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Amino Acid Regulation of Synthesis of Ribonucleic Acid and Protein in the Liver of Rats

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Weanling (23-day-old) rats were fed on either a low-protein diet (6% casein) or a diet containing an adequate amount of protein (18% casein) for 28 days. Hepatic cells from animals fed on the deficient diet were characterized by markedly lower concentrations of protein and RNA in all cellular fractions as compared with cells from control rats. The bound rRNA fraction was decreased to the greatest degree, whereas the free ribosomal concentrations were only slightly less than in control animals. A good correlation was observed between the rate of hepatic protein synthesis *in vivo* and the cellular protein content of the liver. Rates of protein synthesis both *in vivo* and *in vitro* were directly correlated with the hepatic concentration of individual free amino acids that are essential for protein synthesis. The decreased protein-synthetic ability of the ribosomes from the liver of protein-deprived rats was related to a decrease in the number of active ribosomes and heavy polyribosomes. The lower ribosomal content of the hepatocytes was correlated with the decreased concentration of essential free amino acids. In the protein-deprived rats, the rate of accumulation of newly synthesized cytoplasmic rRNA was markedly decreased compared with control animals. From these results it was concluded that amino acids regulate protein synthesis (1) by affecting the number of ribosomes that actively synthesize protein and (2) by inhibiting the rate of synthesis of new ribosomes. Both of these processes may involve the synthesis of proteins with a rapid rate of turnover.

Previous studies in our laboratories indicated that dietary amino acids influenced hepatic protein synthesis (1) by affecting the ability of polyribosomes to synthesize protein and (2) by influencing the concentration of cytoplasmic ribosomes (Wannemacher, Cooper & Yatvin, 1968*a*). It was further hypothesized that the former process is related to the concentration of cellular free amino acids, whereas the latter is correlated with the ability of newly synthesized ribosomal subunits to leave the nucleus.

Further, polyribosomal integrity and rates of protein synthesis are dependent on the amino acid concentration in both a system *in vitro* (Baliga, Pronczuk & Munro, 1968; Wannemacher, Cooper & Muramatsu, 1970) and in perfused liver (Jefferson & Korner, 1969). Jefferson & Korner (1969) found that 11 amino acids (arginine, asparagine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, threonine, tryptophan, valine) were essential for hepatic protein synthesis. Likewise, incorporation of leucine into cardiac proteins is closely correlated with the cellular free amino acid content in the myocardium of the hypotrophic heart (Wannemacher & McCoy, 1969). One of the objectives of the present study was to investigate the relationship *in vivo* between hepatic free amino acid content, the rate of amino acid incorporation into liver protein and the polyribosomal integrity of this tissue.

Shields & Korner (1970) have reported that amino acid starvation can inhibit the rate of appearance of newly synthesized ribosomal subunits into the cytoplasm. The experiments described below were designed to study the effects of protein deprivation on release and appearance in the cytoplasm of newly synthesized ribosomes.

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MATERIALS AND METHODS

Materials. L-[U-^{14}C]Leucine (250Ci/mmol), L-[4,5-^{3}H]leucine (55.5Ci/mmol), [*methoxy*-^{3}H]puromycin dihydrochloride (1.1Ci/mmol), [5-^{3}H]uridine (25Ci/mmol) and [5-^{3}H]uridine triphosphate (tetrasodium salt) (22.2Ci/mmol) were obtained from New England Nuclear Corp., Boston, Mass., U.S.A. ATP, GTP, CTP, UTP, L-amino acids, phosphoenolpyruvic acid, pyruvate kinase, 2-mercaptoethanol and antiserum to horse ferritin were obtained through Calbiochem, Los Angeles, Calif., U.S.A.

Animals. Male weanling (23-day-old) rats of the Wistar strain (Camm Research, Wayne, N.J., U.S.A.) were placed in individual cages and fed an agar-gel diet (Allison, Wannemacher, Banks & Wunner, 1964) *ad libitum* for 28 days. Each experimental group contained 12 animals, of which six were fed on a diet that contained 6% casein, which in effect represented a low-protein diet, whereas the remaining six were fed with an adequate amount of protein (18% casein). At 1 week before they were killed all rats were trained to consume their food between 0830 and 1030 hours. All animals were maintained on a 12h light (06.00–18.00 hours)–12h dark schedule and at a temperature of 25–26°C with 40% relative humidity. In conducting the research described in this report, the investigators adhered to the 'Guide for Laboratory Animal Facilities and Care', as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences–National Research Council.

