Amino Acid Composition of Thymus Nucleohistone.* (20152)

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The isolation of a nucleohistone from chick erythrocyte nuclei and the microbiological analysis of this protein for its amino acid composition has been described in a previous paper(1). In this communication we wish to report a similar study of nucleohistones derived from bovine thymus gland by a modification of the method of Mirsky and Ris(2) for the isolation of chromosomes.

Experimental.† One hundred grams of fresh calf thymus were minced with scissors and suspended in 450 ml of 0.14 M NaCl. The solution was then homogenized in a Waring Blendor for 7 minutes. A drop of octyl alcohol was added to minimize foaming. The solution was removed from the Blendor and immediately cooled to 0°C, after which the solution was centrifuged for 5 minutes at 1500 G. Following centrifugation, 2 distinct layers of solid material were noted, an upper layer of chromosomal material, and a lower layer of debris and unbroken nuclei. The turbid supernatant liquid contained lighter cellular elements and cytoplasmic material. It was discarded along with the uppermost part of the chromosomal layer. The centrifugate was again suspended in 4 times its volume of isotonic saline and centrifuged for 3 minutes at 1200 G. The clean, white layer of chromosomal material was then separated from the debris with a suction pipet and transferred to a clean test tube where it was resuspended in 4 times its volume of 0.14 M NaCl and washed 4 additional times. The thoroughly washed chromosomal material was then filtered in the cold and approximately 20 ml of 0.2 N HCl were added to extract the histone. The HCl extraction was continued until all of the histone had gone into solution. The acid extract was dialyzed against distilled water overnight, after which 0.05 N NaOH was added to maximum precipitation at pH 10.4. The precipitated nucleohistone was filtered, washed twice with ethanol and once with ether, and finally dried *in vacuo* at room temperature. The dried product was analyzed for total nitrogen by the method of Hiller, Plazin, and Van Slyke(3) and then hydrolyzed in an all-glass reflux condenser. One sample was hydrolyzed with 20% HCl and another, for determination of tryptophan, with 5 N NaOH(4). The hydrolyzates were neutralized and analyzed microbiologically for 18 amino acids by methods previously described(1). Analyses for amino nitrogen were also performed on samples from the hydrolyzates using the method of Van Slyke *et al.*, based on the gasometric measurement of carbon dioxide evolved after reaction of the hydrolyzates with ninhydrin(5).

Results and discussion. The results of the analyses for the total nitrogen and the amino nitrogen of the proteins are shown in Table I. The amino acid analyses on 2 individual samples of bovine thymus nucleohistones, designated T-1 and T-2, are reported in Table II. The serine and threonine figures have been corrected for decomposition of 5 and 10%, respectively, as suggested by Rees(6) who

TABLE I. Nitrogen Analyses of Thymus Nucleohistones, g/100 g.

	Total N.		α-amino N.	
	A.	B.	C.	D.
Sample No.	By Kjeldahl analysis	Calc. from amino acid content (Table II)	By ninhydrin analysis	Calc. from amino acid content (Table II)
T-1	16.35	16.14	11.71	12.64
-2	16.31	16.37	10.42	11.52

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† All procedures were carried out at 0°C unless otherwise stated. The centrifuge used was an International refrigerated centrifuge, Model PR-1. When using the Waring Blendor it was placed in the refrigerator at 0°C.

TABLE II. Amino Acid Composition of 2 Samples of Thymus Nucleohistone.

Amino acid	Amino acid per 100 g protein		Contribution of each amino acid to total N. of protein*	
	T-1	T-2	T-1	T-2
	g		%	
Alanine	9.5	10.0	9.5	9.7
Arginine	12.0	12.9	23.5	25.4
Aspartic acid	6.8	6.4	4.4	4.1
Cysteine/cystine	—	—	—	—
Glutamic acid	11.4	11.0	6.6	6.4
Glycine	4.8	4.6	5.5	5.3
Histidine	3.0	3.1	5.0	5.2
Isoleucine	6.6	6.6	4.3	4.3
Leucine	9.6	7.7	6.3	5.0
Lysine	13.2	13.4	15.5	15.8
Methionine	1.0	0.8	0.5	0.4
Phenylalanine	2.4	2.4	1.2	1.3
Proline/OH proline(?)	3.4	4.0	2.5	3.0
Serine	3.2	4.0	2.6	3.3
Threonine	6.0	6.3	4.3	4.5
Tryptophan	0	0	0	0
Tyrosine	4.4	4.1	2.1	1.9
Valine	4.8	4.4	3.5	3.2
Ammonia			1.4	1.6
Totals			98.7	100.4

* These figures were obtained by calculation from the amino acid percentage composition of the protein as shown in the first 2 columns of this table and total N. obtained by Kjeldahl analysis (Table I).

observed this degree of destruction under similar conditions of hydrolysis.

