shows (fig. 1) that only peak 3, fractions 128–160, reacted only with anti Apo A and fused with apo A and native HDL. The HDL peak was concentrated in an Amicon filter and chromatographed on Sephacryl-200 in the presence of NaDOC. Figure 2 shows the presence of 3 protein peaks between fractions 100 and 146. The protein fractions contained no detectable phospholipids or cholesterol. The phospholipids and cholesterol eluted between fractions 150 and 184 and no protein was detected in the eluant. If lower concentration of NaDOC (< 10 mg/mg lipoprotein) were added some of the cholesterol esters and triglycerides eluted with the protein. The concentration of bile salt and NaDOC used in these experiments removed all detectable lipids from HDL. Bile salts are known to form small mixed micelles with lipids such as cholesterol[11], phospholipids[12] and glycerides[13], which can be removed by gel filtration[4].

Resolution of apo-HDL into 3 components (fig. 2) suggests that the polypeptide constituents of HDL have separated. Studies on the characterization and distribution of apolipoproteins have shown that the major density classes are heterogeneous not only with respect to particle size but also with respect to apolipoprotein composition[14–16]. Apo-HDL contains several distinct apoproteins namely A-1, A-11, C-1, C-11, C-111, D[17]. The present studies indicate that the detergent sodium deoxycholate may aid in the resolution of the apoproteins of apo-HDL. Similar resolution of the polypeptide of apo-HDL with another detergent, sodium dodecyl sulfate, was demonstrated previously[15]. The use of Sephacryl-200 instead of Sephadex G-200 commonly employed for the separation of apoproteins[4,18] improved the separation of apo-HDL from HDL lipids and increased the flow rate 5-fold.

The bile salt was removed by gel filtration on a Sephadex G-50 in a detergent-free medium (fig. 3). When the eluted protein was tested against anti-HDL serum the lipid-free apo-HDL gave a precipitin line which fused with that given by native HDL and apo-A (fig. 3). No precipitin lines were formed when the apo-HDL was tested against anti apo-B serum.

In the procedures described, HDL and apo-HDL were obtained by gel chromatography. The detergent NaDOC formed small mixed micelles with the lipids[11] which were separated on Sephacryl-200 packed columns; in addition, longer columns were necessary to obtain satisfactory separation. The apo-HDL obtained gave one precipitin line with anti-HDL which fused with the line obtained with native HDL. These qualitative studies indicate that the apo-HDL carries all the major immunological determinants of the intact molecule of HDL, and that the apo-HDL has not undergone conformational changes resulting in detectable immunochemical alterations.

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Modulation of an 'equilibrium enzyme': Ecological evidence

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Summary. The activity of D-lactate dehydrogenase in the foot of the snail, *Helix pomatia*, is closely correlated with time of year and mean daily temperature, and increases strikingly after the animals have been exposed to a nitrogen atmosphere for 24 h.

Within a given tissue, differences in enzyme activity are usually considered to be 'adaptive' only if they concern non-equilibrium reactions and thus, by definition, regulatory enzymes[2,3]. According to this philosophy, lactate dehydrogenase, an enzyme catalyzing an equilibrium reaction, could not be a candidate for supplying information on enzymic mechanisms of metabolic control. Rolleston[4] has emphasized this point quite recently by stating that the kinetic properties of LDH have nothing to do with aspects of metabolic control but are 'accidents of evolution'. However, despite this warning note, LDH and other enzymes, supposed to catalyze equilibrium reactions, have often been used as indicators of adaptive features of metabolism, particularly in poikilothermic organisms[5,6]. This controversy contains aspects which are of general importance for an understanding of the ways the metabolism of organisms responds to external or internal stimuli. It is also clear that there is a lack of quantitative data on which theories can be built.

I report on measurements of the activities of D-lactate dehydrogenase (D-LDH; EC 1.1.1.28) and pyruvate kinase (PK; EC 2.7.1.40) in the foot of the terrestrial snail, *Helix pomatia*. These measurements were carried out between 1977 and 1980 on animals derived from a single locality (Mühlau) near Innsbruck. The enzymes were extracted from the tissues with an efficiency of about 95%[7], and all activities measured at a temperature of 20 °C. Other specimens were exposed to nitrogen (99.99% purity) for 24 h

and the effect of this treatment on D-LDH and PK was determined. The animals were exposed to N_2 in a chamber kept humid with moist filter paper and conditions of anoxia were monitored with a Clark-type electrode. *H. pomatia* is known to respond to periods of anoxia by the accumulation of striking quantities of D-lactate and succinate in the haemolymph[8], and it has been speculated that the ability to tolerate periods of anoxia may be useful at times when the soil becomes water-logged, particularly in spring before the snails leave their hibernating quarters.

