Protein Synthesis during Right-Ventricular Hypertrophy after Pulmonary-Artery Stenosis in the Dog

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(Received 7 January 1977)

The rate of protein synthesis in the heart of normal dogs and those with pulmonary-artery stenosis was measured by a continuous intravenous infusion of [^{14}C]tyrosine. The protein-synthesis rate of both ventricles was the same in normal dogs and averaged 7.5% per day. The right ventricle hypertrophied rapidly after the acute imposition of pulmonary-artery stenosis, the wet weight increasing by 84% after 24 days, with the rate of increase being most rapid over the first 5 days. The left ventricle remained largely unaffected and served as an internal control. During hypertrophy there was an increased incorporation of [^{14}C]tyrosine into protein without a significant change in the specific radioactivity of free tyrosine in the ventricles. After 5 days of stenosis the synthesis rate of the total mixed proteins of the right ventricle had increased to 13.6% per day, compared with 6.2% in the control left ventricle. This increase in synthesis was reflected in both the myofibrillar and sarcoplasmic proteins. After 5 days the protein-synthesis rate decreased, but still remained significantly elevated above that in the control left ventricle by 24 days of stenosis.

The protein content of a tissue is determined by the balance between the rates of protein synthesis and degradation. In response to pressure or volume overload the mass of the heart increases (Fanburg & Posner, 1968), and this hypertrophy may arise from changes in either protein synthesis or degradation. Amino acid-incorporation studies *in vivo* during hypertrophy of cardiac (Gudbjarnason *et al.*, 1964; Martin *et al.*, 1974; Morkin & Kimata, 1974) and skeletal muscle (Turner & Garlick, 1974; Goldberg, 1969) are compatible with an increased rate of protein synthesis. Morkin & Kimata (1974) report a half-life of 2–3 days for the synthesis rate of cardiac myosin in hypertrophying rat heart, compared with 4–6 days in control animals. Morgan & Rannels (1976) observed a 32% increase in the rate of protein synthesis, with no detectable change in the rate of protein degradation, in isolated perfused hearts from rats with aortic stenosis. In skeletal muscle, Turner & Garlick (1974) report a doubling in the synthesis rate of mixed muscle proteins during hypertrophy of the rat diaphragm 3 days after unilateral division of the phrenic nerve.

In the present study we have measured the rate of protein synthesis and increase in ventricular mass during hypertrophy of the right ventricle after stenosis of the pulmonary artery in dogs. The constant-infusion method of Garlick *et al.* (1973), with [^{14}C]tyrosine, has been used because, first, a steady state of labelling of the free amino acid pool in the tissue is maintained during the incorporation of tyrosine

into protein, and, secondly, reutilization of the labelled amino acid over the time period of the infusion is not significant (James *et al.*, 1975).

Methods

Animals

Mongrel dogs of both sexes with body weights ranging between 7 and 12 kg were studied. Body weights remained stable on equal proportions of goat meat and dry vegetable fibre (Canine Kibbles, Wesfeeds, Bentley, Western Australia, Australia), given each afternoon.

Pulmonary stenosis

Anaesthesia was induced with thiopentone sodium (Pentathal; Abbott Laboratories, Sydney, Australia; 5–10 mg/kg) and pentobarbitone sodium (Sagatal; May and Baker, Sydney, Australia; 15–25 mg/kg) given intravenously, and the anaesthesia was maintained with $\text{N}_2\text{O}/\text{O}_2$ (2:1). Pulmonary-artery stenosis was produced by the method of Hopkins *et al.* (1974). Through a midline sternotomy the pulmonary artery was isolated and a thick silk thread enclosed within polyethylene tubing was tightened around the artery until the right ventricle was maximally distended during diastole without ventricular arrhythmias occurring. Recovery from the operation was assessed by the dog's general appearance and the return of a

normal appetite 24–48 h later; animals that did not eat were rejected from the study. A group of sham-operated dogs underwent all aspects of the operational procedure except for placement of the band around the pulmonary artery. The right-ventricular pressure was measured by using a size 5F Swan Ganz catheter (Edwards Laboratories, Red Hill Ave., Santa Ana, CA, U.S.A.) inserted into the ventricle through an external jugular vein 24 h before the [^{14}C]tyrosine infusion. In eight animals right-ventricular systolic pressure increased as a result of stenosis to 76 ± 6 mmHg compared with 40 ± 5 mmHg in four sham-operated dogs.

