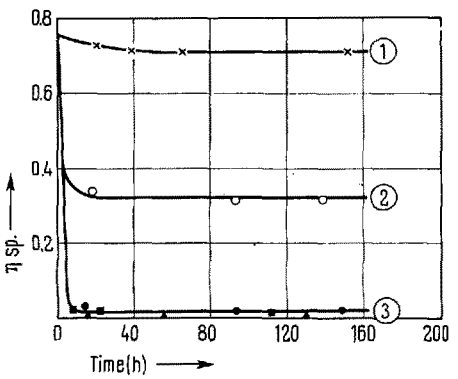
of DNA[4]. The degradation of the DNA was checked by viscosity measurements[4]. Curve 1 shows that addition of 0.1% cysteamine hydrochloride to the DNA solution has no effect on its viscosity, whereas the same amount of L-cysteine causes a distinct decrease of the viscosity (curve 2). The viscosity decrease 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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Physikalisch-chemische Forschungsabteilung, F. Hoffmann-La Roche & Co. AG, Basel (Switzerland), October 7, 1963.

[1] P. Holtz and G. Triem, Z. physiol. Chem. *248*, 5 (1937). – P. Alexander, Z. M. Bacq, S. F. Cousens, M. Fox, A. Herve, and J. Lazar, Radiation Res. *2*, 404 (1955). – D. L. Gilbert, Radiation Res., Suppl. *3*, 49 (1963).

[2] P. Holtz and G. Triem, Z. physiol. Chem. *248*, 1 (1937). – O. Schales, Ber. dtsch. chem. Ges. *71*, 447 (1938). – D. L. Gilbert, R. Gerschman, J. Cohen, and W. Sherwood, J. Amer. chem. Soc. *79*, 5677 (1957).

[3] S. Sakuma, Biochem. Z. *142*, 68 (1923). – O. Warburg, Biochem. Z. *187*, 255 (1927). – L. Michaelis, J. biol. Chem. *84*, 777 (1929). – K. A. C. Elliott, Biochem. J. *24*, 310 (1930). – N. W. Pirie, Biochem. J. *25*, 1565 (1931). – M. Schubert, J. Amer. chem. Soc. *54*, 4077 (1932). – D. L. Leussing, J. P. Mislan, and R. J. Goll, J. phys. Chem. *64*, 1070 (1960).

[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).

[11] The degradation of iron-containing DNA by hydrogen peroxide can be inhibited by the addition of cysteine [K. Berneis, M. Kofler, W. Bollag, P. Zeller, A. Kaiser, and A. Langemann, Helv. chim. Acta *46*, 2157 (1963)] whereas either of the two agents alone will degrade the DNA. The inhibiting effect of cysteine towards hydrogen peroxide or *vice versa* can be completely eliminated by the addition of either of the above mentioned chelating agents.

[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.

standing phosphorus metabolism in any living cell, since it is the energy liberated on ATP hydrolysis, drives all the energy requiring processes of life. Since the hydrolysis of ATP is catalysed by ATPase, we realized the importance of undertaking a systematic investigation on the variation of ATP concentration and ATPase activity in *Tribolium confusum*, Duval at the various stages of its life cycle[3,4], and the results are reported in this communication. In the first part of this communication the conditions for optimum ATPase activity determination have been described.

