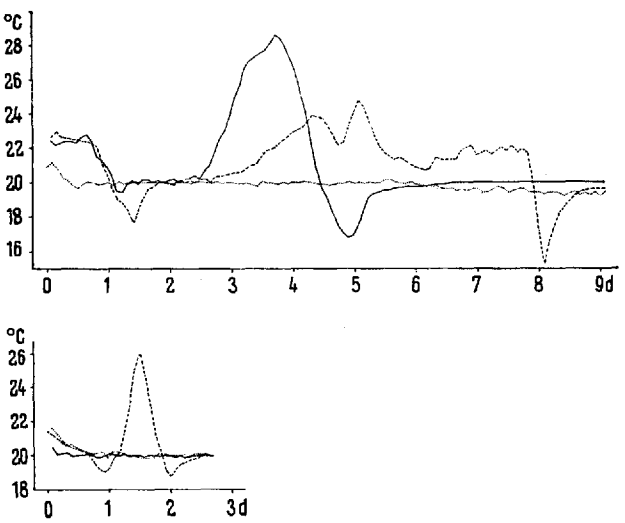
cescens verursachten Erkrankung gelegentlich einen geringfügigen Extremitätentremor.

Für unsere Versuche verwendeten wir ausschliesslich männliche Imagines. Die Tiere wurden vor Versuchsbeginn bei Zimmertemperatur gehalten. Die Applikation der Erreger (kultiviert auf Merck-Standard-I-Nähragar) erfolgte unter CO_2-Narkose intrapericardial (im Abdomen), um eine rasche Verbreitung der Bakterien im Wirt zu erreichen. Es wurde jeweils mit 1 bzw. 2 00-Insektennadeln Kultur infiziert. Auf eine genaue Dosierung verzichteten wir, da beim Punktieren des Rückengefässes häufig Hämolymphe austritt, die unkontrollierbare Erregermengen wieder ausschwemmt. Als Kontrollen dienten unbehandelte Tiere und «leer» punktierte. Während des Versuches befanden sich die Tiere in einem Thermostaten bei 20°C und 50% relativer Luftfeuchtigkeit. Sie waren einzeln an Nadeln mit Kolophoniumwachs thorakal fixiert. Spangengloben boten Laufmöglichkeit. Die Temperaturmessung erfolgte mit Hilfe von punktgeschweissten Thermoelementen (Kupfer/Konstantan). Der Durchmesser der Drähte betrug je 0,02 mm, wobei der Messspitzendurchmesser durch Säureeinwirkung auf 50 µ reduziert war. Die Meßspitze wurde in das 2. Thorakalstigma eingeführt, das Gegenthermoelement konstant bei 0°C gehalten. Ein Mehrfarben-Punkteschreiber registrierte zweistündlich die Thermospannung. Vor dem Versuch eichten wir die Spannungen der einzelnen Temperaturstufen ein, nach dem Versuch überprüften wir sie. Die Empfindlichkeit des Schreibers wurde so einreguliert, dass 1°C 12 mm entsprach. Die Temperaturregistrierung begann etwa 1 h *post infectionem* und erstreckte sich über maximal 10 Tage. Bei einem Teil der Versuche liessen wir zur Kontrolle die Thermostattemperatur mitschreiben. Die Versuche liefen meist an vier Tieren gleichzeitig (1 unbehandelte Kontrolle, 1 «leer» punktierte Kontrolle, 2 Versuchstiere).



Verlauf der Temperatur von gesunden und infektionskranken *Periplaneta americana* (jeweils Einzeltiere). Oben: ····· unbehandelte Kontrolle, ----- 1 Nadel *Serratia marcescens*, —— 2 Nadeln *Serratia marcescens*. Unten: —— unbehandelte Kontrolle, ····· «leer» punktierte Kontrolle, ----- 2 Nadeln *Pseudomonas aeruginosa*.

