A Decreased Aminoacyl-Transfer-Ribonucleic Acid-Binding Capacity of 40S Ribosomal Subunits Resulting from Hypophysectomy of the Rat

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A technique that permitted the reversible dissociation of rat liver ribosomes was used to study the difference in protein-synthetic activity between liver ribosomes of normal and hypophysectomized rats. Ribosomal subunits of sedimentation coefficients 38S and 58S were produced from ferritin-free ribosomes by treatment with 0.8M-KCl at 30°C. These recombined to give 76S monomers, which were as active as untreated ribosomes in incorporating phenylalanine in the presence of poly(U). Subunits from normal and hypophysectomized rats were recombined in all possible combinations and the ability of the hybrid ribosomes to catalyse polyphenylalanine synthesis was measured. The results show that the defect in ribosomes of hypophysectomized rats lies only in the small ribosomal subunit. The 40S but not the 60S subunit of rat liver ribosomes bound poly(U). The only requirement for the reaction was Mg^{2+} , the optimum concentration of which was 5mM. No apparent difference was seen between the poly(U)-binding abilities of 40S ribosomal subunits from normal or hypophysectomized rats. Phenylalanyl-tRNA was bound by 40S ribosomal subunits in the presence of poly(U) by either enzymic or non-enzymic reactions. Non-enzymic binding required a Mg^{2+} concentration in excess of 5mM and increased linearly with increasing Mg^{2+} concentrations up to 20mM. At a Mg^{2+} concentration of 5mM, GTP and either a 40–70%-saturated- $(NH_4)_2SO_4$ fraction of pH 5.2 supernatant or partially purified aminotransferase I was necessary for binding of aminoacyl-tRNA. Hypophysectomy of rats resulted in a decreased binding of aminoacyl-tRNA by 40S ribosomal subunits.

Ribosomes from mammalian sources can be reversibly dissociated into subunits by treatment with high concentrations of univalent cations at elevated temperatures (Martin & Wool, 1968). This technique permits formation of hybrid ribosomes, which can be used to locate specific ribosomal functions or defects. Ribosomes prepared from the liver of hypophysectomized rats have a decreased ability to incorporate amino acids into proteins in the presence or absence of an excess of poly(U), compared with ribosomes of normal animals (Korner, 1968). We report here the ribosomal subunit location of the defect in ribosomes of hypophysectomized rats and that this defect results in an impaired ability to bind aminoacyl-tRNA. A preliminary report of some of this work has been published (Barden & Korner, 1969).

Materials and Methods

Materials

[3H]Phenylalanine (3020mCi/mmol or 21 Ci/mmol) and L-[U- ^{14}C]phenylalanine (459mCi/mmol) were

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obtained from The Radiochemical Centre, Amersham, Bucks., U.K.; GTP (disodium salt), ATP (disodium salt) and hydroxyapatite were obtained from the Sigma Chemical Co., St. Louis, Mo., U.S.A. 3H -labelled poly(U) (36 μ Ci/ μ mol of phosphorus) and unlabelled poly(U) were from Miles Chemical Corp., Elkhart, Ind., U.S.A.

Animals

Male Sprague-Dawley rats of 150–170g wt. range were used. Hypophysectomized rats of the same strain, sex and weight range were obtained from Charles River Laboratories, Wilmington, Mass., U.S.A. Rats were starved overnight before use.

Preparation of ribosomes and subunits

Ribosomes that were to be used for the derivation of ribosomal subunits were prepared by homogenizing liver in 2.5vol. of ice-cold 0.25M-sucrose containing medium P (20mM-tris-HCl, pH 7.6, 4mM- $MgCl_2$ and 6mM-2-mercaptoethanol). (The Mg^{2+} ion content of media was frequently altered, and where different from 4mM the final concentration is shown after the letter denoting the medium.) The supernatant obtained by centrifugation for 10min at

