serum total lipids, free fatty acids and cholesterol. Changes in the serum phospholipids levels were not significant after fracture. Maximum increase in total lipids and serum cholesterol levels was observed 5 days after fracture. Whereas the peak increase in serum free fatty acids was noticed 6 days following fracture. In our earlier studies also, we have observed similar changes in lipid metabolism in response to stress produced by electric shock in rabbits[9].

JOHNSON and SVANBORG[10] have shown elevated levels of cholesterol following ischemia of limb. In vitro studies of DEMATTIES[11] demonstrated ten-fold increase in the incorporation of (2-C^{14}) acetate into cholesterol of liver slices 24 h after trauma, and suggested an increase in the rate of breakdown and synthesis of cholesterol following physical injury. The elevated levels of free fatty acids and other lipid fractions observed by us and other workers following trauma could probably be due to accelerated mobilization of fat from adipose tissues. Following trauma or stress, the blood levels of Adrenaline and ACTH are elevated which may be responsible for mobilizing the lipids at a higher rate. Surgical operations are also accompanied by increased levels of free fatty acids and triglycerides[4,5]. Increased mobilization of fat following trauma or stress is needed to meet the increased requirements of energy[12]. The insignificant changes in serum phospholipids levels noticed in the present study may be due to the non-involvement of phospholipids in the fat transport between its depots and other tissues.

Zusammenfassung. Nach Knochenverletzung beim Kaninchen kommt es im Blut zu einer drastischen Erhöhung der gesamten Lipide, freien Fettsäuren und des Cholesterols, während die Veränderungen des Serum-Phospholipids nur geringfügig ist. Die Erhöhung bleibt bis zum 9. Tag nach erfolgtem Knochenbruch.

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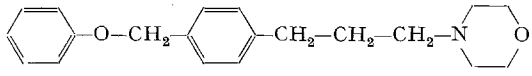
The Dearalkylation of Fomocaine Isomers by Microsomal Monooxygenase System of Rabbit Liver

Fomocaine (Panacaine®) as a basic ether represents a new type of chemical structure with a local anaesthetic activity[1]:



The pharmacological properties of this substance have been reported[2], as well as its application in pharmacotherapy[3]. Recently the metabolites of fomocaine detected in the urine of rats and guinea-pigs have been described[4], and better therapeutical properties of the *ortho* isomer of fomocaine were found[5].

Monooxygenase system of smooth endoplasmic reticulum (SER) contained in microsomes prepared from liver of various animal species has been known and commonly accepted as a most important enzyme system specifically involved in metabolism and biotransformation reactions of drugs[6]. In this paper we report on experiments concerned with the biotransformation reactions of fomocaine and its isomer in the microsomes of rabbit liver.

Experimental. The microsomal fraction of the homogenate from the liver of rabbits (male, chinchilla, 3–4 kg, Velaz n.p.) was prepared according to REMMER et al.[7]. The incubation mixture contained 0.8 ml of 0.5 *M Tris* phosphate buffer pH 8.5, nicotineamideadeninedinucleotidephosphate (30 μmoles), adenosinetriphosphate (10 μmoles), glucoso-6-phosphate Na salt (100 μmoles), glucoso-6-phosphate dehydrogenase (E.C. 1.1.1.49. – 0.52 IU) and the substrate under study (4–100 μmoles). The reaction was started by addition of 2.0 ml of microsomal suspension. The total volume of the sample was 7.5 ml. The experimental blank contained 0.8 ml of 0.5 *M Tris* phosphate buffer pH 8.5, 4.5 ml water, substrate and 2.0 ml of microsomes. The mixture was incubated in Dubnoff incubator at 37°C for 10–60 min in open flasks and then 0.5 ml $ZnSO_4$ (20%) and 0.5 ml $Ba(OH)_2$ (saturated) were added. The precipitated proteins were separated by centrifugation (Janetzki T 23, 5000 rpm,

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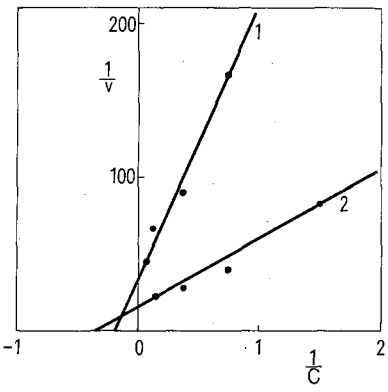
Relation between the rate of phenol formation and the concentration of *o*- and *p*-fomocaine, respectively

Substrate concentration [C, m*M*]		0.667	1.330	3.330	6.670	13.330
Reaction velocity (v, nmoles $C_6H_5OH \times$ nmoles^{-1} cytochrome P-450 $\times$ min^{-1})	*ortho*	—	0.006	0.011	0.015	0.023
	para	0.012	0.025	0.035	0.041	—

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.

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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-