In conclusion, our results do not support either Melnick's or our own previous assumption that any modification in the guanidine molecule is incompatible with its antiviral activity.

Riassunto. La 2-guanidino-pirimidina solfato e la 4-guanidino-2,6-dimetilpirimidina solfato inibiscono *in vitro* la moltiplicazione del poliovirus e del Coxsackie B_3 ma non quella del virus vaccinico e dell'adenovirus 5. L'analogia di comportamento con la guanidina è documentata oltre che da ciò, dalla esistenza di una resistenza e di una dipendenza crociata. Si dimostra cioè che non tutte le sostituzioni nella molecola della guanidina comportano una perdita dell'attività antivirale, come ritenuto in precedenza.

B. Loddo, A. Garzia, and W. Ferrari

Istituti di Farmacologia, Igiene e Microbiologia dell'Università di Cagliari (Italy), July 17, 1963.

The Specificity of Histones in Chicken Erythrocytes

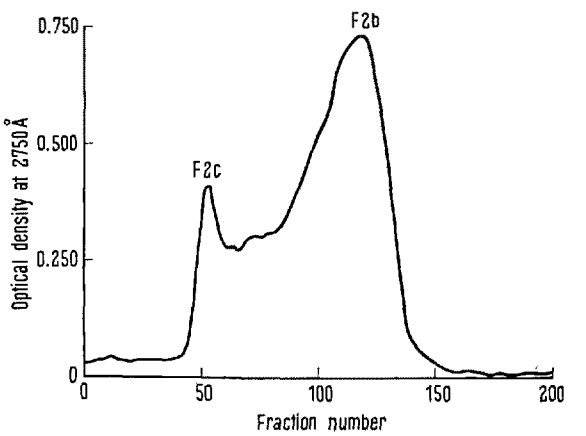
Because of their location in cell nuclei and their ability to interact strongly with deoxyribonucleic acid (DNA), histones may play a key role in the structural organization of DNA and may affect the regulatory functions of DNA. If histones act as gene inhibitors, as was suggested by Stedman and Stedman[1,2], then the histones from different species should show specificity of chemical composition and physicochemical behavior. However, species and cell specificity of histones is controversial; many histones prepared from different tissues of the Vertebrata genus are strikingly similar. Neelin and Butler[3,4] have reported specific elution and starch gel electrophoretic patterns for histones from different tissues of chicken, but gave no analytical data to support this claim of specificity. Stedman and Stedman[1,2] found that, when purified to constant composition, the main and subsidiary histones from the erythrocytes and thymocytes of the fowl differed in their arginine contents. In their more recent paper, Mauritzen and Stedman[5] reported the specificity of amino acid composition of the arginine-rich β-histones from erythrocytes, spleen and liver of domestic fowl. The differences, however, are rather small. To obtain more data, experiments on the isolation and purification of histones from chicken erythrocytes were initiated.

For the present study, fresh blood was collected from decapitated chickens and washed three times with 0.14 *M* NaCl containing 0.01 *M* trisodium citrate. The red cells were sedimented by centrifugation and then hemolyzed by freezing and subsequent thawing. Disrupted erythrocytes were homogenized in a Waring blendor with 0.14 *M* NaCl containing 0.01 *M* trisodium citrate and centrifuged[6]. This washing procedure was repeated several times until the supernatant fluid was clear. The yellowish sediment was then washed with 80% ethanol and extracted with ethanol-HCl and with 0.2 *N* HCl[6,7], yielding fractions F2aF3 and F1F2b, respectively.

The two main fractions, arginine-rich (F2aF3) and lysine-rich (F1F2b), were analyzed by electrophoresis in starch gel[6-8]. A pattern consistent with that described for the similar fraction in mammalian tissues, i.e. two main bands close to the origin (F3), and two bands with high electrophoretic mobility (F2a) resulted from the arginine-rich F2aF3 fraction electrophoresis. Electrophoresis of the lysine-rich group F1F2b revealed the presence of a band absent in mammalian tissue electrophoresis, located between the two sharp zones of very lysine-rich histone F1 on one side and the broader band of N-terminal proline fraction F2b on the other[6,7].

