SUMMARY

It has been established that when defatted tomato seeds are extracted with a 0.2% solution of NaOH and the protein is precipitated by acidification to pH 5.5 the yield of protein amounts to 11% on the weight of the initial raw material.

In terms of quality, the tomato protein belongs to the moderately balanced plant isolates.

LITERATURE CITED

1. Ya. M. Gol'denberg and A. F. Fan-Yung, in: The Use of Wastes in the Preserving Industry [in Russian], Moscow (1971).
2. Ya. M. Gol'denberg, Konservn. Ovoshchesush. Promst'., 8 (1967).
3. Ya. M. Gol'denberg, Konservn. Ovoshchesush. Promst'., 1 (1968).
4. P. W. Edwards, R. K. Eskew, and A. Hoersch, Food Technol., 6, 383 (1952).
5. C. H. Perrin, Anal. Chem., 25, No. 6, 968 (1953).
6. Handbook on Methods of Investigation, Technical and Chemical Control, and Accounting in the Oils and Fats Industry [in Russian], Leningrad, Vol. II (1965), p. 303.
7. V. N. Orekhovich (ed.), Analytical Methods of Protein Chemistry [Russian translation], Moscow (1963), p. 468.

IDENTIFICATION AND FRACTIONATION OF THE TOTAL HISTONE OF THE COTTON PLANT

G. F. Kasymov, N. I. Koryakina,
T. P. Kopitsya, and V. K. Burichenko

UDC 547.96

In order to isolate individual fractions of the histones of the cotton plant of variety 108-F and to investigate them further, the total histone has been chromatographed on Acrylex P-60 and Bio-Gel P-30. It has been shown that the most complete fractionation is achieved on a Acrylex P-60. By electrophoresis of the fractions in 15% PAAG in the presence of 6.25 M urea and on the basis of amino acid compositions, the following order of elution of the histones has been established: H1, H2B + H2A, H3, and H4. In a comparison of the amino acid composition of the H3 and H4 histones of the cotton plant with histones enriched with arginine from other species, a somewhat higher lysine : arginine ratio in these fractions has been found. On comparing the electrophoretic mobilities of the histone fractions from the cotton plant with the histones of calf thymus, some difference is observed which is apparently connected with a difference in their molecular weights.

Histones, basic proteins of the cell nucleus, are assigned a leading role in the structural organization and functioning of the chromosomes, which is responsible for the great attention devoted to their investigation.

At the present time, a considerable amount of material has been accumulated on histones of animal origin [1-4]. Information relating to plant histones is less detailed, although interest in their isolation and study is increasing.

The majority of methods for isolating histones do not always enable homogeneous fractions to be obtained because of cross-contamination [5-7].

We have previously [8] fractionated cotton-plant histones by a modification of Johns' method. This method enabled us to obtain the individual histones H1, H2B, and H4. Further purification was required for the other fractions. However, the chromatography of several plant histones on Bio-Gels enables individual fractions to be isolated without additional purification [9-12].

V. I. Nikitin Institute of Chemistry, Academy of Sciences of the TadzhSSR, Dushanbe.
Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 832-838, November-December, 1979. Original article submitted June 11, 1979.

