

## THE HISTIDINE CONTENT OF MEAT

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During the investigation of the amino acid composition of meat in the authors' laboratory, it was found that samples of the same kind of meat taken from different individual animals always had very nearly the same content of arginine, lysine, tryptophan, methionine, threonine, phenylalanine, valine, leucine, and isoleucine. It was also found that the protein in a given type of tissue had practically the same content of these amino acids, whether it came from beef, pork, or lamb. This latter finding suggests that the composition of the protein tissue structure is essentially the same in the three species of animals. Such a concept is in agreement with the findings of Beach, Munks, and Robinson (1). The similarity in the amino acid composition of animal muscle tissues has also been noted by Block and Bolling (2).

In the initial phases of the present investigation, it became apparent that there was a very significant difference in the histidine content of beef and lamb muscle tissue. Likewise, there was much less uniformity in the histidine content of different samples of the same kind of muscle tissue than there had been in the case of the amino acids previously studied. Findings such as these might be expected if a substantial part of the histidine content of muscle tissue were in some form other than protein.

At least four chemical compounds related to histidine have been found in muscle tissue. Ergothioneine and histamine are present in too small quantities to be of significance in relation to this problem. Carnosine and anserine are present in larger quantities. The relationship of these two compounds to the determination of the total histidine content of meat constitutes a part of the present investigation.

A microbiological method for the determination of histidine in meat and in other food materials is presented herewith; *Streptococcus faecalis* R is used as the test organism.

### EXPERIMENTAL

*Preparation and Hydrolysis of Meat Samples*—The dehydrated and defatted samples previously prepared for studies on the methionine content

of meat (3) were used. The ratio of the dried solids to the fresh meat was known from nitrogen determinations on the fresh and dehydrated materials. In all cases where the extracted fat contained as much as 1 per cent of the total nitrogen, this was taken into consideration in calculating the equivalence values.

Except where otherwise stated, hydrolysis of the dehydrated samples was effected by refluxing 0.5 gm. of material with 100 ml. of 6 N hydrochloric acid for 24 hours. Most of the hydrochloric acid was distilled off at reduced pressure and the solution neutralized with sodium hydroxide. Histidine determinations were also carried out on hydrolysates prepared in the same way directly from fresh meat, 2.5 to 3.0 gm. samples of finely ground meat being taken. In all cases, obvious fat and gristle were removed.

When the two procedures were applied to several samples of meat, the difference in the histidine values was well within the experimental error of the microbiological assay method.

*Determination of Histidine with Streptococcus faecalis R*—The medium for the determination of histidine with *S. faecalis R* is given in Table I. The composition of the salt solutions is as follows: Salts 1, 25 gm. of  $K_2HPO_4$ , 25 gm. of  $KH_2PO_4$ , 250 ml. of water; Salts 2, 10 gm. of  $MgSO_4 \cdot 7H_2O$ , 0.5 gm. of NaCl, 0.5 gm. of  $MnSO_4 \cdot 4H_2O$ , 250 ml. of water; Salts 3, 0.5 gm. of  $FeSO_4 \cdot 7H_2O$ , 250 ml. of water. The composition of the medium as indicated was adopted after critical comparison of variously modified media. Consistently good results were obtained when this medium was used for both histidine and threonine determinations with *S. faecalis R*. (For the determination of threonine, the threonine in the medium is replaced by an equal weight of histidine.)

The use of sodium succinate as a buffer in place of sodium acetate results in greater acid production by this organism, probably because succinate buffers the medium in a range nearer to neutrality. It has been shown by Guirard, Snell, and Williams (4) that acetate is an important nutrient for a number of lactic acid bacteria; for this reason, acetate should always be included in the medium.

Stock cultures of the organism are carried on the tryptone, peptone, tomato juice, and agar previously described (5). Solid stabs are made at monthly intervals. Weekly transfers are made from the solid medium to a liquid medium of the same composition, and serial transfers are made daily throughout the week on the liquid medium for the preparation of inocula. Cultures for inocula are incubated 18 hours and then refrigerated a few hours until used. For the preparation of inocula, the bacteria are separated from the liquid culture by centrifugation, washed twice with sterile saline, and finally diluted with sterile saline until turbidity is just

perceptible. 1 drop of the very dilute suspension is used to inoculate each assay tube.