A definite weight, usually from 80–100 mg, of 12–13-day-old larva was washed twice with 0.85% cold saline and then a 1.0% homogenate in cold glass-distilled water was prepared in an Elvehjem-Potter homogenizer at 0°C, filtered through 6 layers of fine cheese cloth, then subjected to freezing and thawing three times and finally diluted with an equal volume of cold water. It was again homogenized in an all-glass homogenizer and subsequently used for the determination of ATPase activity. The insect used in this work was taken from a pure stock continually reared for the last ten years on a diet composed of wheat flour, previously passed through a No. 80 sieve, and 5% dried brewer's yeast. The cultures were kept in a room maintained at 28 ± 1°C and at constant humidity of 70 ± 5%. The ATPase activity was determined according to the method previously described by SARKAR, SZENT-GYÖRGYI and VARGA[5] by measuring the 'P' liberated from ATP in a reaction mixture of 1 ml, containing 30 μmoles of tris, pH 8.5, 2.5 μmoles of Na-salt of ATP, 0.025 μmoles of EDTA, 1.25 μmoles of Mg^{++} or 2.5 μmoles of Ca^{++} and 0.5 ml of the diluted homogenate. The reaction mixture was incubated at 28 ± 5°C for 30 min and the reaction was terminated by adding an equal volume of 10% cold TCA. On standing for 30 min at 2 to 4°C, it was centrifuged at 0°C for 20 min at 2500 r.p.m. The clear TCA-extract was used for 'P' determination. Phosphorus was determined by KING's[6] modification of Fiske and Subbarow's method, and protein by the method of LANG[7]. The ATPase activity was expressed as mg of 'P' liberated per 0.5 ml of the homogenate.

The results listed in the Table indicate that ATPase activity depends on either Mg^{++} or Ca^{++} and Mg-activated ATPase is 15–18% more active than Ca-activated ATPase under the best conditions of the experiment. These two metals have also been found to antagonize each other as is known to be the case with muscle ATPase. Addition of DNP at optimum concentration, enhances the ATPase activity by only 10–12%; it is, however, active over a wide range of its concentration. EDTA, on the other hand, suppresses the hydrolysis of ATP and at $2.5 \times 10^{-3} M$ concentration about 80–85% of ATP hydrolysis is inhibited. ADP and AMP are also hydrolyzed to the extent of 40–45% and 10–12% respectively, calculated on the basis of phosphorus, liberated when ATP acts as the substrate. α-Glycerophosphate is also hydrolyzed by the enzyme present in this homogenate, but only to a very limited extent. Optimum concentrations for Mg^{++} and Ca^{++} as found are $1.25 \times 10^{-3} M$ and $2.5 \times 10^{-3} M$ respectively. At concentrations above these, they both tend to inhibit the ATPase activity. Mn^{++} and Zn^{++}, can also to some extent activate the ATP hydrolysing enzyme, Ni^{++} on the other hand, blocks the ATP hydrolysis to the extent of 85–90%.

The pH-activity curve as shown in Figure 1 reveals that there are two pH optima for Mg-activated ATPase, one at pH 7.5 and the second one at pH 8.5. For Ca-activated ATPase there also appears to exist two pH optima; one at 7.5 and the other at 8.5. Although there are more than one pH optimum, the measurements of ATPase activity were carried out at pH 8.5 in all cases unless otherwise mentioned, since ATPase activity is greater at pH 8.5 than at pH 7.5.

Figure 2 shows that the rate of hydrolysis of ATP increases linearly with the increase in the substrate concentration, and using LINEWEAVER and BURK's equation[8]

ATPase activity in *Tribolium confusum*, Duval

mg of phosphorus liberated $\times 10^2$		mg of phosphorus liberated $\times 10^2$	
Complete system	54–56	Complete system	43–46
$-Mg^{++}$	8–10	$-Ca$	6– 7
$-ATP$	5– 6	$-ATP$	4– 6
$+EDTA$ (0.5 μmoles)	40–42	$+EDTA$ (0.5 μmoles)	34–36
(2.0 μmoles)	9–10	(2.0 μmoles)	6– 8
$-ATP + ADP$ (5 μmoles)	22–25	$-ATP + ADP$ (5 μmoles)	18–20
$-ATP + AMP$ (5 μmoles)	5– 6	$-ATP + AMP$ (5 μmoles)	3– 5
$+Mn^{++}$ (2 μmoles)	36		
$+Zn^{++}$ (2 μmoles)	40		
$+Ni^{++}$ (2 μmoles)	2.6		

The complete system contains ATP 5 μmoles, Mg^{++} (as chloride) 1.25 μmoles, or Ca^{++} (as chloride) 2.5 μmoles, EDTA 0.025 μmoles (routinely added), tris buffer pH 8.5, 20 μmoles and 0.5 ml of 0.5% homogenate of 12 to 14 days old larva, previously subjected to freezing and thawing three times before use, incubated at 28 ± 0.5°C for 30 min. The reaction was terminated by adding 10% cold TCA.

