incorporate amino acid, both *in vivo* and *in vitro*, and are intended to throw some light on the nature of the apparatus for protein synthesis during amphibian oogenesis.

MATERIALS AND METHODS

Chemicals. The disodium salt of ATP, the sodium salt of GTP, creatine phosphate, creatine phosphokinase and high-purity tris were obtained from Sigma Chemical Co., St Louis, Mo., U.S.A. Pure-grade reduced glutathione (sodium salt) and β-mercaptoethanol were obtained from L. Light and Co. Ltd., Colnbrook, Bucks. Pancreatic ribonuclease, sodium deoxycholate, unlabelled amino acids, toluene and ethylene glycol monoethyl ether (Cellosolve) were obtained from British Drug Houses Ltd., Poole, Dorset. 2,5-Diphenyloxazole and 1,4-bis-(5-phenyloxazol-2-yl)benzene came from Thorn Electronics Ltd., Surbiton, Surrey.

Radioactive amino acid. L-[U-^{14}C]Leucine (170mc/m-mole) was obtained from The Radiochemical Centre, Amersham, Bucks.

Animals. Adult female *Xenopus laevis laevis* Daudin were obtained from The South African Snake Farm, P.O.

Box 6, Fish Hoek, Cape Province, South Africa. They were kept in glass tanks with 6in. of stood tap water at 18–20°, and fed on chopped bovine liver twice a week in the mornings. The tanks were cleaned out in the afternoons.

Preparation of microsomes. All preparative stages were carried out in a cold room at 4°. The liver and ovaries were removed and weighed in ice-cold medium (medium A) containing tris buffer (50mM) adjusted to pH7·8 with HCl, KCl (25mM), $MgCl_2$ (5mM) and sucrose (0·25M) (Hoagland, Stephenson, Scott, Hecht & Zamecnik, 1958). The tissues (10–15g.) were chopped into small pieces with scissors and gently homogenized (three strokes at 1000–2000 rev./min.) in 50ml. of medium A in a Teflon–glass Potter–Elvehjem homogenizer (A. H. Thomas Co., Philadelphia, Pa., U.S.A.). The tissue suspension was centrifuged at 12500*g* for 10min. at 0–4° in an MSE 8×50ml. angle-head centrifuge to remove cell membranes, nuclei, mitochondria and unbroken cells. The pellet was discarded and the supernatant centrifuged at 76000*g* for 2hr. at 0° in an MSE 8×50ml. angle-head centrifuge to give a microsomal pellet and PMS.* The PMS was decanted and retained as a source, after dialysis, of transfer RNA and activating enzymes for addition to the cell-free system. The microsome pellet was washed gently with medium A and then resuspended in medium A (1·0ml./g. of tissue); before use in the cell-free system the microsome suspension was recentrifuged at 12500*g* for 10min. at 0–4°.

The microsome pellet was observed to be composed of two layers: the lower layer was deep yellow from liver but pale yellow from ovary; the upper layer was deep red-brown in both cases. The upper layer was easily removed from the lower one and from ovary it contained 50–60% of the total microsomal RNA, but from liver it only contained 10–20% of the total microsomal RNA.

Preparation of polysomes. A polysome fraction was prepared by the method of Wettstein *et al.* (1963) except that there was no 0·3M-sucrose layer between the microsome preparation and the 2M-sucrose. The microsome suspension was treated with an equal quantity of freshly prepared 2% (w/v) sodium deoxycholate in medium A and layered over 5ml. of 2M-sucrose in medium A. The tube was centrifuged at 0° for 4hr. at 105000*g* in an MSE 3×20ml. swing-out-head centrifuge. The polysome pellets obtained were colourless and the red and yellow colours of the microsome pellets were observed as bands at the interface. The pellet was resuspended in a volume of medium A equal to that of the microsome suspension from which it was derived.

The mean percentage recoveries of microsomal RNA in polysome fractions and standard errors of the means in five experiments were for liver 34·3±8·3% and for ovary 4·3±0·9%.

Incubation mixture. In the experiment shown in Fig. 1,

* Abbreviation: PMS, post-mitochondrial supernatant.

