sorbing material present in irradiated dilute aqueous solution of sugars.

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Relationship between Tannin Levels, Rat Growth, and Distribution of Proteins in Sorghum

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Three high tannin varieties of sorghum gave significantly lower growth responses in weanling rats when compared with three low tannin varieties. Whole kernel and hand-separated endosperms were fractionated by the Landry and Moureaux method to yield five soluble fractions. Nitrogen recoveries ranged from 83 to 96%. Distribution of proteins among the five fractions was distinctly different in the high tannin and the low tannin sorghums, especially in fractions I and V.

The discovery that the opaque-2 gene in corn improves protein quality (Mertz *et al.*, 1964) has stimulated considerable interest among breeders, agronomists, nutritionists, and biochemists in improvement of protein quality and quantity of other cereal grains. Sorghum grain ranks fifth in acreage of crops of the world and forms the basic food in many parts of Africa and Asia. In order to improve the quality of sorghum grain, it is desirable to separate the proteins of sorghum and study the individual fractions in detail. Published literature has been very limited because of the insoluble characteristics of sorghum grain proteins. The procedure that has been employed for the fractionation of sorghum proteins by other workers (Haikerwal and Mathieson, 1971; Jones and Beckwith, 1970; Skoch *et al.*, 1970; Virupaksha and Sastry, 1968) is based on the classical procedure of Osborne and Mendel (1914). Some of the problems that have been associated with this procedure have been reported by the above workers, such as low nitrogen recoveries (Skoch *et al.*, 1970), and the inability to work with the alcohol fractions (Jones and Beckwith, 1970) and with the glutelin fractions (Haikerwal and Mathieson, 1971) due to the gelling of these fractions. Thus, Osborne and Mendel's (1914) procedure and

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its modifications appear to be unsatisfactory in selectively extracting different classes of proteins of sorghum due to the insoluble nature of the proteins in the solvent systems employed.

We have employed the procedure of Landry and Moureaux (1970). This procedure solubilizes most of the nitrogen of sorghum and yields five different soluble fractions. In the Osborne and Mendel (1914) procedure, four protein fractions are obtained: albumins, globulins, prolamines, and glutelins. In the present procedure, in addition to extracting the albumins, globulins, and prolamines, three additional fractions are obtained in place of the one glutelin fraction of Osborne and Mendel (1914). We have fractionated the proteins of three high tannin and three low tannin sorghum samples and have compared the various fractions obtained. The possible influence of polyphenols (tannins) on the distribution and availability of sorghum proteins is discussed. A preliminary report has been published (Jambunathan *et al.*, 1972).

MATERIALS AND METHODS

Sample. Six inbred lines of sorghum [*Sorghum bicolor* (L.) Moench] grown at the Purdue Agronomy Farm were chosen for this study. The World Germ Plasm Collection numbers (IS numbers) and the Purdue numbers in parentheses for these samples were as follows: IS 0062 (925006), IS 2283 (925074), IS 2319 (0819), IS 3982 (025042), IS 6992 (025154), and IS 8165 (03701089). Three of the lines—IS 0062, IS 3982, and IS 2319—were low pigment varieties, yellow or light yellow and white chalky in color, respectively, and the other three lines—IS 2283, IS 6992, and IS 8165—were pigmented bird-resistant varieties, brown to red brown in color. In this paper only the IS numbers will be used to identify the sorghum samples.

The endosperm, germ, and pericarp (bran) fractions were separated by soaking the grains in cold water for 2 hr and dissecting them manually using a scalpel (Hubbard *et al.*, 1950). The separated fractions were air dried at room temperature to a constant weight and the percentage distributions of each of the fractions were calculated. This was not performed for samples 2319 and 8165. The endosperm portion and the whole kernels were ground in a Wiley mill and were defatted with *n*-butyl alcohol. They were then air dried and ground to a fine powder in a small ball mill. Nitrogen in the powder was determined by the standard micro-Kjeldahl procedure.

Rat Feeding Experiments. Six male weanling rats of the Wistar strain (40 to 50 g, 21 days old) were used to evaluate the growth response of each of the four sorghum samples (0062, 3982, 2283, and 6992). The rats were housed in individual aluminum cages and were supplied water and food *ad libitum*. The diet consisted of 95% ground sorghum, 4% minerals (Hawk Oser Salt Mixture No. 3, Nutritional Biochemicals), and 1% vitamin fortification mix (General Biochemicals). Weekly food intake and growth data were obtained for 28 days.