Preparation of homogenate. The rats were decapitated without anaesthesia by a guillotine. After decapitation the rats were perfused with cold 0.9% NaCl until the livers were cleared of all blood. The livers were quickly removed and placed immediately in 5vol. of ice-cold 0.25M-sucrose in TKM buffer (0.05M-tris–HCl, pH7.6 at 25°C, 0.065M-KCl, 0.01M-$MgCl_2$). All subsequent operations were performed at a temperature near 0°C. Individual livers were blotted dry, weighed and passed through a tissue press; most of the connective tissue was thereby removed. Then 2vol. of ice-cold 0.25M-sucrose in TKM buffer was added to 1vol. of liver pulp and homogenized by 10 strokes at 800rev./min in a Potter–Elvehjem homogenizer with a motor-driven Teflon pestle.

Preparation of nuclear-free ribosomal, total ribosomal and non-sedimentable fractions. The nuclei in 1ml of homogenate were sedimented by the procedure of Blobel & Potter (1966) and the number of nuclei in this fraction was counted by means of an electronic counter (Wannemacher, Muramatsu & Cooper, 1968*b*). From the assumption of Mariani, Migliaccio, Spadoni & Ticca (1966) that the cytoplasmic mass of binuclear cells was twice that of mononucleated cells, it was possible to use the nuclear counts to calculate the results on a per-cell basis. The post-nuclear supernatant was utilized for the separation of free-ribosomal, total ribosomal and non-sedimentable fractions, as described by Blobel & Potter (1967).

Estimation of rates of hepatic protein synthesis in vivo. Rats were injected intraperitoneally with L-[4,5-^{3}H]leucine (10μCi/100g body wt.) 1h before they were killed. The uptakes of radioactivity into the total protein and non-protein fractions of the liver were measured by previously described methods (Wannemacher *et al.* 1968*a*) and these results were utilized to estimate the rate of hepatic protein synthesis *in vivo* and to make corrections for the size of the leucine pool (Wannemacher, 1967).

Analysis of ribosomal amino acid-incorporating ability, polyribosomal pattern and number of active ribosomes. A sample (6ml) of liver homogenate was diluted with 2ml of purified rat antibody to horse ferritin (0.4mg of precipitable antibody) and centrifuged for 15min at 15000rev./min (15000*g*) in a Spinco no. 40 rotor. The post-mitochondrial supernatant was treated with Triton X-100 (final concn. 2.0%), layered over 1.0M-sucrose–TKM buffer and centrifuged for 4h at 40000rev./min (106000*g*). The resulting precipitate (ribosomes) was dissolved in 0.25M-sucrose–TKM buffer and analysed for RNA content, ability to incorporate amino acids, polyribosomal pattern and number of active ribosomes. The amino acid-incorporating system of Cooper, Muramatsu & Wannemacher (1968*a*) was utilized in these experiments and the reaction was stopped after 2, 5, 10 and 15min of incubation. Polyribosomal patterns were separated on a 10–40% linear sucrose gradient and analysed as described by Yatvin & Lathrop (1966). The number of active ribosomes was determined by the [^{3}H]peptidyl-puromycin technique of Wool & Kurihara (1967).

Incorporation of uridine into RNA in vivo. Rats were injected intraperitoneally with [5-^{3}H]uridine (100μCi/100g body wt.) and were killed at $\frac{1}{4}$, $\frac{1}{2}$, 1, 2, 4, 12, 24, 72, 120, 168 or 216h after injection. The radioactivity in RNA of the total nuclear and ribosomal fractions was measured by the procedure of Blobel & Potter (1968). To overcome the possible differences in the size of UMP pool, the incorporation of uridine into the nuclear and ribosomal RNA was expressed as percentage of total RNA radioactivity.