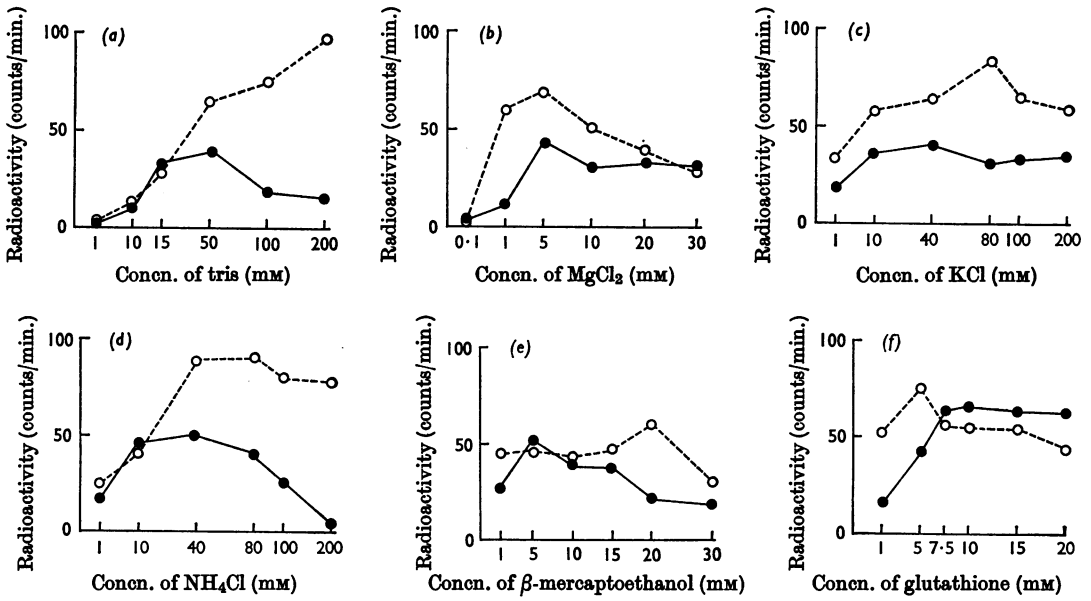

Fig. 1. Incorporation of L-[U-^{14}C]leucine by liver and ovary microsomes at different concentrations of (*a*) tris buffer, pH7·8, (*b*) $MgCl_2$, (*c*) KCl, (*d*) NH_4Cl, (*e*) β-mercaptoethanol and (*f*) reduced sodium glutathione. In each case the non-variable components were tris buffer, pH7·8, 135mM, $MgCl_2$ (13·5mM), KCl (67·5mM) and β-mercaptoethanol (9mM). In the experiments with NH_4Cl and reduced glutathione these substances completely replaced KCl and β-mercaptoethanol respectively. Other additions per tube were ATP (1μmole), GTP (0·03μmole), creatine phosphate (5μmoles), creatine phosphokinase (2μg.), all unlabelled amino acids (0·05μmole) except leucine, L-[U-^{14}C]leucine (0·1μc, specific activity 170μc/mole). ●, 0·1mg. of liver microsomal RNA and liver PMS (3·4mg. of protein, 0·17mg. of RNA); ○, 0·3mg. of ovary microsomal RNA and ovary PMS (3·1mg. of protein, 0·48mg. of RNA).

liver and ovary microsomes were incubated for 15min. at 30° with liver and ovary PMS respectively. ATP (1μmole), GTP (0·03μmole), creatine phosphate (5μmoles) and creatine phosphokinase (2μg.) were added to each tube as an energy-generating system, which is a requirement of the system (Table 1). The concentrations of the components when not being varied were as follows: tris buffer, pH7·8 (135mM); $MgCl_2$ (13·5mM); KCl (67·5mM); β-mercaptoethanol (9mM). In experiments with NH_4Cl and glutathione these substances replaced KCl and β-mercaptoethanol respectively.

The incubation mixture for all subsequent experiments was based on the results shown in Fig. 1 and was made up as follows: 0·1ml. of microsome or polysome preparation in medium A; 0·1ml. of energy-generating system, as defined above, in distilled water; 0·1ml. of complete amino acid mixture (each amino acid 0·5mM) minus leucine in distilled water; 0·1ml. of L-[U-^{14}C]leucine (0·1μC/tube) in distilled water; 0·1ml. of buffer [tris buffer, pH7·8 (0·2M); $MgCl_2$ (20mM); KCl (0·45M); reduced glutathione (75mM)]; 0·5ml. of PMS dialysed for 6–12hr. with continuous stirring against 3l. of the buffered salt solution of medium A, replaced by the same volume of fresh medium after the first 3hr.

The final volume was 1ml. and the final concentrations were: tris (50mM); $MgCl_2$ (5mM); KCl (60mM); reduced glutathione (7·5mM). All incubations were for 15min. at 30° in a constant-temperature water bath. Tubes were covered with Parafilm during the incubation.

The amount of PMS protein added per tube was always between 2·5 and 4·0mg. for both liver and ovary PMS, and the amount of RNA was between 0·15 and 0·23mg. for liver PMS and between 0·35 and 1·25mg. for ovary PMS.

The results in Figs. 2(*a*) and 2(*b*) show that the incorporation in this system is proportional to the amount of RNA up to a limit. Figs. 2(*a*) and 2(*b*) also point to a difference in activity between liver and ovary PMS.

Fig. 3 shows that the time-course for incorporation in this system is linear for the first 15min. at least.

Labelling of protein in vivo by L-[*U*-^{14}C]*leucine.* An adult female toad was injected via the dorsal lymph sac with 20μC of L-[U-^{14}C]leucine. The animal was killed 30min. later and the liver and ovaries were removed. A microsome preparation was made in the way described above.

Separation of bound and free ribosomes by sucrose-density-gradient centrifugation. To determine the ratio of bound to free ribosomes the whole microsome preparation was subjected to density-gradient centrifugation in sucrose by the method recommended by Campbell *et al.* (1965) as a modification of the method of Henshaw *et al.* (1963), except that the Mg^{2+} concentration of the gradient was 1mM. The microsome pellet was resuspended in a medium (medium