The assay is carried out as follows: Graded amounts of pure L-histidine are added to a series of 18 mm. tubes which are to be used as standards. The range of the standards is from 0 to 50  $\gamma$  of histidine at 5  $\gamma$  intervals. Assay tubes are similarly prepared by the addition of graded amounts of neutral hydrolysates. Each assay is carried out at five different test

TABLE I  
*Medium for Determination of Histidine with Streptococcus faecalis R\**

	gm.		mg.
Glucose	40	DL-Alanine	400
Succinic acid	20	L-Arginine	400
Sodium acetate (anhydrous)	6	DL-Aspartic acid	800
	mg.	L-Cystine	400
Adenine sulfate	10	L-Glutamic acid	400
Guanine	10	Glycine	400
Uracil	10	DL-Isoleucine	400
Xanthine	10	DL-Leucine	400
Riboflavin	1	L-Lysine	400
Niacin	2	DL-Methionine	400
Pyridoxamine	0.8	DL-Phenylalanine	400
Thiamine chloride	0.4	L-Proline	400
Calcium pantothenate	0.4	DL-Serine	400
	$\gamma$	DL-Threonine	400
Biotin	2	DL-Tryptophan	400
p-Aminobenzoic acid	2	DL-Tyrosine	400
Folic acid (synthetic)	10	DL-Valine	400
	ml.		
Salts 1	10		
" 2	10		
" 3	10		

Add 12 gm. of NaOH pellets and finish neutralizing with NaOH solution, dilute to 1 liter

\* Medium for 200 cultures of 10 ml. final volume (5 ml. of above medium per culture).

levels. The standard tubes as well as the assay tubes are all prepared in duplicate. 5 ml. of the medium are added to each tube followed by water to make a total volume of 10 ml. The contents of the tubes are then mixed by shaking. Unless this is done before the tubes are autoclaved, irregularities in the results are likely to occur. The tubes are covered with aluminum caps, sterilized at 15 pounds pressure for 10 minutes, cooled, inoculated, and incubated in a constant temperature water bath at 35° for 72 hours. After incubation, the bacteria are centrifuged and 5 ml. aliquots

are titrated with 0.1 N sodium hydroxide. A typical standard curve is shown in Fig. 1.

*Determination of Histidine by Other Methods*—The medium used for the determination of histidine with *Leuconostoc mesenteroides* P-60 was the same as that described by Dunn *et al.* (6), except that norvaline, norleucine, and hydroxyproline were omitted. The general procedure for conducting the tests and for handling the bacteria was the same as that described in the present paper for the determination of histidine with *Streptococcus faecalis* R, with the exception that the incubation period was 4 days.

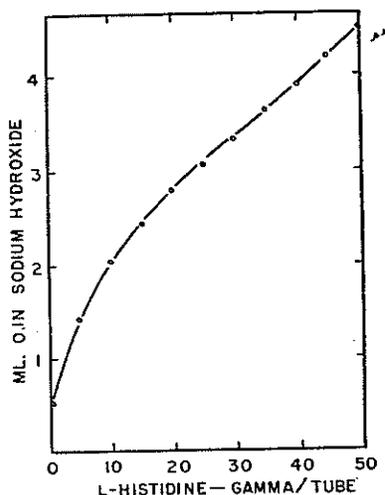


FIG. 1. Standard curve for the determination of histidine with *Streptococcus faecalis* R. Titration values are for 5 ml. aliquots from 10 ml. cultures.

Histidine was determined by the use of *Lactobacillus fermenti* 36, according to the procedure of Dunn, Shankman, and Camien (7).

Histidine was determined chemically (Pauly reaction) as described by Macpherson (8), save that the sulfanilic acid was dissolved in 5 per cent, instead of 10 per cent, hydrochloric acid. Twice the recommended amount of the sulfanilic acid solution was used in each test and, accordingly, double the amount of sulfanilic acid was employed, although the total amount of hydrochloric acid was the same as that used by Macpherson. Until this modification of the procedure was made, the values obtained for the histidine content of the copper anserine preparations did not agree at different test levels.

