rats that were anesthetized with ether and the hearts were isolated and perfused via the coronary circulation with Krebs-Henseleit Bicarbonate buffer[6], gassed with oxygen at 37°C in either the presence or not of 10^{-5} *M* DNP. The perfusate flows by gravity from a reservoir 70 cm above the heart. After 10 min the hearts were frozen in liquid nitrogen and the content of phosphorylase *a* and total (a + b) was determined, as above. The determination of the content of lactic acid in hearts[7] was carrying out in normal and DNP-poisoned rats that were anesthetized with ether and the hearts rapidly removed, weighed and ground in 3% perchloric acid solution in the proportion of 6 ml/g of muscle. Aliquots of 0.2 ml of the acid supernatant were diluted to 3 ml with solution containing (final volume): 0.2 *M* glycine buffer pH 10.0, 0.2 *M* semi-carbazide 0.0025 *M* NAD and 50 µg of lactic dehydrogenase crystallized from beef heart[8], after $^1/_2$ h of incubation at 30°C the optical density was determined at 340 nm in Beckman DB spectrophotometer.

Table II shows that perfused hearts of animals poisoned by DNP also show an increase of the content of phosphorylase *a* from phosphorylase *b*; at the same time the content of lactic acid is increased about 4 times, showing that the DNP per se is responsible by that increased rate of the glycogenolysis. The content of phosphorylase in experiments shown in Table II is higher when compared with that of Table I; this is probably due to the pretreatment of the extract with Norit A which, by the adsorption of nucleotides, gives less imprecise estimation of the fraction in the form of phosphorylase *a*, according to CORNBLATH et al.[6]. In our case the increase of the content of phosphorylase *a* mantained the same proportion in both normal and reserpinized animals. These results suggest that the push mechanism for accelerating glycogenolysis[9], that seems to be controlled by the content of phosphorylase *a*, could explain the role of the DNP as glycogenolytic agent. On the other hand, one can consider that DNP is an ATPasic agent, and the content of ATP of the cell is low in poisoned animals, and the pull mechanism could be also involved, once phosphofructokinase, the enzyme responsible for this mechanism, is highly sensitive to low concentration of the ATP. The action of DNP can be compared with that of glucagon and the anoxia in cardiac muscle[6]. Thus glucagon was found to stimulate glycogenolysis and lactate production, and to increase the phosphorylase *a* up to 50%, and DNP, like anoxia, produces a still faster rate of glycogenolysis but a smaller increase in phosphorylase *a* activity up to about 30%. By the other hand, with glucagon neither the rate of glycogenolysis nor the phosphorylase *a* basal level shows any important increase in skeletal muscle. Our findings strongly suggest that the injection of DNP simulates the anoxia, as concerns its effect on the glycogenolysis in the skeletal and cardiac muscle.

Table II. Content of phosphorylase *a* and total and of lactic acid in perfused hearts with $10^{-5}M$ DNP

Determination	Normal perfused hearts	DNP-poisoned perfused hearts
Phosphorylase *a* (10 rats)	18.0 ± 4.5[a]	72.0 ± 11.5
Total phosphorylase (+ AMP) (10 rats)	168.0 ± 21.0	192.3 ± 25.0
Ratio Phosphorylase *a*/ Total phosphorylase × 100	10.7 ± 1.8	37.5 ± 7.4
Lactic acid (6 rats)	1.4 ± 0.2	6.0 ± 0.4

[a] S.E.M. The activity of phosphorylase is expressed in units/g of heart and the lactic acid in µmol/g of heart.

Résumé. L'effet du DNP dans la glycogénolyse du cœur du rat a été étudié en mesurant le taux de phosphorilase *a*, phosphorilase totale et phosphorilase *b*-quinase. On a obtenu des résultats semblables à ceux qui ont été observés pendant l'anoxie. L'occurrence possible de deux mêchanismes d'accélération de la glycogénolyse («push» et «pull»), dans les animaux emploisonés par le DNP, est discutée.

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Alterations in Lipid Metabolism Following Trauma in Rabbits

During the recent years several attempts have been made to investigate the endocrine and metabolic response to injury, largely in reference to protein and carbohydrate metabolism[1-3]. However, changes in lipid metabolism following trauma have not been extensively studied. WALDSTROM[4] observed raised plasma concentrations of unesterified fatty acids following surgical operations. Similarly plasma free fatty acid levels are also known to be increased in such cases[5].

The present study has been undertaken to elucidate the changes in the lipid metabolism following experimentally produced fracture of femur in rabbits.

10 healthy rabbits of either sex weighing around 1.5 kg were selected and a closed fracture of shaft of the right femur was produced in all of them. 24 h prior to fracturing, blood samples were collected through the ear vein which served as the control. In the fractured animals

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blood was collected in same way at intervals of 4 h and 1, 2, 3, 4, 5, 6, 7, 8 and 9 days. From these samples total lipids, plasma free fatty acids, serum phospholipids and serum cholesterol were estimated.