The protein-synthesis rate was determined in both ventricles 5, 12 or 24 days after the stenosis or sham operation.

Continuous infusion of [^{14}C]tyrosine

The animals were last fed 16 h before, but allowed to drink freely up to, the start of the infusion. A polyethylene cannula (internal diam. 0.8 mm) was inserted into a forelimb vein, taped to the limb and connected to a Braun continuous-infusion pump (B. Braun Apparatebau, Melsungen, W. Germany). L-[U- ^{14}C]Tyrosine (483 mCi/mmol) was diluted with 0.9% NaCl, containing heparin (50 units/ml), to a radioactivity of 1.67 $\mu\text{Ci/ml}$. Unlabelled carrier tyrosine was added to the infusate to give a concentration of 33 μM , which was the average concentration of tyrosine in dog plasma. The solution was infused at a rate of 5 ml/h for 6 h. The animals were fully conscious and completely unrestrained during the infusion, and in general rested quietly. After 6 h the dog was killed rapidly by an intravenous injection of pentobarbitone sodium (326 mg/ml).

Tissue treatment

After death, the heart was rapidly excised and rinsed in an ice-cold modified Krebs solution (Lulich *et al.*, 1976) for several seconds to remove entrapped blood. The heart was then placed on ice and subsequent procedures were performed in a cold-room at 4°C. The ventricles were isolated, the right-ventricular free wall was dissected from the left ventricle (the septum remaining with the left ventricle) and each was weighed. Five samples (each approx. 100 mg) were taken from each ventricle and dried overnight at 120°C for tissue-water determination. Samples (5 g) from each ventricle were homogenized in ice-cold 10% (w/v) trichloroacetic acid with a no. 25 Kontes Duall glass homogenizer (Kontes Glass Co., Vineland, NJ, U.S.A.). The homogenate was then centrifuged at 1000g for 10 min at 2°C and the supernatant was assayed for its free tyrosine specific

radioactivity (Garlick & Marshall, 1972). The mixed protein precipitate was washed another three times in 25 ml of 10% trichloroacetic acid, followed by single washes of acetone, ethanol and then diethyl ether.

For isolation of the myofibrillar and sarcoplasmic protein fractions, a further 3 g of tissue from both ventricles was homogenized with a Kontes Duall glass homogenizer in 20 mM-phosphate buffer (16 mM- Na_2HPO_4 /4 mM- KH_2PO_4), pH 7.3, and centrifuged at 8000g for 10 min at 2°C. The proteins from the supernatant were precipitated by the addition of an equal volume of 20% (w/v) trichloroacetic acid, washed three times with the same, and are referred to as the sarcoplasmic-protein fraction. The pellet remaining after homogenization in the phosphate buffer was washed once in the buffer and resuspended in 0.6 M-KCl/3 mM-MgATP/20 mM-phosphate buffer, pH 7.0, and agitated gently for 3 h at 4°C. After centrifugation at 8000g for 10 min at 2°C, the protein was precipitated from the supernatant by the addition of 10 vol. of 20 mM-phosphate buffer, pH 7.0, and is referred to as the myofibrillar-protein fraction. The tyrosine specific radioactivity of this myofibrillar protein remained unchanged when two further cycles of redissolving in 0.6 M-KCl and precipitation by dilution were performed. Results of polyacrylamide-gel electrophoresis of this protein, after dissociation in 1% (w/v) sodium dodecyl sulphate/1% (w/v) mercaptoethanol at 37°C for 1 h, on 6% (w/v) polyacrylamide gel (Weber & Osborn, 1969), are shown as a densitometric scan (Fig. 1). There was no change in either the band pattern or the relative intensities of the bands after two additional dilution-precipitation cycles. The proteins were identified by electrophoresis of purified standards run concurrently with the myofibrillar protein fraction. Purified myosin was prepared by the method of Starr & Offer (1971), actin by the method of Rees & Young (1967), and tropomyosin was a gift from E. F. Woods (Division of Protein Chemistry, CSIRO, Parkville, Melbourne, Australia).