*Fractionation of Meat Samples*—Fresh meat was finely ground by the use of the Latapie grinder. Samples were taken for nitrogen determination

and for direct hydrolysis. A weighed portion (approximately 100 gm.) was stirred mechanically with 2 volumes of water and the protein was precipitated by the addition of 4 volumes of acetone. The precipitate was removed by centrifuging, dried *in vacuo* at 80°, and ground in a hammer mill. The supernatant liquid was filtered, concentrated at reduced pressure to remove acetone, adjusted to pH 4 with hydrochloric acid, and extracted with Skellysolve B. The aqueous phase was concentrated to dryness *in vacuo* and the residue was taken up in water, neutralized, and diluted to a suitable volume. Aliquots were taken for nitrogen determination, direct analysis, and hydrolysis.

*Preparation of Anserine*—Copper anserine was prepared from rabbit muscle tissue, as described by Schenk *et al.* (9).

TABLE II  
*Effect of Conditions of Hydrolysis on Liberation of Histidine from Meat*

	Time of hydrolysis	L-Histidine found in crude protein*
	hrs.	per cent
Refluxed with 6 N HCl	4	3.87
“ “ 6 “ “	8	3.87
“ “ 6 “ “	12	3.88
“ “ 6 “ “	16	3.90
“ “ 6 “ “	24	4.00
Autoclaved at 15 lbs., 3 N HCl	2	3.89
“ “ 15 “ , 3 “ “	4	4.03
“ “ 15 “ , 3 “ “	6	4.00

\* This is equivalent to calculating to 16 per cent protein.

### Results

Unless otherwise indicated, the data were obtained by the use of the histidine method described above in which *Streptococcus faecalis* R is the test organism.

The liberation of histidine from meat samples by hydrolysis with hydrochloric acid under various conditions was studied as a preliminary to this investigation. The data in Table II show that histidine is very readily liberated from meat samples and that it is not destroyed by prolonged hydrolysis. Values close to the maximum were obtained after autoclaving the samples for 2 hours with 3 N hydrochloric acid and also after refluxing them for 4 hours with 6 N hydrochloric acid.

Table III shows the results of recovery experiments in which pure histidine was added to hydrolysates of proteins and meat samples. The recoveries which ranged from 96 to 100 per cent were considered to be satisfactory.

The histidine content of several proteins is given in Table IV. The value of 3.04 per cent obtained for the histidine content of casein is in satisfactory agreement with the values obtained by other workers who have used microbiological methods. Guirard, Snell, and Williams (10) obtained a value of 3.2 per cent (moisture-free basis) by the use of both *Leuconostoc mesenteroides* and *Lactobacillus delbrueckii*. Dunn and Rockland (11) came to the conclusion that the histidine content of casein

TABLE III  
Recovery of Histidine Added to Hydrolysates\* (0.250 Mg. of L-Histidine Was Added to Each Hydrolysate)

Hydrolysate	L-Histidine		Recovery of added histidine <i>per cent</i>
	Present in hydrolysate <i>mg.</i>	Total found <i>mg.</i>	
Bovine serum albumin.....	0.155	0.405	100.0
Fibrin.....	0.207	0.457	100.0
Beef tongue.....	0.099	0.339	96.0
"  spleen.....	0.096	0.343	98.8
"  liver.....	0.208	0.449	96.4
Lamb chop.....	0.128	0.372	97.6

\* The figures are averages of values obtained at five different test levels.

TABLE IV  
Histidine Content of Some Proteins

Protein	Nitrogen content*	Histidine content*
	<i>per cent</i>	<i>per cent</i>
Casein (Difco isoelectric).....	15.56	3.04
Fibrin (Wilson Laboratories).....	16.07	2.32
Bovine serum albumin (Armour).....	16.15	3.38
Zein (commercial).....	15.20	1.22

\* Moisture-free, ash-free basis.

is 3.00 per cent (16 per cent nitrogen). Stokes *et al.* (12) reported a value of 2.8 per cent (oven-dried casein). On the other hand, the values obtained for the histidine content of casein by chemical methods are, in general, considerably lower (see Vickery and Winternitz (13)).