Determination of RNA polymerase. Samples (0.5ml) of a nuclear suspension (approx. 1×10^{6} nuclei in 0.25M-sucrose) were added to a Mg^{2+}-dependent system, containing in 0.5ml: 50μmol of tris–HCl, pH8.5 at 25°C, 5μmol of $MgCl_2$, 5μmol of cysteine hydrochloride, 5μmol of 2-mercaptoethanol, 6μmol of NaF, 0.6μmol each of ATP, GTP and CTP, 0.03μmol of UTP and 0.00018μmol of [5-^{3}H]UTP (4μCi); or a Mn^{2+}-dependent system, containing in 0.5ml: 50μmol of tris–HCl, pH7.5 at 25°C, 4μmol of $MnCl_2$, 400μmol of $(NH_4)_2SO_4$, 5μmol of 2-mercaptoethanol, 6μmol of NaF, 0.6μmol each of ATP, GTP and CTP, 0.03μmol of UTP and 0.00018μmol of [5-^{3}H]UTP (4μCi). After incubation for 30min at 37°C, the reaction was stopped by placing the sample in an ice bath and by the immediate addition of 0.2ml of a solution containing 25μmol of UTP plus 10mg of RNA ml followed by 5ml of 0.1M-$Na_4P_2O_7$–0.4M-$HClO_4$. The RNA precipitate was washed four times with 5ml of the 0.1M-$Na_4P_2O_7$–0.4M-$HClO_4$ reagent, then dissolved in 0.5ml of 0.3M-KOH and added to 10ml of scintillation mixture (Wannemacher, Banks & Wunner, 1965). The radioactivity in zero-time blanks was subtracted from the 30min samples and the results were expressed as amount of uridine incorporated into RNA per 10^{6} nuclei or per μg of DNA. Incorporation of radioactivity by the Mg^{2+}-dependent system was linear over the 30min period, whereas the $Mn^{2+}/(NH_4)_2SO_4$ system approached a plateau after 25min of incubation.

Radioactivity measurements. Samples were added to 10ml of *p*-dioxan scintillation solution, which contained 10g of 2,5-diphenyloxazole, 0.25g of 1,4-bis-(4-methyl-

5-phenyloxazol-2-yl)benzene and 700g of naphthalene in 1 litre of *p*-dioxan. Samples were counted for radioactivity in a Packard scintillation spectrometer at 6°C and were corrected for quenching by the internal-standard technique. The background counting rate was 20–30c.p.m. with an efficiency of 10–20% for ^3H and 50–60% for ^{14}C.

Analysis of RNA, DNA, protein and free amino acids. Samples of liver homogenate and cellular fraction were analysed for RNA, DNA and protein content by previously described procedures (Wannemacher *et al.* 1968*a*). Samples of liver homogenate were treated with sulphosalicylic acid and the resulting filtrate was analysed for free amino acids by ion-exchange chromatography (Wannemacher *et al.* 1968*a*).

Statistics. Group means were compared by Student's *t* test, and the difference between two means was considered significant at $P<0.01$ under the null hypothesis.

RESULTS

Effect of amino acid deprivation on the protein and RNA content of various fractions from liver cells. The results in Table 1 illustrate the markedly lower protein and RNA content of the liver cells from animals fed on a 6%-casein diet. These results are in agreement with the observation of Wannemacher *et al.* (1968*a*) on decreased cell number and size of livers from rats fed on the low-protein diet.

The protein concentration of the various cellular fractions from the liver of protein-deprived animals was approx. 50% of that found in the control rats, except for the free ribosomes, which exhibited only a 29±2% (mean±S.E.M.) decrease. A similar change was observed in RNA content of the whole liver cells from rats fed on the 6%-casein diet, but the percentage decrease in RNA concentration was greatest in the bound-ribosomal fraction, 57±3% (mean±S.E.M.), and least in the free ribosomal fraction, 20±1% (mean±S.E.M.). The free/bound rRNA ratio was increased from 0.231±0.015 (mean±S.E.M.) in control rats to 0.438±0.048 (mean±S.E.M.) in the protein-deprived animals. In both the free and bound ribosomal fractions the RNA/protein ratio was approx. 0.40–0.50 and was not affected by diet.

Protein synthesis and ribosomal integrity in vivo. When the values for rates of leucine incorporation *in vivo* into hepatic protein of a rat were plotted against the corresponding concentrations of cellular protein, a significant correlation ($P<0.01$) was observed between these two determinations (Fig. 1*a*). The values for the rate of protein synthesis and concentrations of cellular protein for the 6%-casein group were all less than those for the control rats.

The individual free amino acid concentrations in liver cells from both groups of rats were very similar to those reported previously (Wannemacher *et al.* 1968*a*). When the rate of protein synthesis *in vivo* for a rat was plotted against the sum of concentrations of the 11 hepatic free amino acids which Jefferson & Korner (1969) found to be essential for maximal rate of amino acid incorporation and stability of polyribosomal profiles, a significant correlation ($P<0.01$) was observed (Fig. 1*b*). The concentration of essential amino acids and the rate of protein synthesis were all less for liver cells from each of the protein-deprived animals as compared with the control group.