The activities of D-LDH and PK in crude tissue homogenates were correlated with 5 variables explained in the legend to the table. These variables are strongly correlated with each other, thus in order to illustrate the structure of the relationship a stepwise multiple regression analysis was applied. The most clear-cut example of the correlation between LDH activity and environmental factors is set out in figure 1. From the data as summarized in the table the following conclusions may be drawn:

The activity of the D-LDH in the foot of *H. pomatia* is closely correlated with time of year. It is highest in early spring, lowest in late summer ($R = -0.73$, time of year being expressed by number of days, starting with January 1st). In winter animals the activity of this enzyme is high again[7]. The correlation between enzyme activity and time of year differed somewhat from year to year. However, when the mean temperature on the day of collection is taken into account some of these differences disappear

Statistics for correlation between activity of D-LDH (in μmoles $\cdot$ min^{-1} $\cdot$ g f.w^{-1}) and each of 5 environmental factors: t_y = time of year, expressed by number of days, starting with January 1st; T1d = mean temperature on day of collection; T5d = mean temperature during 5-day period before collection; P_w1d = mean water vapour pressure (in mm Hg) on day of collection; P_w5d = mean water vapour pressure during 5-day period before collection. N = 113

a) Simple correlation coefficients

Variable No.	Signature	R
1	t_y	−0.73
2	T1d	−0.65
3	T5d	−0.62
4	P_w1d	−0.60
5	P_w5d	−0.66

b) Stepwise multiple linear regression analysis for environmental factors against activity of D-LDH

Variable entered	DF	F-level	R^2
1	111	125.50	0.531
2	110	14.38	0.585
4	109	1.98	0.592
3	108	0.97	0.596
5	107	0.08	0.596

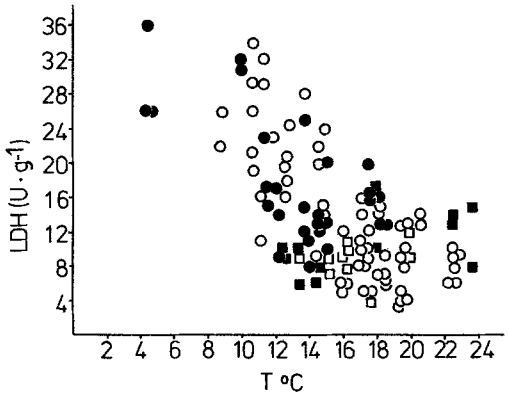

Figure 1. Maximum activity of D-LDH measured at 20 °C plotted against mean temperature on day of collection. Each symbol represents 1 animal the tissue of which was dissected out and frozen at − 70 °C within 2 h after collection. Full squares: 1977; full circles: 1978; open squares: 1979; open circles: 1980. After thawing at 4 °C the tissue was homogenized in 0.033 M phosphate buffer pH 6.5 with an Ultraturrax mixer and centrifuged at 12.000 × g for 40 min. For enzyme determinations the following assay was used: Phosphate buffer 0.067 M pH 6.0 0.88 ml; NADH 3.75 mM 0.04 ml; supernatant of tissue homogenate 0.04 ml; Na-pyruvate 25 mM 0.04 ml. The disappearance of NADH was followed at 340 nm in a Zeiss PM 6 recording spectrophotometer.