10000g and 0°C was removed and Triton X-100 added to a final concentration of 1% (v/v). The Mg^{2+} ion concn. was adjusted to 44mM and the material maintained at 0°C for 40min to permit ribosomal aggregation (Takanami, 1960). Ribosomes were formed into pellets by carefully layering 15ml of this material over 15ml of 0.5M-sucrose, containing medium P-14mM- Mg^{2+} , and centrifuging for 15min at 29000g. The ribosome pellet was resuspended in medium M-5mM- Mg^{2+} (20mM-tris-HCl, pH 7.6, 100mM-KCl, 40mM-NaCl, x mM-magnesium acetate and 6mM-2-mercaptoethanol) by gentle agitation and used immediately. Ribosomal subunits were prepared, separated on sucrose density gradients and recombined as described by Barden & Korner (1969).

A Spinco model E ultracentrifuge fitted with u.v.-absorption optics was used for analysis of ribosomal subparticles. A cell with 4° aluminium sector and 12mm path-length was used in all cases. The photographic plate was scanned with a Joyce-Loebl automatic recording densitometer mk. IIC and sedimentation coefficients were calculated by the method of Markham (1960).

Conditions for incorporation of amino acid

The incubation conditions were as previously described (Munro *et al.*, 1964), scaled down to a final volume of 0.2ml, except that 0.125 μ Ci of radioactive amino acid was added per tube. Poly(U), if present, was at a concentration of 0.5mg/ml. Cell-sap was prepared as described by Munro *et al.* (1964) and a ratio of cell-sap protein to ribosomal protein of between 25:1 and 50:1 used in incubations. Incubations, performed in quadruplicate, were stopped by addition of ice-cold 0.5M-perchloric acid containing unlabelled amino acid corresponding to the radioactive amino acid used.

Precipitated proteins were spun down, redissolved in 0.5ml of 0.3M-NaOH and RNA was hydrolysed at 37°C for 1h. Proteins were reprecipitated by cold 5% (v/v) trichloroacetic acid, collected on Whatman glass-fibre membranes and washed with cold 5% trichloroacetic acid.

Preparation of aminoacyl-tRNA

The 165000g supernatant, from rat liver homogenized in 0.25M-sucrose-containing medium M-10mM- Mg^{2+} , was passed through a column of Sephadex G-25 (coarse) equilibrated with medium M-10mM- Mg^{2+} and the effluent adjusted to pH 5.2 with 1.0M-acetic acid. The precipitate obtained after centrifugation was divided into two: one half was resuspended in 10mM-tris-HCl, pH 9.5, and 6mM-2-mercaptoethanol and the aminoacyl-tRNA discharged by incubation at 37°C for 15min (Gatica *et al.*, 1966). After reprecipitation at pH 5.2 the two

half-pellets were recombined and resuspended in medium M-10mM- Mg^{2+} (15ml/50g of liver). Aminoacylation was effected by incubation at 37°C for 15min in the presence of 5mM-ATP and [3 H]phenylalanine (1mCi) and labelled aminoacyl-tRNA was extracted with water-saturated phenol as described by Moldave (1963). The specific radioactivities of 3 H-labelled aminoacyl-tRNA preparations used are quoted in the text.

Preparation of enzyme fractions

A 40-70%-satd.-(NH_4) $_2$ SO $_4$ fraction of pH 5.2 supernatant was resuspended in medium M containing the appropriate concentration of Mg^{2+} and dialysed against the same medium before use. Partially purified aminotransferase I was prepared by a method similar to that of K. Moldave (Schneir & Moldave, 1968; Gasior & Moldave, 1965). pH 5.2 supernatant was passed down a Sephadex G-25 (coarse) column equilibrated with 0.05M-tris-HCl, pH 8.0, 6mM-2-mercaptoethanol and the effluent stirred with hydroxyapatite (2g/50g of liver) for 1h at 2°C.

After the recovered hydroxyapatite was washed in 0.175M-potassium phosphate buffer, pH 6.8 (3 $\times$ 80ml), partially purified aminotransferase I was obtained from 0.25M-potassium phosphate buffer, pH 6.8, washings of the hydroxyapatite by adding (NH_4) $_2$ SO $_4$ to 65% saturation. Aminotransferase I was resuspended in, and dialysed against, medium M before use. All enzyme fractions were prepared from normal rats.

