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The Effect of Chick-Liver Ribonucleic Acid on Amino Acid-Incorporation Systems from Rat Liver

By ALEXANDRA VON DER DECKEN
The Wenner-Gren Institute for Experimental Biology, University of Stockholm, Sweden

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1. Rat-liver microsomes, ribonucleoprotein particles and a fraction mainly consisting of microsomal membranes were tested for their ability to incorporate amino acids into protein in the presence of ATP, GTP, phosphoenolpyruvate and pyruvate kinase. Addition of polyuridylic acid or of ribonucleic acid from rat-liver nuclei stimulated the incorporating activities. 2. These protein-synthesizing systems were found to be susceptible to ribonucleic acid from chick-liver nuclei as well. The biological activity of the ribonucleic acid from chick liver was measured by its capacity to stimulate amino acid incorporation. 3. In the presence of chick-liver ribonucleic acid, the ribonucleoprotein particles from rat liver showed an increased radioactivity in ribosomal units with a sedimentation constant higher than 70s. 4. This was investigated by sucrose-gradient centrifugation or by column chromatography on agarose suspensions.

When rat-liver ribonucleoprotein particles are incubated with cell sap from chick liver in the presence of labelled amino acids and a source of energy, radioactivity is incorporated into a protein with the immunological properties of chick serum albumin (Decken, 1963*a*,*b*). It seemed possible that messenger ribonucleic acid present in the chick-liver cell sap could be a factor of major importance for this labelling. A way of testing this possibility would be to replace the cell-sap factor by isolated messenger ribonucleic acid obtained from chick-liver nuclei. As an introduction to this study the stimulatory effect of chick-liver ribonucleic acid on the general protein synthesis in rat-liver systems was investigated. To my knowledge no studies have been published dealing with the effect of heterologous animal ribonucleic acid on liver systems.

Since the stimulatory effect of messenger ribonucleic acid on ribosomes is very sensitive to ribonuclease even at very low concentrations, attempts were made to reduce the enzyme activity to a minimum in the course of the preparations. The general susceptibility to ribonucleic acid of the various rat-liver preparations thus obtained was determined by use of artificial messenger(polyuridylic acid) and homologous rat-liver ribonucleic acid. Under the conditions finally adopted it was found that chick-liver ribonucleic acid stimulated the protein-synthetic activity of rat-liver preparations and induced a certain formation of active polysomes.

A preliminary report of this work has appeared (Decken, 1964).

MATERIALS AND METHODS

Materials. DL-[1-^{14}C]Leucine (20mc/m-mole) and DL-[1-^{14}C]phenylalanine (4·5mc/m-mole) were obtained from New England Nuclear Corp., Boston, Mass., U.S.A. ATP (disodium salt), GTP (sodium salt), tris–HCl and tris base, bovine serum albumin and catalase, twice-crystallized, were obtained from Sigma Chemical Co., St Louis, Mo., U.S.A. Cadmium-free horse-spleen ferritin was obtained from Pentex Inc., Kankakee, Ill., U.S.A., pyruvate kinase from C. F. Boehringer und Soehne G.m.b.H., Mannheim, Germany, Siliclad from Clay–Adams Inc., New York 10, N.Y., U.S.A., membrane ultrafilter of pore size 250–450mμ from Membranfilter A.-G., Goettingen, Germany, Lubrol W from Imperial Chemical Industries Ltd., Manchester, and polyuridylic acid from Miles Laboratories, Stoke Poges, Slough, Bucks. Deoxycholate and yeast RNA were E. Merck A.-G. preparations (Darmstadt, Germany). Bentonite was purified essentially as described by Petermann & Pavlovec (1963). The sediment obtained between 10000 and 15000*g* was used. Lauryl sulphate was purified as described by Crestfield, Smith & Allen (1955). Phosphoenolpyruvate was synthesized as described by Clark & Kirby (1963) and recrystallized three times as a monocyclohexylammonium salt. Before use the salt was dissolved in 0·2M-tris–HCl buffer, pH7·8 (0·5ml./30mg. of salt), and adjusted to pH7 (on indicator paper) with 0·7N-KOH. All solutions were made up in glass-distilled water and the media used for preparation of liver fractions, of RNA, for sucrose-gradient centrifugation and for column chromatography were sterilized for 15min. in a Sky-line pressure cooker. The preparation procedures were carried out at 4°.

Preparation of fractions from rat liver

Sprague–Dawley male or female rats (150–180g.) were starved over night. The rats were killed by a blow on the

head, decapitated, bled and the livers removed and placed in ice-cold medium A (35mM-tris–HCl buffer, pH 7·8 at 25°, 25mM-KCl, 0·05M-NH_4Cl, 0·01M-$MgCl_2$ and 0·25M-sucrose).

