The influence of the nutritive value of proteins on the level of protein synthesis in vitro in rat skeletal muscle

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1. The amino acid incorporating activity of skeletal muscle ribosomes was studied in rats under various nutritional conditions using labelled amino acids.

2. Ribosomes were obtained from rats that were given a protein-free diet for 5 d followed by a high-protein diet containing casein, gelatin or wheat gluten for 16·5 h and from others that were given one of these protein-containing diets or one containing protein from polished rice for 6 d.

3. The level of isotope incorporation relative to RNA was somewhat higher when the protein source given for 16·5 h was a good-quality protein such as casein than with gelatin or wheat gluten, but fine discrimination between proteins was not considered to be feasible with this system.

4. In the rats that were given the protein-containing diets for 6 d the differences were more pronounced and the amino acid incorporating activity was correlated with the biological value of the protein.

In previous work (von der Decken, 1967, 1968*a*, *b*, 1969*a*, *b*) it was demonstrated that the activity of mammalian ribosomes in incorporating amino acids into protein is sensitive to short-term protein starvation of the animals. Feeding rats with protein after the depletion period enhanced the incorporating activity. It was observed that not only the liver ribosomes, but also the ribosomes prepared from skeletal muscle, were affected by the diet (von der Decken & Omstedt, 1970). In liver the effect of a protein-free carbohydrate-rich diet in depressing protein synthesis is masked to a certain extent by a simultaneous increase in enzymes involved in liver gluconeogenesis (Kaplan & Pitot, 1970). On the other hand, in skeletal muscle the changes are less complex and the influence of protein malnutrition on the mechanism of polypeptide formation is better defined in its effect.

Whereas in earlier work a comparison was made between a protein-free diet and a diet rich in protein of high biological value (BV), the present investigation is concerned with the effect on protein synthesis of diets containing proteins of different BV. As a preliminary, the nutritional conditions have been established that give statistically significant differences in the specific activity of the incorporation of amino acids into protein after a reasonably short period of feeding with the test diets.

EXPERIMENTAL

Materials. Chemicals were of AR grade wherever possible. The sources were given in detail previously (von der Decken, 1968*a*, 1969*a*). In addition, L-[U-^{14}C]-phenylalanine (specific activity 477 mCi/mmol) and DL-[3-^{14}C]phenylalanine (specific activity 20 mCi/mmol) were obtained from The Radiochemical Centre, Amersham,

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Table 1. *Percentage composition of the experimental diets*

Ingredient	Protein diet	Protein-free diet
Protein	60*	0
Glucose	15	54
Sucrose	7	27
Maize oil	10	10
Salt mixture	5	5
Vitamin mixture	0·5	0·5
Cellulose powder	3	3

* Casein, gelatin, wheat gluten, or protein from polished rice.

Bucks., UK; L-[U-^{14}C]valine (specific activity 200 mCi/mmol) was obtained from New England Nuclear Corp., Boston, Mass., USA; polyuridylic acid from Miles Laboratories Inc., Kankakee, Ill., USA; Triton X-100 from Packard Instrument Co., Inc., Downers Groove, Ill., USA; Vitamin Diet Fortification Mixture, Salt Mixture R.H. and vitamin-free casein (pH 5·8) from Nutritional Biochemicals Corp., Cleveland, Ohio, USA; D-glucose anhydrous from Mallinckrodt Chemical Works, St Louis, Mo., USA; gelatin, wheat gluten and protein from polished rice from General Biochemicals, Chagrin Falls, Ohio, USA; cellulose powder from The Swedish Cellulose Powder and Woodflour Mills Ltd, Gothenburg, Sweden.

Animals and experimental treatments. Male rats about 35 d of age and weighing 100–130 g were used. Control rats were given a conventional stock diet. All animals were given water and food *ad lib.* The experimental rats received 50 kcal (0·209 MJ) of food every day at 16.00 hours. They were not fasted before decapitation at 08.30 hours the next day.

Expt 1. Three groups of rats were given a protein-free diet for 5 d and then one of three high-protein diets containing 60% casein, gelatin or wheat gluten for 16.5 h. (For the numbers of rats used, see Tables 2 and 3.)

Expt 2. Groups of animals were given high-protein diets for 6 d. Three of the diets were the same as those used in Expt 1 and the fourth contained 60% protein from polished rice. (For the number of rats used, see Table 4.)

The protein and protein-free diets were isocaloric; their compositions are given in Table 1.

Preparation of liver cell sap. The liver was placed into ice-cold liver medium (0·035 M-tris–HCl, pH 7·8 (measured at 25°), 0·1 M-KCl, 9 mmol-$MgCl_2$, 0·25 M-sucrose). Liver cell sap was prepared as described earlier (von der Decken, 1968*a*) and, in order to remove free amino acids, was passed through a column of Sephadex G-25 (von der Decken, 1968*a*) previously equilibrated with liver medium.