Binding assays

Both poly(U) and aminoacyl-tRNA-binding assays were based on the Millipore technique (Nirenberg & Leder, 1964). In the poly(U)-binding assay, ribosomal subunits were bound to Millipore HA membranes (2.5cm diam.: 0.45 μ m pore size); all remaining binding sites were saturated with bovine serum albumin (5ml of a 1mg/ml soln. in medium M) (Moore, 1966) and the desired amount of 3 H-labelled poly(U) was passed through and washed with medium M. All operations were carried out at 0°C. Unless otherwise stated the 3 H-labelled poly(U) was diluted with medium M to 0.1 μ g/ml and unlabelled poly(U) added at a concentration of 0.2 μ g/ml.

For assay of aminoacyl-tRNA binding, ribosomal subunits were incubated with 0.5mM-GTP, a stated amount of 3 H-labelled aminoacyl-tRNA and, where indicated, poly(U) (0.5mg/ml) and transfer factors. The ionic composition was that of medium M-5mM- Mg^{2+} unless otherwise stated. Aminoacyl-tRNA binding reached a plateau level after 10min at 37°C and in the assays reported here the reaction was

terminated after 15 min by addition of ice-cold medium M containing the appropriate concentration of Mg^{2+} ions; the contents of the tube were then poured through a Millipore HA filter. Unbound aminoacyl-tRNA was washed through with cold medium.

Bound poly(U) or aminoacyl-tRNA was estimated by scintillation counting. Results are expressed as c.p.m. bound/mg of ribosomal RNA for poly(U) binding and pmol of tRNA bound/mg of ribosomal RNA after taking certain control incubations into consideration. For purposes of calculation the mol. wt. of tRNA was taken as 27×10^3 , and 1.9% of the tRNA preparation was assumed to be phenylalanyl-tRNA (Allen *et al.*, 1969). In poly(U) binding the control was the counts retained by a filter treated with bovine serum albumin and through which an amount of 3H -labelled poly(U) was passed, corresponding to the amount used in the experimental system. For aminoacyl-tRNA binding the complete incubation mixture was maintained at $0^\circ C$, and a mixture minus ribosomal subunits was incubated at $37^\circ C$ and account was taken of whichever gave the highest blank.

Estimation of radioactivity

Millipore HA filters or Whatman glass-fibre membranes were dried and counted in toluene scintillator [3.5 g of 2,5-diphenyloxazole and 0.55 g of 1,4-bis-(4-methyl-5-phenyloxazol-2-yl)benzene in 1 litre of toluene] by using a Packard Tri-Carb scintillation spectrometer. Counting efficiency for 3H label was 38% and 86% for ^{14}C . Aqueous solutions of 3H -labelled aminoacyl-tRNA were counted in Triton-toluene scintillator (5 g of 2,5-diphenyloxazole in 300 ml of Triton X-100 and 700 ml of toluene) (Patterson & Greene, 1965). Sufficient counts were recorded to decrease the standard error to 3% or less.

Determination of RNA and protein

RNA was determined from extinction at 260 nm. An extinction coefficient of 23.5/mg of RNA per ml per cm was used for intact ribosomes and 60S ribosomal subunits, and one of 29.9/mg of RNA per ml per cm for 40S ribosomal subunits was calculated by the method of Fleck & Munro (1962). Protein content was measured by the method of Lowry *et al.* (1951).

Results

Formation and recombination of ribosomal subunits

Ribosomes prepared by precipitation with Mg^{2+} ions were of activity in a cell-free system comparable with those prepared by the normal method of differential centrifugation. However, Mg^{2+} -precipitated

ribosomes were free from ferritin contamination and this resulted in better resolution on sucrose density gradients in the region of the 60S ribosomal subunit. The ferritin content of ribosome preparations varies considerably (Helgeland, 1968) and, although it often presented little difficulty with ribosomes prepared from normal rats, the proportion present in ribosomes prepared from hypophysectomized rats was invariably higher and necessitated its removal.

