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.

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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

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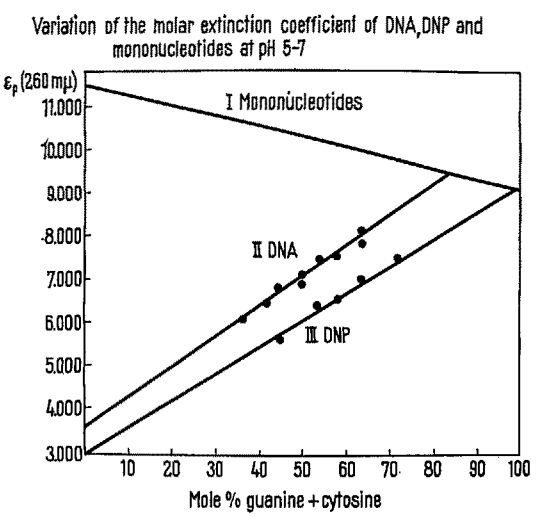
Extinction coefficients of DNA's from diverse sources

Source	G+C	E_p (260)	Designation	Reference
Yeast	36	6100	DNA	CHARGAFF et al.[6]
Calf thymus	42	6500	DNA	JORDAN[7] FREDERICQ et al.[3]
Drosophila eggs	42	6450	DNA	KIRBY[8]
Salmon sperm	42	6500	DNA	CHARGAFF et. al.[1]
Chicken blood	44	6810 5650	DNA DNP	This paper This paper
Escherichia coli	50	6900 7140	DNA DNA	CHARGAFF et al.[9] This paper
Avian tubercle bacilli	54	7500 6400	DNP DNA	CHARGAFF and SAIDEL[10] VISCHER et al.[11]
Achromobacter Sp.	54	6400	DNP	MASUI et al.[12]
Aerobacter aerogenes	58	6590 7570	DNA DNA	This paper This paper
Bovine tubercle bacilli	64	8200 7900 7000	DNA DNP DNA	TSUMITA and CHARGAFF[13] TSUMITA and CHARGAFF[13] TSUMITA and CHARGAFF[13]
Micrococcus lysodeikticus	72	7550	DNA	This paper

studies on the hypochromism of microbial DNAs and to correlate the results with theories regarding the hydrogen bonding of base pairs.

The Table presents a compilation of data for DNAs and DNPs of different base compositions. Values were determined for *Escherichia coli*, *Aerobacter aerogens* and *Micrococcus lysodeikticus* DNA isolated by the following procedure (see MARMUR[14]). A suspension of bacteria in 0.15 *M* NaCl was stirred for 10 min at 60°C with 10% recrystalized sodium dodecyl sulfate to produce a viscous solution. The solution was cooled to 5°C and 0.1 *M* versene pH 4.5 was added to yield a gel which was separated by centrifugation at 3000 *g* for 30 min. A fibrous precipitate was obtained by the addition of 0.54 vol of isopropanol to the supernatant. This product was further purified by treatment with ribonuclease, a repetition of the detergent procedure, and three alcohol precipitations. Optical densities were determined for samples in 0.3 *M* NaCl in a Beckman DB Spectrophotometer equipped with a Photovolt No. 43 logarithmic recorder. Phosphates were determined by the absorbance of their molybdate complex at 620 mμ in Elon buffer after a 1 h digestion of a $^1/_2$ ml sample at 195°C in 70% perchloric acid. This procedure yielded preparations of consistently high E_p values: 7550 for *M. lysodeikticus* and 7570 for *A. aerogenes*. Omission of the versene step resulted in a lower value for *A. aerogenes* (6590), and *M. lysodeikticus* (6270) but did not effect the value of *E. coli* (7140). Also included in the Table is the E_p of chicken blood nucleoprotein (DNP) isolated as described by JORDAN[7]. The same value (5650) is obtained by either the low ionic strength method or the method of Doty and Zubay. The E_p of these preparations was observed to increase to the value characteristic of the DNA (6810) after freezing and thawing, treatment with 1 *M* NaCl, or after standing in the refrigerator for one month.

The values are plotted graphically in the Figure. Line I graphs the extinction coefficient expected for a mixture of mononucleotides of the indicated base composition



Variation of the molar extinction coefficient of DNA, DNP, and mononucleotides at pH 5–7.

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taken from the data of FREDERICQ et al.[2]. The bottom two lines are regression lines calculated for the values which fall upon them. The mathematical formulae for these lines are:

$$(E_p260)_1 = 11\,500 - 2325\ (G+C)$$
$$(E_p260)_2 = 6965\ (G+C) + 3595$$
$$(E_p260)_3 = 6250\ (G+C) + 2970$$

where (G + C) is the mole fraction of guanine + cytosine. Almost all the points on line II represent samples designated by the authors as DNA, and the 'goodness of the fit' of the line is sufficient to conclude that the samples falling upon it correspond to what is generally termed 'native DNA'. All the points on line III represent samples designated as DNP or DNA which have been inadequately deproteinized. It has already been mentioned that a sample designated as DNP may, by appropriate treatments, give a value characteristic of DNA.