 0009-3130/79/1506-0736$07.50

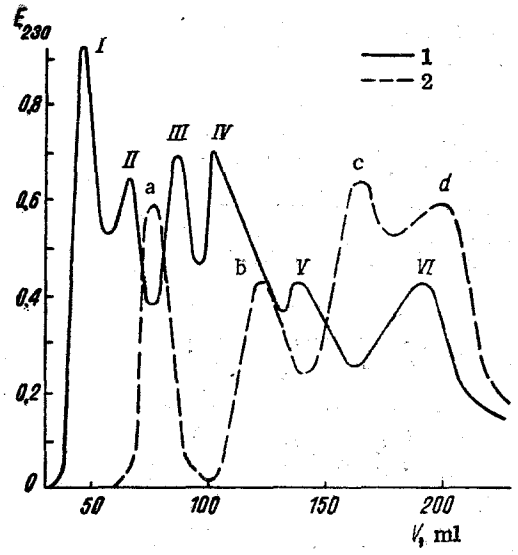

Fig. 1

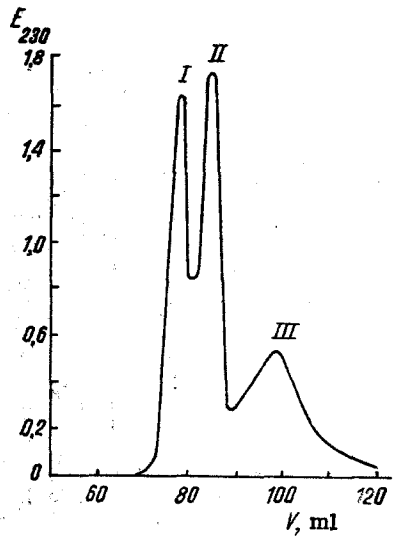

Fig. 2

Fig. 1. Fractionation of the total histone on Acrylex P-60: 1) cotton plant; 2) calf thymus.

Fig. 2. Fractionation of the total cotton plant histone on Bio-Gel P-30.

The aim of the present investigation was to find the optimum conditions for separating the total histones of the cotton plant of variety 108-F into fractions by gel chromatography.

To separate the total histones of the cotton plant, we used the conditions of fractionation on Acrylex P-60 [11] and Bio-Gel P-30 [12]. For comparison, under similar condition, we chromatographed the total histone of calf thymus on Acrylex P-60.

The total histone and the fractions after chromatography were characterized by their electrophoretic mobilities in 15% PAAG (method of Panyim and Chalkley [13]) and by their amino acid compositions (JLC-6AH analyzer, Japan). The amino acid compositions of the histones of the cotton plant were compared with literature information for the histones of calf thymus [14], of Euglena gracilis [15], rye shoots [16], and wheat germ [17] (Tables 1 and 2).

The results of gel chromatography (Figs. 1 and 2) showed that the most complete fractionation of the histones was achieved on a column with Acrylex P-60 if the elution buffer used was 0.02 N HCl containing 0.02% of sodium azide and 0.05 M NaCl and the solvent for the histones was an 8 M solution of urea containing 1% of β-mercaptoethanol and 10% of sucrose (pH 5.0). The conditions for fractionating plant histones on Bio-Gel P-30 [12] (eluent and solvent for the histones 0.01 N HCl) did not permit complete separation of the cotton plant histones to be achieved.

In the electrophoretic comparison of the total histones of the cotton plant and of calf thymus, some similarity in fractional composition was observed. Both histones contain five main fractions, although they differ somewhat in mobility. The cotton plant histones are less mobile and on chromatography they were eluted earlier than the calf thymus histones. This behavior of the histones is probably due to a difference in their molecular weights. This hypothesis is confirmed by literature information showing a difference in the molecular weights of histones of plant and animal origin [16].

The electrophoretic characteristics of the histones obtained after fractionation on Acrylex P-60 show that nonhistone proteins are eluted first (absence of bands in gel 1, Fig. 3A), these making up ~10% of the total amount of combined histone. Fraction I is characterized by a high content of acidic and a relatively low content of basic amino acids (Table 1).

The high lysine content and low arginine content permit fraction II of the cotton plant histone to be considered as lysine-rich. Furthermore, in its mobility it corresponds to histone H1. But on comparison with the amino acid compositions of histone H1 from other tissues, it can be seen that the amount of lysine in fraction II is low, apparently because of its partial contamination with acidic proteins. This was confirmed by the preliminary

TABLE 1. Amino Acid Composition (moles/100 moles of Amino Acids) of Histones H1, H2B, and H2A of the Cotton Plant Isolated After Chromatography on Acrylex P-60 in Comparison with Some Literature Figures