Treatment of rat liver ribosomes with 0.8M-KCl at $30^\circ C$ and subsequent centrifugation on sucrose gradients containing high KCl dissociated some of them into subunits. A final concentration of 0.8M-KCl was found to be optimal for subunit formation in the presence of 5 mM- Mg^{2+} . Fractions of sucrose gradients corresponding to each of the two subunits were collected and their purity was ascertained in the analytical ultracentrifuge (Fig. 1). Small ribosomal subunits, with sedimentation coefficient of $38.0 \pm 0.6 s_{20,w}$, were free from contamination. The larger ribosomal subunit preparation of sedimentation coefficient $58.5 \pm 1.1 S$ contained 10–15% of undissociated monomers. When mixed in an E_{260} ratio of 2.5:1, 60S and 40S subunit preparations recombined to give a major particle sedimenting at 76S and a minor particle sedimenting at about 50S (Fig. 1). The ratio in which 60S and 40S subunits were mixed was not critical to the subsequent activity of re-associated ribosomes, and an E_{260} ratio of between 2:1 and 3:1 (60S:40S) produced ribosomes of very similar activities. Similar characteristics have been reported for the recombination of reticulocyte ribosomal subunits (Bonanou & Arnstein, 1969).

Activity of subunit fractions and reassociated ribosomes in protein synthesis

Neither of the two ribosomal subunit preparations nor reassociated ribosomes had any activity when assayed for ability to catalyse protein synthesis in the absence of poly(U), but in the presence of an excess of poly(U) reassociated ribosomes exhibited an activity greater than that of undissociated control ribosomes. The 40S ribosomal subunit preparations had no ability to catalyse synthesis of polyphenylalanine, but 60S ribosomal subunit preparations had an activity equal to 10–15% of that of reassociated ribosomes (Table 1).

Subunits prepared from ribosomes of normal and hypophysectomized rats were recombined in all possible combinations and the ability of the homologous and hybrid reassociated ribosomes to catalyse synthesis of polyphenylalanine was measured (Table 2). Reassociated ribosomes composed of a 40S and 60S subunit from normal rats had the same activity as a hybrid reassociated ribosome containing a normal 40S subunit with a 60S subunit from hypophysectomized rats. Any homologous or hybrid re-associated ribosomes containing a 40S subunit from

Fig. 1. Purity of subunit fractions and reassociated ribosomes

All figures are the densitometer traces of photographs obtained with the u.v. optical system of the analytical ultracentrifuge. Photographs were taken at 4 min intervals after maximum speed was achieved. Sedimentation in all cases is from left to right. (a) 40S ribosomal subunits ($1.0 E_{260}$ units/ml) in medium M-5 mM- Mg^{2+} . Temperature of the run was 24°C and maximum speed 36000 rev./min. (b) 60S ribosomal subunits ($3.8 E_{260}$ units/ml) in medium M-5 mM- Mg^{2+} . Temperature of the run was 4°C and maximum speed 34000 rev./min. (c) Reassociated ribosomes ($1.9 E_{260}$ units/ml) in medium M-10 mM- Mg^{2+} . Temperature of the run was 24°C and maximum speed 34000 rev./min.

Table 1. Activity of subunit fractions and reassociated ribosomes

Preparations of 40S subunits, 60S subunits, re-associated ribosomes [E_{260} ratio (40S:60S) = 1:2.5] and undissociated ribosomes (10–16 μ g of RNA), all from normal rats, were incubated in a total volume of 0.2 ml with cell-sap, 5 mM-ATP, 0.1 mM-GTP and 0.125 μ Ci of [14 C]phenylalanine (459 mCi/mmol) for 30 min at 37°C. The ionic composition was that of medium M-10 mM- Mg^{2+} . Poly(U) (110 μ g) was added where indicated. In the absence of poly(U) the 19 other unlabelled protein amino acids were present at a concentration of 7.5 μ M. The results were corrected for incorporations in the absence of ribosomes or subunits.

