

Es ist denkbar, dass auch die starke Hemmung der Pyridinnucleotid-Transhydrogenase eine der Ursachen für die Änderung der Fettsäuresynthese ist: Pyridinnucleotid-Transhydrogenase ist für den verglichen mit NAD hohen Reduktionsgrad des NADP in gekoppelten Mitochondrien<sup>16,17</sup> verantwortlich. Eine Hemmung des Enzyms bedingt daher erhöhtes NADH-Angebot, was zu einer Steigerung der intramitochondrialen (NADH-abhängigen) de novo-Synthese und zur Kettenverlängerung von Fettsäuren führen kann<sup>18</sup>.

Tatsächlich konnten wir bei einer kürzlich durchgeführten klinischen Studie feststellen, dass Patienten bei einer extrem hohen Dosierung von Benzbromaron (400–600 mg/Tag) eine deutliche Vermehrung der Freien Fettsäuren und Triglyceride im Plasma aufweisen<sup>8</sup>. Unsere hier berichteten in vitro Versuche bieten eine mögliche Erklärung für diese klinische Beobachtung.

**Summary.** The uricosuric drug benzbromarone inhibits a number of membrane-bound mitochondrial enzymes. A

strong inhibition of succinate dehydrogenase,  $\alpha$ -glycerophosphate dehydrogenase and pyridinnucleotide transhydrogenase was found (approximately 28, 48, 45  $\mu$ M benzbromarone for 50% inhibition respectively). Some possible consequences of these results for the lipid metabolism in patients during benzbromarone are discussed.

R. KRAMAR und M. M. MÜLLER

Medizinisch-Chemisches Institut der Universität Wien,  
Währingerstrasse 10, A-1090 Wien (Österreich),  
4. September 1972.

<sup>16</sup> L. DANIELSON und L. ERNSTER, *Biochem. Z.* 338, 188 (1963).

<sup>17</sup> M. KLINGENBERG und W. SLENCZKA, *Biochem. Z.* 337, 486 (1959).

<sup>18</sup> A. F. WHEREAT, M. W. OROSHIMO und J. NELSON, *J. biol. Chem.* 244, 6498 (1969).

## The Phosphorylases System of the Cardiac Muscle of Normal and Reserpinized Rats Poisoned with 2,4-Dinitrophenol

The glycogenolytic effect of 2,4-dinitrophenol (DNP) on the skeletal muscle in vivo, was recently explained by, among other factors, the increase of the activity of phosphorylase *b* kinase, the enzyme that catalyzes the conversion: phosphorylase *b* to *a*, and consequently the content of phosphorylase *a* is found increased<sup>1</sup>. The effect of glycogenolytic agents such as adrenalin and other catecholamines seem to be mediated by increasing the production of adenosine 3', 5' phosphate which increases the activity of phosphorylase *b* kinase<sup>2</sup>. Recent studies, however, show that this nucleotide is not increased either in DNP-poisoned animals<sup>3</sup> or in anoxia<sup>4</sup>. In order to accumulate evidence of the similarities of the action of the DNP with that of anoxia on the glycogenolysis, we performed determinations of phosphorylase *a*, phosphorylase *b* kinase and lactic acid in the heart of rats poisoned with DNP. Experiments were also made in reserpinized animals. The comparison between the effect of DNP and those of glucagon and anoxia is discussed.

The content of phosphorylases in rat hearts in vivo was determined in adult rats that were injected i.p. with 2.5 mg/100 g DNP. After 1 h they were anesthetized with

ether and the hearts were rapidly removed, weighed and ground in ice-cold solution containing 0.001 *M* EDTA – 0.02 *M* Sodium fluoride pH 6.8 in the proportion of 25 ml/g of muscle. After the treatment of the extract with either norit A or not, 20 mg/ml, 1.0 ml of the centrifuged extract were taken and incubated with glycogen for 20 min at 30°C and aliquots of 0.4 ml were then treated with glucose-1-phosphate, and either water or AMP as previously described<sup>1</sup>. The activity of phosphorylase *b* kinase was determined in male rats that were either injected or not with DNP 2.5 mg/100 g. After 1 h they were anesthetized with ether and the blood was drained from the jugular vein. The hearts were cooled for 30 min in crushed ice, then weighed and ground in sand with ice-cold solution of 0.002 *M* EDTA pH 7.0 in proportion of 2 ml/g heart. The homogenate was then centrifuged at 4000  $\times$  *g* for 30 min and the supernatant was filtered through glass wool and the activity of phosphorylase *b* kinase was determined immediately, as described<sup>1</sup>.