Amino acid	Acidic proteins of the cotton plant, fr. I	H1						H2B					H2A			
		calf thymus [14]	Euglena gracilis [15], fr. A	rye shoots [16]	wheat germ	cotton shoots, fr. II	cotton plant CMC histone, fr. II	calf thymus	Euglena gracilis, fr. C	rye shoots	wheat germ	cotton shoots, fr. III	calf thymus	Euglena gracilis, fr. B	rye shoots,	cotton shoots, fr. IV
Lys	9,75	27,9	35,0	25,2	26,3	20,09	23,54	16,6	18,2	14.7	17,9	17,29	12,9	16,7	14,3	15,70
His	1,97	0,0	0,9	1,0	1,3	0,99	0,68	2,2	1,9	1,5	1,3	1,61	2,7	0,7	1,5	1,49
Arg	4,09	1,7	2,3	2,7	3,5	2,85	1,38	6,2	6,1	4,4	6,2	5,34	9,4	6,1	7,1	6,08
Asp	10,96	2,0	0,1	3,5	3,0	6,29	5,43	4,9	5,7	5,5	5,0	6,73	5,6	5,8	6,2	6,95
Thr	5,74	5,5	3,4	5,9	6,1	6,59	7,46	6,1	5,3	5,2	6,2	4,37	5,0	5,4	3,8	5,50
Ser	8,72	6,9	4,5	3,8	3,7	8,99	8,93	10,7	7,4	7,1	6,4	6,59	5,0	5,9	4,6	6,92
Glu	14,53	3,4	3,2	3,9	4,0	10,80	9,42	7,6	7,2	9,0	8,9	11,43	9,0	7,1	6,2	13,90
Pro	2,85	10,2	10,1	11,4	11,1	7,38	10,42	4,9	3,2	4,9	6,0	5,03	4,0	6,1	5,3	2,56
Gly	8,90	6,9	3,8	4,8	3,7	5,68	5,24	5,5	8,4	9,0	8,3	9,39	9,1	11,3	11,8	8,21
Ala	10,33	25,3	22,6	26,5	27,1	16,58	16,22	10,1	12,0	10,8	13,7	12,62	13,2	12,5	16,2	12,81
Cys	—	0,0	0,0	—	0	—	—	0,0	0,0	—	0,2	—	0,0	0,0	—	—
Val	5,03	4,3	5,5	4,1	5,0	3,74	2,10	7,0	5,4	5,6	5,5	5,14	6,0	5,6	7,1	4,40
Met	2,37	0,0	0,6	0,2	0,5	0,72	1,91	1,5	1,9	1,4	0,8	1,29	0,2	0,4	0,2	1,02
Ile	2,77	0,8	1,1	2,2	1,5	1,57	1,21	4,9	5,7	6,9	4,4	2,62	4,3	2,4	3,3	3,17
Leu	7,29	4,3	4,3	3,0	1,0	4,86	4,15	4,9	6,6	7,6	7,5	7,14	10,2	10,1	9,6	7,49
Tyr	1,65	0,5	1,6	0,7	0,9	1,13	0,86	3,8	1,5	3,2	2,1	1,67	2,3	1,9	2,0	1,31
Phe	3,04	0,5	1,2	0,9	0,8	1,70	1,05	1,6	3,5	3,2	2,0	1,75	1,0	1,8	0,7	2,50
Lys/Arg	2,38	16,4	15,2	9,3	7,51	7,04	17,06	2,7	3,0	3,3	2,89	3,23	1,4	2,7	2,0	2,58
basic/acidic	0,62	5,4	11,6	3,8	4,44	1,40	1,72	2,0	1,8	1,3	1,83	1,33	1,7	1,8	1,7	1,11

TABLE 2. Amino Acid Compositions (moles/100 moles of Amino Acids) of the Arginine-rich Histones of the Cotton Plant Isolated after Chromatography on Acrylex P-60 in Comparison with Literature Figures