	$10^{-3} \times$ Incorporation (c.p.m./mg of RNA)	
	-poly(U)	+poly(U)
40S subunit preparation	0	0
60S subunit preparation	0	17.5
Reassociated ribosomes	0	168.3
Control, undissociated ribosomes	12.5	124.2

hypophysectomized rats had less ability to incorporate [14 C]phenylalanine into protein than re-associated ribosomes containing the normal 40S subunit. There was no correlation between activity of the reassociated ribosomes and source of the 60S ribosomal subunit.

Conditions for binding of poly(U)

The binding of poly(U) to ribosomes or subunits is critically dependent on the Mg^{2+} concentration (Takanami & Okamoto, 1963; Moore, 1966; Wolfe & Weisberger, 1965), the optimum for which depends upon the particular assay conditions. The use of medium M containing between 0.0 and 20.0 mM- Mg^{2+} at each stage of the assay procedure described in the Materials and Methods section indicated an optimum Mg^{2+} concentration of 5 mM for the binding of poly(U) to 40S ribosomal subunits. An amount of poly(U) necessary to saturate fully the binding potential of 40S ribosomal subunits was found to be present when the ratio of ribosomal RNA to poly(U) in the binding assay was 16:1 (w/w) or less. Binding of poly(U) to 60S ribosomal subunit preparations did not occur to an extent greater than that, which may be explained by the monomer contamination of such fractions.

Poly(U) binding to 40S ribosomal subunits of normal and hypophysectomized rats

Addition of poly(U) to a cell-free protein-synthesizing system containing ribosomes from hypophy-

sectomized rats results in at least as great a stimulation of incorporation of phenylalanine as occurs when ribosomes from normal rats are used, but the absolute level of incorporation is lower (Korner, 1965). One interpretation of this is that ribosomes from hypo-

physectomized rats have an impaired ability to bind poly(U). For this reason the binding of poly(U) to 40S ribosomal subunits of normal and hypophysectomized rats was assayed (Table 3). No difference was detected in the abilities of 40S ribosomal subunits from normal and hypophysectomized rats to bind poly(U) under the conditions of the Millipore assay.

Table 2. *Activity of homologous and hybrid reassociated ribosomes formed from subunits of normal and hypophysectomized rats*

Reassociated ribosomes formed by combining subunits from normal and hypophysectomized rats in all possible combinations [E_{260} ratio (60S:40S) = 2.5:1] and control undissociated ribosomes (8–15 μ g of RNA) were incubated in a final volume of 0.2 ml containing cell-sap, 5 mM-ATP, 0.1 mM-GTP, poly(U) (0.5 mg/ml) and 0.125 μ Ci of [14 C]phenylalanine (459 mCi/mmol) for 30 min at 37°C. The results of several separate experiments are shown as means $\pm$ S.E.M. with the total number of observations in parentheses. The incorporation of [14 C]phenylalanine/mg of ribosomal RNA is expressed as a percentage of the mean value for undissociated ribosomes from normal rat. The difference between the means of incorporation by reassociated ribosomes containing 40S subunits from either normal or hypophysectomized was tested by Student's *t* test and found to be <0.001 . N, Normal; H, hypophysectomized.

Sample	Incorporation (% of control)
Control undissociated ribosomes	
Normal rat	100.0 $\pm$ 4.8 (12)
Hypophysectomized rat	69.5 $\pm$ 4.6 (12)
Reassociated ribosomes	
N 40S, N 60S	161.6 $\pm$ 8.8 (12)
N 40S, H 60S	160.3 $\pm$ 4.3 (12)
H 40S, H 60S	125.0 $\pm$ 3.9 (12)
H 40S, N 60S	124.3 $\pm$ 4.3 (12)