Preparation of ribosomes from skeletal muscle. The ribosomes were prepared according to Peters, Richardson, Small & White (1970) and von der Decken & Omstedt (1970) with several minor modifications. The following media were used. Medium A: sucrose 0·25 M, tris–HCl buffer, pH 7·8 (measured at 25°) 0·1 M, KCl 0·185 M, $MgCl_2$ 9 mmol. Medium B: sucrose 0·35 M, tris-HCl buffer, pH 7·8 (measured at 25°) 0·035 M, KCl 0·185 M, $MgCl_2$ 9 mmol. Medium C: sucrose 0·25 M, tris-HCl buffer,

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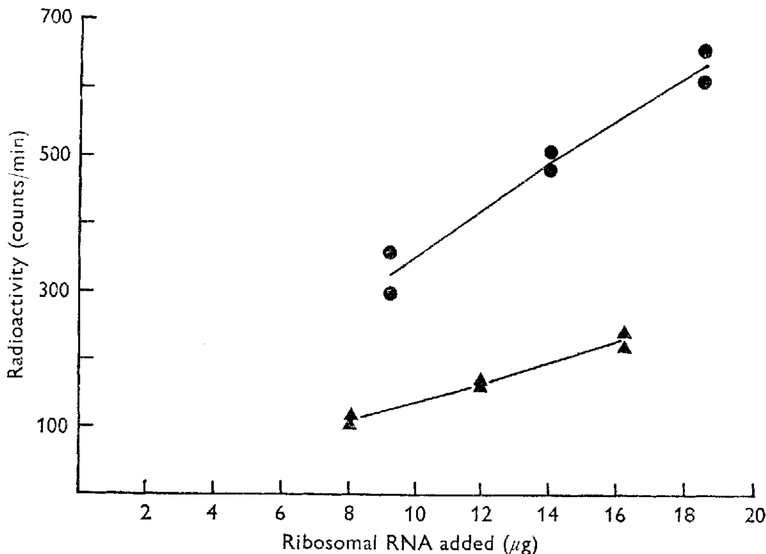

Fig. 1. Incorporation of [^{14}C]phenylalanine into protein by skeletal mucle ribosomes from rats. Conditions of evaluation are given on p. 470. Each ribosomal concentration was run in duplicate. ●, casein diet supplemented with 0·3 % L-methionine; ▲, gelatin diet. Both diets were given for 6 d.

pH 7·8 (measured at 25°) 0·035 M, KCl 0·1 M, $MgCl_2$ 9 mmol. The skeletal muscle (approximately 3 g) was minced and homogenized in medium A as described by Peters *et al.* (1970). The suspension was centrifuged for 10 min at 1000 g_{av}. The supernatant fraction was decanted off into a beaker with a magnetic stirrer and 10% Triton X-100 was added to give a final concentration of 1%. The suspension was centrifuged for 20 min at 15000 g_{av} and the supernatant fraction was layered in 6 ml portions over 5 ml medium B. The ribosomes obtained after centrifugation for 2 h at 165000 g_{av} were suspended in 1 ml of medium C per 3 g original weight of muscle. A Dounce homogenizer (Kontes, Glass Company, Vineland, New Jersey, USA) with pestle B was used.

Amino acid incorporation in vitro. The incubation mixture contained phosphoenolpyruvate 10 mmol, ATP 1 mmol, GTP 0·1 mmol, pyruvate kinase 40 μg/ml, amino acid mixture 12·5 μg/ml (von der Decken, 1968*a*), [^{14}C]amino acid (as indicated in Fig. 1 and the tables) 0·05 μCi of the L-form, $MgCl_2$ 7 mmol, KCl 0·08 M, tris-HCl buffer 0·035 M, sucrose 0·25 M, Sephadex-treated cell sap (0·5 mg protein) and varying amounts of ribosomes (8–25 μg RNA). The mixture of amino acids included the labelled one, the final volume being 130 μl. When the poly U dependent synthesis of polyphenylalanine was studied the system contained also 25 μg of poly U. The $MgCl_2$ concentration was increased to 10 mmol and 0·05 μCi of [^{14}C]phenylalanine of the specific activity given in Table 3 was added. After incubation for 12 min at 35°, 100 μl were transferred to filter-paper discs, extracted (Mans & Novelli, 1961) and counted in a Packard, model 314 EX, Tri-Carb Liquid Scintillation Photometer System at 40% efficiency.