Samples of the total mixed muscle proteins, sarcoplasmic and myofibrillar proteins were hydrolysed in 6 M-HCl for 20 h at 110°C in sealed tubes, and the tyrosine specific radioactivity in each protein was determined by the method of Garlick & Marshall (1972). By this method bacterial L-tyrosine decarboxylase is used to convert tyrosine into tyramine and the latter is selectively extracted for the specific-radioactivity determination.

^{14}C radioactivity was determined with a Nuclear-Chicago Isocap-300 liquid-scintillation counter by using a scintillant containing 35% (w/v) Triton X-100 and 0.6% (w/v) 2,5-diphenyloxazole in toluene. Radioactivity of the samples was determined to give an s.d. of 2% or better, and the efficiency of counting was 89%.

Fig. 1. Densitometric tracing of myofibrillar protein from dog heart after polyacrylamide-gel electrophoresis. Myofibrillar protein was extracted from the heart with 0.6M-KCl/3 mM-MgATP/20 mM-phosphate buffer (16 mM- Na_2HPO_4 /4 mM- KH_2PO_4), pH 7.0, and polyacrylamide-gel electrophoresis was performed after dissociation of the protein in 1% (w/v) mercaptoethanol/1% (w/v) sodium dodecyl sulphate at 37°C for 1 h. The major proteins are myosin heavy chains (MH), actin (A), tropomyosin (TM) and the two light chains of myosin (LC1 and LC2).

Calculations

From the ratio of the specific radioactivities of the free (S_i) and protein-bound tyrosine (S_b) after the 6 h infusion, a fractional synthesis rate constant (k_s) was calculated by the method of Garlick *et al.* (1973), by using the equation:

$$\frac{S_b}{S_i} = \frac{R}{(R-1)} \cdot \frac{(1-e^{-k_s t})}{(1-e^{-Rk_s t})} - \frac{1}{(R-1)}$$

where R is the ratio of protein-bound to free tyrosine in the heart. The equation was solved graphically for k_s .

Statistical methods

Results are expressed as the means $\pm$ S.E.M. with the numbers of observations in parentheses. Statistical evaluation was performed by analysis of variance or by using the paired Student's t test. Exponentials were fitted by the method of least squares.

Reagents

L-Tyrosine decarboxylase (type 1 from *Streptococcus faecalis*) was obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.); ^{51}Cr -EDTA in sterile aqueous solution (0.5–1.5 mCi/mg of Cr) and L-[U- ^{14}C]tyrosine (483 mCi/mmol) were from The Radiochemical Centre (Amersham, Bucks., U.K.);

Fig. 2. Specific radioactivity of plasma tyrosine during a continuous intravenous infusion of [^{14}C]tyrosine into a dog. During a continuous intravenous infusion of [^{14}C]tyrosine, blood samples were taken from a long cannula inserted into the femoral vein via a superficial branch. The plasma was separated and the plasma proteins were precipitated with trichloroacetic acid. The trichloroacetic acid supernatant was assayed for tyrosine specific radioactivity (c.p.m./ μmol).

1-nitroso-2-naphthol (commercial grade) was from Ajax Chemicals (Auburn, N.S.W., Australia); tyrosine (Analytical Reagent) was from Calbiochem (Australia) Pty. (Carlingwood, N.S.W., Australia); Triton X-100 (purified) and 2,5-diphenyloxazole were obtained from Koch-Light Laboratories (Colnbrook, Bucks., U.K.); toluene (commercial grade) was from Shell Chemicals (Australia) Pty. (Perth, Western Australia, Australia); all other reagents were obtained from BDH Chemicals (Australia) Pty. (Collingwood, N.S.W., Australia) and were of the highest available purity.

Results

Specific radioactivity of tyrosine in plasma

The rapid rise to a plateau of the plasma tyrosine specific radioactivity during an infusion of one dog is illustrated in Fig. 2. In six dogs the average rate constant (λ_p) for rise to plateau of the plasma tyrosine was $72 \pm 17 \text{ days}^{-1}$, which was obtained by fitting the data to an exponential of the form $S_p = S_{p\text{max}} \times (1 - e^{-\lambda_p t})$, where S_p is the specific radioactivity of the plasma amino acid, $S_{p\text{max}}$ is the value of S_p when the plateau value has been reached, and t is the time. In some dogs fluctuations in the plateau value occurred, with one or two of the hourly points showing a variation of not more than 20% above or below the average specific radioactivity. Garlick *et al.* (1976) found it was necessary to feed pigs during infusions of [^{14}C]tyrosine to obtain a plateau of the amino acid