Bei sämtlichen infektionskranken Versuchstieren kam es nach einer Inkubationszeit von 1–2,5 Tagen zu einer signifikanten Erhöhung der Körpertemperatur, die bis zu einer maximalen Differenz von 12,6°C zur Umgebungstemperatur fortschreiten konnte. Wie aus der Figur (oben) zu ersehen ist, kann eine Erhöhung der Temperatur, wenn auch unter Schwankungen, bis zu einem Zeitraum von 5 Tagen beibehalten werden. Während der Inkubationsphase auftretende Temperaturunterschiede zur Umgebung vermögen wir noch nicht zu typisieren, sie sind teilweise auch bei Kontrolltieren zu beobachten. Da es durch die Narkose und sonstigen Manipulationen zu einer vorübergehenden Erregung der Tiere kommt, mögen sie zum Teil dadurch bedingt sein, zum Teil könnte es sich dabei auch um Verletzungsfolgen handeln. Die Akinese der Versuchstiere kann mit dem Höhepunkt der Temperaturkurve zusammenfallen, sie kann auch zu einem späteren Zeitpunkt erfolgen. Ein Tier, das eine Infektion mit *S. marcescens* überlebte, zeigte ebenfalls eine typische Temperaturerhöhung, die jedoch maximal lediglich 2,1°C betrug. Später kam es bei dem Tier wieder zu einer Normalisierung der Temperatur. Die in der Figur wiedergegebenen Temperaturkurven scheinen den von uns bereits in unseren Vorversuchen beobachteten akuteren Verlauf der durch *Ps. aeruginosa* verursachten Bakteriose im Vergleich zu der von *S. marcescens* hervorgerufenen zu bestätigen[9].

Summary. It is shown that two bacterial diseases of *Periplaneta americana* are accompanied by a significant increase of body temperature of the insect.

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[9] Die verwendeten Bakterienstämme verdanken wir Herrn Prof. Dr. DIMMLING vom Institut für Hygiene und Mikrobiologie der hiesigen Universität und Herrn Dr. O. LYSENKO vom Laboratory of Insect Pathology, Prag.

Phosphodiesterase from *Thiobacillus thioparus*

During studies of the nucleolytic enzymes in extract of the autotrophic bacteria *Th. thioparus*, on the basis of identification of the products of hydrolysis of yeast ribonucleic acid (RNA) and other observations, the presence of at least three different enzymes was established, *viz.* 5′-nucleotidase, phosphodiesterase and ribonuclease. Ribonuclease was isolated and its properties partly investigated[1]. In the present paper some properties of phosphodiesterase, which was separated from ribonuclease and partly purified, are reported.

Phosphodiesterase activity was determined, using Ca-[bis(*p*-nitrophenyl)-phosphate]$_2$ as substrate[2]. The reaction mixture contained 100 µl 0.01 *M* substrate in 0.1 *M* Tris-HCl buffer of pH 8.5, 100 µl 2×10^{-3} *M* solution of $MnSO_4$ and 10 µl of enzyme solution. After incubation at 37° for 60 min, the reaction was stopped by adding 2.8 ml of 0.1 *N* NaOH. The precipitate, if any, was centrifuged, and extinction of *p*-nitrophenol split off was determined at 400 mµ with the Uvispec spectrophotometer (Hilger Co., London). Enzyme activity was expressed in µ*M* of *p*-nitrophenol split off by 1 ml of enzyme solution at 37° during 60 min.

[1] W. OSTROWSKI and Z. WALCZAK, Acta biochim. Polon., in press.
[2] R. L. SINSHEIMER and J. F. KOERNER, J. biol. Chem. *198*, 293 (1952).

The moist mass of bacteria, growing on a standard medium previously described[3], was ground up with 5 vol 0.1 *M* citrate-phosphate buffer of pH 7.0 in a mortar with carborundum. After centrifuging, the supernatant was dialyzed overnight against distilled water and any precipitate formed was discarded. Protamine sulphate in aqueous solution was next added in amounts of 75 mg protamine/100 mg nitrogen in the extract. After centrifuging, the supernatant was then fractionated with ammonium sulphate, fraction precipitated between 0.25 to 0.55 saturation being retained. The ammonium sulphate fraction was first dialyzed against distilled water and then against 0.01 *M* Na-phosphate buffer of pH 7.6 and submitted to DEAE-cellulose column chromatography[4]. Elution was carried out with NaCl solutions of various concentrations buffered with 0.01 *M* phosphate buffer, pH 7.6. Phosphodiesterase activity was found to be concentrated chiefly in the fraction desorbed at 0.2 *M* NaCl at pH 7.6 (Figure 1). In peak 5 almost all 5′-nucleotidase activity present in ammonium sulphate fraction was concentrated. The fractions exhibiting the highest specific activity of diesterase when pooled contained approximatively 60% of the total activity adsorbed on the column and a 30-fold concentration as compared with crude extract. The preparation thus obtained is contaminated with 5′-nucleotidase causing hydrolysis of 5′-phosphonucleosides to the corresponding nucleosides. 3′-Phosphonucleosides are completely resistant to the action of this enzyme. The preparation of phosphodiesterase above described was used in further experiments.