A linear rate of amino acid incorporation was observed over the 15min incubation for the ribosomal system for protein synthesis *in vitro*. The rate of leucine incorporation into protein was

Table 1. *Distribution of proteins and RNA in liver cells*

Livers were from 51-day-old rats that had been fed on either a 6%-casein or an 18%-casein diet for 28 days. Animals from each dietary group were killed 3h after ingestion of a meal. The livers were fractionated by the procedure of Blobel & Potter (1967). Cell protein and RNA were estimated by using the assumption that binuclear cells have twice the cytoplasmic mass of mononuclear cells. Results are the mean±S.E.M. of six rats per group. Values in parentheses are the percentage change between 18%- and 6%-casein groups.

Fraction	Concn. of protein (pg/cell)		Concn. of RNA (pg/cell)	
	6% casein	18% casein	6% casein	18% casein
Whole cell	253±25 (−54)	550±28	25.3±2.8 (−44)	45.3±1.0
Nuclei	19.6±1.4 (−51)	39.9±3.6	2.72±0.18 (−35)	4.17±0.26
Bound ribosomes	24.1±3.6 (−56)	54.5±3.4	11.0±1.9 (−57)	25.6±1.2
Free ribosomes	10.6±1.2 (−29)	14.9±15	4.87±0.49 (−20)	6.12±0.33
Soluble	193±30 (−55)	425±28	6.63±0.68 (−24)	8.76±0.42

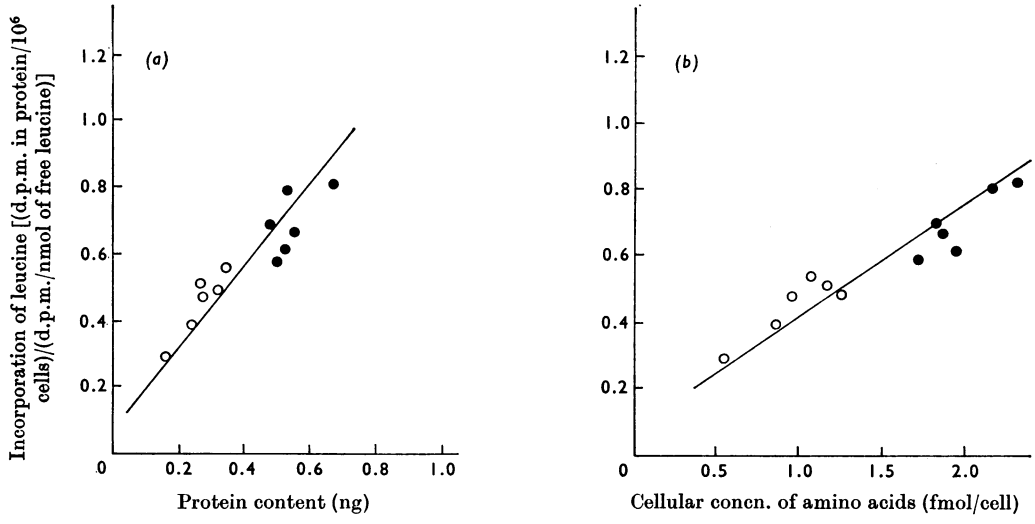

Fig. 1. Rate of incorporation of radioactive leucine into proteins of hepatic cells *in vivo* (*a*) (corrected for specific radioactivity of the precursor pool; Wannemacher, 1967) plotted against the protein content of a liver cell or (*b*) plotted against the sum of the cellular concentrations of the 11 free amino acids that Jefferson & Korner (1968) found essential for the maximal rate of protein synthesis in liver tissue. Rats were fed as described in Table 1. Each point is the result for one rat; ○, rats fed on a 6%-casein diet; ●, rats fed on the 18%-casein diet. The line was drawn from the slope calculated by regression analysis. A correlation coefficient (*r*) was calculated for the results: (*a*) 0.836; (*b*) 0.853.

0.89±0.06 (mean±S.E.M.) and 2.05±0.13nmol/h per mg of rRNA respectively for the hepatic ribosomes from the rats fed on 6%- and 18%-casein diets. This represented a 57% decrease in the rate of protein synthesis for the ribosomes from the protein-deprived rats as compared with controls. A 21±2% (mean±S.E.M.) and 54±4% (mean±S.E.M.) decrease in control values was noted for the percentage of polyribosomes and number of active ribosomes respectively (Table 2) in the liver of protein-deprived rats.