Abbreviations: ATP—adenosine triphosphate, ATPase—adenosine triphosphatase, ADP—adenosine diphosphate, AMP—adenosine monophosphate, DNP—2,4-dinitrophenol, EDTA—ethylenediaminetetraacetate, trishydroxymethyl aminoethane.

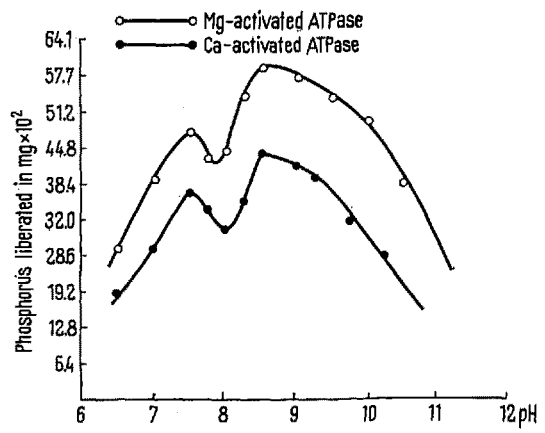

Fig. 1. The conditions of the experiment were the same as described at the bottom of the Table, except that buffers of same ionic strength but of different pH were used.

[3] The life cycle of *Tribolium confusum*, Duval is divided into five well defined phases such as: (a) egg (−6 to 0 days), (b) larval stage (0 to 13 days), (c) prepupal stage (14 to 17 days), (d) pupal stage (18 to 21 days), and (e) adult stage.

[4] ANIMA DEVI, A. LEMONDE, UMA SRIVASTAVA, and N. K. SARKAR, Exp. Cell. Res. *29*, 443 (1963).

[5] N. K. SARKAR, A. E. SZENT-GYÖRGYI, and L. VARGA, Enzymologia *14*, 288 (1950).

[6] E. J. KING, Biochemical J. *26*, 292 (1932).

[7] C. A. LANG, Anal. Chem. *30*, 1692 (1958).

[8] H. LINEWEAVER and D. BURK, J. Amer. chem. Soc. *56*, 658 (1936).

the value for Km, the Michaelias-Menten constant, was found to be at $0.07 \times 10^{-3} M$ of the substrate.

The changes in the ATPase activity measured at the various morphological stages of the insect, from egg to adult stage, appear to be inversely related to the variation of ATP concentrations at any one stage of its life cycle, as shown in Figure 3. The low ATP concentration in the larva is as should be expected, because during this period synthesis of RNA, proteins and lipids are known to occur most rapidly[9] and these energy-requiring processes of life derive the necessary energy from ATP hydrolysis and hence ATP concentration cannot be high. This is also the period of most active growth of the insect[4]. During the latter period of the larval stage, the insect is most active, and therefore requires energy for such purposes. This energy comes from ATP hydrolysis. The ATPase activity is therefore expected to be high during this period, which has been found to be indeed the case. During the pupal period the insect does not eat or excrete any waste product of metabolism to any measurable extent, and during this period there is no appreciable change in the weight of the insect. Naturally, therefore, the ATPase activity should be comparatively low, and that is what has been found. In conclusion, it can be said that the hydrolysis of ATP by ATPase occures most extensively during the period when the insect grows most actively. ATP concentration is low during the most active growth period, as during this period ATP is extensively used up for such synthetic reactions as RNA, proteins, lipids etc. essentially required for the growth of the insect.

