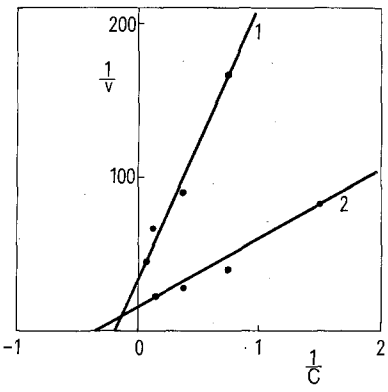
10 min), the supernatant was adjusted to pH 9.0 and 12.0, respectively, and extracted with ether. The ether layer was evaporated to dryness, the residue dissolved in 0.2 ml of ethanol and analyzed by thin layer chromatography. (DC-Fertigplatten Kieselgel F_{254}, Schichtdicke 0.25 mm, Merck; solvent system: acetone-ethanol 1:1; detection: iodine[8], Gibbs reagent[9]).

For quantitative determination of phenol, the supernatant was adjusted to pH 9.0 and extracted twice with 50 ml of ether each, the extracts were mixed and washed with 20 ml 2 *N* HCl to remove the excess of unreacted substrate, and the ether layers were dessicated with sodium sulphate. The extracts from 2 parallel experiments were combined and evaporated on a rotary evaporator in special conical tubes with calibration and made to 0.10 ml with methanol. The phenol contained in 0.75 µl of this methanolic solution was isolated by thin layer chromatography (Kieselgel GF_{254} Merck, plates 20×20 cm, thickness 0.3 mm; activation 1 h at 105°C, washed with methanol; solvent system: benzene-ethanol 95:5; detection: UV-light and Gibbs reagent[9]). The areas containing phenol were quantitatively transferred in glass tubes, extracted with 3.0 ml methanol and to the clear solution 0.05% Na_2CO_3 was added up to 9.5 ml; then 0.5 ml of 4-aminoantipyrine (2%) and 0.25 ml $K_3Fe/CN/_6$ (8%) followed. The absorbancy of the well mixed samples was determined after 5 min at 500 nm[10] (Spectrophotometer Spekol, VEB C. Zeiss, Jena) against the blank which passed through the same isolation procedure.

In control experiments boiled microsomes (100°C, 5 min) were incubated under standard conditions, in other experiments the incubation proceeded anaerobically (in oxygen free N_2) or in a CO and O_2 (1:1) mixture as a gas phase. Protein concentration in microsomes was determined according to[11], and cytochrome P-450 content according to[12].



The Lineweaver-Burk plot for *o*-fomocaine (1) and *p*-fomocaine (2). K_m for *1* = $5.33\times10^{-3}M$, for *2* = $2.85\times10^{-3}M$, V for *1* = 3.0×10^{-2}, for *2* = $6{,}6\times10^{-2}$ nmoles $C_6H_5OH\times$nmoles^{-1} cytochrome P-450 × min^{-1}.

Results and discussion. The microsomal fraction of rabbit liver homogenate was shown to set free phenol from the molecule of fomocaine. The phenol was identified by thin layer chromatographiy (Rf = 0.38), and its formation was linear with time during the first 30 min. The relation between the phenol formation rate and the concentration of fomocaine was studied with microsomal preparation containing 26.3 mg protein × ml^{-1} and 1.05 nmoles of cytochrome P-450 × mg^{-1} protein. Analogous experiments were carried out with *ortho*-fomocaine using a preparation which contained 32.3 mg protein × ml^{-1} and 1.64 nmoles of cytochrome P-450 × mg^{-1} protein. The results are given in the Table. From a double reciprocal plot (Lineweaver – Burk) the values of K_m and V for both isomers were found (Figure). The dearalkylation of fomocaine was inhibited by carbon monooxide, anaerobiosis and absence of NADPH. The dearalkylation of fomocaine and its *ortho* isomer has to be taken as the biotransformation reaction of general importance for the compounds of this chemical structure. Recently it has been found[13] that the model substance of this type, phenylbenzylether, undergoes the same detoxication mechanism.

Zusammenfassung. Oxidative Spaltung der Äther-Bindung in Fomocain (Panacain®) durch das mikrosomale Monooxygenase-System der Kaninchenleber wurde nachgewiesen. Aus der Kinetik dieser Dearylalkylierung wird geschlossen, dass das *para*-Isomere eine doppelt so grosse Affinität zum mikrosomalen Enzymsystem besitzt als das *ortho*-Isomere und doppelt so schnell metabolisiert wird. Die Dearylalkylierung scheint eine allgemeine Biotransformationsreaktion für derartige strukturelle Typen zu sein.

A. JINDRA JR.[14], O. HELIA and A. JINDRA

Biochemical Institute, Comenius University, Bratislava (Czechoslowakia), 26 April 1972.