Fig. 3. Effect of pulmonary-artery stenosis on mass of left and right ventricles of the dog heart

The percentage increase in mass of the right (●) and left (○) ventricle after stenosis of the pulmonary artery was calculated from the ventricle-wet-weight/body-weight (g/kg) ratio. The bars denote $\pm$ S.E.M. The right-ventricle/body-weight ratio was 1.27 ± 0.07 (13) in normal animals and increased to 1.87 ± 0.19 (5) after 5 days' stenosis, 2.05 ± 0.09 (5) after 12 days' stenosis and was 2.33 ± 0.15 (5) after 24 days. The left-ventricle/body-weight ratio in normal animals was 3.91 ± 0.27 (8) and did not significantly increase during the 24 days of pulmonary stenosis. The body weight of the animals did not change over the period of stenosis.

in plasma. Feeding was not necessary, however, for the attainment of a plateau during infusions of [14 C]-tyrosine into dogs.

Right-ventricular hypertrophy

The right-ventricular/body-weight ratio was 1.87 ± 0.19 (5) g/kg after 5 days of pulmonary stenosis, 2.05 ± 0.09 (5) after 12 days and 2.33 ± 0.15 (5) after 24 days, compared with 1.27 ± 0.07 (13) g/kg in normal dogs, body weight remaining constant throughout the time. Right-ventricular weight increased therefore most rapidly during the first 5 days of stenosis, averaging 48% above normal by the end of that time ($P < 0.01$) and 84% above normal by 24 days (Fig. 3). Sham-operated dogs had a right-ventricular/body-weight ratio of 1.25 ± 0.10 (4) g/kg 5 days after the operation, and this ratio was not significantly different from normal. Left-ventricular mass averaged 14% above normal 24 days after stenosis, but this increase was not significant. The increase in total protein content of the right ventricle of dogs with stenosis paralleled the increase in mass. The water content of the myocardium increased significantly from 78.9 ± 0.4 (10)% in normal dogs to

Fig. 4. Fractional synthesis rates of mixed myocardial protein in the left and right ventricles of dogs after pulmonary-artery stenosis

Dogs were infused intravenously with [14 C]tyrosine for 6 h and the fractional synthesis rate was determined from the ratio of the specific radioactivity of tyrosine in the total mixed protein and in the free amino acid pool in the ventricle. The synthesis rate of the right ventricle (●) was significantly elevated above that in the control left ventricle (○) at days 5 and 12 ($P < 0.001$) and at day 24 ($P < 0.02$). The left-ventricle protein-synthesis rate did not change significantly from that of the normal group during the 24 days' stenosis. Each point is the mean $\pm$ S.E.M. of five observations for animals with stenosis and of six observations in the normal group (day 0).

80.1 ± 0.6 (5)% after 5 days' stenosis ($P < 0.01$), but had returned to normal by 12 days' stenosis.

Synthesis rate of mixed muscle proteins

In six normal dogs the synthesis rate of total mixed muscle proteins in the left and right ventricle were the same, 7.6 ± 0.7 (6)% and 7.5 ± 0.8 (6)% per day respectively. The synthesis rates were also the same in the ventricles from four sham-operated animals. The mean estimate for the synthesis rate obtained by pooling values from the left and right ventricles from normal dogs was 7.5 ± 0.5 (12)% per day, which corresponds to a half-life for the turnover of dog myocardium of 9.2 days. A marked increase in the protein-synthesis rate in the right ventricle occurred in animals with pulmonary stenosis (Fig. 4). After 5 days the protein-synthesis rate had more than doubled to 13.6 ± 0.7 (5)% per day ($P < 0.001$), compared with a rate of 6.2 ± 0.4 (5)% in the corresponding left ventricle. At 12 days the synthesis was still significantly elevated at 9.6 ± 0.1 (5)% per day ($P < 0.001$) and remained at this value until at least 24 days. This increase in synthesis rate was associated with an increase in the specific radioactivity of the protein-bound tyrosine (S_b), without a significant change in the specific radioactivity of the free tyrosine

Table 1. Fractional synthesis rates of sarcoplasmic and myofibrillar proteins from the left and right ventricles of dogs after pulmonary-artery stenosis

The fractional synthesis rate was calculated as in Fig. 4. Each value is the mean $\pm$ S.E.M. and the number of animals in each group is shown in parenthesis. The statistical significance of the difference between the left and right ventricles of each group is shown by * $P < 0.01$ and † $P < 0.001$.