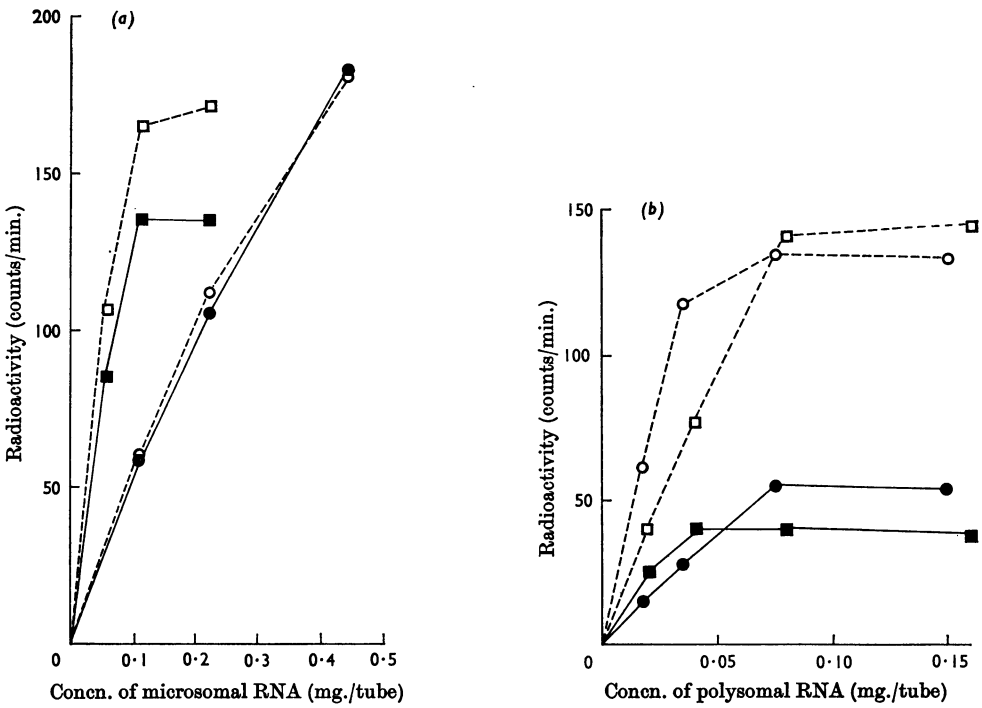

Fig. 2. Incorporation of [^{14}C]leucine by liver and ovary (*a*) microsomal RNA and (*b*) polysomal RNA at various concentrations. The cell-free system consisted of the standard incubation mixture plus liver PMS (3·4mg. of protein, 0·27mg. of RNA) or ovary PMS (3·35mg. of protein, 0·47mg. of RNA) as indicated. ○, Ovary microsomes or polysomes and ovary PMS; ●, ovary microsomes or polysomes and liver PMS; □, liver microsomes or polysomes and ovary PMS; ■, liver microsomes or polysomes and liver PMS.

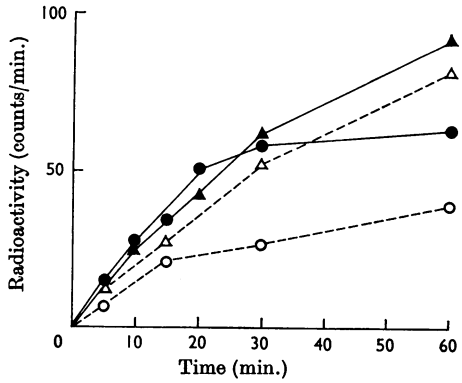

Fig. 3. Time-course of [^{14}C]leucine incorporation by liver and ovary microsomes and polysomes. The standard cell-free system was used as described in the Materials and Methods section, plus liver PMS (●, ▲) (3·8mg. of protein, 0·21mg. of RNA), or ovary PMS (○, △) (3·5mg. of protein, 0·52mg. of RNA). ●, Liver microsomes (0·07mg. of RNA); ▲, liver polysomes (0·03mg. of RNA); ○, ovary microsomes (0·23mg. of RNA); △, ovary polysomes (0·02mg. of RNA).

B) containing $MgCl_2$ (1mM), KCl (25mM) and tris buffer, pH7·8 (35mM). A 1ml. portion of this suspension was layered over 16ml. of a linear 5–20% (w/v) sucrose gradient in medium B. The gradient was prepared by layering 8ml. of 5% sucrose over 8ml. of 20% sucrose, leaving to stand overnight (12hr.) in a 37° incubator and then at 4° in a refrigerator until used. Reproducible results were obtained with this method. Just before use 3ml. of a 50% (w/v) sucrose solution in medium B was introduced under the gradient as a cushion, by means of a syringe passed carefully down one side of the tube. Centrifuging times are indicated on the Figures. All centrifuging was performed in an MSE Superspeed 50 centrifuge with temperature probe attachment (the setting was for 0° with calibration +1°).

After centrifugation the top of the tube was plugged with a Perspex stopper having a cone-shaped entrance to a narrow-bore polythene tube from which 25-drop fractions were collected by upward displacement of the gradient with heavy sucrose injected from a syringe. The volume of each fraction was calculated by dividing the volume of the gradient by the number of fractions obtained. A 0·1ml. portion was removed from each fraction, diluted to 0·5ml. with distilled water and E_{260} determined. For selected fractions a continuous spectrum was recorded from 400 to 220mμ on a Unicam SP.800 recording spectrophotometer with 1cm. light-path micro-cells (volume 0·45ml.).

Estimation of RNA. The amount of RNA was determined by u.v. absorption. Corrections were made for light-scattering and for the presence of ferritin. The correction for scattering was calculated from the fact that scattering increases inversely as the fourth power of the wavelength (Tanford, 1965). The E_{360} value was multiplied by a factor (1·6 for 320mμ, 2·73 for 280mμ, 3·68 for 260mμ and 5·40 for 237mμ) to give the scattering correction. The correction for ferritin was that suggested by Munro, Jackson & Korner (1964). The E_{320}, corrected for scattering, was multiplied by a factor (1·5 for 260mμ and 1·4 for 280mμ) to give the ferritin correction. The concentration of RNA was then determined from the nomograph (distributed by the California Corp. for Biochemical Research, Los Angeles, Calif., U.S.A.) based on the extinction coefficients for enolase and nucleic acid given by Warburg & Christian (1942).