Enzymic and non-enzymic binding of aminoacyl-tRNA to 40S ribosomal subunits

At low concentrations of Mg^{2+} the binding of aminoacyl-tRNA by rat liver ribosomes is completely dependent upon both GTP and a soluble enzyme fraction (Ibuki & Moldave, 1968), whereas at higher concentrations of Mg^{2+} binding can occur in their absence (Siler & Moldave, 1969). The active component of the soluble enzyme fraction is amino-transferase I (Schneir & Moldave, 1968; McKeehan & Hardesty, 1969), and this is specifically bound by the 40S ribosomal subunit (Rao & Moldave, 1969). [3 H]Phenylalanyl-tRNA was bound to isolated 40S ribosomal subunits in both enzymic and non-enzymic reactions. At 5 mM- Mg^{2+} binding of aminoacyl-tRNA was entirely dependent upon an enzyme fraction, whereas at higher concentrations of Mg^{2+} non-enzymic binding also occurred. Non-enzymic binding increased linearly with Mg^{2+} concentration at least as far as 20 mM, whereas enzymic binding showed signs of reaching a plateau level at this Mg^{2+} concentration.

At 5 mM- Mg^{2+} , saturation with enzyme fraction was achieved when the concentration of the 40–70% satd.-(NH_4)₂SO₄ fraction was in excess of 0.5 mg/ml. If partially purified aminotransferase I was used, a concentration of 300 μ g/ml was sufficient to give maximum binding. The binding of phenylalanyl-tRNA showed an absolute requirement for the presence of poly(U).

Table 3. *Poly(U) binding to 40S ribosomal subunits of normal and hypophysectomized rats*

The 40S ribosomal subunits prepared from normal (8.1 μ g of RNA) and hypophysectomized rats (8.0 μ g of RNA) were bound to Millipore filters. After filling remaining binding sites with bovine serum albumin the binding of saturating (0.925 μ g) and unsaturating (0.154 μ g) amounts of 3 H-labelled poly(U) was determined as described in the Materials and Methods section. Results are expressed as means $\pm$ S.E.M.

40S ribosomal subunit derivation	Degree of saturation with poly(U)	Concn. of Mg^{2+} (mM)	$10^{-3} \times$ Incorporation (c.p.m./mg of RNA)
Normal rat	Unsaturated	5	59.3 $\pm$ 3.9
Hypophysectomized rat	Unsaturated	5	59.6 $\pm$ 6.7
Normal rat	Saturated	5	162.3 $\pm$ 16.7
Hypophysectomized rat	Saturated	5	165.0 $\pm$ 8.9

Table 4. *Binding of aminoacyl-tRNA by 40S ribosomal subunits of normal and hypophysectomized rats*

The 40S ribosomal subunits (8.3 µg of RNA) of normal or hypophysectomized rats were incubated with [³H]-phenylalanyl-tRNA (40 µg; 597 × 10⁴ c.p.m./mg), 0.1 mM-GTP and poly(U) (0.5 mg/ml) in a final volume of 0.2 ml for 15 min at 37°C. Aminotransferase I, if present, was at a concentration of 300 µg/ml and a 40–70% satd.-(NH₄)₂SO₄ fraction at 1.5 mg/ml. Results are given as means ± S.E.M.

Source of 40S subunit	Enzyme fraction	Binding of phenylalanyl-tRNA (pmol/mg of RNA)
Normal rat	Aminotransferase I	118.9 ± 6.2
Normal rat	40–70% satd. (NH ₄) ₂ SO ₄ fraction	111.6 ± 2.9
Hypophysectomized rat	Aminotransferase I	83.4 ± 2.0
Hypophysectomized rat	40–70% satd. (NH ₄) ₂ SO ₄ fraction	76.2 ± 2.5

Effect of hypophysectomy of rats on the binding of aminoacyl-tRNA to 40S ribosomal subunits

The binding ability of 40S ribosomal subunits from normal and hypophysectomized rats was measured and the results are shown in Table 4. The 40S ribosomal subunits from normal rats enzymically bound phenylalanyl-tRNA to a significantly greater degree than did those from hypophysectomized rats.