When the sum of the concentrations of these same 11 free amino acids was plotted against the protein-synthetic ability of the ribosomes *in vitro*, a highly significant correlation ($P<0.001$) was observed (Fig. 2*a*). The values for the livers of the animals fed on 6% casein were all less than those for the controls fed on 18% casein.

Effects of amino acid deprivation on RNA synthesis and catabolism. A highly significant ($P<0.001$) correlation was observed between the cellular concentration of the 11 free amino acids and the total rRNA content (Fig. 2*b*). The amino acid and RNA contents of the livers of the protein-deprived group were all less than those for the control group.

At short intervals (up to 3h) after injection of [^{3}H]uridine, the hepatic RNA from the rats fed on the 6% casein contained significantly more radioactive uridine than those from control rats. By 12h after injection of the uridine, there was no difference between the results for the two groups of animals (Fig. 3). The loss of radioactivity from hepatic RNA was similar for both groups of rats, with a calculated half-life of 100±9h (mean±S.E.M.).

When the cellular distribution of radioactive RNA was measured at various times after the injection of [^{3}H]uridine, the nuclear fraction contained most of the bound radioactive nucleotides at the early time-periods and the rRNA slowly became labelled, so that by 12h it contained most of the radioactivity (Fig. 4). In liver cells from the protein-deprived rats, the release of newly synthesized RNA from the nucleus to cytoplasm was much slower than in control animals (Fig. 4). Thus 12h after the injection of [^{3}H]uridine the rRNA fraction in the liver of rats fed on 6% casein contained 61%±5 (mean±S.E.M.) less radioactivity compared with controls.

The RNA polymerase activity of isolated liver nuclei from the rats fed on 6% casein was similar or slightly elevated when compared with rats fed on 18% casein (Table 3). Similar results were observed when the Mg^{2+}-dependent or Mn^{2+}-plus-high-salt system was used, irrespective of whether the results were expressed on a DNA or on a nuclear basis.

Table 2. *Effect of diet on the protein-synthetic activity and composition of liver ribosomes*

Ribosomes were prepared by centrifuging the post-mitochondrial supernatant through 1 M-sucrose as described in the Materials and Methods section. The protein-synthetic system *in vitro* contained in 1 ml: 40 μg of rRNA, 400 μg of cell-sap protein, 250 μmol of sucrose, 65 μmol of KCl, 10 μmol of $MgCl_2$, 50 μmol of tris–HCl buffer, pH 7.6 at 25°C, 5 μmol of ATP, 0.1 μmol of GTP, 19 L-amino acids naturally occurring (minus leucine) in the concentrations found in liver cells (Wannemacher *et al.* 1970), and 0.8 nmol of L-[U-^{14}C]leucine (0.2 μCi). For the determination of active ribosomes the reaction mixture contained, in 1.02 ml: 50 μmol of tris–HCl, pH 7.8, 80 μmol of KCl, 12.5 μmol of $MgCl_2$, 10 μmol of 2-mercaptoethanol, 5 μmol of ATP, 0.05 μmol of GTP, 1 μmol of phosphoenolpyruvate, 10 μg of pyruvate kinase, 0.0045 μmol of [^{3}H]puromycin (5 μCi) and 150 μg of rRNA. The mixture was incubated at 37°C for 30 min. The [^{3}H]peptidyl-puromycin was separated and its radioactivity counted by the Millipore-filter method of Wool & Kurihara (1967). Polyribosomes were separated on 10–40% sucrose gradients by the procedure of Yatvin & Lathrop (1966). Rats were fed as described in Table 1. Results are recorded as the means ± S.E.M. of six animals and percentage change from 18%-casein-fed controls (values in parentheses).

