comptés à partir de la pointe de la racine; les vieux tissus formant la coiffe étant éliminés) du DCP, du DNP, du DNC et du Mn^{++} est étudiée en fonction de leurs concentrations. Le pH, dont l'importance a été démontrée[14] est rigoureusement maintenu à 6,1, valeur qui correspond au maximum d'activité des auxines-oxydases[15]. On peut observer que le Mn^{++}, à fortes concentrations, inhibe, alors que le DCP, le DNP et le DNC activent toujours, mais plus ou moins fortement, les auxines-oxydases.

Essai 3 (Fig. 3): L'action combinée du DCP et du Mn^{++}, pour des tissus méristématiques (0,5 à 3,0 mm du sommet) est examinée. On peut remarquer que si le Mn^{++} est un inhibiteur des auxines-oxydases lorsqu'il est appliqué seul ou avec de faibles concentrations de DCP, son action devient nettement stimulatrice en présence de fortes doses de DCP. Ces résultats confirment donc, sur un autre matériel, les observations antérieures et suggèrent que le Mn^{++} doit agir essentiellement, en ce qui concerne l'inactivation de l'ABIA, en relation directe avec les composés phénoliques des tissus.

L'emploi de ces diverses substances fournit des perspectives nouvelles quant au problème du vieillissement des tissus qui paraît être placé sous le contrôle des auxines endogènes[16] et par conséquent des systèmes auxines-oxydasiques.

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Summary

2,4-dichlorophenol (DCP), 2,4-dinitrophenol (DNP) and 2,4-dinitro-*o*-cresol (DNC) applied at a concentration of $1 \cdot 10^{-7}$ to $1 \cdot 10^{-3}$ *M*, produce a dramatic increase in IAA-oxidase activity in young cells of *Lens* root. IAA destruction by 'breis' from root tissues was inhibited by Mn^{++} ion at low concentrations of DCP ($1 \cdot 10^{-\infty}$ to $1 \cdot 10^{-7}$ *M*), but was enhanced by Mn^{++} ion at higher ($1 \cdot 10^{-5}$ to $1 \cdot 10^{-3}$ *M*) DCP levels.

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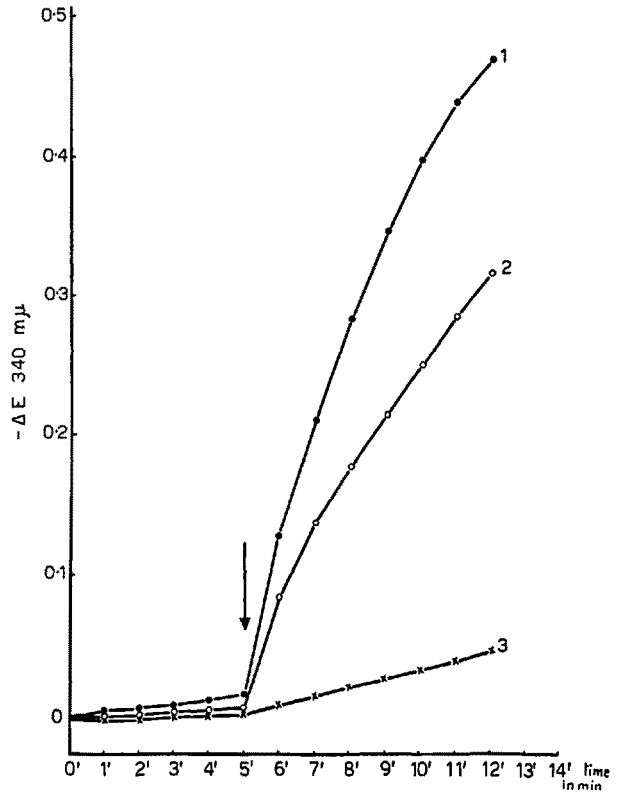
Enzymatic Activities in the Perilymph Phosphohexoseisomerase and Lacticdehydrogenase

In some previous papers on the chemical composition of horse perilymph, we reported data on the amino acid, keto acid and protein content of this biological fluid[1].

The object of the present investigation was to study whether enzymatic activities could be detected in the perilymph. For this purpose we have analyzed phosphohexoseisomerase (PHI) and lacticdehydrogenase (LD) activities of horse perilymph compared with the blood serum and cerebro-spinal fluid activities[2].

Methods. The perilymph, serum and liquor were collected as previously described from horses immediately (15–30 min) after death. In order to obtain the necessary amount of perilymph, samples of this fluid from 25/30 animals were mixed together. The same operation was performed, to obtain comparable results, for serum and liquor. Enzymatic activities were determined in duplicate on fluids thus collected from three groups of animals. The liquids, stored when necessary at 0°C for a maximum of 24 h, were centrifuged before use at 3000 r.p.m. for 20 min.



Lacticdehydrogenase activity of serum (1), perilymph (2), and liquor (3). Same conditions as in the test; in this experiment the reaction was started by adding (at arrow) the piruvate.

PHI activity was tested according to the reaction: glucose-6-phosphate (G6P) ⟶ fructose-6-phosphate (F6P), determining the amount of F6P formed in 1 h at 37°C in an incubation mixture made up as follows: 0·3 ml 0·045 *M* G6P (4·5 mg K salt, obtained according to Seegmiller from Ba salt 'Sigma')[3]; 0·3 ml sodium diethylbarbiturate buffer 0·1 *M*, pH 7·8; 0·2 ml of the fluid under examination.