An attempt was made to separate this fraction represented by a band of intermediate electrophoretic mobility in starch gels from the F1 and F2b components. The whole F1F2b mixture was chromatographed on a carboxymethyl cellulose column (Cellex, Calbiochem) using potassium acetate buffer pH 4.2 as eluent[9]. The very lysine-rich fraction F1 was eluted with 0.33 *M* KCl, and the intermediate band emerged from the column together with the F2b fraction, suggesting their close similarity.

Further separation of the intermediate band protein from the F2b histone was achieved by chromatography on Sephadex G 75 (medium grade). Columns 2.6 × 120 cm were filled with the G 75 Sephadex made up in 0.01 *N* HCl. Protein, 200 mg in 2 ml of 0.01 *N* HCl, was applied to the column and HCl of the same normality was used as eluent, and 1.0 ml fractions were collected every 10 min. The separation of the F2b histone into two peaks is shown in the Figure. Fractions No. 42–62 and No. 95–140



Elution pattern for histones of chicken erythrocytes F2b after chromatography on Sephadex G 75 in 0.01 *N* HCl.

[1] E. Stedman and E. Stedman, Nature *166*, 780 (1950).
[2] E. Stedman and E. Stedman, Phil. Trans. Roy. Soc. B *235*, 565 (1951).
[3] J. M. Neelin and G. C. Butler, Canad. J. Biochem. Physiol. *37*, 843 (1959).
[4] J. M. Neelin and G. C. Butler, Canad. J. Biochem. Physiol. *39*, 845 (1961).
[5] C. M. Mauritzen and E. Stedman, Proc. Royal Soc. B *150*, 299 (1959).
[6] L. S. Hnilica and H. Busch, J. biol. Chem. *238*, 918 (1963).
[7] E. W. Johns and J. A. V. Butler, Biochem. J. *82*, 15 (1962).
[8] E. W. Johns, D. M. P. Phillips, P. Simson, and J. A. V. Butler, Biochem. J. *80*, 189 (1961).
[9] L. S. Hnilica, C. W. Taylor, and H. Busch, Exp. Cell Res., Suppl. *9*, 367 (1963).

comprising the peaks F2c and F2b were pooled and analyzed. The amino acid analysis and electrophoresis in starch gel showed that the second peak was the F2b fraction; the first peak represented the intermediate band protein which was labeled F2c.

The F2b histone from chicken erythrocytes did not differ from the F2b histones of mammalian origin in amino acid composition, in N-terminal proline, or in starch gel electrophoretic pattern[9].

Amino acid composition and N-terminal amino acids of the lysine-rich histone fractions from chicken erythrocyte nuclei[a]

Fraction	F1	F2b	F2b	F2c	F2c
Amino acid	Amino acid composition	Amino acid composition	N-terminal amino acids	Amino acid composition	N-terminal amino acids
Lysine	25.1	16.5	2.9	21.0	12.7
Histidine	0.2	2.5	–	1.8	–
Arginine	2.6	7.0	–	10.6	–
Aspartic acid	4.0	4.2	–	2.3	–
Threonine	3.5	6.8	–	4.2	52.0
Serine	6.5	10.1	7.0	11.9	8.8
Glutamic acid	4.3	8.3	–	5.3	–
Proline	7.7	4.6	87.2	6.7	13.2
Glycine	7.7	6.5	1.7	5.4	3.0
Alanine	27.1	10.3	2.1	15.1	8.4
Valine	5.4	6.1	–	4.6	–
Methionine	–	1.3	–	0.2	–
Isoleucine	1.1	5.2	–	3.4	2.0
Leucine	4.3	5.4	–	4.7	
Tyrosine	0.5	3.4	–	1.7	–
Phenylalanine	0.5	1.4	–	0.7	–

[a] All values are expressed as moles/100 moles of all amino acids found. Amide was not determined.