	Fractional rate of protein synthesis (%/day)			
	Myofibrillar protein		Sarcoplasmic protein	
	Left ventricle	Right ventricle	Left ventricle	Right ventricle
5-day sham (4)	5.6 $\pm$ 0.4	6.2 $\pm$ 0.6	9.7 $\pm$ 0.4	10.6 $\pm$ 0.7
5-day stenosis (5)	4.2 $\pm$ 0.4	9.4 $\pm$ 1.5*	8.6 $\pm$ 0.6	14.7 $\pm$ 1.1†
12-day stenosis (5)	5.6 $\pm$ 0.3	7.7 $\pm$ 0.3†	8.8 $\pm$ 0.7	10.9 $\pm$ 0.5*
24-day stenosis (5)	6.8 $\pm$ 0.4	8.2 $\pm$ 0.7*	9.1 $\pm$ 0.5	11.2 $\pm$ 0.8†

(S_1) from normal. The protein-synthesis rate in the left ventricle remained unchanged throughout the 24 days' stenosis.

Myofibrillar and sarcoplasmic protein synthesis

The myofibrillar protein-synthesis rate in sham-operated dogs after 5 days was the same in both ventricles and averaged 5.9 ± 1.1 (8)% per day. The sarcoplasmic protein-synthesis rate in both ventricles of these animals was also the same and averaged 10.1 ± 0.4 (8)% per day, the synthesis rate of sarcoplasmic protein being significantly higher than that of myofibrillar protein ($P < 0.001$). The synthesis rate of both these protein fractions in the right ventricle was markedly increased by stenosis (Table 1). That of sarcoplasmic protein was 71% above that in the corresponding left ventricle at 5 days ($P < 0.001$) and remained significantly elevated to a lesser extent throughout the 24 days. Synthesis of myofibrillar protein was similarly stimulated.

Discussion

The fractional synthesis rate of 7.5%/day for mixed muscle proteins of the normal dog myocardium reported here is only slightly higher than the rate of 6.8%/day in pig myocardium (Garlick *et al.*, 1976), both these estimates being obtained with the amino acid tyrosine. During the continuous infusion of [^{14}C]tyrosine the specific radioactivity of the plasma rapidly reached a steady state or plateau (Fig. 2), and this is an advantage when measuring the rate of protein synthesis (Garlick *et al.*, 1973; James *et al.*, 1975). The synthesis rate of the sarcoplasmic protein fraction was almost double that of the myofibrillar protein fraction in the heart, and the rate for total mixed muscle proteins was intermediate. Halliday & McKeran (1975) have labelled human skeletal muscle (vastus lateralis) by a continuous

infusion of [^{15}N]lysine and found a synthesis rate for sarcoplasmic protein of 3.8%/day and for myofibrillar protein of 1.46%/day. Smaller differences, however, in the synthesis rates of these two fractions have been reported in rat skeletal muscle by Millward & Garlick (1972), but no difference was found by Sender & Garlick (1973) in a heart perfused *in vitro*.

Right-ventricle hypertrophy

The greatest increase in the protein-synthesis rate during hypertrophy was observed 5 days after pulmonary-artery stenosis, and thereafter the synthesis rate declined to a value which approached that in the control left ventricle by 24 days. Although the right ventricle hypertrophied in these animals, the left ventricle was largely unaffected and served as an internal control. Although this work shows that the average synthesis rate of the total mixed protein is greatest at day 5, it is likely that the maximum rate is attained at an earlier time. Skosey *et al.* (1972) pulse-labelled rats with [^{14}C]lysine at various time points after aortic stenosis and found that the incorporation into non-collagen protein and myosin 4 h after each pulse was maximal after 4 days of cardiac hypertrophy.