Discussion

Reassociated ribosomes were devoid of any ability to catalyse polypeptide synthesis unless poly(U) was added to the system, and must therefore lack any active endogenous mRNA (Table 1). Their activity in the presence of poly(U) exceeded that of control undissociated ribosomes, as would be expected if the endogenous mRNA content of the latter interferes with attachment of ribosomes to poly(U).

It has been argued that the difference in activity between ribosome preparations from normal and hypophysectomized rats is not a reflection of a difference in endogenous mRNA content (Korner, 1965), and the fact that the difference still persisted after dissociation and reassociation, when no active natural mRNA remains, supports this view. The difference must lie in the ribosomes themselves and was in fact located in the 40S ribosomal subunit (Table 2).

The activity of undissociated ribosomes from hypophysectomized rats was approx. 70% of that of ribosomes from normal rats, whereas reassociated ribosomes containing a 40S subunit from hypophysectomized rats exhibited an activity of 77% of reassociated ribosomes containing a normal 40S subunit. This discrepancy may result from the presence of endogenous mRNA in undissociated ribosomes or from the fact that not all ribosomal particles dissociate on treatment with KCl, and the subunit fractions are not thus uniformly derived from the total spectrum of particles present before treatment with KCl.

Foster & Sells (1969) have reported that reassociated ribosomes from livers of normal and hypophysectomized rats have the same ability to catalyse synthesis of polyphenylalanine. However, they prepared subunits from the polysomes of both types of rat, and it has been shown that very little, or no, difference in activity exists between polyribosomes of normal and hypophysectomized rats (Korner, 1964). In contrast, the ribosomal subunits used here were derived mainly from monomers. It is notable that ribosomes of diabetic rats, which are less active than normal ones, have a defect in the 60S ribosomal subunit (Martin & Wool, 1968).

It is possible that the defect in the 40S ribosomal subunit of hypophysectomized rats results from a decreased interaction with 60S ribosomal subunits. However, this view is not supported by the behaviour of ribosomes of hypophysectomized rats during dissociation, since they show no greater tendency to form subunits than do ribosomes of normal rats, and the difference most probably lies in one of the functions of the 40S ribosomal subunit in protein synthesis.

Our results show that poly(U) is bound by the small, but not by the large, ribosomal subunit of rat liver, and appear to offer no support for the differences in activity of ribosomes from normal and hypophysectomized rats resulting from differences in the binding of this polynucleotide. However, we cannot rule out the possibility of a difference in the functional binding of poly(U), and although identical amounts of poly(U) are bound by 40S ribosomal subunits of control and hypophysectomized animals the polynucleotide may not be associated at the correct site on the ribosomes of hypophysectomized rats.

Liew & Korner (1969) have reported a decreased binding of aminoacyl-tRNA by intact unwashed ribosomes of hypophysectomized rats both in the presence and the absence of poly(U). No enzyme fraction was added to the binding assay, but inhibition of the reaction by 5'-guanylmethylenediphosphonate suggests that the ribosomes were contaminated by transfer factors and peptide-bond and

polypeptide formation probably occurred. It could be argued that the differences reported could have arisen because more transfer factors remained attached to ribosomes of normal rats than to those of hypophysectomized rats, rather than by differences in the ribosomes themselves. The use of 40S ribosomal subunits and added transfer factors in the present work overcomes these difficulties, since if peptidyl-transferase is an integral part of the large ribosomal subunit (Monro, 1967) the formation of di-, tri- or poly-peptides is extremely unlikely. The results reported here show that differences do exist between the aminoacyl-tRNA-binding potentials of 40S ribosomal subunits of normal and hypophysectomized rats and confirm the conclusion of Liew & Korner (1969).

We propose that the impaired ability of ribosomes from hypophysectomized rats is a result of a defect in the 40S ribosomal subunit and is manifested by a decreased ability of this particle to bind aminoacyl-tRNA. We do not know whether this difference between 40S ribosomal subunits of normal and hypophysectomized animals results from a change in the degree of affinity for aminoacyl-tRNA or from a change in the proportion of subunits which are capable of binding aminoacyl-tRNA or from a difference in the functional binding of polynucleotides.

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