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The Effect of Temperature on Ca^{2+} Uptake and Ca^{2+}-Activated ATP Hydrolysis by Cardiac Sarcoplasmic Reticulum

The ATP dependent Ca^{2+} uptake by sarcoplasmic reticulum (SR) fractions prepared from skeletal[1-4] or heart muscle[5-7] is stoichiometrically linked to the Ca^{2+}-activated ATP hydrolysis. The effect of temperature on Ca^{2+} uptake and Ca^{2+}-dependent ATP splitting by SR of skeletal muscle have been carefully investigated in the presence and absence of oxalate[3]. The temperature dependence of Ca^{2+} uptake by cardiac SR was investigated[8,9], whilst the effect of temperature on the Ca^{2+}-activated ATPase activity has not been studied. The present communication deals with the influence of temperature on both Ca^{2+} uptake and Ca^{2+}-activated ATP hydrolysis by cardiac SR and the energies of activation (E_a) of both processes.

Methods. Cardiac SR was prepared from dogs. Hearts were homogenized by a Waring blender (Buehler, type ho) in 3 volumes of 0.3 *M* sucrose containing 2 m*M* ascorbic acid and 20 m*M* *Tris*-HCl (pH 7.2) for 20 sec. The homo-

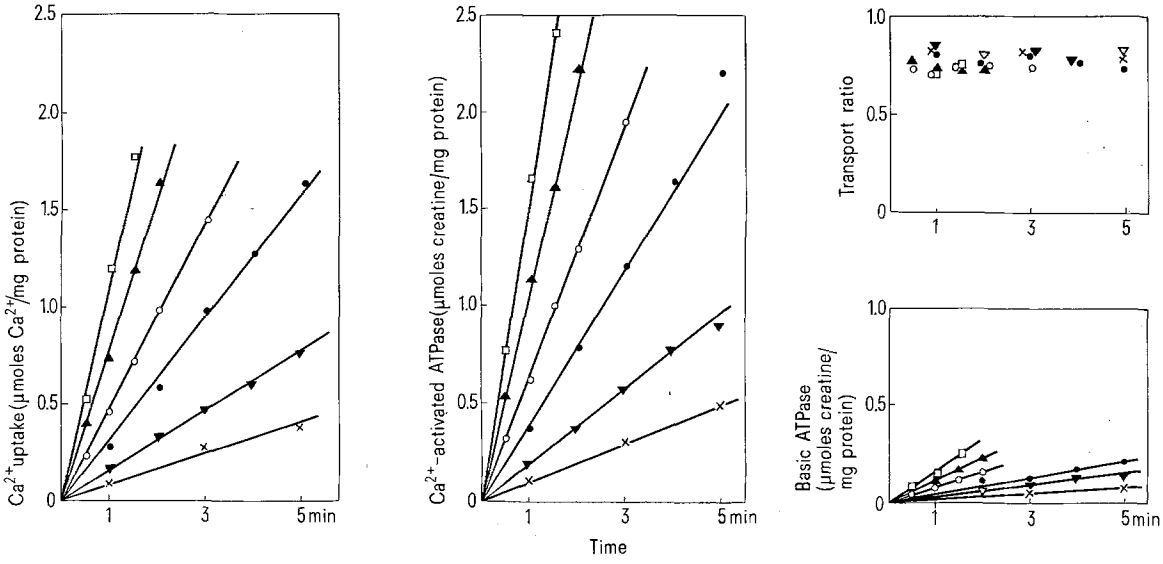

Fig. 1. Temperature dependence of Ca^{2+} uptake, Ca^{2+}-activated and Ca^{2+}-independent (basic) ATP hydrolysis by cardiac SR. Conditions of incubation are given in the methods. Transport ratio = the rate of Ca^{2+} uptake divided by the rate of the Ca^{2+}-activated ATP splitting. Temp. (°C): □—□, 35°; ▲—▲, 30°; ○—○, 25°; ●—●, 20°; ▲—▲, 15°; ×—×, 10°.

genate was centrifuged at 1,000 ×*g* (20 min), 12,000 ×*g* (20 min and 10 min) and 40,000 ×*g* (60 min) as described previously[10]. The 12,000–40,000 ×*g* fraction, which contains the SR, was extracted with 0.6 *M* KCl for 60 min, recentrifuged and washed once in the homogenization medium. SR fractions were also prepared by the addition of KCl to the supernatent of the 12,000 ×*g* fraction (final concentration 0.2 *M*); the fraction was centrifuged at 40,000 ×*g* and the pellet washed once. The pellets were resuspended in 0.3 *M* sucrose containing 10 m*M* Histidine-buffer (pH 7.2) and stored below −20 °C.