Liver microsomes and cell sap. These were prepared by a method that followed closely that of Zamecnik & Keller (1954). The liver was homogenized in 2·5 times its weight in the medium A. The homogenate was centrifuged for 10min. at 15000*g*. The supernatant obtained was centrifuged in a Spinco model L preparative centrifuge (no. 40 rotor) for 60min. at 105000*g*, when preparing microsomes, or for 90min. at the same speed for the preparation of cell sap. Only the clear part of the supernatant was used as cell sap. The microsomal pellet obtained after centrifugation for 60min. was rinsed with medium A and suspended by addition of 1·6ml. of medium A (0·4ml./g. of original liver) and gently homogenized in an all-glass Potter-type homogenizer.

Isolation of ribonucleoprotein particles. The RNP* particles were isolated by the method of Rendi & Hultin (1960) as described in detail by Decken & Campbell (1962*b*). In addition, all media contained 0·05M-NH_4Cl, and the 0·15M-sucrose was replaced by 0·25M-sucrose. If not otherwise indicated, the RNP particles from 1g. of original liver were suspended in 0·1ml. of medium A.

Preparation of the membrane fraction. The method of Ernster, Siekevitz & Palade (1962) was employed. The livers were homogenized in 2·5 times their weight of 0·25M-sucrose. After centrifugation of the homogenate for 10min. at 15000*g*, 10ml. of the supernatant was added to no. 40 rotor Spinco tubes, which contained 1·1ml. of 2·6% deoxycholate. The tubes were gently inverted three or four times. The mixture was centrifuged for 90min. at 105000*g*. The so-called 'M fraction' was then recovered as described by Ernster *et al.* (1962), transferred to new Spinco tubes, diluted with medium A to 11ml. and centrifuged for 60min. at 105000*g*. The supernatant was discarded, the tubes were rinsed with medium and the pellet was gently suspended in 1·6ml. of medium A (0·4ml./g. of original liver).

Preparation of RNA from a liver fraction containing nuclei. Chick liver from 5–8-day-old chicks, or rat liver from rats weighing 150g., were used. After decapitation of the animals the livers were removed and chilled in ice-cold 0·25M-sucrose. They were then minced and homogenized in a loose-fitting Potter-type all-glass homogenizer in 10 times their weight of 0·25M-sucrose. The homogenate was passed through a double nylon cloth of pore size 63μ^2. The filtrate was centrifuged for 10min. at 700*g*, the supernatant discarded and the loose pellet suspended in lauryl sulphate at 4° at a final concentration of 0·8%. After 3min. an equal volume of freshly distilled ice-cold 90% phenol was added. The mixture was kept at 65° for 15min. (Georgiev & Mantieva, 1962) while gently homogenized. The mixture was then centrifuged for 20min. at 8000*g* in the cold, the water phase sucked off and the RNA of the water phase precipitated by the addition of 2·5vol. of ethanol at −15°. The mixture was stored at −15° over night, the precipitated RNA collected by centrifugation at −10° and dried *in vacuo*. The RNA was dissolved in water before fractionation by a sucrose gradient or in 5% sucrose when applied to columns of Sephadex G-200.

* Abbreviations: RNP, ribonucleoprotein; s-RNA, transfer ribonucleic acid; m-RNA, messenger ribonucleic acid; poly U, polyuridylic acid.

Sucrose-gradient centrifugation

A linear sucrose gradient was used throughout. The concentration of the sucrose–salt media varied as indicated in the Figures, depending upon the purpose of the experiment. Most of the gradient centrifugations were conducted in the no. 30 rotor of the Spinco model L centrifuge (Charlwood, 1963). The tubes were siliconized with Siliclad. After centrifugation, fractions of about 1ml. were collected from the bottom of the sucrose gradient by puncturing the tube with a hypodermic needle. Sedimentation constants were calculated according to the method of Martin & Ames (1961), catalase or horse ferritin being used as a standard. When RNA fractions, obtained from the gradient centrifugation, were tested for stimulation of amino acid incorporation, 0·075–0·15ml. samples were removed from different fractions of the gradient and added directly to the amino acid-incorporation systems.

Molecular sieving

Sephadex G-200. The ethanol-precipitated RNA of the phenol-extracted nuclei was dried *in vacuo*, and dissolved in 1–2ml. of 5% sucrose. RNA (5–10mg.) was applied to a 2·3cm. × 55cm. Sephadex G-200 column (Porath & Flodin, 1959) equilibrated with 35mM-tris–HCl buffer, pH 7·8, in 1mM-$MgCl_2$ and the RNA eluted with the same buffer. The flow rate of the column was adjusted to 6ml./hr. Samples of 2–3ml. were collected and the extinction was determined in a Beckman model DU spectrophotometer at 260mμ. The combined RNA of the first and second peaks was reprecipitated by addition of 2·5vol. of ethanol. The suspension was maintained at −15° for 1 or more days and collected again by centrifugation at −10°. The RNA containing sediment was dried *in vacuo*, dissolved in a volume of water as small as possible and used immediately to test its effect on polysome formation. When the stimulatory effect on amino acid incorporation of RNA fractions from the column was tested, portions (0·075–0·15ml.) were removed from the different fractions and added to the amino acid-incorporation systems.