Diesterase from the cells of *Th. thioparus*, in distinction to diesterase of snake venom[5,6], is strongly activated by Mn^{++} ions with the optimal concentration at 10^{-3} *M*, when bis(*p*-nitrophenyl)-phosphate is used as substrate.

[3] W. Ostrowski and A. Krawczyk, Acta biochim. Polon. *4*, 249 (1957).
[4] H. A. Sober and E. A. Peterson, Fed. Proc. *17*, 1116 (1958).
[5] G. Schmidt in *The Nucleic Acids* (Acad. Press Inc., Publishers, New York 1955), vol. I, p. 585.
[6] W. E. Razzell and H. G. Khorana, J. biol. Chem. *234*, 2105 (1959).

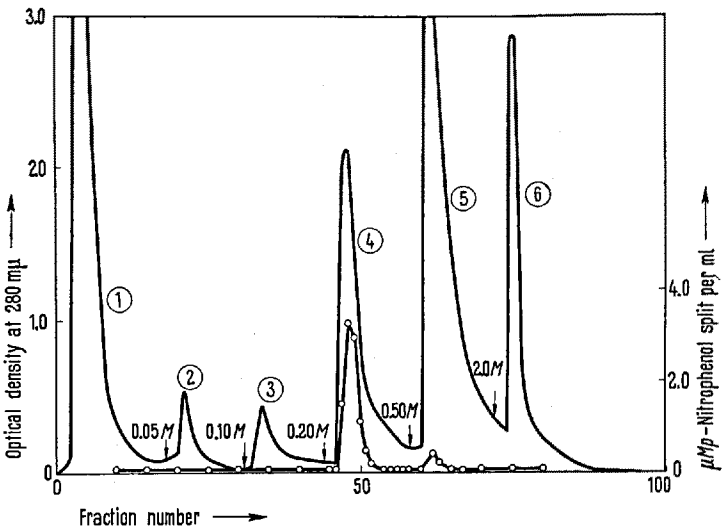

Fig. 1. Stepwise separation of ammonium sulphate fraction on DEAE-cellulose column. The sample (600 E_{280} units) and column (1.4 × 18 cm) were separately equilibrated with 0.01 *M* Na-phosphate buffer, pH 7.6. The elution was performed by passing NaCl solutions of various concentrations buffered with 0.01 *M* phosphate buffer, pH 7.6. Fractions (9 ml) collected at 120 ml/h, temperature 5° —— absorbancy at 280 mμ, o——o phosphodiesterase activity.