The sorghum samples 2319 and 8165 were each fed to ten male weanling rats for a period of 28 days. The diet was adjusted to provide 8.8% protein (based on micro-Kjeldahl value), 5% total fat, 5% mineral mixture (including ash contents), and 2% fiber. The remainder of the diet was cornstarch and 1% vitamin fortification mix.

Amino Acid Analysis. Approximately 25 mg of the sorghum kernel and endosperm samples was hydrolyzed with 100 ml of 6 *N* HCl under reflux for 24 hr. The acid was removed *in vacuo* using a rotary evaporator and the residue was dissolved in pH 2.2 buffer and made up to a known volume. Aliquots of the hydrolysate were applied to the column of a Beckman Model 121 automatic amino acid analyzer. A rapid accelerated method (2 hr) was employed for the separation of amino acids.

Tryptophan. Tryptophan was determined on the whole kernels by ion exchange chromatography after barium hydroxide hydrolysis (Slump and Schreuder, 1969).

Table I. Composition of Whole Grain Sorghums (Percent of Whole Kernel, Dry Basis)

Sample	Endosperm	Germ	Pericarp (bran)
IS 0062	85.3	10.9	4.3
IS 3982	86.0	9.4	4.3
IS 6992	81.7	9.5	8.7
IS 2283	83.6	10.3	6.5
Average	84.2	10.1	6.0

Fractionation of Sorghum Proteins. Two-gram portions of the powdered defatted kernels and endosperms were extracted with 20 ml of the following solvents successively, according to fractionation sequence D of Landry and Moureaux (1970). The duration of time (min) of extraction is indicated after each solvent system used. Fraction I: 0.5 *M* NaCl 60, 30, 30: H_2O 15, 15; Fraction II: 70% isopropyl alcohol (v/v) 30, 30, 30; Fraction III: 70% isopropyl alcohol (v/v) with 0.6% 2-mercaptoethanol (v/v) 30, 30; Fraction IV: borate buffer with NaCl (pH 10, 0.5 *M*) with 0.6% 2-mercaptoethanol (v/v) 60, 30, 15; Fraction V: borate buffer with NaCl (pH 10, 0.5 *M*) with 0.6% 2-mercaptoethanol and 0.5% sodium lauryl sulfate (w/v) 60, 30, 15. The extractions with sodium chloride (Fraction I) were performed at 4° and the remainder were performed at room temperature. The mixtures were stirred with a magnetic stirrer for the specified time, centrifuged, and the supernatants were pooled together for each fraction. Nitrogen in each fraction was determined by the micro-Kjeldahl method.

Tannin Determination. Tannins in grain sorghum and in the extracts were determined according to the method of Burns (1971). A standard curve was prepared with catechin (Sigma Chemical Co.) and the results obtained with the sorghum samples and the extracts were expressed in terms of catechin equivalents. Since this method does not measure the tannins *per se*, the term tannin in this paper refers to polyphenolic compounds.

RESULTS AND DISCUSSION

Table I gives the percent of endosperm, germ, and pericarp (bran) expressed as percent of whole kernel. Samples 2283 and 6992, which were pigmented bird-resistant varieties, contained a higher percentage of pericarp and lower percentage of endosperm when compared with the low pigmented varieties 0062 and 3982. Pericarp fraction of the high tannin sorghums probably includes the testa layer of the cells containing the tannin compounds. Table II gives the results of the rat feeding experiments. Rats which were fed the low pigment varieties 0062 and 3982 gained an average of 34.8 and 28.7 g per rat, respectively, and those fed the pigmented bird-resistant varieties 6992 and 2283 gained an average of 7.3 and −0.6 g per rat, respectively. Tannins were determined (Table II) on the whole kernels and the values suggest an inverse relation between catechin and growth response of sorghum. Since no attempt was made to feed the rats at equal protein levels, the lower protein contents of sorghums 6992 and 2283 (Table III) may have been responsible for the poor growth of the rats. The effect of tannin on the growth response of rats is clearly demonstrated if we look at the results obtained with sorghum samples 2319 and 8165. Since these two diets were prepared to contain equal amounts of protein, oil, minerals, and fiber, the poor growth response of rats fed on 8165 can be attributed to the high tannin content of the sorghum sample. It may be noted that the average feed intake of rats on sorghum 8165 (high tannin) is greater than that of rats on sorghum 2319 (low tannin). The protein efficiency ratios of 0.71 and 1.28 obtained with samples 8165 and 2319, respectively, were signifi-