Protein estimation. Protein was estimated by the method of Lowry, Rosebrough, Farr & Randall (1951) with bovine γ-globulin as standard.

Extraction of protein for assay of radioactivity. The incubations were terminated by addition of 1ml. of 10% (w/v) trichloroacetic acid containing 4g. of unlabelled L-leucine/l. The precipitate was collected by centrifugation (15min. at 6000*g* at 0°), dissolved in 0·25ml. of N-NaOH and reprecipitated with 0·5ml. of 10% trichloroacetic acid. The alkaline-washing procedure was repeated twice more, after which the precipitate was washed successively with ethanol, ethanol–ether (1:1, v/v) and ether. The precipitate was finally dissolved in 0·2ml. of 50% (v/v) formic acid.

Fractions from gradients were treated in the same way except that, before the first precipitation by trichloroacetic acid, 2mg. of bovine γ-globulin/tube was added as co-precipitant, and the alkaline washing was done only once.

Determination of radioactivity. The radioactivity was determined by using a method similar to that suggested by Hall & Cocking (1965) for high-efficiency liquid-scintillation counting. The sample to be counted was dissolved in 0·2ml. of 50% formic acid and transferred to a 10ml. vial for use in the IDL liquid-scintillation head 2022 (Isotope Developments Ltd., Beenham, Berks.). The sample tube was washed out with 1·3ml. of Cellosolve, which was added to the counting vial. Then 3ml. of toluene containing 0·3g. of 1,4-bis-(5-phenyloxazol-2-yl)benzene/l. and 5g. of 2,5-diphenyloxazole/l. was pipetted into the counting vial and mixed thoroughly by shaking. The vial was placed in the dark for at least 2hr. before counting.

The efficiency of counting in this system was calculated by adding a known amount of [^{14}C]leucine to the tubes after initial counting of the activity in the sample. The results were consistent from one experiment to another and gave a value of 55±1·5% (S.E.M.; 30 measurements) with the following setting on the coincidence unit HV_1 1180v, HV_2 1300v, upper gate 40v and disk bias (on the 1700 scaler) 10v. All samples were counted for 1000sec.

RESULTS

Comparison of the abilities of liver and ovary microsomes to incorporate [^{14}C]*leucine into protein.* Fig. 1 shows that the two systems have slightly different requirements for optimum incorporation. Table 1 shows that both systems are dependent on energy, PMS, unlabelled amino acids and microsomes for maximum incorporation. Table 2 shows that, in the system used, with concentrations of RNA that give a linear response (see Fig. 2*a*) the radioactivity incorporated by 1mg. of liver microsomal RNA is 11·9–13·1 times the radioactivity incorporated by 1mg. of ovary microsomal RNA.

Comparison of the abilities of liver and ovary polysomes to incorporate [^{14}C]*leucine into protein.*

Table 1. *Dependence of* L-[*U*-^{14}C]*leucine incorporation on components of the cell-free system*

Liver and ovary microsomes were incubated with liver and ovary PMS respectively under the conditions defined in the Materials and Methods section. Different components of the cell-free system were omitted, as indicated, and replaced by the appropriate volume of distilled water. Results are expressed as means ± S.E.M. of the counts/min. above zero-time control per mg. of microsomal RNA, with the numbers of experiments in parentheses.

	Liver	Ovary
Complete system	703±179 (8)	84±20 (8)
ATP, GTP and energy-generating system omitted	0 (5)	0 (5)
Amino acids omitted	145±54 (5)	21±6 (5)
PMS omitted	72±23 (5)	12±4 (5)
Microsomes omitted	0 (5)	0 (5)

Table 2. *Incorporation of* L-[*U*-^{14}C]*leucine into liver and ovary microsomes and polysomes in the cell-free system*

Liver and ovary microsomes and polysomes were incubated with liver and ovary PMS, as described in the Materials and Methods section, with the standard cell-free system. In all experiments the amount of microsomal or polysomal RNA added per tube was within the limits defined in Figs. 2(*a*) and 2(*b*). Liver and ovary PMS were made equal with respect to protein concentration in any one experiment, values for different experiments being between 2·5 and 4·0 mg. of PMS protein per tube. The results are expressed as means ± S.E.M. of the counts/min. above zero-time control per mg. of microsomal or polysomal RNA added per tube, with the numbers of experiments in parentheses.

Source of PMS	Liver	Ovary
Source of microsomes		
Liver	703±179 (8)	996±216 (8)
Ovary	54±11 (8)	84±20 (8)
Source of polysomes		
Liver	1197±354 (7)	2030±547 (6)
Ovary	1960±707 (6)	4673±979 (6)

Assuming that protein synthesis takes place on ribosomes bound into polysomes, and that there is no difference in the rate of peptide-bond formation/mg. of active polysomal RNA or of the amount of leucine in the proteins synthesized by the liver and ovary, then substitution of polysomes for microsomes should remove the differences in the incorporation by liver and ovary microsomes. The results in Table 2 indicate that ovary polysomes incorporate at least as much [^{14}C]leucine/mg. of polysomal RNA as liver polysomes. The percentage recovery of polysomal RNA from microsomes is eight times as great from liver as from ovary; this could be because there is about seven times more RNA bound to membranes in liver than in ovary (Figs. 4 and 5). These results are consistent with the idea that a large amount of ovary microsomal RNA is inactive in protein synthesis.