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General aspects of the incubation system. To standardize the experimental conditions the following procedure was worked out. The rats were given the various diets, and ribosomes from their skeletal muscle were isolated. As a source of soluble enzymes, cell sap was prepared from the livers of control rats kept on a conventional stock diet. The activity of the ribosomes was measured by adding them in increasing amounts to the system. As shown in Fig. 1, there was a linear increase in radioactivity proportional to the amount of ribosomes added from the two types of animal. Three concentrations of ribosomes were each run in duplicate. As a control, an incubation without ribosomes was made. The resulting radioactivity, between 20 and 50 counts/min, was subtracted from the activity with ribosomes present. From the six incubations made with each rat, the mean radioactivity was expressed as counts/min per mg ribosomal RNA. The mean value obtained from each series of incubations was used for the comparison of the different proteins (see Tables 2–4).

Analytical methods. Protein was determined by the method of Lowry, Rosebrough, Farr & Randall (1951) with bovine serum albumin as a standard. RNA was extracted with 0·4 M-$HClO_4$, and the absorption at 260 nm was measured (Ogur & Rosen, 1950). The RNA concentration was calculated on the basis of the extinction coefficient of 34·2 mg^{-1} cm^2.

Evaluation of the results. The results of Expt 1 represent the mean values for the experiments which were run simultaneously under the described dietary conditions. The ribosomes of the casein-fed rats were compared on the same day with those of the gelatin-fed rats. In Expt 2 the mean value of the observations made with each rat are given.

RESULTS

Expt 1. Incorporating activity of the ribosomes after 16·5 h *on a high-protein diet.* As shown in Table 2, the ribosomes of the casein-fed rats had a higher incorporating activity than those from the gelatin-fed animals. This was so with both the [^{14}C]amino acids tested, although the differences obtained were more pronounced with [^{14}C]phenylalanine. The ratio in amino acid incorporating activity between ribosomes from casein- and gluten-fed animals was not more than 1·20. When the poly U-directed polyphenylalanine synthesis was studied, the difference in activity between the ribosomes from casein- and gelatin-fed rats was negligible (Table 3).

Expt 2. Incorporating activity after 6 *d on a diet containing either good-quality or poor-quality proteins.* A preliminary comparison of casein- and gelatin-fed rats gave a ratio in activity of about 2·4. This large difference suggested that smaller differences in BV might be detectable. Therefore, the amino acid incorporating activity was measured after feeding the rats with casein, protein from polished rice, wheat gluten or gelatin. The results are presented in Table 4. The results for rats which were fed simultaneously are shown in the same row in the table. The mean value of radioactivity for six incubations is shown for each rat. In all instances there were significant differences in activity between the pair of diets that were compared. In test no. 5 the incorporating activity of the casein-fed rat was somewhat higher; that of the gelatin-fed animal was within the range of the diet. The results were included, however, to indicate the variation in response to which biological systems may be subjected.

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Table 2. *Incorporation of [^{14}C]amino acid into protein by skeletal muscle ribosomes of rats starved of protein for 5 d followed by 16·5 h on a protein-rich diet containing casein or gelatin*

(Mean values with their standard errors)

	Radioactivity (counts/min per mg ribosomal RNA)		
[^{14}]Amino acid	Casein	Gelatin	Ratio, casein:gelatin
Phenylalanine	28750 ± 230	19200 ± 565	1·50
Valine	5900 ± 550	4430 ± 330	1·33

The incubation system was as described in Fig. 1, with 0·05 μCi of the labelled amino acid indicated. There were six rats in each group. They were divided equally and tested for incorporation with [^{14}C]phenylalanine and [^{14}C]valine.

Table 3. *Polyphenylalanine synthesis by skeletal muscle ribosomes of rats kept on a protein-free diet for 5 d followed by a protein-rich diet containing casein or gelatin for 16·5 h*

	Radioactivity (counts/min per mg ribosomal RNA)		
[^{14}C]Phenylalanine	Casein	Gelatin	Ratio, casein:gelatin
20 mCi/mmol	36200	36900	0·98
477 mCi/mmol	311000	287500	1·08

The incubation system was as shown in Fig. 1. Each observation is based on two animals.

Table 4. *Incorporation of [^{14}C]phenylalanine into protein by ribosomal cell sap from rats after 6 d on a diet containing protein of various biological values*

Test no.	Casein*	Rice†	Wheat gluten	Gelatin
1	41 830	—	—	27 020
2	34 500	—	25 900	—
3	34 150	—	25 230	—
4	34 280	—	20 220	14 120
5	53 300	—	—	17 200
6	—	—	21 680	10 850
7	—	26 300	—	16 920
8	—	24 830	—	16 720
9	—	—	23 100	9 090
Biological value‡	80–85	64	58	20–30

Levels of significance of comparisons between diets: casein *v.* gelatin (tests nos. 1, 4, 5) $P < 0·001$; casein *v.* wheat gluten (tests nos. 2–4) $P < 0·001$; rice *v.* gelatin (tests nos. 7, 8) $P < 0·001$; wheat gluten *v.* gelatin (tests nos. 4, 6, 9) $P < 0·001$.