Table II. Weight Gain of Rats Fed on a Sorghum Diet for a Period of 4 Weeks

	IS 0062	IS 3982	IS 6992	IS 2283	IS 8165[a]	IS 2319[a]
Total average gain/rat (g)	34.8	28.7	7.3	−0.6	11.6	19.6
Total average feed consumed/rat (g)	225.7	197.3	163	156	181	171.6
Total average protein consumed/rat (g)	24.0	23.6	14.3	11.4	15.9	15.1
Tannin % (catechin equivalents)	0.52	0.47	4.62	6.88	2.69	0.51
Protein efficiency ratio					0.71[b]	1.28[b]

[a] Isonitrogenous diet fed to ten rats (see text for details). [b] Significantly different from each other at the 1% level.

Table III. Amino Acid Composition of Grain Sorghums[a]

Amino acid	IS 0062	IS 3982	IS 6992	IS 2283	IS 8165	IS 2319	Average of 522 lines[b]
Lysine	2.8	2.5	2.6	2.3	2.3	2.5	2.14
Histidine	2.3	2.3	2.2	2.1	2.1	2.1	2.01
Arginine	4.5	4.0	4.5	3.6	4.1	4.1	3.59
Aspartic acid	7.7	6.7	7.2	7.0	7.3	7.5	7.83
Threonine	3.3	3.3	3.3	3.2	3.2	3.3	3.26
Serine	4.2	4.4	4.5	4.4	4.4	4.3	4.52
Glutamic acid	22.8	21.8	22.1	20.8	25.6	25.7	23.22
Proline	7.7	7.8	6.9	6.2	7.9	8.0	8.16
Glycine	3.3	3.4	3.6	3.4	3.0	3.1	3.07
Alanine	9.2	9.2	9.5	8.5	9.9	9.7	9.89
Cystine	1.3	1.5	1.3	1.5	1.0	1.0	0.92
Valine	5.1	5.2	5.4	4.8	5.1	5.5	5.35
Methionine	1.7	1.8	1.9	1.3	1.8	1.6	1.03
Isoleucine	4.0	4.0	4.3	3.8	4.2	4.3	4.08
Leucine	13.2	13.2	13.7	12.2	13.7	13.9	14.27
Tyrosine	4.3	4.5	4.7	4.4	4.4	4.9	4.50
Phenylalanine	5.1	5.0	5.4	4.9	5.3	5.5	5.19
Tryptophan	1.3	1.3	1.2	1.4		1.1	1.3[c]
Isoleucine/leucine	0.3	0.3	0.3	0.3	0.3	0.3	0.3
% protein	11.2	12.6	9.2	7.7	10.3	12.7	12.61
% tannin	0.52	0.47	4.62	6.88	2.69	0.51	

[a] Grams per 100 g of protein. [b] Data kindly supplied by R. C. Pickett and J. D. Axtell, Department of Agronomy, Purdue University. [c] Average for nine lines.

Table IV. Amino Acid Composition of Sorghum Endosperms[a]

Amino acid	IS 0062	IS 3982	IS 6992	IS 2283
Lysine	1.8	2.2	2.1	2.1
Histidine	2.4	2.7	2.1	2.5
Arginine	3.5	3.7	3.6	3.2
Aspartic acid	8.4	8.0	8.0	6.4
Threonine	3.5	3.7	3.4	3.2
Serine	4.9	5.0	4.7	4.6
Glutamic acid	27.5	26.4	26.0	22.9
Proline	8.8	9.3	8.4	7.9
Glycine	3.0	2.9	3.0	3.0
Alanine	11.1	10.8	10.8	9.5
Cystine	1.4	2.1	0.7	1.2
Valine	5.7	5.7	5.6	4.9
Methionine	1.2	1.5	1.4	1.6
Isoleucine	4.6	4.6	4.7	4.0
Leucine	17.1	16.3	16.4	14.2
Tyrosine	5.3	5.3	5.1	4.4
Phenylalanine	6.0	6.0	6.0	5.2
Isoleucine/leucine	0.3	0.3	0.3	0.3
% protein	10.0	8.6	9.5	7.2

[a] Grams per 100 g of protein.

cantly different from each other at the 1% level of correlation.