Average values for the histidine content of different kinds of meat are given in Table V. It will be noted that the histidine content of the protein in kidney, heart, liver, and tongue from beef, pork, and lamb was very nearly the same. A substantially higher histidine content was found in

those kinds of meat which consist primarily of skeletal muscle tissue. The statistically significant difference between the histidine content of beef loin and lamb chop is of particular interest. In contrast to this difference, data showing the similarity of these two kinds of meat with respect to their content of a number of other amino acids are given in Table VI. The figures in Table VI are averages of values obtained by the analysis of three or more samples which were known to have been taken

TABLE V  
*Histidine Content of Meat*

Kind of tissue	No. of samples	Average protein content	L-Histidine in fresh tissue			L-Histidine in crude protein,† average values
			Minimum	Maximum	Average*	
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Beef loin.....	10	21.62	0.74	0.91	0.81 ± 0.018	3.74
“ brisket.....	3	20.48	0.75	0.90	0.84	4.10
Pork loin.....	8	20.45	0.67	0.96	0.77 ± 0.032	3.76
Lamb chop.....	7	20.10	0.58	0.69	0.63 ± 0.013	3.14
Beef liver.....	4	18.88	0.49	0.52	0.50	2.64
Pork “.....	3	19.48	0.49	0.58	0.54	2.66
Lamb “.....	3	21.31	0.55	0.60	0.58	2.72
Beef tongue.....	3	17.13	0.43	0.45	0.44	2.57
Pork “.....	3	15.91	0.42	0.46	0.44	2.76
Beef heart.....	7	17.77	0.43	0.50	0.46 ± 0.010	2.59
Pork “.....	3	16.94	0.42	0.46	0.44	2.60
Lamb “.....	3	16.43	0.42	0.43	0.43	2.61
Beef kidney.....	6	17.52	0.41	0.50	0.45 ± 0.016	2.56
Pork “.....	3	15.53	0.39	0.41	0.40	2.58
Lamb “.....	3	15.68	0.40	0.41	0.41	2.61
Beef brain.....	3	10.58	0.27	0.29	0.28	2.65
“ thymus.....	3	15.93	0.27	0.29	0.28	1.76
“ spleen.....	2	18.45	0.45	0.49	0.47	2.55

\* Average values ± standard error.

† This is equivalent to calculating to 16 per cent nitrogen.

from different animals. For details of the methionine studies see Lyman *et al.* (3). Details of the determination of the other amino acids will be given in a later publication.

In order to obtain information with respect to the amount of histidine contained in animal tissues which is not combined in the form of protein, samples of finely ground fresh meat were suspended in water and the protein was precipitated with acetone. The precipitated protein and the acetone-soluble material were analyzed for histidine, the latter both before and after hydrolysis.

Fractionation of skeletal muscle tissues in this manner gave acetone-soluble fractions which contained relatively large amounts of histidine. In the case of Pork Loin 1, this amounted to a little more than one-third of the total histidine content of the original sample. As compared to beef and pork, rat muscle tissue gave an acetone-soluble fraction which contained much less histidine. The corresponding values for samples of lamb chop were intermediate. In Table VII, the histidine values for the acetone-soluble fractions are those obtained after hydrolysis.

Carnosine and anserine are typical components of skeletal muscle tissue, only small amounts of these compounds having been found in the other organs. Skeletal muscle tissue from different species varies in the relative proportion of the two compounds which they contain. Carnosine, which

TABLE VI  
*Amino Acid Composition of Muscle Tissue Expressed As Per Cent of  
Crude Protein*

Amino acid	Beef loin	Lamb chop
L-Valine.....	5.29	5.40
L-Isoleucine.....	5.84	5.74
L-Threonine.....	4.50	4.75
L-Phenylalanine.....	4.23	4.33
L-Arginine.....	6.22	6.19
L-Lysine.....	9.07	8.75
L-Methionine.....	2.47	2.41
L-Tryptophan.....	1.25	1.23
L-Histidine.....	3.74	3.14

yields natural L-histidine on hydrolysis, predominates in beef and pork, while rat muscle tissue contains relatively large amounts of anserine but very little carnosine (14-17). On hydrolysis, anserine yields methylhistidine; it is therefore important to know whether methylhistidine behaves like histidine with the microorganisms generally used for the assay of this amino acid.

Anserine was accordingly prepared from rabbit muscle tissue. After three recrystallizations of the copper derivative, the product possessed a satisfactory nitrogen and copper content.