Agarose suspension. A suspension of 1% agarose (kindly given by Dr S. Hjertén, Uppsala, Sweden) was used. The column size was 1·6cm. × 50cm. The flow rate was adjusted to 5ml./hr. Details for the use of agarose columns have been published by Hjertén (1962). The medium for equilibration and elution of the column contained 35mM-tris–HCl buffer, pH 7·8 at 25°, 25mM-KCl, 0·05M-NH_4Cl and 5mM-$MgCl_2$.

Amino acid incorporation in vitro

Effect of RNA fractions. When the effect of RNA preparations on amino acid incorporation was studied, the final volume of incubation usually was 0·5ml. The microsomes, RNP particles or the membrane fractions, each suspended in medium A, were added in 0·1ml. portions, and the cell sap in 0·1–0·3ml. portions. The concentration of ATP was 1mM, of GTP 0·2mM, of phosphoenolpyruvate 10mM. The amount of pyruvate kinase added was 20μg., of L-[^{14}C]leucine 0·006μmole (0·125μc) or L-[^{14}C]phenylalanine, 0·05μmole (0·25μc). Different amounts of RNA or poly U were added together with the labelled amino

acid after various periods of incubation as indicated in the Figures and Tables. After incubations of varying duration, as indicated, trichloroacetic acid was added to a final concentration of 5% (w/v) and the proteins were extracted (Decken & Campbell, 1964). The dried proteins were dissolved in 50% formic acid, transferred to polyethylene disks (2 cm.^{2}), dried in a desiccator and counted at infinite thinness in a windowless gas-flow counter (Nuclear–Chicago Corp.). The weight of the proteins as determined by weighing was between 1·5 and 2 mg. Radioactive leucine (0·01 μc) dissolved in formic acid and counted at infinite thinness gave 4900 counts/min., indicating a counting efficiency of about 22%.

Effect of RNA on formation of polysomes. RNP particles from 3–4 g. of liver corresponding to 3–4 mg. of RNA were suspended in 1–2 ml. of medium A by gentle homogenization. ATP (4 μmoles), GTP (0·8 μmole), phosphoenolpyruvate (40 μmoles), phosphoenolpyruvate kinase (80 μg.) in a final volume of 0·64 ml. were added and the mixture was preincubated for 15 min. at 35° to make the particles susceptible to the RNA to be added additionally (Fessenden, Cairncross & Moldave, 1963; Nakamoto, Conway, Allende, Spyrides & Lipmann, 1963). The mixture was chilled in ice and centrifuged for 1 min. at 5000*g* to remove denser aggregates.

(a) Sucrose gradient. The supernatant was divided into four parts and 0·1 ml. of water, or 150–300 μg. of RNA in 0·1 ml. of water, was added. The mixture was supplemented with ATP (1 μmole), GTP (0·2 μmole), phosphoenolpyruvate (10 μmoles), pyruvate kinase (20 μg.), L-[^{14}C]leucine (1 μc, 0·05 μmole) and, when indicated, 0·4 ml. of cell sap (4·5 mg. of protein). After incubation for 5 min. at 4° the mixtures were layered on a sucrose gradient (10–25% sucrose in 35 mM-tris buffer, pH 7·2 at 25°, 25 mM-KCl, 0·05 M-NH_4Cl, 5 mM-$MgCl_2$). Usually, four parallel sucrose-gradient centrifugations were carried out, two in the absence and two in the presence of RNA. Additional tubes containing RNA alone, or markers for determination of sedimentation constants, were run in parallel. Centrifugation was conducted for 90 min. at 78000*g* in the no. 30 rotor of the Spinco model L centrifuge. Samples (1 ml.) were collected, and the extinction was measured at 260 mμ and 280 mμ. An equal volume of 10% (w/v) trichloroacetic acid was added and the suspension passed through a membrane filter of pore size 250–450 mμ. The membrane filters were washed twice with 5% (w/v) trichloroacetic acid at 90° with weak suction. The membrane filters were transferred to planchets, dried and counted in a windowless gas-flow counter (Nuclear–Chicago Corp.) at 22% counting efficiency.

(b) Agarose columns. To the 5000*g* supernatant, ATP (1 μmole), GTP (0·2 μmole), phosphoenolpyruvate (10 μmoles), pyruvate kinase (20 μg.) and 0·2 ml. of water or 1·0–1·5 mg. of RNA in 0·2 ml. of water were added, followed by 1 μc of L-[^{14}C]leucine (0·05 μmole). Incubation was for 5 min. at 4°. The mixture was applied then to agarose columns. The elution medium consisted of 35 mM-tris–HCl, pH 7·8 at 25°, 25 mM-KCl, 0·05 M-NH_4Cl and 5 mM-$MgCl_2$. The flow rate was 5 ml./hr. Samples of 1·5 ml. were collected and the extinction was determined at 260 mμ and 280 mμ. An equal volume of 10% (w/v) trichloroacetic acid was added and the proteins were treated on membrane filters as described in the preceding section.