The last column of Table III gives the mean amino acid composition of 522 different lines of sorghum obtained from the World Sorghum Collection grown at Lafayette, Ind. The amino acid values reported here give the best averages thus far obtained for sorghum samples grown at one location. The values for cystine and methionine are probably too low, since no correction was made for losses due to oxidation during hydrolysis. The amino acid compositions of whole kernels of the six lines used in this study are also shown in Table III. When individual amino acid values for the whole kernels are compared, no major differences seem to exist. It may be noted that the tryptophan content of the whole kernel is more than 1% in all samples. These values suggest that sorghum grain is not deficient in tryptophan. When the amino acid values of the six lines (Table III) are compared with the average values reported for 522 lines, no major differences are noticeable.

Table IV gives the amino acid composition of sorghum endosperms. No obvious differences in amino acid values were observed among the four endosperm samples analyzed. When these values are compared with the values of whole kernels in Table III, it is clear that the endosperm contains lesser amounts of lysine, arginine, and glycine, and higher amounts of glutamic acid, proline, alanine, leucine, isoleucine, and phenylalanine, indicating the effect of germ and pericarp in the distribution pattern of amino acids. The endosperms of 3982 and 6992 were obtained from a different stock of the whole kernels.

Table V gives the percentage distribution of nitrogen in the five fractions obtained from the whole kernels and the endosperms. Fraction I represents the saline soluble proteins (albumins and globulins). Fraction II represents the alcohol-soluble proteins (kafirins). Fraction III, which represents the proteins soluble in isopropyl alcohol containing a reducing agent, should be similar to Fraction II in its solubility characteristics and amino acid composition.

Table V. Nitrogen Distribution in Whole Kernels (WK) and Endosperms[a] (E)

Fraction	IS 0062[b]		IS 3982[b]		IS 2319,[b] WK	IS 6992[c]		IS 2283[c]		IS 8165,[c] WK
	WK	E	WK	E		WK	E	WK	E	
I (saline)	16.8	8.0	15.4	6.6	16.1	5.6	2.8	4.1	2.9	8.5
II (isopropyl alcohol)	18.4	19.9	10.6	13.6	14.8	5.9	9.2	2.5	13.3	6.2
III (isopropyl alcohol + 2-mercaptoethanol)	18.9	35.1	18.2	29.7	14.9	11.5	27.0	13.8	28.1	7.6
IV (borate buffer + 2-mercaptoethanol)	6.1	6.4	6.4	9.2	4.4	15.0	10.6	17.3	11.2	8.1
V (borate buffer + 2-mercaptoethanol + sodium dodecyl sulfate)	29.9	26.5	34.2	24.0	40.5	54.8	45.0	49.3	35.7	57.3
Total Nitrogen Extracted	90.1	95.9	84.8	83.1	90.7	92.8	94.6	87.0	91.2	87.7

[a] Percent of soluble nitrogen. [b] Low tannin. [c] High tannin.

Table VI. Effect of Alkali Dehulling on the Distribution of Nitrogen in Whole Kernels[a]

Fraction	IS 6992	IS 6992, dehulled	IS 2283	IS 2283, dehulled
I (saline)	6.0	9.3	4.1	5.8
II (isopropyl alcohol)	3.8	9.8	2.5	5.2
III (isopropyl alcohol + 2-mercaptoethanol)	6.9	20.9	13.8	18.0
IV (borate buffer + 2-mercaptoethanol)	12.8	7.0	17.3	7.5
V (borate buffer + 2-mercaptoethanol + sodium dodecyl sulfate)	59.9	43.9	49.3	50.4
Total nitrogen extracted	89.4	90.9	87.0	86.9
% protein	9.9	10.4	7.9	7.9

[a] Percent of soluble nitrogen.