Table I shows that the content of phosphorylase *a* in hearts of rats poisoned with DNP is increased when compared with normal rats, by about 3 times, and also that the phosphorylase *a* is in high level, due the activity of the phosphorylase *b* kinase. This effect was also found in skeletal muscle in vivo<sup>1</sup>. By the other hand, as the effect of the DNP could only be detected in vivo<sup>5</sup>, and its action on the phosphorylases system occurs after 1 h from the injection, it was important to know its effect in perfused hearts, to avoid both the action of the metabolic products of the reduction of DNP in liver and the possibility of the increase of adrenalin in the blood circulation. The experiments in perfused hearts were done in adult

Table I. Content of phosphorylase *a* and total and of phosphorylase *b* kinase in hearts of rats poisoned with DNP 2.5 mg/100 g

Determination	Normal rats		DNP poisoned rats	
Phosphorylase <i>a</i> (10 rats)	7.0 $\pm$	1.7 <sup>a</sup>	25.2 $\pm$	3.1
Total phosphorylase (+ AMP) (10 rats)	67.2 $\pm$	7.1	85.3 $\pm$	13.0
Ratio Phosphorylase <i>a</i> /Total phosphorylase $\times$ 100	10.4 $\pm$	1.1	30.7 $\pm$	6.5
Phosphorylase <i>b</i> kinase (10 rats)	1346 $\pm$	480	3384 $\pm$	334

<sup>a</sup> S.E.M. The activity of phosphorylase *a* and total are expressed in units according to CORI<sup>10</sup> per gram of heart. The activity of phosphorylase *b* kinase is expressed in units of phosphorylase *a* formed from phosphorylase *b* in 15 min according to FISHER and KREBS method<sup>11</sup>.

<sup>1</sup> A. FOCESI JR., Q. S. TAHIN, M. M. EL-GUINDY and A. E. VERCESI, *Experientia* 25, 1243 (1969).

<sup>2</sup> G. T. CORI and B. ILLINGWORTH, *Biochim. biophys. Acta.* 27, 105 (1956).

<sup>3</sup> A. FOCESI JR. and A. E. VERCESI, unpublished results.

<sup>4</sup> H. E. MORGAN and A. PARMEGGIANI, in *Control of Glycogen Metabolism* (Ed. W. J. WHELAN and M. P. CAMERON, Ciba Foundation Symposium 1964), p. 254.

rats that were anesthetized with ether and the hearts were isolated and perfused via the coronary circulation with Krebs-Henseleit Bicarbonate buffer<sup>6</sup>, 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<sup>7</sup> 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<sup>8</sup>, after  $\frac{1}{2}$  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 pre-treatment 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 CORNBATH et al.<sup>6</sup>. In our case the increase of the content of phosphorylase *a* maintained the same proportion in both normal and reserpinized animals. These results suggest that the push mechanism for accelerating glycogenolysis<sup>9</sup>, 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 ATPase 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<sup>6</sup>. 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.

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

A. E. VERCESI<sup>13</sup> and A. FOCESI JR.<sup>12</sup>

*Departamento de Bioquímica, Instituto de Biologia, Cidade Universitária de Campinas, Caixa postal 1170, Campinas (São Paulo, Brazil), 28 August 1972.*

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 <i>a</i> (10 rats)	18.0 ± 4.5 <sup>a</sup>	72.0 ± 11.5
Total phosphorylase (+ AMP) (10 rats)	168.0 ± 21.0	192.3 ± 25.0
Ratio Phosphorylase <i>a</i> /Total phosphorylase × 100	10.7 ± 1.8	37.5 ± 7.4
Lactic acid (6 rats)	1.4 ± 0.2	6.0 ± 0.4

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

- <sup>5</sup> T. SEKINO, M. M. EL-GUINDY, Q. S. TAHIN and A. FOCESI JR., XXII<sup>a</sup> S.B.P.C. Meeting, Abstract vol. 1970.
- <sup>6</sup> M. CORNBATH, P. J. RANDLE, A. PARMEGGIANI and H. E. MORGAN, *J. biol. Chem.* 238, 1592 (1963).
- <sup>7</sup> In *Sigma Technical Bulletin*, No. 825-UV.
- <sup>8</sup> G. W. SCHWERT, D. B. S. MILLAR and Y. TAKENAKA, *J. biol. Chem.* 237, 2131 (1962).
- <sup>9</sup> E. HELMREICH, S. KARPATIN and C. F. CORI, in *Ciba Foundation Symposium*, 1964, p. 211.
- <sup>10</sup> G. T. CORI, B. ILLINGWORTH and P. J. KELLER, in *Methods of Enzymology* (Eds. S. P. COLOWICK and N. O. KAPLAN; Academic Press Inc., New York 1955), vol. 1, p. 100.
- <sup>11</sup> E. H. FISCHER and E. G. KREBS, in *Methods of Enzymology* (Eds. S. P. COLOWICK and N. O. KAPLAN; Academic Press, New York 1962), vol. 5, p. 373.
- <sup>12</sup> Acknowledgment. The authors wish to express their gratitude to Mr. A. F. LEITE, J. GUIMARÃES FILHO, Miss ZIMAR FARIA and Miss SAMIRA ABDOUGH for the efficient technical assistance.
- <sup>13</sup> Fellow of F.A.P.E.S.P.

## 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<sup>1-3</sup>. However, changes in lipid metabolism following trauma have not been extensively studied. WALDSTROM<sup>4</sup> 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<sup>5</sup>.

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

- <sup>1</sup> J. M. HOWARD, *Ann. Surg.* 141, 321 (1955).
- <sup>2</sup> J. M. KINNEY, *Br. J. Surg.* 54, 435 (1962).
- <sup>3</sup> H. T. RANDAL, *J. Am. med. Ass.* 202, 117 (1967).
- <sup>4</sup> L. B. WALDSTROM, *Acta chir. scand. Supp.* 238 (1959).
- <sup>5</sup> S. P. ALLISON, K. PROWSE and M. J. CHAMBERLAIN, *Lancet*, 1, 478 (1967).