Labelling of s-RNA. This was done as described by Decken & Campbell (1962*a*).

Analyses

RNA was determined by the orcinol method of Mejbaum (1939), hydrolysed yeast RNA being used as a standard. Proteins were determined either by dry weight or by the method of Lowry, Rosebrough, Farr & Randall (1951), bovine serum albumin being used as a standard.

RESULTS

Three different fractions of rat liver were used to study the effect of RNA on the incorporation of amino acids into protein: microsomes, RNP particles and a preparation mainly consisting of microsomal membranes (membrane fraction). The RNA/protein ratio of the preparations was 0·27 and 1·0 for microsomes and RNP particles respectively (Decken & Campbell, 1964). For the membrane fraction the ratio was 0·04–0·06.

As a first approach, the effect of poly U on the incorporation of [^{14}C]phenylalanine into protein was tested. After Lubin (1963) had found a stimulation by NH_4^+ on the effect of poly U on [^{14}C]-phenylalanine incorporation a beneficial effect by this ion on amino acid incorporation in general was observed. Thereafter, 0·05 M-ammonium chloride was included in all preparative media. The concentration of magnesium chloride was 10 mM, as has been suggested by Campbell, Cooper & Hicks (1964). With the batch of poly U used, 50 μg. stimulated the incorporation of [^{14}C]phenylalanine about eight times, both for microsomes and the membrane fraction. Poly U was added 2 min. after incubation had started. The activity of the membrane fraction and the stimulatory effect by poly U suggested the presence of ribosomes in that fraction. When RNP particles were tested, an approximately 30-fold increase of the incorporation of [^{14}C]phenylalanine into polypeptide was obtained, provided that no more than 0·4 mg. of RNP-RNA was incubated with 50 μg. of poly U and that at least 4·5 mg. of cell-sap protein was added. The time of incubation before the addition of poly U could be extended to 15 min. without increasing the phosphodiesterase activity present in microsomes (Decken & Campbell, 1964).

Activity of rat-liver nuclear RNA

Addition of 25 μg. of total rat-liver nuclear RNA to RNP particles containing 1 mg. of RNP-RNA stimulated the incorporation of [^{14}C]leucine into protein nearly three times (from 110 counts/min. to 300 counts/min.). The RNA was prepared in the presence of bentonite. However, since the s-RNA present in the nuclear RNA preparation could account for the stimulatory effect, the total RNA was fractionated either on a sucrose gradient or by

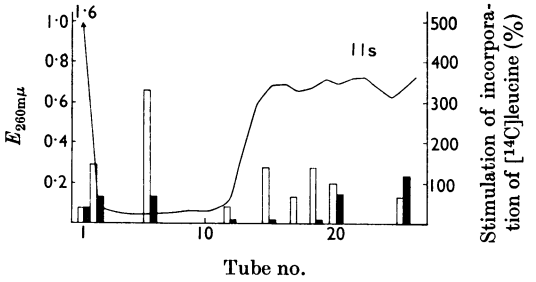

Fig. 1. Sucrose-gradient centrifugation of rat-liver nuclear RNA and the effect of various fractions on [^{14}C]leucine incorporation into protein by rat-liver microsomes (filled bars) or RNP particles (open bars). —, Extinction at 260 mμ. The sucrose gradient (5–20% sucrose) was run for 7 hr. in the swinging bucket SW 25 of the Spinco model L centrifuge. Tube no. 1 is from the bottom of the gradient, and tube no. 26 is from the top. The RNA was applied in the presence of bentonite. Of the fractions indicated 0·15 ml. was added to the amino acid-incorporation system containing ATP, GTP, phosphoenolpyruvate, pyruvate kinase and [^{14}C]-leucine as described in the Materials and Methods section. Microsome experiment: final volume was 0·8 ml. Microsomes (1·2 mg. of protein) and cell sap (4·5 mg. of protein) were added without any preincubation. Period of incubation was 30 min. at 35°. The total radioactivity of the control (incubated in the absence of RNA) was 435 counts/min. RNP particle experiment: final volume was 0·6 ml. RNP particles (0·3 mg. of protein) and cell sap (2·5 mg. of protein) were incubated with all additions except [^{14}C]-leucine and RNA for 15 min. at 35° and after addition of [^{14}C]leucine and RNA for another 45 min. at 35°. Total radioactivity of the control (incubated in the absence of RNA) was 570 counts/min.