Ca^{2+} uptake was measured by the Millipore filtration technique[11]. The incubation medium contained 20 m*M* Histidine-buffer (pH 7.0), 100 m*M* KCl, 5 m*M* $MgCl_2$, 5 m*M* Na_2-oxalate, 5 m*M* Na_2-ATP, 1.25 m*M* Na_2-creatine phosphate, creatinekinase 0.15 mg/ml, 5 m*M* NaN_3, 0.1 m*M* ^{45}(Ca) $CaCl_2$ and 0.05 mg SR protein/ml. Assays were also performed in the above medium, except ATP was 0.5 m*M* and 0.1 m*M* EGTA (ethylene-*bis*-(-2-aminoethyl)-N, N′-tetraacetic acid) was present. The pH of 7.0 was carefully adjusted at each temperature. Total ATPase activity of the SR was determined simultaneously with Ca^{2+} uptake by measuring the rate of creatine liberated[12]. The basic ATPase activity was measured in the absence of Ca^{2+} and the presence of 0.2 m*M* EGTA. The Ca^{2+}-activated ATPase (extra ATPase) was calculated by subtracting the basic from the total ATPase activity. Protein was measured by the Folin method standardized against bovine serum albumine[13].

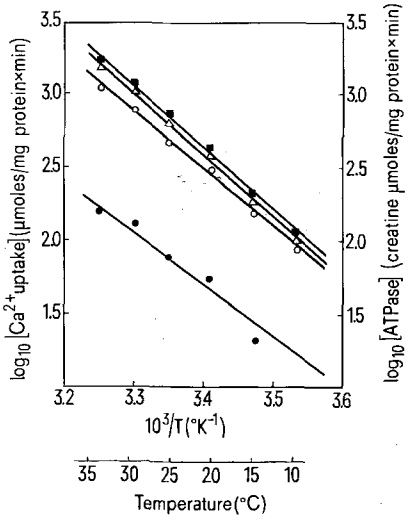

Fig. 2. Arrhenius plots for Ca^{2+} uptake, Ca^{2+}-activated, total- and basic ATP hydrolysis by cardiac SR. The rates of Ca^{2+} uptake and ATP hydrolysis were calculated from the data in Figure 1. The slopes were calculated using regression analysis. Symbols: ○—○, Ca^{2+} uptake; △—△, Ca^{2+}-activated ATPase; ■—■, total ATPase; ●—●, basic ATPase.

Results and discussion. The data of a typical experiment measuring Ca^{2+} uptake, Ca^{2+}-activated ATP hydrolysis and Ca^{2+}-independent ATP hydrolysis of cardiac SR at various temperature are shown in Figure 1. The increase in the rate of Ca^{2+} uptake at higher temperatures correlates with the increase in the rate of the Ca^{2+}-activated ATP splitting. The small variations of the transport ratio (the

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rate of Ca^{2+} uptake divided by the rate of the Ca^{2+}-activated ATP hydrolysis) at different temperatures are within the experimental error. Transport ratios obtained from various SR preparations ranged from 0.7 to 0.95, suggesting a stoichiometry of Ca^{2+} uptake and Ca^{2+}-dependent ATP hydrolysis of 1. Similar values have been obtained previously for SR fractions prepared from calves[10] and rabbits[14,15], which agree with transport ratios reported from several laboratories[5-7]. The Ca^{2+}-activated ATPase of the SR fractions represents about 90% of the ATPase activity (Figure 1) assayed in the presence of 5 m*M* azide. Azide does not influence the Ca^{2+} uptake of Ca^{2+}-dependent ATP splitting by the SR[10], however the Ca^{2+}-independent ATPase activity is partially inhibited.

Straight lines were obtained by plotting the logarhythms of the rate of Ca^{2+} uptake or the rates of the total-, basic- and Ca^{2+}-activated ATP hydrolysis against the reciprocal of the absolute temperature (Arrhenius plot; Figure 2). The E_a for Ca^{2+} uptake and Ca^{2+}-activated ATP hydrolysis obtained from 4 different SR preparations are practically the same and slightly lower for the Ca^{2+}-independent ATP hydrolysis (Table). Since the latter is partially inhibited by azide, a comparison with the values obtained for Ca^{2+}-dependent ATP splitting is not warranted.

Energies of activation for Ca^{2+} uptake and ATP hydrolysis by cardiac SR

	Energies of activation (Kcal/mole^{-1})
Ca^{2+} uptake	16.65 ± 0.87 (14.04–14.66)
Ca^{2+}-activated ATPase	17.93 ± 0.49 (16.83–19.22)
Total ATPase	17.50 ± 0.67 (15.82–19.22)
Basic ATPase	14.05 ± 1.29 (10.56–16.69)

The values are means ± SEM obtained from 4 different SR preparations. The minimal and maximal values are given in parentheses. The E_a were calculated from the equations: $\log V = -A/T + B$ and $E_a = R \times 2,303 \times A$. Arrhenius plots were prepared and the slopes (A) determined by regression analysis. E_a, activation energy (Kcal/mole^{-1}); R, 1,987 $\times 10^{-3}$ Kcal/°K^{-1}/mole^{-1}.

