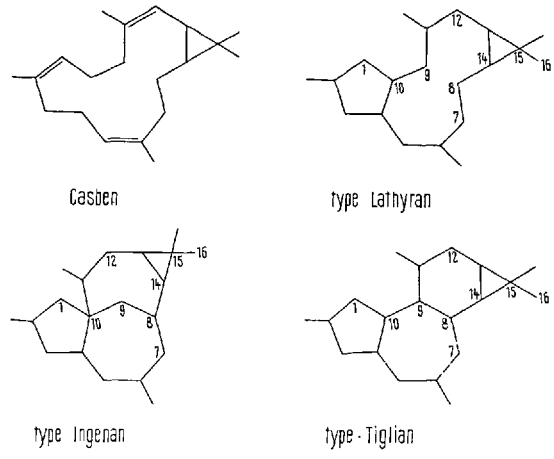
Ester L_8, m.p. 198–203 °C, contains nitrogen: $C_{30}H_{37}O_7N$ (MS) and is the diacetate-pyridine-3′-carboxylate IV of lathyrol. UV (MeOH): λ_{max} = 218,5 271,5 nm, ε = 14700, 15400. IR (KBr): 1740, 1715 (CO), 1647, 1623 (C = C–CO), 1590 (C = N), 902 (C = CH_2), 740, 700 cm^{-1} (C_5H_4N). With the exception of the signals of the aromatic protons, the NMR spectrum of IV ($CDCl_3$) is identical with that of I. Instead of the multiplet of the benzoyl group as in I, the NMR spectrum of IV shows the signals of the protons of nicotinic acid: H′-2: 9,25, D ($J_{2,4}$ = 1 cps); H′-6: 8,75, DD ($J_{5,6}$ = 2,5 cps, $J_{4,6}$ = 1 cps); H′-4: 8,25, M ($J_{4,5}$ = 4 cps, $J_{4,6}$ = 1 cps, $J_{2,4}$ = 1 cps); H′-5: 7,4 ppm, DD ($J_{4,5}$ = 4 cps, $J_{5,6}$ = 2,5 cps). The relative positions of the three ester groups in IV remain to be established.

In the acetone extract of latex of *E. lathyris* collected from plants in their second year, apparently none of the 8 diterpene esters L_1–L_8 isolated from the seed oil is present. Especially, no esters of lathyrol type diterpenes were found. However, a mixture of ingenol esters with highly unsaturated fatty acids (C_{10}: 2⊿ and 3⊿; C_{12}: 2⊿ and 3⊿, mass-spectrometrically) was isolated.

The esters of ingenol with a free hydroxyl group in 20-position exhibit considerable irritant (L_5, L_6 and mixture of esters from latex) and cocarcinogenic activities (L_5) in the mouse (see [1, 2, 6]). L_4 i.e. ingenol-20-hexadecanoate and also the esters of lathyrol and its derivatives (L_1, L_2, L_3, L_7, L_8) are inactive in the biological assays mentioned above (see [1, 2, 6]).

Recently an a further derivative of the macrocyclic parent hydrocarbon lathyran has been isolated from an Euphorbiacea: Bertyadionol[11] form a Bertya species. The structural relationship between the macrocyclic skeletons of casbene[10], lathyran, tiglian and ingenan[3–6], as visualized in the scheme above, may indicate the existence of hitherto unknown biosynthetic pathways of diterpenes in Euphobiaceae: for example from geranyl-geraniol-pyrophosphate they may form parent alcohols of the lathyran type (*E. lathyris*, Bertya sp.[11]), tiglian type (*Croton tiglium*[1], *E. triangularis*[8], *E. cooperi*[9]) and ingenan type (*E. lathyris*, *E. ingens*[7]).



Zusammenfassung. Ein Diacetat-Benzoat und ein Diacetat-Nicotinoat des neuen Diterpens «Lathyrol» sowie weitere Ester des Diterpens Ingenol wurden aus dem Samenöl bzw. dem Latex von *Euphorbia lathyris* L. isoliert. Die chemische Struktur von Lathyrol wurde mittels spektraler Daten aufgeklärt. Lathyrol ist die Muttersubstanz der beiden bereits früher aus *E. lathyris* isolierten makrozyklischen Diterpene 6.20-Epoxy-lathyrol und 7-Hydroxy-lathyrol. Es wird ein Biogeneseweg für Diterpene aus Euphorbiaceen vorgeschlagen.

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Circadian Variations of Muscle Metabolites

On the basis of several reports, it could be established that glycogen[1] and protein[2] metabolism in muscle has a very fast turnover and also that it is under hormonal control. Nevertheless, even when it is known that the levels of circulating hormones are far from being constant[3–6], there is a scarcity of available data concerning the spontaneous variation of muscle metabolites concentration during the 24 h period. This information might provide important clues for the choice of any experimental design schedule. Therefore, we considered it important to establish whether the content of substances such as DNA, total protein and glycogen in muscle tissue vary or remain unchanged during the 24 h period. The results obtained are presented in this paper.

Material and methods. Female mice of the C3H-S strain, 6 weeks old, from the Instituto de Embriología, Biología e Histología, Facultad de Ciencias Médicas, Universidad Nacional de La Plata, were used throughout the experiments. In their 3rd week of age they were caged in groups of 10 in a room at a temperature of 25 ± 1°C with water and food ad libitum and illumination (fluorescent light 40 W) from 06.00 to 18.00 h alternating with 12 h darkness. Mice have nocturnal habits, and feed during the dark period, as has been previously demonstrated[7]; thus, the dark (18.00–06.00) and light (06.00–18.00) periods are named activity and rest periods, respectively.