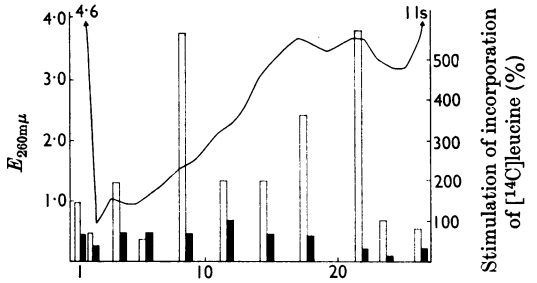

Fig. 2. Sucrose-gradient centrifugation of chick-liver nuclear RNA and the effect of various fractions on [^{14}C]-leucine incorporation into protein by RNP particles (open bars) or membrane fraction (filled bars) from rat liver. —, Extinction at 260 mμ. The sucrose gradient (10–25% of sucrose in 35 mM-tris–HCl and 1 mM-$MgCl_2$) was run in the no. 30 rotor of the Spinco model L centrifuge for 5 hr. Tube no. 1 is from the bottom of the gradient, and tube no. 26 from the top. Of the fractions indicated, 0·15 ml. was added to the amino acid-incorporation system having a final volume of 0·5 ml. and containing ATP, GTP, phosphoenolpyruvate, pyruvate kinase and [^{14}C]leucine as described in the Materials and Methods section. RNP particle experiment: particles (0·06 mg. of protein) and cell sap (1·2 mg. of protein) were incubated with all additions except [^{14}C]-leucine and RNA for 15 min. at 35° and after addition of [^{14}C]leucine and RNA for another 45 min. at 35°. The total radioactivity of the control (incubated in the absence of RNA) was 70 counts/min. Membrane fraction: this fraction (0·6 mg. of protein) and cell sap (1·2 mg. of protein) were incubated with all additions except [^{14}C]leucine and RNA for 2 min. at 35° and after addition of [^{14}C]leucine and RNA for another 30 min. at 35°. The total radioactivity of the control incubated in the absence of RNA was 38 counts/min.

gel filtration. Fig. 1 shows the fractionation of rat-liver nuclear RNA on a sucrose gradient between 5% and 20% sucrose. Different fractions were tested for their ability to stimulate the incorporation of [^{14}C]leucine into protein by microsomes or RNP particles. The microsomal systems were slightly stimulated by the RNA and the systems containing RNP particles showed a stronger response. A few micrograms of a fraction near the bottom of the density gradient (tube 6) stimulated the incorporation more than three times. In addition, the nucleic acid of the size of ribosomal RNA (tubes 15–20) and the RNA of the 4 s region (tubes 25–26) had a stimulatory effect on amino acid incorporation. When calculated per extinction unit added the stimulation by the light RNA was less marked.

Activity of chick-liver nuclear RNA

Sucrose gradient. A somewhat different fractionation on a sucrose gradient was performed with chick nuclear RNA. The gradient between 10% and 25% sucrose was run in an angle rotor instead of the swinging bucket and the RNA was prepared in the absence of bentonite. Fig. 2 shows the pattern of fractionation and the effect of some of the fractions on the incorporation of [^{14}C]leucine by both RNP particles and the membrane fraction from rat liver. Again the fractions near the bottom of the gradient and the nucleic acid of the size of ribosomal RNA (tubes 16–22) and of the low-molecular-weight RNA had a stimulatory activity. The RNP particles showed a better response to the RNA added than did the membrane fraction. More than fivefold stimulation was obtained by the RNA of tube 9 and tube 22.

Fractionation by gel filtration. To separate s-RNA from RNA of higher molecular weight the cross-linked dextran gel Sephadex G-200 was used. This column has the advantage of separating out any ribonuclease that might remain after the preparation of the RNA. In addition, the capacity is much greater than that of a sucrose gradient. The

distribution of RNA along the column is dependent upon the size and the shape of the material added (Porath & Flodin, 1959). The high-molecular-weight RNA (mol.wt. > 200000) will move in the front. The distribution pattern of nuclear RNA is not comparable with that obtained by sucrose-gradient centrifugation. The Sephadex G-200 is not able to separate the high-molecular-weight RNA into several fractions, but a better separation of the low-molecular-weight RNA can be obtained.

Fig. 3 represents the distribution pattern of total chick nuclear RNA on a Sephadex G-200 column. Three main peaks can be distinguished. The peak in the front (tubes 32–43) was the high-molecular-weight RNA and the last peak (tubes 90–100) had the size of s-RNA. It was able to bind [^{14}C]leucine (0·02mμmole/10μg. of RNA) when the pH 5-precipitable fraction of cell sap from rat liver was used to activate the amino acid. The peak between the high- and the low-molecular-weight RNA probably corresponds to a 10s RNA. This fraction as well as the high-molecular-weight RNA were not able to bind activated amino acids. The stimulatory effect of different fractions on the incorporation of [^{14}C]phenylalanine or [^{14}C]leucine into protein by RNP particles or the membrane fraction from rat liver are shown in Fig. 3. In agreement with the results obtained by sucrose-gradient centrifugation an increase of activity was observed on addition of most of the fractions.