$C_{10}H_{16}N_4O_3CuO$ . Calculated, N 17.5, Cu 19.8; found, N 17.1, Cu 19.9

Copper was removed with hydrogen sulfide and the sample was hydrolyzed with hydrochloric acid and analyzed for histidine by several different methods. Because of their similar chemical nature, anserine preparations are likely to contain small amounts of carnosine (14). The amount of

L-histidine present in the hydrolysate from this source was determined by the Pauly reaction (18), since methylhistidine does not produce a color with diazotized sulfanilic acid (14, 19).

TABLE VII  
*Histidine Distribution in Meat*

Meat sample	L-Histidine from 100 gm. fresh meat				L-Histidine in crude protein*		
	Original sample	Fraction precipitated by acetone	Fraction soluble in 60% acetone	Total accounted for	Original sample	Precipitated by acetone	Soluble in 60% acetone
	mg.	mg.	mg.	per cent	per cent	per cent	per cent
Beef Loin 1	781	567	194.9	97.6	3.74	2.97	10.60
“ “ 2	796	598	192.7	99.3	3.68	3.00	11.71
“ “ 3	760	549	176.7	95.5	3.65	2.75	8.73
Pork “ 1	960	620	328.8	98.8	4.36	3.07	18.70
“ “ 2	684	519	170.0	100.7	3.43	3.86	9.66
“ “ 3	754	563	166.2	96.7	3.56	2.89	9.18
Lamb Chop 1	690	516	139.3	95.0	3.24	2.66	7.53
“ “ 2	632	505	96.9	95.2	3.11	2.73	5.13
Rat muscle	503	461	36.3	98.9	2.30	2.28	2.16
Beef liver	502	481	9.8	97.8	2.47	2.49	0.98
“ kidney	398	404	11.0	104.3	2.37	2.60	0.84
“ heart	447	443	7.5	100.8	2.44	2.60	0.60
“ brain	264	254	7.0	98.9	2.54	2.63	0.93

\* This is equivalent to calculating to 16 per cent nitrogen.

TABLE VIII  
*Histidine Content of Hydrolyzed Anserine Preparation As Determined by Different Methods*

Analytical method	Histidine found*
Chemical (Pauly reaction)	per cent 1.62
Microbiological	
<i>Leuconostoc mesenteroides</i> P-60 used as test organism	1.66, 1.63
<i>Streptococcus faecalis</i> R “ “ “ “	2.10, 2.15
<i>Lactobacillus fermenti</i> 36 “ “ “ “	1.67

\* Values expressed as per cent of histidine in copper anserine preparation. Where two values are given, separate hydrolysates were prepared and analyzed.

The data in Table VIII show that methylhistidine has no histidine activity at all for *Leuconostoc mesenteroides* P-60 nor for *Lactobacillus fermenti* 36, although it is slightly active for *Streptococcus faecalis* R. The

activity of methylhistidine for this latter organism is, however, too small to cause a significant error in the determination of histidine in meat.

One of the most useful methods of evaluating the reliability of microbiological assays is to make determinations by the use of more than one organism. Table IX shows the results of analyzing various kinds of meat and fractions obtained therefrom by the use of *Streptococcus faecalis* R and *Leuconostoc mesenteroides*. In only one case is there marked disagreement between the values obtained by the two methods. The higher value

TABLE IX  
Comparison of Values for Histidine Content of Meat and Meat Fractions  
Obtained by Use of Different Test Organisms

Material analyzed	L-Histidine content of 100 gm. fresh meat or fraction derived therefrom	
	Test organism	
	<i>Streptococcus faecalis</i> R	<i>Leuconostoc mesenteroides</i>
	mg.	mg.
Beef loin	781	755
Protein from beef loin precipitated by acetone	567	576
Fraction from beef loin soluble in 60% acetone, before hydrolysis	19.7	2.8
Fraction from beef loin soluble in 60% acetone, after hydrolysis	195	195
Beef liver	502	511
Protein from beef liver precipitated by acetone	481	500
Fraction from beef liver soluble in 60% acetone, before hydrolysis	6.8	6.8
Fraction from beef liver soluble in 60% acetone, after hydrolysis	9.8	8.7
Beef brain	281	275
Lamb heart	423	432

obtained for the histidine content of the unhydrolyzed acetone-soluble fraction from beef loin by the use of *Streptococcus faecalis* R suggests that this organism can make partial use of histidine in the form of carnosine. It will be noted that, after hydrolysis, the histidine values obtained by the two methods were identical. The 10-fold and greater increase in the value for the histidine content of the acetone-soluble fraction after hydrolysis indicates that most of the histidine in the acetone-soluble fraction was present in a combined form. Very little free histidine was present before hydrolysis.