The reaction was stopped by adding 3 ml of 10% trichloroacetic acid. After centrifugation, 2 ml of the supernatant were used for the F6P determination, by Roe's method[4].

A standard of fructose was made each time under the same experimental conditions, and corrections for F6P values were made according to Umbreit *et al.*[5]

LD activity was followed according to the reaction:

$$DPNH + \text{piruvate} + H^+ \rightarrow DPN^+ + \text{lactate}$$

determining the amount of DPNH oxidized from the decrease in optical density at 340 mμ.

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The DPNH was prepared from DPN 'Sigma' by enzymatic reduction with alcohol and alcohol dehydrogenase, crystallized according to RACKER[6].

Table I

PHI activity of perilymph, liquor and serum expressed in mg F6P formed per hour

	Perilymph	Liquor	Serum
mg F6P/ml/h .	2·51 ± 0·1	0·53 ± 0·07	1·08 ± 0·04
mg F6P/mg protein/h . .	0·82 ± 0·016	0·36 ± 0·05	0·016 ± 0·001

In a cuvette of the Beckman spectrophotometer were mixed: 0·48 μM DPNH in 0·1 ml; 3 μM piruvate in 0·1 ml; 0·3 ml $NaHCO_3$ 0·02 Mol; 2·5 ml phosphate buffer 0·1 Mol, pH 7·8. The reaction was started by adding 0·25 ml of the fluid under examination, and the decrease in optical density followed, at room temperature, for 10 min, taking readings every minute.

Proteins were determined in the fluids before each enzymatic test by GOA's micromethod[7].

Table II

LD activity of perilymph, liquor and serum expressed in Mol × 10^7 lactate formed per minute

	Perilymph	Liquor	Serum
Mol × 10^7 lactate/ml/min .	1·75 ± 0·07	0·22 ± 0·03	2·63 ± 0·2
Mol × 10^{-7} lactate/mg protein/min . .	0·58 ± 0·03	0·15 ± 0·025	0·04 ± 0·003

Results. In Table I and II the values for PHI and LD activities in perilymph, liquor and blood serum are reported. From these data it is evident that PHI and LD activities are present in the perilymph to a considerable degree. With reference to the protein content, much higher activities are found for perilymph than for liquor and serum. The activities for milligrams of protein are in perilymph, liquor and serum in the ratio 100:44:2 for PHI, and 100:26:7 for LD.

These results contribute to differentiate the perilymph from both blood serum and cerebro-spinal fluid.

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Institute of Biochemistry and Institute of Oto-rhino-laringology, University of Rome, July 20, 1956.

Riassunto

È stata messa in evidenza nella perilinfa di cavallo la presenza di notevole attività fosfoesosoisomerasica e latticodeidrogenasica. In rapporto al contenuto proteico tali attività enzimatiche risultano maggiori nella perilinfa che nel siero di sangue e nel liquor.

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Physical Properties of Deoxyribonucleic Acid Isolated from Ehrlich Ascites Tumor Cells by Various Methods

The requirement for tumor cell deoxyribonucleic acid (DNA) to be used in certain biological studies in progress in this laboratory led to an examination of various methods for the isolation of this cell component.

The Ehrlich ascites carcinoma was employed throughout the investigations. DNA has been isolated from cells of this tumor by four different methods, two of which involved the use of surface active agents. In addition, DNA has been separated from deoxyribonucleoprotein isolated from this tumor by means of chloroform-octyl alcohol and by extraction with hot concentrated salt solution.

All samples were subjected to analysis for nitrogen and phosphorus, and a number of the physical properties of all samples were measured. A description of these physical properties comprises the main subject of this report.

Experimental. The Ehrlich ascites carcinoma was grown in Swiss albino mice. Seven to nine days after the intraperitoneal implantation of 10–12 × 10^6 tumor cells, the mice were sacrificed and ascitic fluids aspirated from the peritoneal cavities. The cells were freed of erythrocytes by washing in physiological saline in the manner described by MCKEE, LONBERG-HOLM, and JEHL[1]. The washed cells were transferred to a mortar set in an alcohol-dry ice bath, and when thoroughly frozen were ground to a fine, homogeneous powder.

The powder was placed in a Waring blendor containing isotonic sodium chloride, pH 7·0 and 0·001 M with respect to sodium citrate, and was blended at low speed for 10–15 min. Nuclei were sedimented from the suspension by centrifugation at 2000 rpm for 20 min. The supernatant was discarded and the nuclei washed three additional times in saline-citrate solution.

DNA was isolated directly from such nuclei by the sodium xylene sulfonate method of SIMMONS, CHAVOS, and ORBACH[2] (SCO) and the sodium dodecyl sulfate method of KAY, SIMMONS, and DOUNCE[3] (KSD).

Two more samples were obtained by first isolating deoxyribonucleoprotein from the nuclei by a technique derived from the methods of STERN[4] and GAJDUSEK[5]. In brief, this consisted of extracting the nucleoprotein by shaking the nuclei for 16–24 h in the cold with distilled water, precipitating the protein from the filtered extract at 0·14 M sodium chloride concentration, and further purifying it by three cycles of dissolution in distilled water and precipitation at isotonic sodium chloride concentration.

One sample of DNA was isolated from the nucleoprotein by the MIRSKY and POLLISTER[6] modification of Sevag's chloroform-octyl alcohol technique[7]. A second was prepared by extracting the protein with 10% sodium

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