Possible formation of rat-liver polysomes by chick nuclear RNA. The stimulation of amino acid incorporation obtained by the addition of chick-liver RNA may also involve an increased formation of polysomes. This approach was studied with sucrose-gradient centrifugation or column chromatography on agarose suspensions. Before addition to the RNP particles, the chick RNA was fractionated on a column of Sephadex G-200 to separate the low-molecular-weight RNA from the bulk of RNA and to remove any ribonuclease, as already described in reference to Fig. 3. The RNA, corresponding to fraction 31–84 in the Figure, was collected and reprecipitated, and the incubation and fractionation were carried out as described in the Materials and Methods section. Since RNP particles are able to incorporate amino acids into protein to a reasonable extent without addition of cell sap (Decken, 1961) the possible formation of polysomes and their amino acid-incorporating activity could be studied in the absence or the presence of rat-liver cell sap.

Sucrose gradient. The pattern of fractionation of RNP particles after incubation in the absence (Fig. 4*a*) and in the presence (Fig. 4*b*) of chick RNA showed slight differences. The distribution pattern of the added chick RNA is indicated in

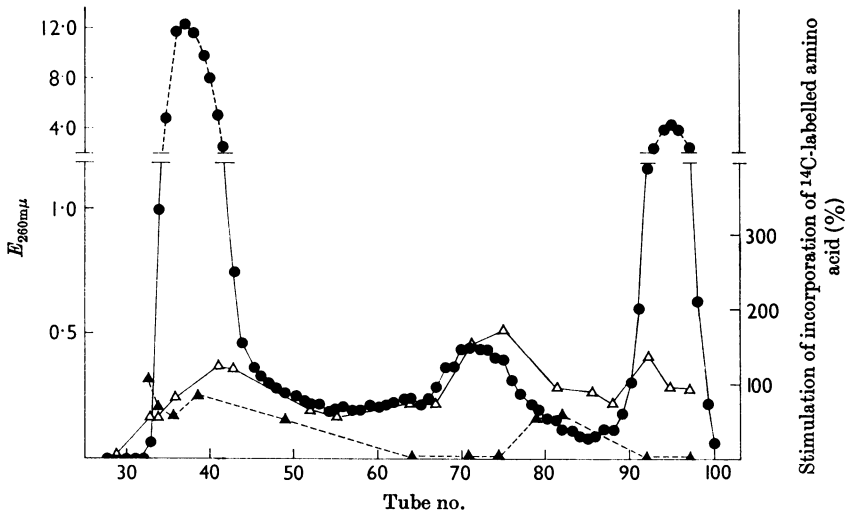

Fig. 3. Distribution pattern on Sephadex G-200 of chick-liver nuclear RNA. The flow rate of the column (2·3cm. × 55cm.) was adjusted to 6ml./hr. Samples (2·5ml.) were collected. Portions (0·15ml.) were taken out where indicated and added to an amino acid-incorporation system as described in Fig. 2. In the membrane fraction [^{14}C]leucine was replaced by [^{14}C]phenylalanine (0·25μc). In control experiments with incubation in the absence of RNA the RNP particles had a total radioactivity of 45counts/min. and the membrane fraction 29counts/min. △——△, Radioactivity incorporated by RNP particles; ▲---▲, radioactivity incorporated by membrane fraction; ●——●, extinction at 260mμ.

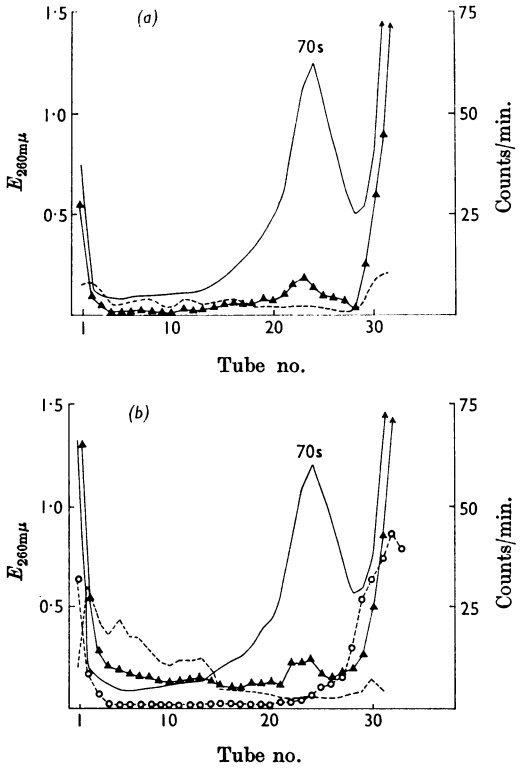

Fig. 4. Effect of chick-liver nuclear RNA on polysome formation as tested by sucrose-gradient centrifugation. Tube no. 1 is from the bottom of the gradient, and tube no. 32 is from the top. Incubation was as described in the Materials and Methods section. (*a*) Incubation in the absence of RNA. (*b*) Incubation in the presence of RNA. ——, Extinction at 260 mμ of incubation mixture; O---O, extinction at 260 mμ of the chick RNA added to the system; ▲——▲, radioactivity of the proteins. Results are duplicates of parallel experiments. Broken lines indicate counts/min./10 μg. of RNP–RNA. (For the calculations the RNA extinction coefficient 22 mg.$^{-1}$ was used.)