The incubation system was as described in Fig. 1.

* Supplemented with 0·3% L-methionine.

† Protein from polished rice.

‡ Taken from FAO (1970).

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Table 5. *RNA: protein ratio (w/w) of ribosomal preparations obtained from rat skeletal muscle after 6 d on a diet containing proteins of various biological values*

(Mean values with their standard errors; numbers of independent observations are given in parentheses)

Casein	Wheat gluten	Gelatin
0·119 ± 0·002 (7)	0·099 ± 0·002 (7)	0·081 ± 0·002 (8)

Levels of significance of comparisons between diets: casein *v.* wheat gluten, $P < 0·001$; casein *v.* gelatin, $P < 0·001$; wheat gluten *v.* gelatin, $P < 0·001$.

RNA to protein ratio of the ribosomal preparations. It is known that the RNA content of liver changes with dietary conditions (Munro, 1968). The method of preparation of skeletal muscle ribosomes was such that they contained proteins other than those of ribosomal origin. This was reflected to some extent in the ratio of RNA to protein of the ribosomal preparations obtained after the various dietary treatments. When the ratios were compared after 6 d on a protein diet, the casein-fed rats had the highest RNA content relative to protein (Table 5), whereas the ribosomes of gluten-fed and gelatin-fed rats contained less RNA in proportion to protein. On the other hand, after 5 d of protein-free diet followed by 16·5 h of a protein diet the ratios were fairly constant and were between 0·088 and 0·094 for the different treatments.

DISCUSSION

In earlier experiments (von der Decken & Omstedt, 1970) rats were given one meal of high-quality protein after several days of protein starvation or they were kept throughout on the protein-free diet. A comparison was then made of the amino acid incorporating activity by skeletal muscle ribosomes. The ability to incorporate amino acids into protein increased considerably after one meal of high-quality protein. Waterlow & Stephen (1968) have shown that protein deprivation caused reduction in the rate of muscle protein synthesis as measured by continuous infusion of labelled lysine into the rat. It was thought that muscle protein synthesis may play an important part in the process of adaptation to low-protein intake.

In the present investigation the sensitivity of the protein synthetic activity of skeletal muscle ribosomes to dietary proteins of various BV was studied. When the proteins were given after 5 d of protein starvation there were measurable differences in ribosomal activities between high- and low-quality proteins. The increase in activity was more pronounced when phenylalanine rather than valine was used. It is known from in vivo studies (Waterlow, 1969) that different amino acids vary in the extent of their reutilization, and this may be reflected in in vitro systems. However, further work is needed to elucidate this question. It was obvious that under these conditions statistically significant differences between dietary proteins of more similar BV would be difficult to achieve.

A change from 16·5 h to 6 d of protein feeding increased the differences between high- and low-quality proteins and it was found that amino acid incorporation was

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sensitive also to proteins of intermediate BV. It seemed that the functional sites for polypeptide formation were not activated to the same extent by the various proteins.

The differences in protein synthesis were completely abolished when the poly U-directed polyphenylalanine synthesis rate was measured. Apparently the ribosomes from rats fed with poor- or good-quality proteins could utilize poly U to the same extent, indicating that the ribosomes themselves were not impaired by the dietary treatment. The effect on ribosomal activity in the absence of poly U might be an effect on initiation or termination of polypeptide formation. At the 10 mmol-$MgCl_2$ concentration which has to be used in a poly-U-dependent system, initiation is independent of ribosomal factors which otherwise catalyse this reaction (Shafritz, Prichard, Gilbert & Anderson, 1970).

It is known that in total starvation the ribosomal RNA in rat liver is degraded quickly and that there is a change in the rate of RNA turnover in relation to intake of protein (Enwonwu & Munro, 1970). Similar alterations may take place in skeletal muscle. The ribosomal preparations used here were obtained by treatment with Triton X-100 and showed a good amino acid incorporating activity, but the protein content of the preparations was high when the rats were given protein in their diet for only 16·5 h. It was less when a high-protein diet was given for 6 d. In fact, the difference in the ratio RNA: protein were statistically significant and showed a relation to the intake of protein.

The results presented in Table 4 show that there is a correlation between the BV of the protein and the amino acid incorporating activity of the skeletal muscle ribosomes. There was some variation between rats fed on a similar diet in the level of amino acid incorporation. Apart from the biological variation between the animals, this may have been due to the environmental and seasonal factors such as, for example, light. It was preferred, therefore, to keep the rats on the diet to be compared for the same period of time and to carry out the comparative experiment on the same day. The diets showed a significant influence on the differences in amino acid incorporation.

In summary, the results of our experiments support the view that the amino acid incorporating activity of skeletal muscle ribosomes is dependent on the BV of the dietary protein fed to the animals for the previous 6 d.

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