Diet	Rate of protein synthesis *in vitro* (d.p.m./min per μg of RNA)	Percentage of active ribosomes	Percentage of polyribosomes
6% Casein	9.0 ± 0.5 (−57)	10.9 ± 2.1 (−54)	37.0 ± 1.4 (−21)
18% Casein	20.7 ± 2.5	25.2 ± 2.3	46.6 ± 2.2

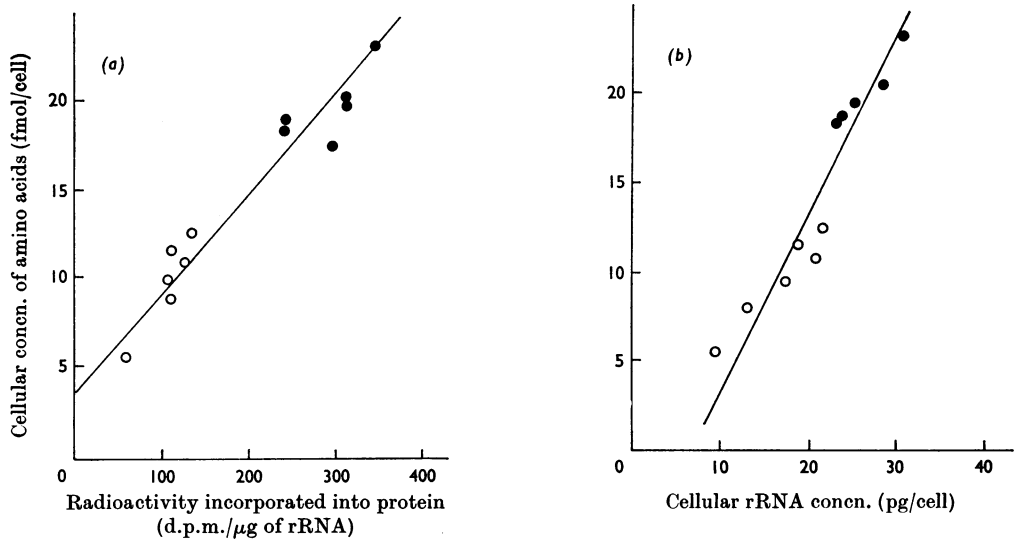

Fig. 2. Sum of the cellular concentrations of the 11 hepatic free amino acids that are essential for protein synthesis (Jefferson & Korner, 1969) plotted against (*a*) the radioactivity incorporated into the protein isolated from the 15 min incubation of the protein-synthetic system *in vitro* (see Table 2 for details), or (*b*) the cellular concentration of total rRNA (isolated by the procedure of Blobel & Potter, 1967). The symbols and statistical analysis are the same as described in Fig. 1(*a*). The correlation coefficients for results are: (*a*) 0.967; (*b*) 0.941.

DISCUSSION

Cellular distribution of protein and RNA. The results showing that there is a higher proportion of free ribosomes in the liver cells of rats fed on the protein-deficient diet are in agreement with previous observations (Mandel, Quirin, Bloch & Jacob, 1966; Wannemacher *et al.* 1968*a*).

Several investigators (Redman, 1969; Hicks, Drysdale & Munro, 1969; Ganoza & Williams, 1969) have suggested that free ribosomes synthesize mostly internal proteins of the hepatic cells, whereas

bound ribosomes synthesize mainly serum proteins (such as albumin and transferrin), which are secreted from the liver. Since the liver cells from the protein-deprived rats contained a larger proportion of free ribosomes, one could assume that the synthesis of certain serum proteins is markedly decreased in these animals. This hypothesis is supported by the observations *in vivo* in the dog (Wannemacher, Russell & Allison, 1963), rat (Freeman & Gordon, 1964), rabbits (Rothschild, Oratz, Mongelli & Schreiber, 1968) and man (Hoffenberg, Black & Brock, 1966) that nitrogen deficiency and malnutrition will depress the rate of albumin synthesis. Further, in the isolated perfused liver, albumin, fibrinogen, haptoglobin, α_1-acid glycoprotein and α_2-(acute phase)-globulin synthesis is extremely sensitive to the concentration of certain amino acids (Rothschild, Oratz, Mongelli, Fishman & Schreiber, 1969; John & Miller, 1969).

Relationship between free amino acid content and rate of protein synthesis. The amount of cellular protein in liver is proportional to the rate of protein synthesis *in vivo*, which in turn is correlated with the total cellular concentration of the group of free amino acids that are essential for

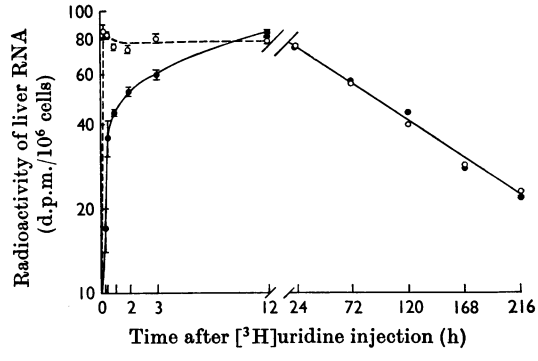

Fig. 3. Plot of logarithm of the radioactivity (d.p.m.) of the total cellular liver RNA at various times after a single intraperitoneal injection (100μCi/100g body wt.) of [5-^{3}H]uridine. The RNA was extracted by the procedure of Blobel & Potter (1968). Symbols are the same as in Fig. 1(*a*). Each point is the mean$\pm$S.E.M. of six animals. The mean values of 24–216h after injection of [5-^{3}H]uridine were essentially the same for both the 18%- and 6%-casein-fed rats, therefore their S.E.M. was not plotted.