In order to determine whether the acetone-soluble fraction from beef

muscle tissue contained considerable amounts of other amino acids in a combined form, the analyses indicated in Table X were carried out. Although Table X does not include data on all of the amino acids, it appears that histidine occupies a somewhat unique position. The increase in the amount of free glycine and glutamic acid after hydrolysis was probably due to the presence of glutathione. The amino acid analyses were all carried out by microbiological procedures.

TABLE X  
*Amino Acid Content of Acetone-Soluble Fraction from Beef Loin*

Amino acid determined	Amount of amino acid found	
	In non-hydrolyzed solution*	After hydrolysis*
	mg.	mg.
L-Arginine.....	15.5	15.2
L-Aspartic acid.....	1.2	5.7
L-Glutamic ".....	16.2	45.4
Glycine.....	12.7	42.6
L-Histidine.....	14.2	176.7
L-Isoleucine.....	7.9	9.5
L-Leucine.....	10.3	11.6
L-Lysine.....	3.2	6.7
L-Methionine.....	4.4	5.5
L-Phenylalanine.....	9.7	6.1
L-Proline.....	4.1	6.6
L-Threonine.....	15.4	20.1
L-Valine.....	11.3	16.3

\* Fraction obtained from 100 gm. of meat.

#### DISCUSSION

Since the hydrolysis of carnosine yields L-histidine, this fraction of the histidine in muscle tissue will be determined by any histidine method, provided that the determinations are carried out on the meat samples as a whole and not on the protein material after separation of non-protein nitrogen and other substances.

Most of the earlier investigations were designed to study the histidine content of tissue proteins and not the total histidine content of the samples. Usually the materials were treated in such a way that part or all of the carnosine content must have been lost. For example, Beach, Munks, and Robinson (1) extracted their meat samples with hot water. Osborne and Jones (20) extracted theirs first with large volumes of water saturated with toluene, then with 95 per cent alcohol, and finally with absolute alcohol. Rees (21) extracted his samples by boiling them with alcohol,

dilute acid, and finally with water. Block and Bolling (22), in one of their investigations, extracted the tissues with acetone, hot alcohol, hot benzene, and ether. It is apparent that the purpose for which the investigation was designed must be kept in mind in evaluating studies on histidine in material from animal sources.

Kuen (17) analyzed two samples of beef muscle for carnosine by several different methods and obtained values ranging from 0.380 to 0.354 per cent, depending on the method used. In terms of mg. of histidine per 100 gm. of fresh tissue, these values become 211 and 252 respectively. It thus appears that beef muscle contains sufficient carnosine to account for all of the combined histidine found in the present investigation in the acetone-soluble fractions from beef loin.

It has been shown that methylhistidine (derived from anserine) does not give rise to a significant error in the determination of histidine by any of three microbiological methods. The fact that skeletal muscle tissue from different species differs in the relative proportions of carnosine and anserine present is the probable explanation for the statistically significant difference between the average values obtained for the histidine content of beef loin and lamb chop.

#### SUMMARY

A microbiological method for the determination of histidine is described. The method was applied to the determination of histidine in meat. The meat samples studied included beef loin, round, brisket, liver, heart, kidney, tongue, brain, thymus, and spleen, as well as pork loin, liver, heart, kidney, and tongue, and lamb chops, liver, kidney, and heart.

The histidine content of the skeletal muscle tissues was higher than that in the organs such as kidney and liver. A substantial part of the histidine of the muscle tissues was found to be in a combined form which was not a part of the tissue protein. The carnosine content of muscle appears to account for this fraction of the histidine.

With the exception of skeletal muscle tissue, there was no substantial difference in the histidine content of the same kind of tissue from beef, pork, and lamb. Furthermore, the organs such as kidney, liver, heart, and tongue all had about the same histidine content.

It was found that the presence of methylhistidine does not give rise to a significant error in the determination of histidine in meat by any of three different microbiological procedures.

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