Comparison of the abilities of liver and ovary post-microsomal supernatants to stimulate incorporation of [^{14}C]*leucine into liver and ovary microsomes and polysomes.* Table 2 indicates that there is a consistent difference between the abilities of liver and ovary PMS to promote incorporation of leucine into liver and ovary microsomes and polysomes. The cause of this difference is not apparent at present but might be due to one or more of the following: (i) The amount of unlabelled leucine available for protein synthesis in ovary PMS is smaller than in liver PMS. (ii) Some rate-limiting factor, such as transfer RNA or activating enzymes, is present in greater quantity in the ovary PMS. It is known that the RNA/protein ratio of the ovary PMS is much larger than that of liver PMS. In the experiments ovary and liver PMS were standardized relative to protein, so that each tube received the same amount of liver and ovary supernatant protein, but different amounts of soluble RNA. (iii) Some factor, such as free ribosomes or messenger RNA, that would be expected to enhance incorporation is present in greater quantities in ovary PMS.

Estimation of the relative amounts of free and bound ribosomes in liver and ovary. The method used to study the ribosome content of the microsome fractions isolated from *Xenopus* liver and ovary is that described by Campbell *et al.* (1965) except that the Mg^{2+} concentration of the gradients was 1 mM not 0·1 mM. The results of one such experiment are shown graphically in Figs. 4 and 5, which show that in *Xenopus*-liver microsomes most of the ribosomal material is associated with microsome membranes (Fig. 4*a*, heavy fraction). After treatment with deoxycholate much of this bound material is liberated and can be recovered in the lighter fractions (Fig. 4*b*, light and intermediate fractions). Ribonuclease treatment releases a little of the bound RNA and greatly increases the monomer peak (Fig. 4*c*).

Ovary microsomes (Fig. 5*a*) show a completely different distribution of the RNA in the gradient, most of the RNA sedimenting to a region associated with monomer ribosomes and small polysomes. Deoxycholate treatment removes nearly all of the bound RNA, which is recovered in the light and intermediate regions of the gradient (Fig. 5*b*). Ribonuclease treatment (Fig. 5*c*) does not appreciably alter the distribution of RNA in the gradient; however, there is some loss from the bound region that is recovered in the monomer and light regions of the tube. It must be stressed that the actual values observed for bound and free ribosomes