Fraction IV represents a new class of proteins hitherto unreported for sorghum and Fraction V represents the glutelin fraction. Landry and Moureaux (1970) denoted the Fractions III, IV, and V obtained from maize as alcohol-soluble glutelins, saline-soluble glutelins and zeanines, respectively. The percentage of soluble nitrogen in the first three fractions of the low tannin samples (0062, 3982, and 2319) is higher than in the high tannin samples (6992, 2283, and 8165), whereas the reverse is observed with Fractions IV and V. Though the distribution patterns are similar in both the whole kernel and endosperm fractions, the differences are less marked in the endosperm fractions. In particular, the main difference in the distribution of nitrogen between the high tannin and low tannin samples occurs in Fractions I and V. In the whole kernel, the nitrogen in Fraction I of 0062, 3982, and 2319 represents 16.8, 15.4, and 16.1% of the total, respectively, while in samples 2283, 6992, and 8165, this fraction accounts for only 4.1, 5.6, and 8.5% of the total nitrogen, respectively. On the other hand, while Fraction V of 0062, 3982, and 2319 accounts for 29.9, 34.2, and 40.5% of the total nitrogen, respectively, the identical fractions in the high tannin samples 6992, 2283, and 8165 represent 54.8, 49.3, and 57.3% of soluble nitrogen, respectively. Similar distribution patterns for Fraction I and V of endosperm can also be seen from Table V. The nitrogen recoveries obtained from the whole kernels were from 87 to 92% and from the endosperm were 91 to 96%, except in the case of 3982, where the nitrogen recoveries were 85 and 83%, respectively.

The color of the extracts obtained from the whole kernels and endosperm showed varied patterns, with the whole kernel fractions exhibiting deeper color than the endosperm fractions. This is probably due to the absence of the testa layer in the latter. The first fractions of the whole kernels were colorless or had a yellow tinge, the second fractions of 6992 and 2283 were yellow in color, and those of 0062 and 3982 were either colorless or very pale yellow in color. The third fractions of the whole kernels were colorless. The major difference, however, occurred in the fourth and fifth fractions. Fractions IV and V of 0062 and 3982 were yellow and pale yellow in color, respectively; the fourth and fifth fractions of 2283 and 6992 were deep red and reddish brown in color, respectively. When tannin determinations were made on these extracts, the big difference, as expected, was shown in the values obtained from Fractions IV and V. Fraction IV of the bird-resistant pigmented varieties contained five times more catechin equivalents than the corresponding fractions of the low pigmented varieties, and Fraction V of the pigmented varieties contained twice the catechin equivalents of Fraction V from low pigmented varieties.

In the case of endosperm fractions, the first three fractions of all four samples were colorless. Fractions IV and V of sample 0062 and 3982 were colorless and light yellow in color, respectively, and Fractions IV and V of samples 6992 and 2283 were red and yellow, respectively.

Since the distribution of proteins in various fractions was distinctly different between the high tannin and low tannin varieties, it was tentatively assumed that the pigments and tannins may be the cause for this uneven distribution of proteins. This idea was further strengthened by the fact that the first fraction in the pigmented samples was very low in protein content, although one would expect it to be reasonably high because the first fraction represents the albumins and globulins, which are indispensible for the germination and sustenance of the plant. Also, Fraction V, one of the glutelin fractions, is higher in the pigmented sample. In order to test the assumption that the pigments and tannins may be bound to the proteins, causing this uneven distribution, we used hot 20% sodium hydroxide for removal of the pericarp and testa layer containing the pigments, according to the method of Blessin *et al.* (1971). Sorghum samples 6992 and 2283 were treated according to the procedure, and the resulting kernels obtained after the treatment were white in color.

Very low catechin values were obtained when the dehulled samples were used for tannin determination. Results of fractionation studies carried out on the dehulled samples are compared with those obtained with the pigmented samples in Table VI. Sample 6992 for this study was obtained from a new lot, and the differences in the distribution of protein patterns obtained with this sample may be due to the environmental factors. However, the standard error for this sample ranged from 0.4 for the first fraction to 1.2 for the fifth fraction. When the results of this experiment are compared with those in Table V, we find that the protein patterns obtained from the dehulled samples appear to have redistributed for the better and approach the distributions obtained with the low tannin sorghums. One obvious similarity is that the percentage of nitrogen is higher in the first three fractions and lower in the last two fractions in the sodium hydroxide-treated kernels as compared to the untreated kernel fractions. Fraction V of sample 2283 does not show any difference between the untreated and sodium hydroxide-treated samples. Also, the sodium hydroxide treatment completely removed the color of Fractions IV and V. The same fractions obtained from untreated kernels are deep red and reddish brown, respectively. Preliminary results obtained by feeding rats a high tannin sorghum dehulled with sodium hydroxide indicate that the dehulling improves its nutritional quality (Axtell *et al.*, 1973).