Fig. 4(*b*). Apparently the RNA was incorporated into the RNP particles since the extinction values of the incubation mixture did not increase in proportion to the RNA added. The high extinction of RNA obtained in the bottom of the sucrose gradient could be due to ribosomal aggregates of the nucleus that are resistant to extraction by hot phenol (McCarty & Laszlo, 1964). The radioactivity profile along the gradient obtained in the presence of RNA (Fig. 4*b*) was somewhat different from that obtained in the absence of RNA (Fig. 4*a*). The difference was more pronounced when cell sap was present during incubation.

Column chromatography on agarose suspensions. Since fractionation on sucrose gradient is limited in capacity, it was thought that molecular sieving by column chromatography would give similar results provided that a gel with appropriate characteristics was chosen. Chromatography on agarose suspensions has been used by Hjertén (1962) to separate substances of extremely high molecular weight, suggesting the possibility of using this type of column for the separation of 70s particles from larger units.

As reference, 70s ribosomes previously isolated by sucrose-gradient centrifugation were applied to the column. As shown in the upper part of Fig. 5(*a*), the particles were eluted in a broad band. When the material of tube 54 was re-analysed by sucrose-gradient centrifugation, the sedimentation constant was again about 70s.

The distribution pattern of the incubation mixtures containing RNP particles without RNA or with RNA are shown in Fig. 5(*a*) and Fig. 5(*b*). The extinctions of the two preparations showed slight differences. These, however, could be due to the RNA added, the extinctions of which have been indicated in Fig. 5(*b*). In these experiments no rat-liver cell sap was added. Although an increased formation of particulate aggregates of a size bigger than 70s as measured by extinction was somewhat uncertain, the radioactivity profiles of the eluates were higher after the addition of chick nuclear RNA. Again, these results clearly suggest an increased formation of active polysomes. Owing to the higher capacity of the columns the differences in radioactivity were more marked than was the case with density-gradient centrifugation.

DISCUSSION

To establish the response of different amino acid-incorporating systems from rat liver to chick-liver nuclear RNA, it was necessary to study first the effect of a synthetic homopolymer, poly U, on the incorporation of [^{14}C]phenylalanine into polypeptides. Maxwell (1962) had shown that the incorporation of [^{14}C]phenylalanine by rat-liver microsomes was stimulated by poly U. Fessenden *et al.* (1963) used RNP particles from rat liver to show a similar stimulation. Campbell *et al.* (1964) studied the effect of poly U on different morphological constituents of the microsome fraction from rat liver and their ability to incorporate [^{14}C]phenylalanine. The three preparations described here, the microsomes, the RNP particles and the membrane fraction, were all susceptible to poly U, and incorporation of [^{14}C]phenylalanine was enhanced. The fraction enriched in membranes was not stimulated to a greater extent than the microsomes or RNP particles. These results are in agreement

