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

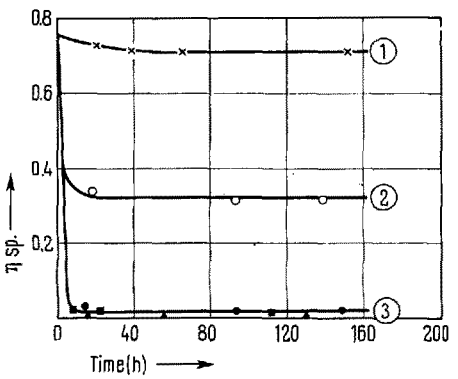
It is known that mercapto compounds can exhibit a pro-oxidative effect[1] which is ascribed to the formation of hydrogen peroxide during their autoxidation[2]. Heavy metal salts are apparently powerful catalysts for the autoxidation of mercapto compounds[3]. In the experiments described, the pro-oxidative 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

of DNA[4]. The degradation of the DNA was checked by viscosity measurements[4]. Curve 1 shows that addition of 0.1% cysteamine hydrochloride to the DNA solution has no effect on its viscosity, whereas the same amount of L-cysteine causes a distinct decrease of the viscosity (curve 2). The viscosity decrease caused by 0.1% L-cysteine is strongly enhanced by the presence of either 0.01% desferrioxamine B[5] or 0.01% E.D.T.A.[6] or 0.001% catalase[7] (curve 3)[8].

According to the experimental results presented, there is a substantial difference between cysteine and cysteamine with respect to their pro-oxidative effect towards DNA. This result may have some relation to the fact that cysteamine, which causes no DNA degradation in our system, is a much better radioprotective agent than cysteine[9].

The promoting effect of the two metal chelating agents mentioned and of catalase towards the DNA degradation by cysteine is very surprising, since the same chelating agents and catalase are strong inhibitors with respect to DNA degradation when hydrogen peroxide is used as the degrading agent[10,11]. Apparently the formation of strong oxidizing agents[1,2] in the course of the autoxidation of cysteine is favoured by the presence of certain chelating agents[12] and by catalase[13].



Effect of mercapto compounds on the specific viscosity of an aqueous solution of 0.07% DNA. (1) 0.1% cysteamine. (2) 0.1% L-cysteine. (3) 0.1% L-cysteine plus the following additives: ▲ 0.01% desferrioxamine B; ■ 0.01% E.D.T.A.; ● 0.001% catalase.

Zusammenfassung. L-Cystein bewirkt in wässriger Lösung unter aeroben Bedingungen einen Abbau von Desoxyribonucleinsäure (DNS), während Cysteamin in derselben Versuchsanordnung diese Wirkung nicht ausübt. Durch Katalase sowie durch die Chelatbildner Desferrioxamin B und E.D.T.A. wird der abbauende Effekt von Cystein gegenüber DNS wesentlich verstärkt. Es liegen somit gerade die umgekehrten Verhältnisse vor wie beim DNS-Abbau durch Wasserstoffperoxyd, welcher durch die erwähnten Chelatbildner und durch Katalase stark abgeschwächt oder sogar unterbunden wird.

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[4] A more detailed description of the experimental conditions is given in: K. Berneis, Helv. chim. Acta *46*, 57 (1963). – K. Berneis, M. Kofler, W. Bollag, A. Kaiser, and A. Langemann, Exper. *19*, 132 (1963). – All experiments and the viscosity measurements were carried out at 37°C.

[5] Desferal®, CIBA Basel (Switzerland).

[6] Ethylenediaminetetraacetic acid disodium salt dihydrate, supplied by Siegfried, Zofingen (Switzerland) (= Komplexon III).

[7] Supplied by Boehringer, Mannheim (Germany).

[8] A promoting effect on the DNA degradation by L-cystein is also exerted by 0.01% phenanthroline. A pro-oxidative effect of the latter additive in other systems has been noted by Tanner et al.[12].

[9] R. L. Straube and H. M. Patt, Proc. Soc. exp. Biol. Med. *84*, 702 (1953). [From J. F. Thomson, *Radiation Protection in Mammals* (Reinhold Publishing Corporation, New York 1962), p. 62.]

[10] K. Berneis, M. Kofler, and W. Bollag, Exper., *20*, in press (1964).

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[12] E. Tanner, W. Schuler, and R. Meier, Helv. chim. Acta *42*, 445 (1959), have investigated the pro-oxidative effect of E.D.T.A. and of other metal chelating agents. – According to Leussing et al.[3] the autoxidation of the iron salts of cysteine proceeds without the formation of oxidizing agents as intermediates. – The iron-catalysed reaction between cysteine and hydrogen peroxide has been investigated by R. G. Neville, J. Amer. chem. Soc. *79*, 2456 (1957).

[13] It is known that catalase in combination with mercapto compounds is capable of oxidizing formate: H. Aebi and E. Frei, Helv. chim. Acta *41*, 361 (1958). – E. Boeri and R. K. Bonnichsen, Acta chem. scand. *6*, 968 (1952). – See also H. Aebi and A. Temperli, Helv. physiolog. Acta *19*, 48 (1961).

The Variation of Adenosine Triphosphate and Adenosine Triphosphatase in *Tribolium confusum*, Duval at the Different Stages of its Life Cycle[1]

Recently Chowdhury and Lemonde[2] reported the results of their determination on the contents of total phosphorus, inorganic phosphorus etc., in *Tribolium confusum*, Duval at the different stages of its growth and development. The determination of total phosphorus or inorganic phosphorus without any knowledge of ATP content and ATPase activity, has much less significance in under-

[1] This work was supported by a grant (A-1388) received from the National Research Council of Canada.

[2] K. D. Chowdhury and A. Lemonde, personal communication.