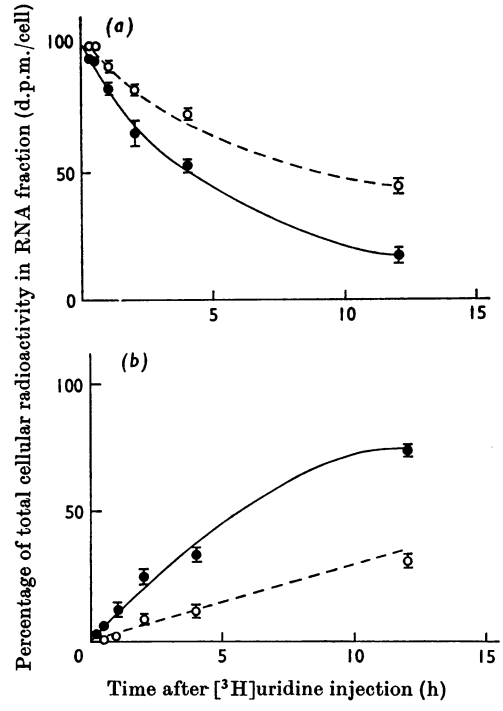

Fig. 4. Percentage of total cellular radioactivity in the nuclear and ribosomal RNA fractions at various times after the single intraperitoneal injection of [5-^{3}H]uridine. Nuclear (*a*) and rRNA (*b*) were isolated by the procedure of Blobel & Potter (1967). The symbols are the same as in Fig. 1(*a*). Each point is the mean$\pm$S.E.M. for six rats.

Table 3. *Effect of diet on RNA polymerase activity*

Nuclei were prepared by the method of Blobel & Potter (1966). The Mg^{2+}- and Mn^{2+}-dependent RNA polymerase activities were determined by procedures described in the Materials and Methods section. The rats were fed as described in Table 1. Results are recorded as the mean$\pm$S.E.M. of six animals and as percentage change from 18%-casein-fed controls (values in parentheses).

Diet	[³H]Uridine incorporated (pmol)			
	Mg^{2+}-stimulated		$Mn^{2+}+(NH_4)_2SO_4$-stimulated	
	(per μg of DNA)	(per 10^6 nuclei)	(per μg of DNA)	(per 10^6 nuclei)
6% Casein	0.167$\pm$0.009 (+49)	6.65$\pm$0.56 (+53)	0.365$\pm$0.039 (+28)	15.06$\pm$2.39 (+37)
18% Casein	0.112$\pm$0.011	4.36$\pm$0.12	0.286$\pm$0.059	11.00$\pm$1.58

protein synthesis (Fig. 1). A similar correlation between the rate of protein synthesis *in vivo* and free amino acid content has been observed in hypertrophic hearts (Wannemacher & McCoy, 1969) and in liver and skeletal muscle of both protein-depleted and hypophysectomized rats (Cooper, Muramatsu, Wannemacher & Leathem, 1968*b*; Cooper, Wannemacher & Raica, 1969). Studies both *in vivo* and *in vitro* have indicated that the removal of certain amino acids from the diet or incubation medium can markedly decrease the concentration of heavy polyribosomes and the rate of protein synthesis *in vitro* (Fleck, Shepherd & Munro, 1965; Wunner, Bell & Munro, 1966; Sidransky, Sarma, Bongiorno & Verney, 1968; Baliga *et al.* 1968; Hogan & Korner, 1968; Wannemacher *et al.* 1970). As reported previously (Wannemacher *et al.* 1968*a*), the rate of protein synthesis *in vitro* is significantly lower when the ribosomes from the liver of the protein-deprived group are compared with those from control animals. Since the rate of synthesis is linear over the 15min incubation period, the ribosomal preparation would appear to be the rate-limiting factor in the system *in vitro*.