Amino acid	H3					H4					
	calf thymus	Euglena gracilis, fraction D	rye shoots	wheat germ	cotton shoots, fr. IV	calf thymus	Euglena gracilis, fr. E	rye shoots	wheat germ	cotton shoots, fr. VI	from cotton shoots after P-30, fr. III
Lys	10,1	12,9	9,7	9,8	13,2	9,8	12,3	9,5	9,2	11,8	11,20
His	2,5	1,9	1,7	2,2	2,05	2,0	2,1	1,8	1,9	2,18	2,32
Arg	13,8	12,3	12,2	11,9	7,83	14,1	11,7	11,6	13 9	10,19	10,72
Asp	4,5	4,3	4,1	4,5	8,19	5,0	4,3	5,9	5,4	7,58	7,34
Thr	6,4	6,0	6,2	6,8	5,22	6,6	6,4	5,3	6,6	5,73	5,90
Ser	3,8	3,5	4,7	3,8	6,90	2,5	3,1	2,6	2,3	5,54	6,77
Glu	10,3	11,8	10,6	12,3	13,37	6,2	9,0	7,1	6,3	11,51	10,92
Pro	4,3	4,4	5,5	4,8	2,09	1,3	1,9	1,1	1,0	2,20	2,43
Gly	5,9	5,9	7,5	6,3	10,74	16,0	14,6	19,3	16,9	13,22	14,41
Ala	13,8	12,1	14,8	12,1	10,88	7,4	7,0	8,2	6,8	9,16	8,55
Cys	0,4	0,6	—	1,1	—	0,0	0,0	—	0	—	—
Val	4,6	5,2	4,5	4,5	4,13	8,0	7,2	7,6	7,9	4,50	4,41
Met	1,5	0,9	0,2	0,1	1,04	1,1	0,2	0,5	0,9	1,43	0,50
Ile	4,8	4,7	4,7	5,4	2,46	5,5	5,9	6 5	5,6	2,70	2,98
Leu	9,1	8,6	8,5	9,3	7,30	8,0	8,5	8,1	8,1	7,08	6,89
Tyr	2,0	1,3	1,5	1,6	1,77	3,5	3,0	2,9	3,9	2,33	2,10
Phe	2,0	3,7	3,5	3,7	2,50	2,2	3,0	2,1	2,3	2,38	2,27
Lys/Arg	0,7	1,0	0,8	0,82	1,68	0,7	1,1	0,8	0,6	1 15	1,04
basic/acidic	1,8	1,7	1,5	1,42	1,07	2,3	2,0	1,9	2,14	1,27	1,33

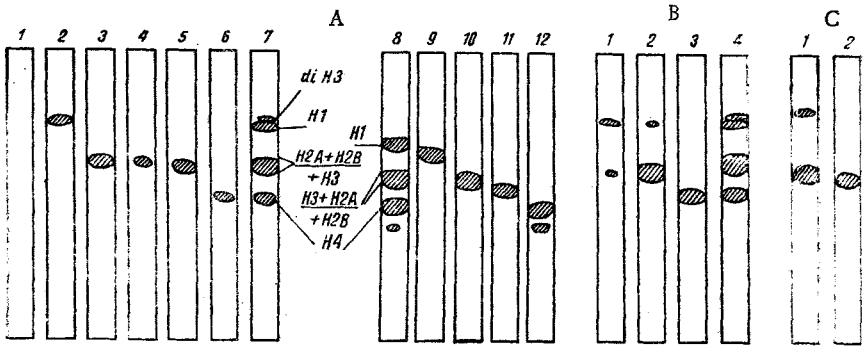

Fig. 3. A. Electrophoretic spectra of the total histones [7) cotton plant of variety 108-F; 8) calf thymus] and of histone fractions isolated after chromatography on Acrylex P-60; fractions of the cotton plant histones: 1) I; 2) II; 3) III; 4) IV; 5) V; 6) VI; fractions of the calf thymus histones: 9) a; 10) b; 11) c; 12) d. B. Electrophoretic spectra of the cotton plant histones after chromatography on Bio-Gel P-30; histone fractions: 1) I; 2) II; 3) III, histone fractions; 4) the total histone of the cotton plant for comparison. C. Electrophoresis of fraction IV of the cotton plant histone isolated after chromatography on Acrylex P-60. Before (1) and after (2) treatment with 5% β-mercaptoethanol.

purification of the total histone (on a column of CM-cellulose), which led to an appreciable increase in the amount of lysine in the H1 fraction.

Fraction III is characterized by a fairly low lysine : arginine ratio. Its amino acid composition and electrophoretic mobility (Fig. 3A) permit it to be assigned to the moderately lysine-rich histone H2B.

An electrophoretic check on the degree of purity of fraction IV (Fig. 3A) revealed the presence of two bands: a less mobile one corresponding to the dimeric form of histone H3, and a more mobile one corresponding to histone H2A. To check this, we carried out the electrophoresis of the histone previously treated with 5% β-mercaptoethanol [18]. In addition, we used the method of specific staining of arginine-rich histones with Bromophenol Blue [19].

As can be seen from Fig. 3B, after treatment with β-mercaptoethanol and staining with Bromophenol Blue, the dimeric form of histone H3 was reduced completely to the monomeric form. Since the amino acid composition of this fraction is similar to that of histone H2A from other species, it may be concluded that histone H2A contaminated with histone H3 was eluted in peak IV (Fig. 1).