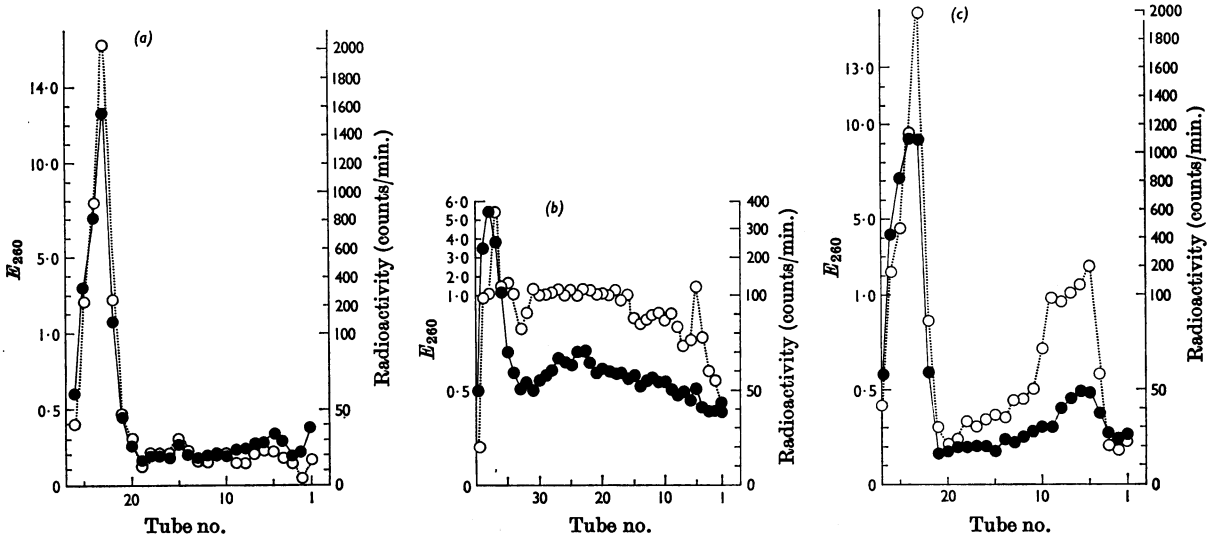

Fig. 4. Distribution of radioactivity and ribosomes among the fractions of sucrose gradients of liver microsomes obtained after incorporation of L-[U-^{14}C]leucine *in vivo* for 30 min. (*a*) Whole-liver microsomes; (*b*) liver microsomes treated with an equal volume of 2% (w/v) sodium deoxycholate; (*c*) liver microsomes treated with pancreatic ribonuclease (1 μg./ml. for 30 min. at 4°). The gradients were linear 5–20% (w/v) sucrose with a cushion of 50% (w/v) sucrose in buffer containing tris, pH 7·8 (35 mM), $MgCl_2$ (1 mM) and KCl (25 mM). The gradients were centrifuged in an MSE 3 × 20 ml. swing-out-head centrifuge at 44000*g* for 140 min. at 0°. Fractions were collected and treated as described in the Materials and Methods section. For calculation of total RNA and radioactivity the gradients were divided into five regions thus: top region, tubes 1–3 (*a*, *b* and *c*); monomer region, tubes 4–7 (*a* and *c*) and 4–8 (*b*); light region, tubes 8–13 (*a* and *c*) and 9–22 (*b*); intermediate region, tubes 14–21 (*a* and *c*) and 23–36 (*b*); heavy region, tubes 22–26 (*a* and *c*) and 37–40 (*b*). The RNA/protein ratios recorded were: (*a*) monomer region, 0·317; light region, 0·116; heavy region, 0·224; (*b*) monomer region, 0·355; light region, 0·448; intermediate region, 0·371; heavy region, 0·347; (*c*) monomer region, 0·331; heavy region, 0·246. ●—●, E_{260}; ○···○, total counts/min. above background.

depend on the microsome preparation (within any one preparation duplicate gradients give very reproducible results). Liver microsomes are much more variable than ovary microsomes: values as low as 45% bound RNA have been recorded for liver microsomes, whereas 7% bound RNA is the lowest recorded for ovary microsomes. The RNA/protein ratios for the various regions of the gradient are recorded in the legends to Figs. 4 and 5 and are comparable with the values of Campbell *et al.* (1965) for rat liver.

It is concluded from this experiment that adult *Xenopus*-liver microsome fraction has a similar distribution of ribosomal RNA to adult rat-liver microsomes as described by Henshaw *et al.* (1963) and Campbell *et al.* (1965), but that ovary microsomes show a completely different pattern.

Incorporation of [^{14}C]*leucine by free and bound ribosomes in* Xenopus *liver and ovary* in vivo. The labelling of protein associated with ribosomal material was investigated after injection of 20 μc of L-[U-^{14}C]leucine via the dorsal lymph sac. The animal was killed 30 min. after injection. Figs. 4 and 5 show the results for this experiment.

In liver most of the radioactivity occurs in the bound ribosomal fraction with, however, little radioactivity in the lighter fractions (Fig. 4*a*). Most of the radioactivity associated with membranes is released by deoxycholate treatment and is recovered in the lighter regions of the gradient (Fig. 4*b*), though some sediments at the top of the tube. Little radioactivity is released by ribonuclease from the bound fraction, but there is an increase in radioactivity in the monomer region as expected (Fig. 4*c*).

In ovary most of the radioactivity is also recovered in the bound ribosome fraction (Fig. 5*a*). In contrast with liver, after deoxycholate treatment most of the radioactivity released from the heavy fraction is recovered in the top fractions, but some is recovered in the light and intermediate fractions (Fig. 5*b*). Ribonuclease increases the radioactivity associated with the monomer and light-polysome regions (Fig. 5*c*).