Whole kernel protein fractionation studies have been reported by Skoch *et al.* (1970) and by Haikerwal and Mathieson (1971). Skoch *et al.* (1970) modified the Osborne and Mendel (1914) procedure by extracting the prolamines with 80% ethanol containing sodium acetate. They used 5% NaCl for the extraction of globulins. Their reported nitrogen recoveries obtained in five sorghum samples varied from 26.4 to 40%. Haikerwal and Mathieson (1971) used the Osborne and Mendel (1914) procedure with no major modifications, except the use of hot ethanol for the extraction of prolamines. They obtained two fractions of glutelins, one extracted with 0.1% NaOH and the other extracted with 0.2% NaOH. However, the second glutelin fraction obtained with 0.2% NaOH and containing 30% nitrogen was gelled by these extraction procedures and in effect they could only solubilize 70% of the nitrogen in sorghum. Skoch *et al.* (1970) reported 5.2 to 10.7% of albumins and globulins in their sample, while in the present study only Fraction I of the pigmented high tannin sample falls within this range. Haikerwal and Mathieson (1971), using one particular sample from Nigeria, reported 28% of the soluble nitrogen in their saline soluble fraction. We have not observed this high percentage of soluble nitrogen in the first fraction of any sample fractionated so far.

Jones and Beckwith (1970) and Virupaksha and Sastry (1968) have reported fractionation studies on sorghum endosperm proteins using the Osborne and Mendel (1914) procedure. Virupaksha and Sastry (1968) reported a range of 2.8 to 14.5% for albumin and globulin fractions in their samples, while we observed a range of 2.8 to 8% for albumin and globulin fractions in our present study. Both Jones and Beckwith (1970) and Virupaksha and Sastry (1968) used a mill to separate the endosperm fractions and they reported contamination of germ with the endosperm fractions. Also, it is not clear from the work of Virupaksha and Sastry (1968) whether their protein recoveries were based on successive extractions. When Fractions II and III obtained from sorghum endosperms are added (see Table V), they represent 36.2 to 55% of the proteins, which is in the range of the values reported for prolamines by these workers. Jones and Beckwith (1970) reported that their alcohol fractions were deep red in color and that they formed a firm gel on standing. We find no such phenomenon in our alcohol fractions and the deep red color appears only in the fourth fraction of pigmented sorghum samples. Recently, Beckwith (1972) reported the isolation of glutelin of sorghum using a modified procedure.

When the values reported in Table V are examined, it is clear that both in the case of kernel and endosperm protein distributions, the sum of Fractions II and III is higher in the low tannin samples when compared with the high tannin samples, and the sum of Fractions IV and V is lower in the former compared with the latter samples. These differences in distribution point out that there is some factor(s) in the high tannin sorghum samples which binds to the protein, causing this uneven distribution, and from the work of Jennings *et al.* (1968) and Loomis and Battaile (1966), we can conclude that they are almost certainly protein–tannin complexes. Strumeyer and Malin (1969) reported the isolation of an amylase inhibitor from the germs of *Leoti* red sorghum, which was capable of inactivating a variety of enzymes. They attributed the properties to a series of oligomeric condensed tannins rather than to a single specific enzyme antagonist. Since we observed the uneven distribution of proteins in the endosperm fractions also, there could be a similar protein–tannin complex existing in the endosperm, causing the uneven distribution on fractionation.

Very little work has been done in the area of pigments and tannins of sorghum, especially with regard to their effect on the biological availability of proteins and on the toxic effect, if any, that may be associated with their presence. Further study of the proteins obtained in Fractions IV and V in high tannin sorghums may yield a clue as to the nature of the inhibitor(s).

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