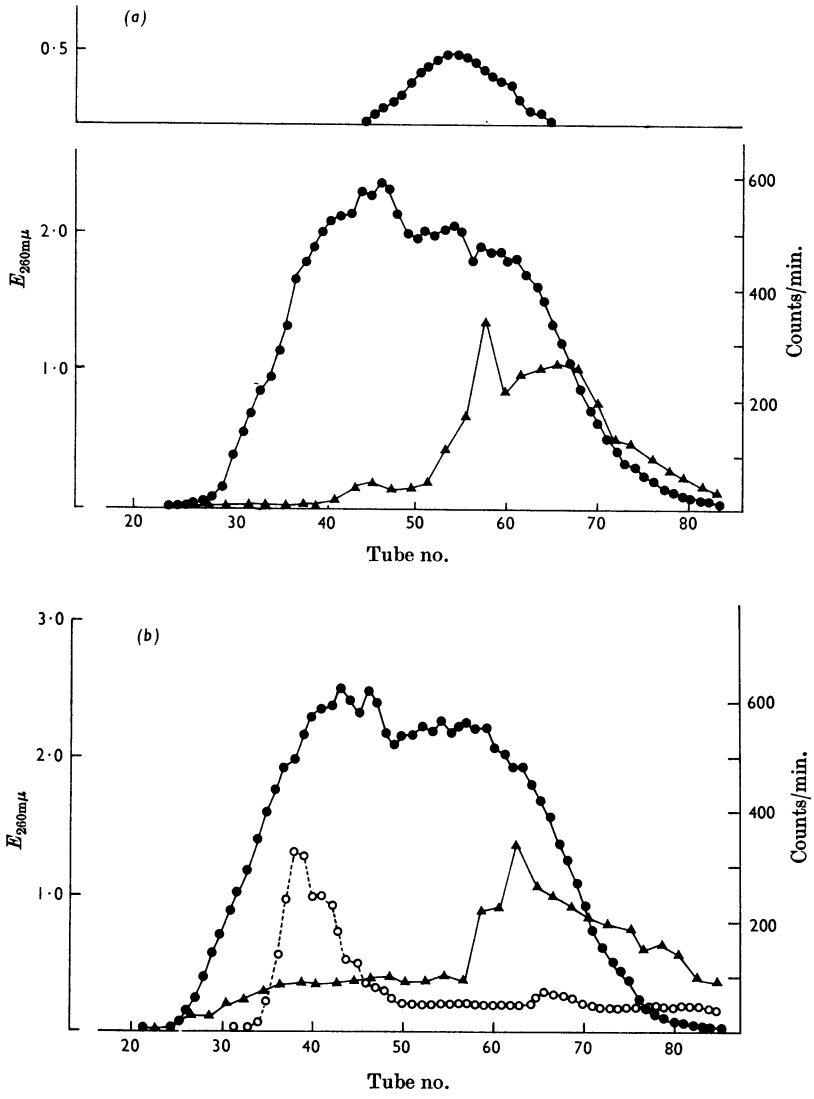

Fig. 5. Effect of chick-liver nuclear RNA on polysome formation as tested by column chromatography on agarose suspension. Incubation of RNP particles from 8–10g. of liver was as described in the Materials and Methods section (see 'Effect of RNA on formation of polysomes', section b). (*a*) Lower part: incubation in the absence of RNA; upper part: distribution pattern of RNP particles of 70s obtained from about 1·5g. of liver and previously isolated by sucrose-gradient centrifugation. (*b*) Incubation in the presence of RNA. ●——●, extinction at 260mμ of the incubation mixture; ○---○, extinction at 260mμ of the RNA added to the system; ▲——▲, radioactivity of the proteins.

with those of Campbell *et al.* (1964), showing that RNA with messenger activity was utilized at least as effectively by RNP particles alone as by a ribosomal fraction rich in membranes. The stimulation by RNA from rat-liver nuclei on a rat-liver system previously shown by Korner & Munro (1963) was also confirmed in the present investigation.

Because of the high stimulation obtained the isolated RNP particles were considered most suitable for studying the effect of chick nuclear RNA. A prerequisite for the stimulation observed

was to keep the amount of ribonuclease at a minimal level. Several precautions have been taken, of which the following may be mentioned. (a) Bentonite, known to have a very strong binding capacity to ribonuclease (Brownhill, Jones & Stacey, 1959), was used occasionally. However, bentonite not only binds ribonuclease but also absorbs 4μg. of chick RNA/mg. This adsorption may be selective. (b) All preparative media were sterilized under pressure, which inactivates pancreatic ribonuclease and may therefore also inactivate ribonuclease present in the materials used. (c) To remove the nuclear-derived ribonuclease, which contaminated the RNA preparations and was inactive in the presence of phenol, the RNA was passed through columns of Sephadex G-200. The molecular weight of ribonuclease is about 13000, and therefore will remain behind the RNA on the column.

It was shown by Dingman & Sporn (1962) that treatment with lauryl sulphate at concentrations higher than 0·1% released deoxyribonucleotides from the nuclei together with RNA. It also was shown by Barondes, Dingman & Sporn (1962) that deoxyribonucleotides inhibited amino acid incorporation in bacterial protein-synthesizing systems. The lauryl sulphate concentration used here in the presence of phenol was 0·4%. At lower concentrations of lauryl sulphate, the RNA that had a stimulatory effect on amino acid incorporation was not extracted. In spite of the high lauryl sulphate concentration there was a stimulatory effect by the RNA on amino acid incorporation. The reason for this may be that protein-synthesizing systems obtained from mammalian tissues are insensitive to deoxyribonucleotides.

It has been pointed out that the capacity of sucrose-gradient centrifugation for the fractionation of polysomes and ribosomes is limited. Agarose columns have been shown to have a higher loading capacity (Hjertén, 1962). However, the very slow flow rate required for a good separation makes the experiments extend over a longer period (about 20hr.).

The present experiments were made in connexion with attempts to label specific chick proteins in rat-liver systems containing RNA from chick-liver nuclei. In a mixed system of this kind an increased incorporation of amino acids into protein especially when obtained in the polysomal region would be evidence for such a synthesis. After sucrose-gradient centrifugation or column chromatography the sedimentation or elution profiles of the labelled proteins indicated that functional polysomal units were actually obtained under the influence of the added RNA. The polysome formation was accentuated when cell sap was added to the system. By increasing the amount of particles, the specific activity of the labelled amino acid and the temperature during incubation, it should be possible to demonstrate a labelling of albumin or of a bigger fragment of it. Experiments of this kind are in progress. However, after purification of the proteins by starch-block electrophoresis followed by immunoelectrophoresis no significant labelling of a protein with the antigen activity of chick serum albumin has been observed so far. Tsugita, Fraenkel-Conrat, Nirenberg & Matthaei (1962), working with the RNA-directed synthesis of a specific protein in bacterial systems, obtained a polypeptide that resembled the protein without, however, being identical to it. It is possible that systems *in vitro* are unable to utilize m-RNA in a proper way.

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