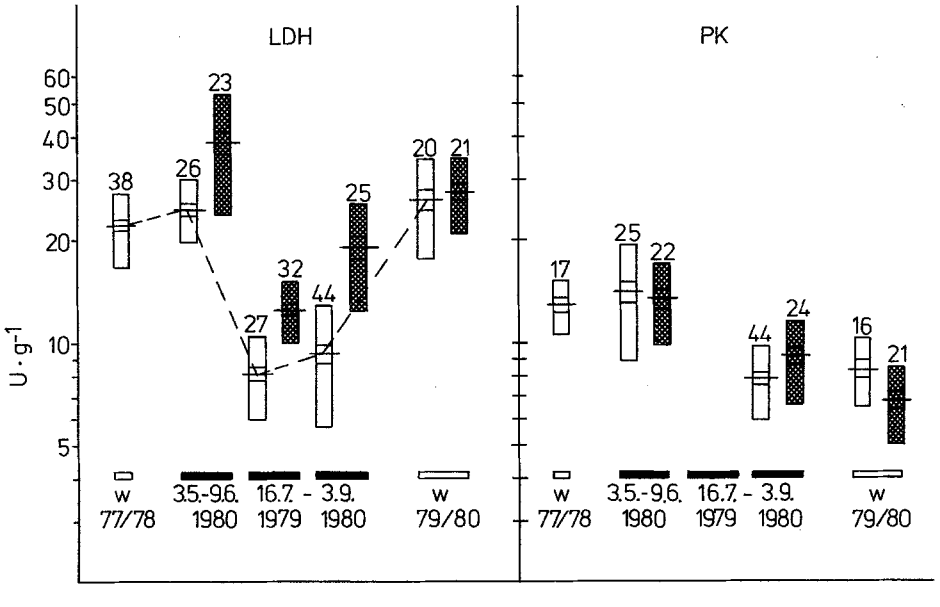

Figure 2. Maximum activity of D-LDH and of PK from the foot of *Helix pomatia* at different times of year. The latter are shown under open (hibernating animals, w) and full (active animals) horizontal bars. Open columns: Control animals treated as described for figure 1 except that before preparation of enzymes they were kept at room temperature in a moist chamber for 24 h. Cross-hatched columns: Animals exposed to 99.99% nitrogen for 24 h (see text). Mean values, SE, SD and number of animals in each series of measurements are indicated. Assay for D-LDH as described for figure 1; assay for PK: Phosphate buffer 0.067 M pH 7.0 0.83 ml; NADH 3.75 mM 0.04 ml; ADP 25 mM 0.04 ml; supernatant of homogenate 0.04 ml; L-LDH 60 units; PEP 50 mM 0.04 ml. All measurements at 20 °C.

(fig. 1). None of the remaining variables made a significant additional contribution to the regression (table).

Exposure of *H. pomatia* to nitrogen for 24 h always leads to a highly significant ($p < 0.001$) elevation of LDH activity in the foot of summer animals, but has no effect on winter animals in which LDH activity is already at its highest. On the other hand, the activity of the non-equilibrium enzyme, pyruvate kinase, is not affected by exposure to a nitrogen atmosphere.

This ecological approach indicates that the activity of an equilibrium enzyme in 1 particular tissue from a species of poikilothermic animals derived from a single locality is modifiable by environmental factors, mainly by time of year, day temperature, and pO_2. The mean variability inherent in this system is approximately 5-fold, ranging from a minimum of 8 to a maximum of 39 units · g f.wt^{-1} but individual minima and maxima cover more than a whole order of magnitude. The sensitive response of LDH to environmental cues can be rationalized by either of 2 assumptions:

Either, the D-LDH of this tissue does not catalyze an equilibrium reaction in vivo; or, the concentrations even of enzymes catalyzing equilibrium reactions are geared to the *maximum flux rates* expected under a given set of conditions. A change of conditions (be it exo- or endogenously determined) may lead to higher flux rates and thus to the induction of additional enzyme activity. As far as LDH is concerned a number of reports suggest that this enzyme can be activated quite rapidly and thus seems to be a site of metabolic control in certain animals[9,10].

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Studies on the chemical constitution and sex pheromone activity of volatile substances emitted by *Dacus oleae*[1,2]

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Summary. Volatile substances from the females of *Dacus oleae* have been submitted to GLC-MS analysis and several components identified. E-6-nonen-1-ol and p-cymene displayed attractive and aphrodisiac effects in both laboratory and field experiments.

Dacus oleae (Gmelin) Diptera, Trypetidae, is a serious pest of olive-trees causing considerable economic damage every year by decreasing olive-oil production, especially in the Mediterranean area.

Up to date only protein degradation products seem to be attractive to this fly, therefore the interest in a more effective and specific attractant for pest preventive control is well justified. Research on a possible pheromone in *D. oleae* started in 1971 with the work of Economopoulos et al.[3]. In recent years Haniotakis et al.[4-6] reported the isolation of a mixture of compounds displaying pheromone activity in both laboratory and field assays; however, no characterization of the components of such mixture has been so far achieved.

In this paper we report on our studies directed towards the identification of some volatile substances emitted by *D. oleae* and the evaluation of their sex-pheromone activity.

The insects were reared in perspex cages ($20 \times 20 \times 20$ cm, nylon net) at 25 ± 5 °C with a 70 ± 10% humidity and a light exposure similar to the natural one. They were fed with a mixture of hydrolyzed soy, sugar and egg-powder. Adults were immediately separated by sex after emergence.

About 3000, 6 days old virgin females were caged in a 100×25 cm perspex cylinder and gently flushed with air over a total period of 9 h (last 3 h of the photoperiod for 3 days). Effluent air and volatile substances were totally condensed in a trapping system cooled by liquid nitrogen. After slow evaporation of the air and salting, the condensate was extracted several times with highly purified ethyl ether. After careful concentration at atmospheric pressure, the extracts were directly used for GLC-MS analysis without any further treatment.

After several attempts, the best gas-chromatographic resolution was obtained by injecting the mixture into a SCOT glass capillary column (CW 20 M; 40 m) working with a programmed temperature (60–200 °C; 5 °C/min). The gas-chromatographic system was connected with an LKB 2091 mass-spectrometer equipped with an LKB 2130 data system.

A typical total ion current plot is represented in the figure and the identified peaks are listed in table 1. All structure attributions were accomplished first by MS-spectra analysis and then by comparison of retention times and fragmentation of each peak with those of pure samples.

Peaks marked with CH refer to aliphatic hydrocarbons which have not been further investigated. Substances marked with an asterisk are not emitted by the insects as could be demonstrated by a GLC-MS analysis in the same conditions of a 'blank' obtained by the above described procedure but in the absence of insects. All of them, together with compounds 9, 10, 14, 16 and 17 were commercially available or easily synthesized.