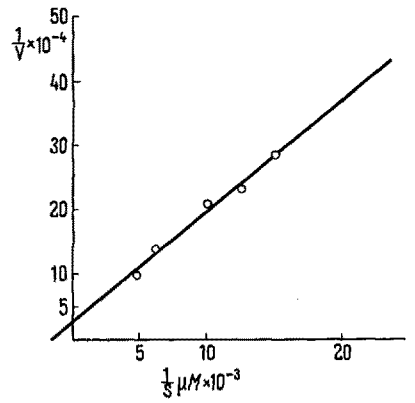

Fig. 2. The conditions of the experiment were the same as described in Figure 1, the only difference was that different concentrations of ATP were used.

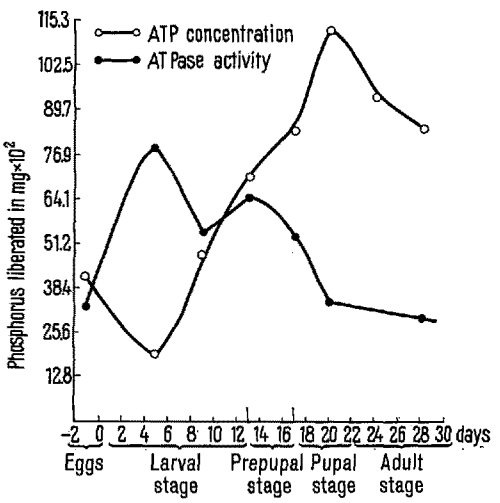

Fig. 3. The conditions of the experiment were the same except that in these experiments homogenates of insects, at various stages of the life cycle from egg to adult stage, were used as enzyme source. The variation of ATP concentration actually represents the 7 min's hydrolyzable phosphorus obtained by subtracting the total inorganic phosphorus (including arginine 'phosphorus') from the phosphorus measured after heating the TCA extract in boiling water for 7 min. It represents all the energy-rich phosphates, di- and tri-phosphates of pridine nucleotides, inosine acid phosphorus, etc. Since ATP is the major component, we have loosely used ATP instead of 7 min's hydrolyzable phosphorus in the text.

Résumé. Un homogénat brut de *Tribolium confusum* Duval peut hydrolyser les molécules d'ATP, d'ADP, d'AMP et d'α-glycérophosphate. L'activité de l'ATPase dépend des ions Mg^{++} et Ca^{++}. La courbe illustrant les variations de l'activité de cette ATPase en fonction du pH, montre deux pH optima, l'un à 7.5 et l'autre à 8.5. L'activité de l'ATPase est élevée durant la période larvaire, alors que la croissance est active et elle est faible durant la période pupale. La concentration de l'ATP est toujours inversement proportionnelle à l'activité de l'ATPase et cela à tous les stades du cycle vital de l'insecte.

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[9] A. DEVI, P. LINDSAY, and N. K. SARKAR, Exper. *19*, 344 (1963).

Über Peptidsynthesen. C-terminale Teilsequenzen des Eledoisins und eledoisinanaloger Verbindungen[1,2]

Bei den bisher bekannt gewordenen blutdruckwirksamen Peptiden wie Angiotensin, Bradykinin oder Kallidin bewirkt der Fortfall von mehr als zwei Aminosäuren stets einen Verlust der Aktivität. Anders verhält sich das Eledoisin (zur Isolierung und Strukturaufklärung dieses Wirkstoffes vgl. [3,4], über eine Synthese vgl. [5], über biologische Eigenschaften vgl. [6–8]). N-terminale Teilsequenzen des Eledoisins sind unwirksam[2]. Dagegen zeigen C-terminale Hexa- und Heptapeptide der originalen Sequenz sowie eledoisinanaloger Verbindungen zum Teil eine erstaunlich hohe biologische Aktivität.

Bei den vorliegenden Teilsequenzen wurde der Asparaginsäurerest in Position 5 gegen Asparagin, Glutamin, Pyroglutaminsäure und Glycin, der Isoleucinrest in Position 8 gegen Valin und Leucin sowie der Methioninrest in Position 11 gegen Alanin ausgetauscht.