The composition of the intermediate band protein labeled F2c is quite specific. It has relatively high contents of lysine, alanine, serine, and arginine (e.g. 21.0%, 15.1%, 11.9% and 10.6%, respectively) and its N-terminal amino acid is threonine (Table).

To our knowledge, threonine as N-terminal in histone has previously not been reported in the literature. The chief N-terminal amino acids of the mammalian histones had been found to be alanine and proline[10]. The biological significance of the threonine N-terminal F2c histone fraction in chicken erythrocytes is unknown at the present time; however, this fraction may become a useful biological marker in studies of developmental mechanisms[11].

Zusammenfassung. Isolierung, qualitative und quantitative Charakterisierung der Histone von Hühnererythrocyten wird beschrieben. Mittels Gelfiltration an Sephadex G 75 wurde eine Fraktion des Histons, charakteristisch für die Hühnererythrocytenzellkerne isoliert und analysiert. Diese Fraktion, als F2c bezeichnet, ist ein Teil des lysinreichen Histons F2, reich an Lysin, Alanin, Serin und Arginin (z.B. 21,0, 15,1, 11,9 und 10,6% respektive). Threonin konnte als N-terminale Aminosäure in dieser Fraktion festgestellt werden.

L. S. HNILICA

Department of Biochemistry, M. D. Anderson Hospital and Tumor Institute, University of Texas, Houston (U.S.A.), August 19, 1963.

[10] D. M. P. PHILLIPS, *Progress in Biophysics and Biophysical Chemistry* (Pergamon Press, London 1962), vol. 12, p. 211.

[11] *Acknowledgments.* This investigation was supported in part by the American Cancer Society Institutional Grant IN-27 and by the American Cancer Society Grant P-328D. The experiments reported in this paper were initiated at Baylor University College of Medicine, Department of Pharmacology. I should like to express my gratitude to Mr. C. W. TAYLOR for invaluable technical assistance.

On the Stability of the Guanine-Cytosine Hydrogen Bond

CHARGAFF and ZAMENHOF[1] have defined the molar extinction coefficient (E_p) of DNA as the absorbance of light of a specific wave length (usually at 260 mμ) in a cuvette having a 1 cm path length, relative to each gram-atom of phosphate per liter present in the sample. Since there is one atom of phosphorus for each base present in DNA, the E_p is the equivalent absorbance per base. This concept is particularly useful as a criterion for assaying the purity and nativeness of DNA preparations. It is known that the presence of protein in a preparation will cause the value to be lower than that of a deproteinized sample and that degradation by enzymatic activity can be revealed by a value higher than anticipated. Furthermore, use of the E_p is advantageous as a reference for studies on the hypochromism of DNA. For example, FREDERICQ et al.[2] have used the E_p as a reference to show that the spectrum of calf thymus in 0.1 *M* acetic acid at pH 3 is almost identical to the spectrum calculated for its constituent nucleotides, an increase at 260 mμ of 50%. In contrast, a number of investigations[3,4] have appeared in the literature recently pertaining to the melting transitions of microbial DNA which present their data in terms of relative absorbance. These studies characteristically show increases of only 30 to 45%, less than would be predicted on the basis of their constituent mononucleotides. The DNA of microorganisms varies from 25 to 75 mole % guanine + cytosine (G + C)[5] and the E_p of a native preparation will depend on its base composition. The purpose of this investigation has been to establish the systematic variation of E_p with base composition, as a reference for

[1] E. CHARGAFF and J. N. DAVIDSON, *The Nucleic Acids*, Chapters 10 and 14 (Academic Press, New York 1955).
[2] E. FREDERICQ, A. OATH, and F. FONTAIN, J. mol. Biol. *3*, 11 (1961).
[3] J. MARMUR and P. DOTY, Nature *183*, 1427 (1959).
[4] J. MARMUR and P. DOTY, J. mol. Biol. *5*, 109 (1962).
[5] N. SUEOKA, J. mol. Biol. *3*, 31 (1961).