Their electrophoretic mobilities in comparison with that of the total histone, and also specific staining with Bromophenol Blue permit fractions V and VI isolated by chromatographing the cotton plant histone on Acrylex P-60 to be assigned to the arginine-rich histones. The amino acid compositions of these fractions (Table 2) resemble those of histones H3 and H4. However, on comparison with the corresponding fractions from other species a somewhat increased lysine : arginine ratio is found in the cotton plant H3 and H4 histones. Similar results were obtained for the arginine-rich fraction (Table 2) and after the chromatography of the total histone on Bio-Gel P-30 (Fig. 2, peak III). It is likely that the slight increase in the amount of lysine in fractions V and VI, classified as arginine-rich, is specific for cotton plant histones.

As already mentioned, the conditions of fractionation on Bio-Gel P-30 did not permit the complete separation of the histones to be achieved in view of their cross-contamination. Electrophoresis of the histones (Fig. 3B) showed that only fraction III, assigned to the arginine-rich histone H4, was homogeneous.

Chromatography of calf thymus histone on Acrylex P-60 (Fig. 1) showed that its elution profile differed somewhat from that of the cotton plant histone. An electrophoretic evaluation of the fractions isolated (Fig. 3) showed the following sequence of elution of calf-thymus histones: H1 (peak a), H3 + H2A (b), H2B (c), and H4 (d).

On the basis of the results obtained it may be concluded that in the nuclei of shoots of the cotton plant of variety 108-F there are five main histone fractions similar in com-

position and properties to the analogous histones isolated from other species of plants and animals. The chromatography of the cotton plant histone on Acrylex P-60 under certain conditions enables individual fractions to be obtained.

EXPERIMENTAL

Isolation of the Total Histone of the Cotton Plant. The total histone was isolated from the cell nuclei of two-day cotton plant shoots. The isolation and purification of the nuclei were effected by a modification of Alfrey's method [20]. The shoots were homogenized in a buffer containing 0.05 M tris, 0.004 M $MgCl_2$, 0.004 M $CaCl_2$, and 0.25 M sucrose, pH 7.2 (buffer A). The homogenate was filtered through a double layer of dense tissue and then through eight layers of Kapron tissue. The nuclei were precipitated by centrifuging (2200 rpm for 20 min) and were washed with 1% of Triton X-100 in the basic buffer A. The nuclei were additionally purified by gradient centrifuging in 1.7 M sucrose at 3000 rpm for 20 min and were washed with 0.14 M NaCl in citrate buffer, pH 7.0. In the following stage of purification, the nuclei were separated from starch granules. The total histone was isolated from the nuclei by extraction with 0.25 N HCl for 18-20 h in the cold. The insoluble part was separated off by centrifuging at 8000 rpm for 20 min. The supernatant was dialyzed against 0.001 N HCl and was freeze-dried. From 2 g of crude nuclei we obtained ~30 mg of total histone.

Fractionation of the Total Histone of the Cotten Plant. The total histone (25 mg) was fractionated at room temperature on a column of Acrylex P-60 (Hungary). The swollen gel was charged into a column with dimensions of 150 × 1.5 cm and was equilibrated with the buffer for elution for two days. Before deposition on the column, the weighed sample of histone was dissolved in 2 ml of 8 M urea containing 1% of β-mercaptoethanol and 10% of sucrose (pH 5.0) and was left in the cold for a day. Elution was performed with 0.02 N HCl containing 0.02% of sodium azide and 0.05 M NaCl. The height of the hydrostatic column was 30 cm. The protein was eluted at the rate of 3 ml/h. Fractions were collected with the aid of an automatic collector (Sweden). The issuance of the fractions was monitored on a Hitachi spectrophotometer (Japan) from the absorption at 230 nm.

To fractionate the total histone on Bio-Gel P-30, a sample of the protein (20 mg) was dissolved in 0.01 N HCl and deposited on a column with dimensions of 140 × 1.8 cm. Elution was carried out with 0.01 N HCl at the rate of 3 ml/h. The collection of the fractions (1 ml each) and the monitoring of their elution were carried out automatically as described above.

Gel Electrophoresis of the Histones. The total histone and the fractions isolated from columns were analyzed by electrophoresis in 15% polyacrylamide gel (PAAG) in the presence of 6.25 M urea by the method of Panyim and Chalkley [13]. A tube with a diameter of 0.55 cm was charged with PAAG to a height of 6.5 cm, 3 M urea was layered above it with the aid of a capillary, and polymerization was carried out at 32°C for 50 min. To eliminate low-molecular weight impurities from the gel, preliminary electrophoresis was carried out for 2 h. Before transfer to the tubes, the histones were dissolved in acetate buffer, pH 3.2, with the addition of a small amount of acrylamide (~15 mg per 0.1 ml of buffer).