The elevated rate of protein synthesis at day 5 was found in both the sarcoplasmic and myofibrillar protein fractions of the right ventricle. The increase of 124% in the rate of myofibrillar protein synthesis in the right ventricle relative to the left at 5 days' stenosis may be slightly overestimated, however, because of a small but statistically insignificant decrease in the synthesis rate in the control left ventricle (Table 1). This may have been the result of a decrease in left-ventricular load early after the production of stenosis. The sarcoplasmic and myofibrillar protein-synthesis rate remained significantly elevated above that in the control left ventricle by an average of 24 and 30% respectively between

days 12 and 24. This result was consistent with the gradual increase in right-ventricular mass which occurred. The estimated myofibrillar protein-synthesis rate is a mean value for the constituent proteins, and a marked increase in synthesis of any one protein relative to the other would be masked. Fig. 1 shows that the myofibrillar protein fraction includes both the heavy and the light chains of myosin, actin and tropomyosin. Zak *et al.* (1971) have reported that the turnover of myosin, actin and tropomyosin from rat heart was similar, with half-lives ranging from 10.5 to 11.5 days. On re-evaluation of these data, however, Zak *et al.* (1976) have concluded that the synthesis of these proteins appears to be non-uniform, the rate of synthesis of myosin heavy chains being approx. 50% faster than for actin.

The precision with which a protein-synthesis rate can be measured is limited by the assumptions involved in the method of Garlick *et al.* (1973) and the accuracy with which the specific radioactivities of the intracellular free tyrosine (S_i) and the protein-bound tyrosine (S_b) can be measured in myocardial tissue. In heart, a source of error in determining the specific radioactivity of the mixed muscle proteins (S_b) could arise from plasma proteins retained in the vascular spaces of the myocardium and precipitated with the muscle proteins during homogenization in trichloroacetic acid. The contribution of plasma proteins to the total mixed protein of heart can be calculated so as to not exceed 1.1% on the basis of a vascular space of 5 ml/100g in dog heart (Rakusan, 1971). The specific radioactivity of the tyrosine in the plasma proteins is, on average, 2.3 times that for total mixed muscle proteins (A. W. Everett, R. R. Taylor & M. P. Sparrow, unpublished work); this contaminant would lead to an overestimate of the synthesis rate of not more than 3%. This minor source of error would not apply to the myofibrillar protein fraction from which plasma proteins are separated. The specific radioactivity of the tissue free tyrosine (S_i) is an average of the extracellular and intracellular free tyrosine in the myocardium. By using ^{51}Cr -EDTA we have estimated the extracellular space to be $17.6 \pm 0.5(4)\%$ in the right ventricle and $14.8 \pm 0.5(4)\%$ in the left ventricle. The specific radioactivity of the total free pool was $72.5 \pm 2.8(20)\%$ that of the plasma, and correction for extracellular tyrosine gives an increase of not more than 10% in the rate of protein synthesis. The use of the specific radioactivity of the intracellular free amino acid as the best estimate of the size of the precursor pool for protein synthesis has been discussed elsewhere (Martin *et al.*, 1974; Wettenthal & London, 1975). We have used the specific radioactivity of the total tissue free tyrosine pool in the above results instead of the specific radioactivity of the intracellular free pool because the corrected value for the protein-synthesis rate is increased by less than 10%.

The rapid increase in myocardial mass in response to the imposition of a pressure load has then been shown to be accompanied by a large increase in the synthesis rate of both myofibrillar and sarcoplasmic protein. We feel it is difficult to draw firm conclusions from our data about protein-degradation rates, but some comment is warranted. Between 5 and 24 days' stenosis the rate of increase of myocardial mass is nearly linear, being close to 1.9% of the control mass per day at the 12-day point (Fig. 1). But the mass by 12 days is 62% above the control mass, and the fractionate rate of increase in mass, and hence in protein, is only about 1.2% per day. The increase in the protein-synthesis rate at 12 days from 6.8%/day in the control left ventricle to 9.6%/day is more than enough to account for the increase in protein. It is certainly not necessary to postulate a decrease in protein degradation accompanying the hypertrophy, and the calculations suggest the possibility of a slight increase in the rate of degradation.

We thank Mr. P. Burrows of the Department of Surgery and Mrs. M. Gray for their technical assistance. This work was supported by grant G1051 from the National Heart Foundation of Australia.

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