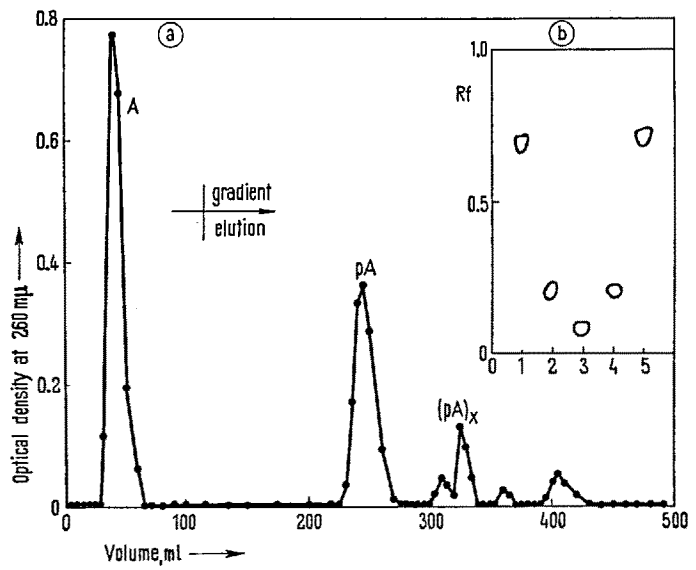

Fig. 2. (a) DEAE-cellulose chromatography of the products of hydrolysis of poly A. Poly A, 12 mg, was incubated in 0.05 *M* Tris-HCl buffer, pH 8.5 with 100 μl phosphodiesterase solution of an activity of 30 μ*M* *p*-nitrophenol/ml in a total volume 1.0 ml for 8 h at 37°. The reaction mixture was deproteinized (isoamyl alcohol-chloroform) and chromatographed on a DEAE-cellulose column (1 × 18 cm). After washing the column with about 100 ml of 0.01 *M* NH_4HCO_3, pH 8.6, the gradient elution was started. The gradient system consisted of a mixing chamber containing 250 ml of 0.01 *M* NH_4HCO_3, pH 8.6, and from the reservoir first 0.05 *M* ammonium bicarbonate, then 0.5 *M*, pH 8.6, was slowly dropped. A flow rate of 1 ml/min was maintained and 5 ml fractions were collected. •——• absorbancy at 260 mμ. A = adenosine, pA = adenosine-5′-phosphate, (pA)x = oligonucleotide, non-identified. – (b) Reproduction of chromatogram illustrating the Rf values of the hydrolysis products of poly A, recovered from the DEAE-cellulose column. The pooled fractions from the column were dried in vacuum, dissolved in water and chromatographed in solvent system: ethanol-1 *M* ammonium acetate, 75:30 v/v, pH 7.5, overnight. The spots on the filter paper were photographed in UV light. – (1) A; (2) pA; (3) (pA)x; (4) (pA)x after hydrolysis with snake venom diesterase for 1 h at pH 8.6; (5) pA after hydrolysis with 5′-nucleotidase for 1 h at pH 8.5.

In contrast to this, Zn^{++} ions at the same concentration inhibit its activity by approximatively 80%. At about five-fold higher concentration of Mn^{++}, the enzyme inhibited by zinc ions regains its original activity, indicating competition between the two ions. Other bivalent metal ions possess non-specific action on the enzyme activity, either inhibition (Ca^{++}, Cu^{++}) or activation (Mg^{++}, Fe^{++}, Co^{++}), but only to a slight degree. Phosphate ions in a concentration of 0.1 *M* decrease the enzyme activity by 70%. In the presence of Versene (0.02 *M*), activity is lowered to 1/7 of that originally present. The optimum pH in the presence of Mn^{++} ions lies at about pH 9.0, and at pH 8.5 in the absence of the activator.

During electrophoresis on a starch block at pH 8.6 diesterase moves toward the anode, indicating the acid character of the enzyme. Most of the inactive proteins are separated from the diesterase activity.

The enzyme is relatively stable at alkaline pH. A loss of approximatively 30% of its activity occurs when it is stored in 0.1 *N* NaOH at 0°, and 80% at 37° during 1 h. In an acid solution (pH less than 4.0) the enzyme is quickly inactivated. It is also thermolabile, losing 4/5 of its original activity when heated to 70° at pH 6.5 for 5 min. Appreciable loss of activity does not take place when the partially purified enzyme is stored in the frozen state (– 12°) for several months and repeatedly thawed and frozen.

Phosphodiesterase causes hydrolysis of yeast RNA, RNA isolated from *Th. thioparus* cells (RNA-Th) by phenol extraction[7], DNA from the thymus (commercial) and the 'core' of yeast RNA obtained by exhaustive digestion of RNA with pancreatic ribonuclease[8]. If one of the above-mentioned highly polymerized polynucleotides is used as the substrate, Mn^{++} ions do not activate the enzyme. The fastest rate of hydrolysis is observed with RNA-Th, and the slowest with the 'core'. The enzyme also digests synthetic polynucleotides[9] such as polyadenylic acid (poly A) and other purine and pyrimidine 3′,5′-oligo-nucleotides. The hydrolysate of poly A was subjected to chromatography on DEAE-cellulose column[10]. The elution pattern of the hydrolysis products present is shown in Figure 2a. As the main products, adenosine (46%) and adenosine-5′-phosphate (31%) were found besides small amounts of oligonucleotides. The adenosine is the result of the presence of 5′-nucleotidase in the preparation of diesterase, as mentioned before. Adenosine and adenosine-5′-phosphate were identified by paper chromatography in solvent system: ethanol-ammonium acetate[11] and Rf values in Whatman 3 MM paper were 0.70 and 0.21 respectively. Adenosine-5′-phosphate, recovered from the column when incubated with 5′-nucleotidase isolated from the cells of *Th. thioparus*, was quantitatively transformed to adenosine (Figure 2b). Adenosine-3′-phosphate was completely resistant to this enzyme.