A close correlation between the E_a for Ca^{2+} uptake and Ca^{2+}-activated ATP hydrolysis by SR from skeletal muscle measured either in the presence or absence of oxalate was reported by INESI and WATANABE[3], however the E_a were higher in the presence of oxalate. E_a for Ca^{2+} uptake in the absence of oxalate reported for cardiac SR (HARIGAYA and SCHWARTZ[8]; Millipore method) and for dog cardiac SR (Enntmann, Allen and Schwartz[9]; Murexide method) were 10 Kcal/mole^{-1} and 6.5 Kcal/mole^{-1}, respectively. These values are clearly lower than those obtained in the present study. It might indicate a different rate limiting step of Ca^{2+} uptake by the cardiac SR measured either in the presence or absence of oxalate, similarly as proposed for skeletal muscle[3].

Zusammenfassung. Der Ca^{2+}-Transport und die Ca^{2+}-aktivierte ATP Hydrolyse (ATP extra Spaltung) durch Membranen des cardialen sarkoplasmatischen Retikulums zeigen die gleiche Temperaturabhängigkeit. Die Aktivierungsenergie der Ca^{2+}-Aufnahme und der ATP extra Spaltung, gemessen bei Anwesenheit von Oxalat, beträgt 16.65 ± 0.87 und 17.93 ± 0.49 Kcal/Mol^{-1}.

J. SUKO

Pharmakologisches Institut der Universität Wien, Währinger Strasse 13a, A-1090 Wien (Österreich), 28 September 1972.

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Beiträge zum Stickstoffmetabolismus in grünen Pflanzen. I. Die reversible Plastidenumwandlung in glucosestimulierten Kulturen von *Spirodela oligorrhiza*, eine Folge von Stickstoffmangel

Das Wachstum von *Spirodela oligorrhiza* auf sterilen, glucosehaltigen Nährlösungen ist – verglichen mit Kontrollen auf glucosefreiem Milieu – gekennzeichnet durch eine nach 15–20 Tagen einsetzende äusserliche Vergilbung und eine auffällige Veränderung der Plastidenfeinstruktur. Dabei verlieren die Chloroplasten ihre charakteristische Thylakoidstruktur und gehen unter Veränderung ihrer typischen Lipidzusammensetzung und unter Anhäufung von Stärke schliesslich in Amyloplasten über[1-3]. Bei Zusatz neuer Stickstoffquellen zur verbrauchten Nährlösung findet unter äusserlicher Wiederergrünung der Pflanzen[1] eine Regeneration des Lamellarsystems der Plastiden statt[2], was bedeutet, dass für die Vergilbung primär der durch einen beschleunigten Verbrauch der Stickstoffquellen der Nährlösung entstandene Stickstoffmangel verantwortlich ist. Ähnliche Veränderungen der Feinstruktur und der stofflichen Zusammensetzung wurden auch in Plastiden von *Chlorella protothecoides* auf acetathaltigem Milieu[4] und von *Chlorella fusca* und *Ankistrodesmus braunii* unter N-Mangelbedingungen erzeugt[5].

Andererseits geht aus Gaswechselmessungen und Markierungsversuchen deutlich hervor, dass in glucosestimulierten Pflanzen von Anfang an die Nettoassimilation von CO_2 herabgesetzt ist[1], und dass die angebotene Glucose in Stärke eingebaut und somit auch in den Stoffwechsel eingeschleust wird[6]. Diese Vorgänge werden bereits unter optimaler Stickstoffernährung induziert und sind als Anzeichen eines Überganges von autotropher zu heterotropher Lebensweise zu werten. Da Spirodela im Dunkeln vollständig heterotroph leben kann[1], wäre denkbar, dass unter Glucoseernährung über genügend lange Zeit diese Umstellung bereits unter Lichtbedingungen erfolgen kann, und dass die beobachtete Vergilbung glucosestimulierter Kulturen somit ausser auf einen N-Mangel teilweise auf eine direkte Wirkung der exogenen Glucose zurückzuführen ist.

Zur Beantwortung dieser Frage haben wir das Verhalten von Pflanzen untersucht, die unter genügender N-Ernährung während längerer Zeit auf glucosehaltiger Nährlösung gezogen wurden. Ferner wurden anhand elektronenoptischer Aufnahmen geprüft, ob eine Rege-

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