Lots of 7 animals each were killed by decapitation at 00.00, 04.00, 08.00, 12.00, 16.00 and 20.00 h on different days. The average body weight in each lot was carefully kept around 20 g. The diaphragms were quickly removed, blotted between filter paper and weighed. Homogenization of the tissue, either for protein or DNA determination, was done in 2 ml of isotonic saline. DNA was extracted

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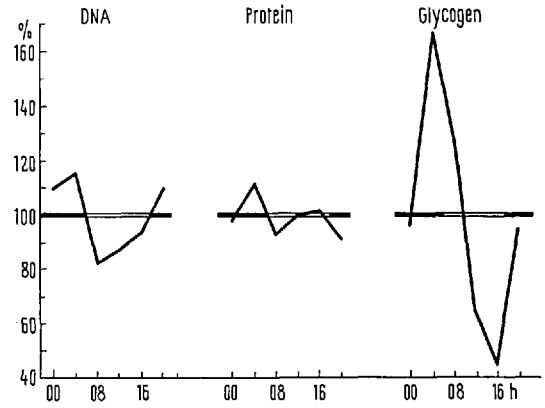
Total DNA, protein and glycogen in mouse diaphragm at each time point

Time	No. of animals	DNA (μg/diaphragm)	Protein (mg/diaphragm)	Glycogen (μg/diaphragm)
00.00	7	114.9 ± 4.5	17.3 ± 0.3	13.4 ± 4.1
04.00	7	121.5 ± 3.8[a]	19.1 ± 0.6[b]	23.3 ± 4.5[c]
08.00	7	87.1 ± 12.5[a]	15.9 ± 0.6	17.8 ± 2.6
12.00	7	92.2 ± 6.9	17.1 ± 0.3	9.2 ± 0.9
16.00	7	98.9 ± 9.0	17.3 ± 0.3	6.2 ± 1.4[c]
20.00	7	114.1 ± 9.7	15.6 ± 0.4[b]	13.3 ± 1.3

Each value represents average ± S.E.M., [a] p between $< 0.025 > 0.02$. [b] $p < 0.001$. [c] p between $< 0.005 > 0.001$.



Circadian percentage variations (see text) of the total DNA, protein and glycogen of mouse diaphragm. The illumination regimen in the animal room is shown in the 100% bar.

following the method of SCHMIDT-THANNHAUSER[8] and measured by the reaction with diphenylamine carried out as described by BURTON[9]; the colours obtained were compared with the one developed by a standard of purified DNA. Protein was determined according to the method of LOWRY et al.[10] in an aliquot of the homogenate kept 12 h at 37°C in 0.1 N NaOH. Glycogen was extracted and estimated colorimetrically according to the method of KRISMAN[11]. The glycogen employed as a standard for this purpose was obtained from our mouse liver strain by the SOMOGYI method[12].

Results. The Table shows the values obtained for total DNA, protein and glycogen in mouse diaphragm at each time point. It can be seen that the peak value of DNA is attained during the last part of the activity period, while the lowest value occurs at the beginning of the rest period. The highest value for total diaphragmatic protein is reached at 04.00 h and the lowest at 200.0 h, the latter appearing close to the beginning of the activity period.

Conversely, the maximum values for muscle glycogen values are separated by a 12 h interval, the first occurring during the last part of the activity period and the second at the end of the rest period.

The changes described above are represented in the Figure as percentage variations, considering the average value obtained throughout the 24 h period as 100% and the values at each time point as deviations from the mean.

Discussion. The present results clearly show a circadian variation in the mouse diaphragmatic DNA, protein and glycogen content. As is shown in the Figure, the percentage range of variation was not of the same extent, i.e. the protein and DNA figures were about 10–15%, whereas the glycogen ones reached a value near the 60% variation. The greatest fluctuations obtained with glycogen might be ascribed to the fact that it represents the main source of metabolic fuel for muscular work. Furthermore, serum glucose levels are not constant throughout the 24 h period, the highest values appearing during the rest period, as has been described[4]. Particularly for the DNA determination, the presence of interferences like those described by EDELMAN[13] could be discarded, since the absorption spectra of both the standard and the extracted DNAs were quite similar. Thus, the observed cyclic variation must be due to real changes in the total muscle DNA.

The 24 h variation observed in the protein, glycogen and DNA content of muscle, closely agrees with other authors' reports concerning the circadian changes of liver DNA[14,15], protein and glycogen[16,17] in several species. The underlying mechanism that controls these changes is beyond our present knowledge. However, all of them rise to their highest values during the activity period, returning to their lowest levels during the rest period. Thus, it could be suggested that they are under the influence of the same circadian oscillator.

Although unexplained, these results demonstrate that the tissue metabolite content of living organisms is under continuous change. This condition deserves a careful control when selecting a given time of the day to perform any experimental design[18].

Resumen. El contenido diafragmático de proteína, glucógeno y DNA fue estudiado a intervalos de 4 h durante las 24 h. Los animales empleados fueron ratones hembras mantenidos en cuartos con períodos de luz-oscuridad de 12 h cada uno. Pudo demostrarse que los tres parámetros presentaron una clara variación circadiana, coincidiendo la aparición de los valores máximos y mínimos para todos ellos durante el período de actividad (oscuridad) y reposo (luz) respectivamente.

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