There is a good correlation between the protein-synthetic ability of the individual ribosomal preparations and the cellular concentration of those essential free amino acids and rates of protein synthesis *in vitro* (Fig. 2*a*). These results are in agreement with the finding *in vitro* of a relationship between the presence of certain amino acids and maintenance of maximal rates of protein synthesis (Baliga *et al.* 1968). These authors suggest that amino acids stimulate formation of heavy polyribosomes and thus influence the protein-synthetic rate. The lower percentage of heavy polyribosomes in the livers of the protein-deprived rat could be accounted for by the decreased cellular concentration of free amino acids. Although the percentage of heavy polyribosomes is lower in the ribosomal preparations from the protein-deprived rats, the decrease in protein-synthetic rate could well be a function of the number of active ribosomes. Such an interpretation is supported by the work of Wool & Kurihara (1967), who observed that in diabetes the rate of protein synthesis in muscle *in vitro* is correlated with the number of active ribosomes but not with the percentage of polyribosomes. These investigators suggest that ribosomes can bind to mRNA and form polyribosomes even when they lack a factor necessary for translation, and that certain stimuli (such as hormones) can trigger the synthesis of 'translation factor(s)', which have a rapid rate of turnover and can activate ribosomes. Thus the cellular concentration of certain amino acids could regulate the release and/or synthesis of 'translation factors', which in turn activate ribosomes. This may be a more important regulator of protein synthesis than the effects on formation of heavy polyribosomes.

Synthesis and release of RNA. The cellular concentration of ribosomes is also closely correlated with the concentration of essential amino acids (Fig. 2*b*). Although the regulatory mechanism for the above phenomenon remains to be elucidated, the results presented in Fig. 3 indicate a more rapid initial synthesis of total RNA in the livers of the protein-deprived rats. Some of this difference may be related to the size of the nucleotide precursor pool (Wannemacher *et al.* 1968*a*), but the kinetics of the early RNA metabolism is different for the two dietary groups. In the control group the [5-^{3}H]-uridine is initially incorporated into RNA at a slower rate than in animals fed on the 6%-casein diet. In the former group, however, the specific radioactivity of RNA continues to increase and reaches a maximum 12h after the initial pulse dose of radioactive uridine. In contrast, the maximum specific radioactivity of the protein-deficient group is found 15min after the pulse dose and remains constant for the next 12h. In the control animals, the results on the rate of synthesis and accumulation of the radioactive nucleotide into hepatic RNA are in good agreement with previous studies (Blobel & Potter, 1968). By calculating the percentage distribution of radioactivity associated with the ribosomal and nuclear RNA fractions at various times after a pulse dose of labelled uridine, it is possible to estimate the relative accumulation of newly synthesized ribosomal units. These results (Fig. 4) show that the number of newly synthesized ribosomal subunits is less in the protein-deprived rats.

Several mechanisms could be postulated to explain the observed difference of hepatic RNA metabolism in the two dietary groups: (1) decreased synthetic rate of RNA; (2) enhanced catabolism of rRNA; (3) decreased appearance of newly synthesized RNA in cytoplasmic ribosomes. The first possibility is unlikely, since nuclear RNA polymerase activity in the liver of protein-deprived rats is slightly elevated in both our study and that of Shaw & Fillios (1968). Our values for the half-life of total hepatic RNA are in agreement with those reported (Gerber, Gerber & Altman, 1960; Erdos & Bessada, 1966; Blobel & Potter, 1968). Since half-life measurements of total cellular RNA mainly reflect changes in rRNA (Blobel & Potter, 1968), it is improbable that the lower concentration of rRNA in the liver of protein-deprived rats reflects an increased catabolic rate. In normal animals little of the rapidly labelled nuclear RNA is stabilized and subsequently able to enter the cytoplasm (Harris, 1964). A decreased RNA stabilization in the hepatic nuclei from protein-deprived rats, as compared with controls, could explain the slower

appearance and lower rRNA content of cytoplasm from these animals.

A similar effect of amino acids on the rate of appearance of newly synthesized ribosomal subunits into the cytoplasm of Landschutz cells has been observed by Shields & Korner (1970). These authors explain their results by suggesting that amino acid starvation exerts its effect through inhibition of the synthesis of proteins with a rapid rate of turnover which are required for ribosome synthesis. A similar mechanism may explain how the concentration of amino acids can regulate the rate of synthesis of ribosomes in liver cells.

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