Total serum lipids, free fatty acids and phospholipids were estimated, adopting the techniques of CHARBROL and CHARONNOT[6], DOLE[7] and YOUNGSBURG and YOUNGSBURG[8] respectively.

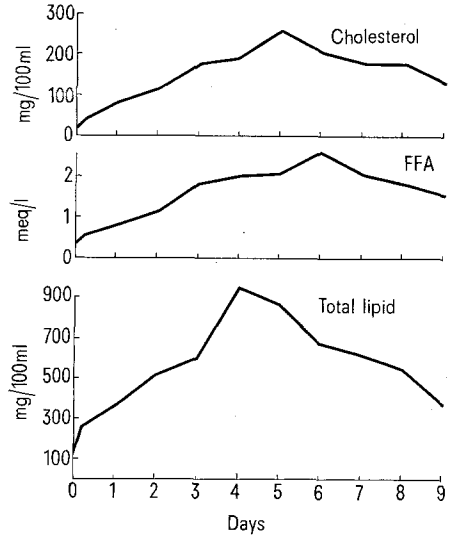
In normal animals, prior fracturing the average serum total lipids level was 142.5 mg/100 ml. After fracture a significant increase in the total lipids was observed which reached its maximum of 967.5 mg/100 ml on 4th day ($P < 0.05$). Subsequently, it gradually decreased upto 9th post fracture day. However, its mean value remained significantly higher than the prefracture value (Figure, Table).

The mean prefracture serum free fatty acid level was found to be 0.385 meq/l. 4 h following fracture it significantly increased to 0.518 meq/l and reached the maximum of 2.521 meq/l on the 6th day ($P < 0.05$). It was accompanied by a gradual fall during the successive days. Although the level remained significantly elevated in comparison to the prefracture level ($P < 0.05$) (Figure, Table).

The average prefracture serum phospholipids level was 20.133 mg/100 ml. Following fracture no significant increase in the levels was observed ($P > 0.1$) (Table).

Average prefracture serum cholesterol level was found to be 39.32 mg/100 ml. After fracture it increased and the elevated trend was maintained in the subsequent days to reach its maximum of 263.02 mg/100 ml on the 5th day ($P < 0.05$). This was followed by a gradual decline. However, the average values remained significantly higher than the prefracture level ($P < 0.05$) (Figure, Table).

In response to the trauma caused by fracture of the femur, the rabbits showed significantly elevated levels of



Shows serum cholesterol, F.F.A. and total lipids levels following different intervals of fracture.

Showing blood levels of total lipids, free fatty acids, cholesterol and phospholipids following different intervals of fractures

	Normal	4th h.	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day	9th day
Total lipid mg/100 ml	142.5 ± 31.6	267.5 ± 42.1 $P < 0.05$	360.0 ± 39.1 $P < 0.05$	520.0 ± 72.1 $P < 0.05$	605.0 ± 51.6 $P < 0.05$	967.5 ± 79.2 $P < 0.05$	876.0 ± 58.1 $P < 0.05$	685.0 ± 73.1 $P < 0.05$	630.0 ± 51.6 $P < 0.05$	463.0 ± 91.6 $P < 0.05$	390.0 ± 69.4 $P < 0.05$
FFA meq/l	0.385 ± 0.1	0.518 ± 0.09 $P < 0.05$	0.788 ± 0.21 $P < 0.05$	1.117 ± 0.28 $P < 0.05$	1.587 ± 0.39 $P < 0.05$	2.089 ± 0.57 $P < 0.05$	2.145 ± 0.70 $P < 0.05$	2.521 ± 0.56 $P < 0.05$	2.154 ± 0.32 $P < 0.05$	1.969 ± 0.41 $P < 0.05$	1.694 ± 0.81 $P < 0.05$
Phospholipid mg/100 ml	20.13 ± 8.6	22.23 ± 7.19 $P > 0.1$	20.55 ± 9.78 $P > 0.1$	20.55 ± 4.21 $P > 0.1$	20.55 ± 10.01 $P > 0.1$	19.51 ± 7.89 $P > 0.1$	22.56 ± 10.78 $P > 0.1$	19.57 ± 8.2 $P > 0.1$	25.17 ± 9.6 $P > 0.1$	19.51 ± 7.12 $P > 0.1$	26.81 ± 11.6 $P > 0.1$
Cholesterol mg/100 ml	39.32 ± 13.1	49.05 ± 10.7 $P > 0.1$	78.7 ± 11.76 $P < 0.05$	116.52 ± 19.1 $P < 0.05$	176.7 ± 21.8 $P < 0.05$	195.49 ± 26.12 $P < 0.05$	263.02 ± 48.6 $P < 0.05$	201.16 ± 31.9 $P < 0.05$	179.7 ± 28.21 $P < 0.05$	178.2 ± 36.78 $P < 0.05$	138.81 ± 41.2 $P < 0.05$

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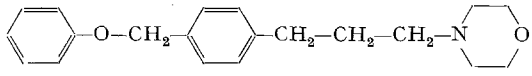
The Dearalkylation of Fomocaine Isomers by Microsomal Monooxygenase System of Rabbit Liver

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	—