Hamer(7) quantitatively analyzed an acid hydrolyzate of thymus histone by the use of starch columns and accounted for 94.7% of the total nitrogen of the protein by 15 amino acids. The NH_3 nitrogen brought the total nitrogen recovered to 99.5%. Methionine, cystine, and tryptophan were not found in the hydrolyzates assayed on the starch column. Neither cystine nor cysteine was detected by a polarographic analysis but a separate colorimetric determination for tryptophan in the protein indicated a content of 0.04%. This corresponds to about 5 γ of tryptophan per ml of the alkaline hydrolyzates which were assayed microbiologically in our laboratory, a quantity well within the sensitivity of our analytical method even after allowing for some decomposition which should be minimal in the alkaline hydrolyzate. However, in none of our thymus nucleohistones was any tryptophan found. A small quantity of methionine was detected, but cystine was either absent or present in trace quantities which were inadequate for a satisfactory assay. In the study of calf thymus reported by Hamer, a great excess of isoleucine over leucine was found, whereas our analyses indicated that these 2 amino acids were present in about the same amount. The sum of the concentrations of isoleucine and leucine in Hamer's samples is approximately those reported in our study. It is possible that complete separation of the 2 amino acids was not obtained in the chromatographed samples on the starched column. Our findings agree with those of Daly *et al.* (8) in showing essentially equal amounts of leucine and isoleucine in the thymus histone samples.

We have previously noted(1) that the chick red cell nucleohistones were characterized by the presence of an excess of lysine residues over arginine, in contrast to the findings of Hamer, or of Daly *et al.* This is also true of the thymus nucleohistones we have studied. The considerable quantities of alanine reported both in the chick and the thymus nucleohistones isolated in our laboratory also contrast with that reported by Hamer or by Daly *et al.* However, the findings with regard to all other amino acids agree well with those of previous workers. Furthermore, the amino acid constitution of the nucleohistones from the thymus is, in general, quite similar to that found by us for corresponding nuclear protein derived from the chick red cell.

Summary. 1. Two samples of histone isolated from bovine thymus nuclei have been analyzed for their content of 18 amino acids by microbiological methods. Approximately 98% of the total N of the protein was accounted for by 16 amino acids. 2. No tryptophan was found in the thymus nucleohistone samples. 3. The presence of methionine in these proteins was confirmed but that of cystine is questionable. 4. In general, the amino acid composition of the thymus nucleohistone resembles that of the corresponding protein derived from chick erythrocyte previously reported by us.

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Liver Xanthine Oxidase Activity in Relation to Availability of Methionine from Soybean Protein.* (20153)

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It is well known that the nutritive value of the protein from soybean meal can be markedly enhanced by methionine supplementation or by proper heat treatment(1). The demonstration that the amount of sulphur(2) or methionine(3) absorbed from the intestinal tract of the rat is the same whether the animal is fed the raw or heated soybean meal has led to the suggestion that the methionine derived from unheated soybean protein must be absorbed in a form(2), or in a manner(3), which cannot be utilized for growth.

Since the growth depression observed in animals fed raw soybean meal is known to be caused by other factors as well as the availability of methionine(4), it appeared desirable to apply a criterion other than growth as a measure of the utility of methionine from raw and heated soybean protein. The xanthine oxidase activity of the liver was selected for this purpose since the activity of this enzyme has been shown to be a sensitive index of the availability of methionine from dietary protein(5,6).

Experimental. Each kg of basal ration used in this study contained the following components: raw or heated (autoclaved at 15 lb for 20 min.) soybean flour,† 400 g; salts (7), 40 g; Crisco, 60 g; sucrose, 500 g; and vitamin supplements(7) modified to include 40 μg vit. B_{12}. When 0.45% DL-methionine supplemented the basal ration it replaced an equal weight of sucrose. Two types of feeding trials were conducted: *ad libitum* feeding (Exp. A), and paired feeding (Exp. B). In Exp. A, young weanling rats (littermates of the same sex) weighing 35-50 g were distributed into the following groups: (I) raw soy; (II) raw soy + methionine; (III) heated soy; (IV) heated soy + methionine. Each group was composed of 3 females and 5 males; within each group rats of the same sex were housed together in separate cages. Food and water were furnished *ad libitum,* and the weight and food consumption were recorded daily for 6 weeks. The design of Exp. B was identical to that of Exp. A except that each animal was housed in an individual cage, and the food intake of each animal in groups II, III, and IV was restricted to that of its littermate in group I. During the last week of the experiment all animals were transferred to individual metabolism cages for a period of 3 days to permit collection of the feces. The feces were dried in an oven (overnight at 105°C), weighed, ground to a fine powder in a Wiley mill, and 2 g samples were analyzed for methionine using the method of Horn *et al.*(8). Representative samples of each ration were likewise analyzed for methionine. From these data, in conjunction with a measure of the food intake during this period, the amount of methionine absorbed was calculated. At the end of 6 weeks, the animals

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† Nutrisoy XXX, Archer-Midland-Daniels Co., Minneapolis, Minn. According to the manufacturer this product has been extracted with hexane and subjected to a minimum amount of heat treatment.