The most likely cause of the hypochromism of DNA is the formation of hydrogen bonds between complimentary base pairs by dipole interaction[15]. The hydrogen bond between adenine and thymine, as postulated by WATSON and CRICK[16] differs considerably in strength from that between guanine and cytosine, which would lead one to expect that the E_p of native DNA would vary as a linear function of the base composition. That is to say, each of the two types of base pairs should contribute to the hypochromism in proportion to the strength of its hydrogen bond. The hypochromism is represented by the increment between line 1 and the observed values on lines 2 or 3. It is largest for a polymer consisting entirely of adenine and thymine residues and gradually diminishes as the G + C content increases. The extrapolated value for a polymer consisting entirely of guanine and cytosine residues is identical to its constituent nucleotides. One would predict from these data that helix formation would not occur under these conditions between polyguanylate and polycytidylate. (Polyguanylate has thus far defied synthesis *in vitro*.) This does not necessarily exclude the existence of a guanine-cytosine hydrogen bond, but its existence in the absence of some structural integrity conferred by presence of the nucleoprotein or by adjacent adenine-thymine bonding seems unlikely.

There has been a controversy for some time as to which base pair was most stable. MARMUR and DOTY[3] presented evidence that DNAs of high G + C content have higher melting transitions than DNAs of low G + C content and interpreted this observation as indicating the guanine-cytosine bond to be stronger. This interpretation was challenged by SCHUSTER[17] who found the guanine and cytosine residues of native DNA reactive to nitrous acid but not the adenine or thymine residues – evidence for a more stable adenine-thymine bond. DEVOE and TINOCO[15] derived mathematical equations from the dipole moments of the constituent bases which indicated that in water an adenine-thymine helix is more stable relative to the coils than is a guanine-cytosine helix. Evidence presented here from the evaluation of molar extinction coefficients corroborates the conclusions of SCHUSTER[17] and of DEVOE and TINOCO[15], but does not explain the melting behavior observed. It has generally been assumed that the melting transitions are a sole consequence of the separation of hydrogen bonded base pairs. This assumption has already been shown to be faulty by the melting transition of deuterated DNA[18]. The substitution of deuterium for hydrogen in proteins and polypeptides results in a significant change in their thermal transitions, as one would predict upon theoretical grounds, but the melting transition of deuterated DNA is virtually identical to that of the hydrogen containing species. Stabilizing forces other than hydrogen bonding must be involved. GEIDUSCHEK and HERSKOVITS[19] have suggested stable hydrophobic 'nuclei' as a result of local regions of high base content of the stronger of the two base pairs (which they assumed to be guanine + cytosine rather than adenine + thymine) while KIRBY[8] believes that small proteinatious units are bound to DNA by chelation of metal ion with the purine bases. Both of these theories are compatible with our results. The consistently lower E_ps found with DNAs which have been inadequately deproteinized and the difficulty in obtaining a completely deproteinized preparation of high G + C content (e.g. *M. lysodeikticus*, 72% G + C) certainly indicates that some protein is tenaciously bound to DNA by extraordinarily stable bonds, while the nature of the variation of E_p with base content supports the contention that regions high in adenine + thymine are further strengthened by the formation of guanine + cytosine bonds.

Zusammenfassung. Die Änderung des molaren Extinktionskoeffizienten natürlicher Desoxyribonucleinsäuren wurde untersucht in Abhängigkeit der Basenzusammensetzung. Aus den experimentellen Befunden wird geschlossen, dass die Wasserstoffbindung zwischen Cytosin und Guanin im Doppelhelix schwach ist und in wässriger Lösung ohne die stabilisierende Wirkung benachbarter Adenin-Tyminbindungen nicht vorliegt. Die mit der Nucleinsäure verbundenen Proteine liefern einen zusätzlichen Stabilisationsfaktor zur Guanin- und Cytosinbindung.

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The Degradation of Deoxyribonucleic Acid by L-Cysteine and the Promoting Effect of Metal Chelating Agents and of Catalase

It is known that mercapto compounds can exhibit a pro-oxidative effect[1] which is ascribed to the formation of hydrogen peroxide during their autoxidation[2]. Heavy metal salts are apparently powerful catalysts for the autoxidation of mercapto compounds[3]. In the experiments described, the pro-oxidative effect of cysteine and of cysteamine towards iron containing sodium deoxyribonucleinate was tested and the influence of metal chelating agents and of catalase on the latter effect was investigated.

In the Figure, results of experiments are presented which were carried out with a 0.07% w/v aqueous solution