The total histone was deposited in an amount of 100-130 μg and the histone fractions in amounts of 50-60 μg per tube. Electrophoresis was performed at 2-4°C for 4.5 h at a current of 1.5 mA per tube for the first 30 min and then at 2 mA.

The gels were stained with a 0.2% solution of Amido Black 10B (AB) in methanol—glacial acetic acid—water (5:1:5) for 16 h. The dye was washed out electrophoretically with 7% CH_3COOH at a voltage of 10 mA per tube.

For the selective staining of the arginine-rich histone fractions, we used a 0.01% solution of Bromophenol Blue, pH 3. The gel was stained for 16 h, and the dye was washed out with a 40% solution of n-propanol at 55°C for 48 h (with five changes of solvent).

Amino Acid Analysis. The sample for amino acid analysis was hydrolyzed in 6 N HCl in evacuated glass tubes at 105-115°C for 22 h. After evaporation of the solvent in a rotary evaporator, the samples were kept in a desiccator over KOH and were then dissolved in 0.01 N HCl. They were analyzed in the long and the short columns of the analyzers. The results of the analysis were recalculated to molar percentages.

SUMMARY

1. Homogeneous fractions of histones H1, H3, and H4 have been isolated by chromatographing the total cotton plant histone of variety 108-F on Acrylex P-60, and their individuality has been shown by electrophoresis and amino acid determinations.

2. It has been shown that fractions H3 and H4 of cotton plant histones differ from the corresponding fractions of other species of plants by a somewhat higher lysine content.

LITERATURE CITED

1. H. Hayashi and K. Iwai, J. Biochem., 72, 327 (1972).
2. S. C. Rall, T. Okinaka, and C. F. Strniste, Biochemistry, 16, No. 22, 4940 (1977).
3. L. A. Bolund and E. W. Johns, Eur. J. Biochem., 35, 546 (1973).
4. P. A. Temussi, J. Theor. Biol., 50, 25 (1975).
5. E. W. Johns, Biochem. J., 92, No. 1, 55 (1964).
6. E. W. Johns and D. M. P. Phillips, Biochem. J., 77, No. 3, 631 (1960).
7. V. V. Sidorova, A. A. Yamaleeva, and V. G. Konarev, Abstracts of Lectures at the Fifth All-Union Symposium on the Structure and Function of the Cell Nucleus [in Russian], Novosibirsk (1975), p. 64.
8. V. V. Sidorova, T. P. Konitsya, G. F. Kasymova, N. I. Koryakina, V. K. Burichenko, and V. G. Konarev, Dokl. Akad. Nauk Tadz. SSR, 21, No. 4, 31 (1978).
9. N. J. Jardine and J. L. Leaver, Biochem. J., 169, 103 (1978).
10. B. Wakim, Arch. Biochem. Biophys., 176, 510 (1976).
11. Yu. A. Tyrsin, I. A. Krasheninnikov, and E. G. Tyrsina, Biokhimiya, 42, No. 5, 898 (1977).
12. M. Tazal and R. D. Cole, J. Biol. Chem., 252, No. 12, 4068 (1977).
13. S. Panyim and R. Chalkley, Arch. Biochem. Biophys., 130, 337 (1969).
14. L. C. Hnilica, M. E. McClure, and T. C. Spelsberg, in: Histones and Nucleohistones, D. M. P. Phillips (ed.), Plenum, London (1971).
15. N. J. Jardine and J. L. Leaver, Biochem. J., 169, 103 (1978).
16. P. Nadeau, D. Pallota, and J. C. Lafontaine, Can. J. Biochem., 55, No. 7, 721 (1977).
17. M. Tazal and R. D. Cole, J. Biol. Chem., 252, No. 12, 4068 (1977).*
18. U. K. Laemmli, Nature (London), 227, 680 (1970).
19. J. D. Barret and E. W. Johns, J. Chromatogr., 75, 161 (1973).
20. V. G. Alfrey, V. G. Littay, and A. E. Mirsky, J. Cell. Biol., 21, 213 (1964).

*As in Russian original — Publisher.