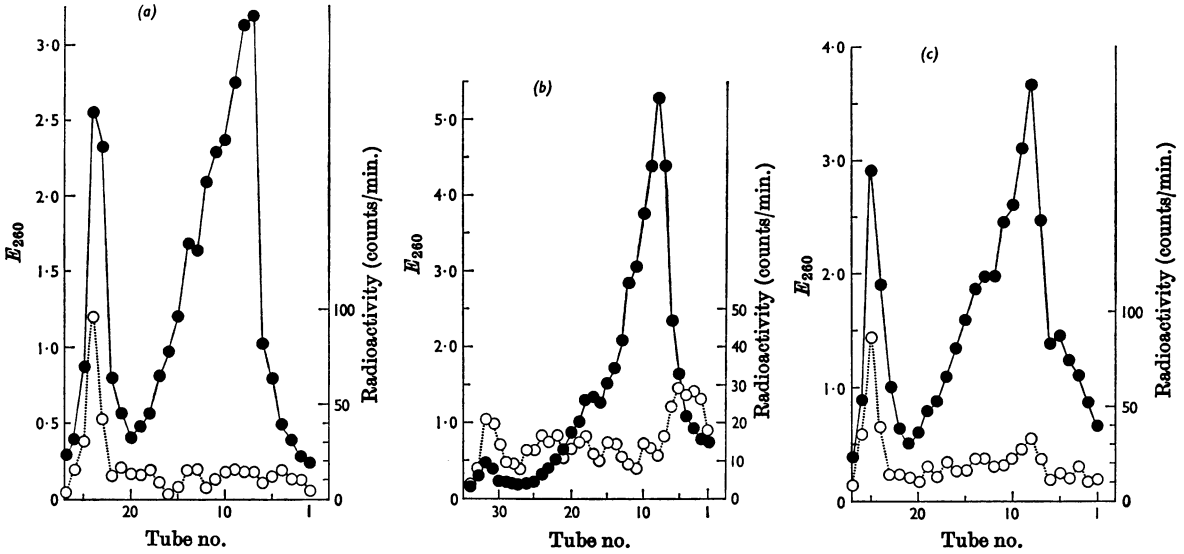

Fig. 5. Distribution of radioactivity and ribosomes among the fractions of sucrose gradients of ovary microsomes obtained after incorporation of L-[U-^{14}C]leucine *in vivo* for 30min. (*a*) Whole ovary microsomes; (*b*) ovary microsomes treated with an equal volume of 2% (w/v) sodium deoxycholate; (*c*) ovary microsomes treated with pancreatic ribonuclease (1μg./ml. for 30min. at 4°). The gradients were prepared as described in the Materials and Methods section. Centrifuging was for 240min. at 44000*g* at 0° in an MSE 3×20ml. swing-out-head centrifuge. Fractions were collected and treated as described in the Materials and Methods section. The gradients were divided into five regions for the purpose of estimating specific activity as in Fig. 4: top region, tubes 1–5 (*a*, *b* and *c*); monomer region, tubes 6–9 (*a*) and 6–10 (*b* and *c*); light region, tubes 10–16 (*a*), 11–20 (*b*) and 11–17 (*c*); intermediate region, tubes 17–22 (*a*), 21–29 (*b*) and 18–23 (*c*); heavy region, tubes 23–25 (*a*), 30–32 (*b*) and 24–26 (*c*). The regions are assumed to be comparable from one gradient to another. The RNA/protein ratios recorded were: (*a*) monomer region, 0·775; light region, 0·648; intermediate region, 0·352; heavy region, 0·272; (*b*) monomer region, 0·915; light region, 0·804; intermediate region, 0·395; heavy region, 0·333; (*c*) monomer region, 0·721; light region, 0·712; intermediate region, 0·362; heavy region, 0·274. ●—●, E_{260}; ○···○, total counts/min. above background.

Table 3. *Specific activity of RNA recovered in the five regions of the gradients shown in Figs. 4 and 5*

Total radioactivities recovered in each fraction, defined in the legends to Figs. 4 and 5, were summed and divided by the total amount of RNA (mg.) recovered from each fraction. The results are expressed as counts/min. above background per mg. of RNA. The recovery of total radioactivity added to the top of the gradient was 60% for liver and 67% for ovary. The recovery of RNA added to the gradient was 66% for liver microsomes and 56% for ovary microsomes.

Fraction	Top	Monomer	Light	Intermediate	Heavy
Whole ovary microsomes	258	37·4	38·0	179	346
Ovary microsomes (deoxycholate-treated)	373	49·5	59·9	344	408
Ovary microsomes (ribonuclease-treated)	108	59·1	72·3	162	406
Whole liver microsomes	1442	744	2210	1854	2097
Liver microsomes (deoxycholate-treated)	7059	5910	3180	2527	1983
Liver microsomes (ribonuclease-treated)	1414	2171	3295	3027	1887

From Table 3 it is clear that the specific activities of RNA in the different regions of the gradient are not the same. In liver microsomes the specific activity of the heavy fraction is greatest, but the light and intermediate fractions have a specific activity approaching this, which indicates that the

Table 4. *Distribution of radioactivity in liver and ovary postmicrosomal supernatant into three fractions*

Two 2ml. samples of the PMS, obtained from the incorporation experiment *in vivo* described in Figs. 4 and 5, were treated with 2ml. of 10% trichloroacetic acid containing 4g. of unlabelled leucine/l. and the precipitate was collected by centrifugation. One precipitate was treated with 0·5ml. of N-NaOH at 20° for 5min. to hydrolyse the amino acid–transfer RNA linkage (Wiseman, 1965), and reprecipitated with 1ml. of 10% trichloroacetic acid. The other sample was not treated with alkali. Both samples were then washed with ethanol, ethanol–ether (1:1, v/v) and ether and dissolved in 0·5ml. of 50% formic acid. Duplicate 0·2ml. samples were then prepared for counting in the way described in the Materials and Methods section. Samples (0·5ml.) of the trichloroacetic acid-soluble material were plated on aluminium planchets, dried and counted in an IDL Geiger–Müller tube MX123 with settings HV 0·7kv and disk bias 5v on the IDL 1700 scaler at an estimated efficiency of 0·8%. The counts recorded were multiplied by 55/0·8 to make them comparable with the counts recorded by the liquid-scintillation method. The amount of RNA in the supernatants was estimated as described in the Materials and Methods section. The results are expressed as counts/min. above background per g. wet wt. of tissue. The specific activity of the supernatant RNA is given by the counts/min. lost during alkaline hydrolysis per mg. of supernatant RNA.

Origin of supernatant	Liver	Ovary	Liver/ovary ratio
Origin of radioactivity			
Trichloroacetic acid-precipitable	14863	2151	6·9
Trichloroacetic acid-precipitable after alkaline hydrolysis	3495	579	6·0
Radioactivity lost during alkaline hydrolysis	11368	1573	7·2
Trichloroacetic acid-soluble	142503	28785	4·95
Specific activity of the supernatant RNA	2755	350	7·9

free polysomes of liver are about equally as active in the incorporation of leucine *in vivo* as bound polysomes. In ovary the heavy fraction also has the greatest specific activity, whereas that of the intermediate fraction is about half this and of the light fraction about nine times less, which indicates that the free polysomes of ovary are very much less active in the incorporation of leucine *in vivo* than the bound ribosomes.

After deoxycholate treatment the specific activities of the intermediate fractions increase, whereas that of the bound fraction shows little change. In both liver and ovary the greatest increase in specific activity is in the top fraction.

Ribonuclease also increases the specific activity of the non-bound regions of the gradient, but has no effect on the specific activity of the bound fractions, in both liver and ovary preparations.