The fraction emerged from the column at higher concentrations of ammonium bicarbonate (labelled (pA)x in the Figure 2a) was first chromatographed on paper in the above solvent system (the Rf was determined to be 0.09), eluted from the paper, incubated with purified snake venom diesterase[12] and then rechromatographed under the same conditions. The spot of adenosine-5′-phosphate was only found (Figure 2b). Because the snake venom diesterase is almost without activity on oligonucleotides with 3′-phosphomonoester end groups[13], it must be assumed that oligonucleotides formed by diesterase from *Th. thioparus* are terminated with 5′-phosphomonoester end groups.

From results reported above it can be concluded that the enzyme from *Th. thioparus* splits off the diester linkages of phosphoric acid in position 3′ of polynucleotide chain. During the initial stage of the enzymic action in the presence of an excess of substrate, chiefly 5′-mononucleotides and small amounts of oligonucleotides were found. Hence, it must be assumed that phosphodiesterase causes gradual degradation of polynucleotide liberating 5′-phosphonucleotides[14].

Investigations on further purification of the enzyme and its specificity are under way and will be published elsewhere.

Résumé. La phosphodiestérase a été isolée des cellules du *Th. thioparus* et partiellement purifiée. Cette enzyme hydrolise les polynucléotides jusqu'à 5′-mononucleotides. Elle est spécifiquement activée par les ions du Mn^{++} et inhibée par les ions du Zn^{++}. Son pH optimum est compris entre 8,5–9,0. L'enzyme est thermolabile et relativement résistante en milieu alcalin.

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Department of Physiological Chemistry, Medical Academy, Cracow (Poland), February 1, 1961.

[7] K. S. KIRBY, Biochem. J. *64*, 405 (1956).
[8] R. J. HILMOE, J. biol. Chem. *235*, 2117 (1960).
[9] The author is indepted to Dr. D. SHUGAR for a preparation of synthetic polynucleotides, poly A, poly G, and poly U.
[10] M. STAEHELIN, E. A. PETERSON, and H. A. SOBER, Arch. Biochem. Biophys. *85*, 289 (1959).
[11] R. BERGKVIST, Acta chem. scand. *11*, 1465 (1957).
[12] F. FELIX, J. L. POTTER, and M. LASKOWSKI, J. biol. Chem. *235*, 1150 (1960).
[13] L. A. HEPPEL, P. J. ORTIZ, and S. OCHOA, J. biol. Chem. *229*, 679, 695 (1957).
[14] I wish to express my best thanks to Professor B. SKARŻYŃSKI for his help and encouragement.

Investigations on Auxin-Gibberellin Interaction on the Growth Mechanism of *Helianthus annuus* Seedlings

The problem of auxin-gibberellin interaction on the mechanism of growth process could be discussed from two different points of view. The aim of the present research is to verify the validity of these two ideas and hence to explain the nature of this interaction on the growth of *Helianthus annuus* seedlings.

Method. The germinating achenes of *Helianthus* were sown in glass tubes containing a mixture of soil and washed sand; these were placed on a vertical klinostat under daylight. After a period of 6 days, the straight hypocotyls, of length approximately 5 cm, were selected to be decapitated. The decapitation was done exactly from the base of the cotyledons which are known to serve as auxin sources to the developing plant. These seedlings were then left in complete darkness for a period of 10 h, to ensure the thorough removal of all endogenous auxins. After this procedure, capillary tubes of length 2.5 cm containing 10^{-5} Mol isomolecular solutions of indole acetic acid (IAA), naphtalene acetic acid (NAA) and gibberellin (GB) respectively, were placed on the apex of the hypocotyls. As control sets, hypocotyls bearing capillary tubes with distilled water were used. For the measurements of the combined action of GB with IAA and NAA, a mixture of equal volumes of isomolecular (10^{-5} Mol) solutions of