Analysis of the radioactivity recovered in liver and ovary post-microsomal supernatant. Since the liver and ovary microsomes show very different specific activities after incorporation of leucine *in vivo* (Table 3) it seemed necessary to decide whether this was due to a differential rate of penetration of label. Table 4 indicates that this is the case, and also that the activity of leucine bound to 'soluble' RNA is 7·2 times as great in liver as in ovary, which is a sufficient difference to account for the differential labelling of the heavy fractions (Table 3), but not for the difference between non-bound ribosomal fractions.

DISCUSSION

The system in vitro. The optimum conditions for incorporation of [^{14}C]leucine by *Xenopus*-liver and -ovary microsomes *in vitro* (Fig. 1) agree very well with the conditions described for incorporation of labelled amino acids by rat- and mouse-liver microsomes *in vitro* into proteins co-precipitating with rat and mouse serum proteins after immunoelectrophoretic separation (Ganoza, Williams & Lipmann, 1965; Williams, Ganoza & Lipmann, 1965). The major difference is in the optimum tris buffer concentrations: 50mM in the *Xenopus* system and 25mM for the rat and mouse systems. Hultin (1961, 1964) used 50mM-tris but 0·25M-potassium chloride for incorporation of amino acids into protein by echinoderm cell-free systems. The *Xenopus* system shows linear incorporation for the first 15min., but incorporation continues at decreased rates up to 1hr. (Fig. 3). The initial rate of incorporation is proportional to the amount of microsomal or polysomal RNA added (Figs. 2*a* and 2*b*). These properties are also features of mammalian systems (Earl & Korner, 1965).

Incorporation of [^{14}C]*leucine by* Xenopus-*liver and -ovary microsomes and polysomes* in vitro. The experiments *in vitro* (Table 2) show that it is possible to isolate microsome fractions from the liver and ovary of *Xenopus* that incorporate [^{14}C]-leucine into trichloroacetic acid-precipitable alkali-stable products. However, *Xenopus*-liver micro-

somes incorporate 12–13 times as much [^{14}C]leucine/mg. of microsomal RNA added as *Xenopus*-ovary microsomes. This difference is abolished by substituting polysomes for microsomes in the incubation mixture.

There are four main factors affecting the amount of labelled amino acid incorporated by equal amounts of ribosomes from two different tissues incubated under identical conditions: (1) The dilution of the labelled amino acid by unlabelled amino acid may be different. In the experiments described here this factor is unlikely to be important, since it is probable that the amount of unlabelled leucine in the microsome pellets is small compared with the amount remaining in the PMS after dialysis. (2) The number of sites available for incorporation of the labelled amino acid may be different in the two cases. This is unlikely since leucine concentration in proteins does not show remarkable variation. Also, the fact that polysome preparations show no appreciable difference suggests that this interpretation cannot be entirely correct. (3) The rate of peptide-bond synthesis/mg. of active ribosomal RNA may be greater in the one tissue than in the other. Again the fact that polysome preparations show no difference suggests that this cannot account entirely for the large difference between the microsome preparations. (4) The proportion of active ribosomes in the two microsomal preparations may vary. The evidence from labelling of protein *in vivo*, the relative amounts of free and bound ribosomes (Figs. 4 and 5) and the recovery of microsomal RNA in polysome fractions indicate that this factor may account for at least part of the difference.

Incorporation of [^{14}C]*leucine* in vivo *by* Xenopus*liver microsomal ribosomes.* The experiment shown in Fig. 4 is very similar to the experiment of Henshaw *et al.* (1963), who followed the incorporation of [^{14}C]arginine *in vivo* by rat-liver microsomal ribosomes. However, their methods of preparation and sucrose-density-gradient analysis of the microsomes were different. The conclusions from the two experiments are the same in that the membrane-bound ribosome fractions are the most heavily labelled, but different in that the free polysomes (intermediate and light fractions; Fig. 4) of *Xenopus*-liver microsomes have a specific activity approaching that of the bound fraction, whereas in rat liver they do not (Fig. 2 of Henshaw *et al.* 1963). The difference can probably be accounted for by the differences in preparative methods and in the solutions used for the sucrose gradients.

Incorporation of [^{14}C]*leucine* in vivo *by* Xenopus*ovary microsomal ribosomes.* Fig. 5 indicates that in *Xenopus* ovary most of the ribosomes are free monomers, inactive in protein synthesis. There is a bound ribosome fraction similar in properties to the bound fraction from liver. This observation indicates that at least a part of the ovary protein is synthesized by the ovary.

It is of importance to know whether the bound ribosome fraction is localized within oocytes or follicle cells. Electron microscopy (Kemp, 1956; Balinsky & Devis, 1963) indicates that there is little if any endoplasmic reticulum in follicle cells, and indeed most of their volume is occupied by nucleus; and in oocytes free ribosomal particles are abundant and bound particles (Wischnitzer, 1964) are scarce. Kessel (1964) describes a structure, called the annulate lamella, in the oocyte of the echinoderm *Thygone briareus* that has ribonucleoprotein particles associated with it, and a similar structure is described for *Xenopus* by Balinsky & Devis (1963) although they do not note the association of ribonucleoprotein particles. Merriam (1966) shows that the presence of follicular epithelium in *Rana pipens* oocytes decreases the incorporation of [^{14}C]-phenylalanine into protein *in vitro*. Together these points argue that the follicle cells have not contributed significantly to the microsome pellets extracted from whole ovary.

The free ribosomes from *Xenopus* ovary are largely monomers and are inactive in protein synthesis at any one time, which is in agreement with previous work on *Xenopus* ovaries (Brown & Littna, 1964) and on echinoderm unfertilized eggs (Gross, 1964).

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