$^{45}Ca^{++}$ and total Ca^{++} in the subcellular fractions from the red and white muscle of the rabbit

	% distribution of $^{45}Ca^{++}$		cpm/mg protein		cpm/m*M* Ca^{++}	
	Red	White	Red	White	Red	White
Mitochondria	8.3 ± 0.9	6.3 ± 0.2	328.0 ± 14.7	253.2 ± 36.1	24.4 ± 0.2	10.2 ± 3.3
Sarcoplasmic reticulum	6.3 ± 0.1	9.2 ± 2.3	7.8 ± 2.1	181.1 ± 17.9	0.4 ± 0.03	6.9 ± 1.6

Data are given ± S.E.

fractions: total Ca^{++} was measured in a Perkin-Elmer Mod. 303 atomic absorption spectrophotometer, in the presence of 1% $LaCl_3$. Protein was determined with a biuret reaction.

A previous study from this laboratory has shown that the distribution of $^{45}Ca^{++}$ among the subcellular fractions after in vivo injection of $^{45}Ca^{++}$ reflects, at least in part, in vivo movements rather than the redistribution of the radioactivity during the fractionation[1]. The Table shows that about 8% of the total Ca^{++} of the red muscle was recovered in the isolated mitochondria, as compared with about 6% in the white muscle. These values were certainly underestimated, since a large number of mitochondria sedimented in the first low speed centrifugation. Upon applying a correction based on the total cytochrome oxidase activity of the homogenates, about $^2/_3$ and 40% of the total $^{45}Ca^{++}$ was found in the mitochondria in the red and white muscle, respectively. The sarcoplasmic reticulum isolated from the red muscle had practically no radioactivity, while the same fraction from the white muscle contained about 10% of the total $^{45}Ca^{++}$ of the muscle. For the reasons mentioned above, also this value was likely to be underestimated. While in the red muscle the amount of $^{45}Ca^{++}$/mg of reticular protein was negligible with respect to mitochondria, in the white muscle it was of the same order of magnitude in the 2 organelles. The amount of $^{45}Ca^{++}$/mg of reticular protein was more than 20 times higher in white than in red muscle, while in mitochondria it was only 25% lower in white than in red muscle. The specific activity of the Ca^{++} in the subcellular organelles could be taken as an indication of the turnover of their Ca^{++}. Clearly, the specific activity of reticular Ca^{++} was much higher in white than in red muscle: in the masseter it was about 60 times lower than that of mitochondrial Ca^{++}, while in the adductor it was of the same order of magnitude. However, the table shows that the specific activity of the mitochondrial Ca^{++} was quite high also in the white muscle. The results thus showed that the Ca^{++} pool associated with the sarcoplasmic reticulum was larger and more active in white than in red muscle, in agreement with the view that sarcoplasmic reticulum is very active in the intracellular movements of Ca^{++}. However, in agreement with the recent studies on heart mentioned above[1], the results have also shown that the specific activity of Ca^{++} in the sarcoplasmic reticulum of the red muscle was negligible with respect to mitochondria, a finding which suggests a role for mitochondria in the Ca^{++} movements linked to the contraction and relaxation of red muscle. However, mitochondria were found to be quite active in the intracellular movements of Ca^{++} also in the white muscle; their participation in the contraction and relaxation cycles of white muscle cannot therefore be ruled out[8].

Riassunto. Dopo iniezione di $^{45}Ca^{++}$ in conigli, radioattività appare nei mitocondri e nel reticolo sarcoplasmico del massetere dell'adduttore magno. La quantità di radioattività, e l'attività specifica del Ca^{++}, sono molto più elevate nei mitocondri che nel reticolo sarcoplasmico del massetere. Sono dello stesso ordine di grandezza nei due organelli dell'adduttore magno. I risultati suggeriscono un ruolo prominente dei mitocondri nei movimenti intracellulari del Ca^{++} nel muscolo rosso, ed un analogo prominente ruolo del reticolo sarcoplasmico nel muscolo bianco.

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Mitogenic Effect of Sodium Phenobarbital and Rat Liver Arginase Activity

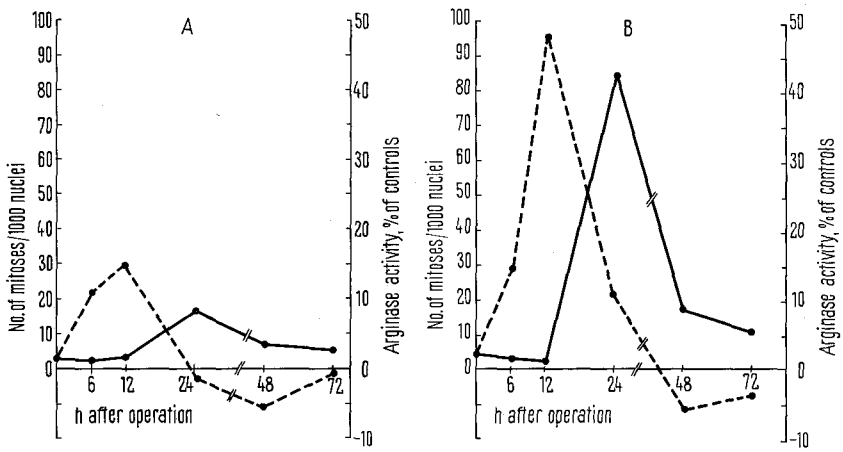
It is well known that administration of phenobarbital to rats brings about dramatic alterations in the biochemical and structural composition of the parenchymal liver cells[1]. Besides that, we have found that phenobarbital has growth-promoting action in both intact and regenerating rat liver due to parenchymal cell hyperplasia[2]. Accordingly, some of the liver metabolic reorganization which follows phenobarbital administration can be assumed to be related to processes associated with growth, rather than to other alterations.

[1] S. ORRENIUS, J. L. ERICSSON and L. ERNESTER, J. Cell Biol. *25*, 627 (1965).

[2] M. JAPUNDŽIĆ, B. KNEŽEVIĆ, V. DJORDJEVIĆ and I. JAPUNDŽIĆ, Exp. Cell Research *48*, 163 (1967).

Arginase activity during liver regeneration of phenobarbital-Na-treated and saline-treated rats

No. of cases	h after operation	Saline-treated rats			Phenobarbital-Na treated rats		
		Arginase activity	% of difference	p values	Arginase activity	% of difference	p value
7	Intact liver	285.9 ± 41.2	–	–	253.9 ± 23.8	–	–
7	6	318.4 ± 29.0	+11.7	< 0.01	295.9 ± 50.0	+16.3	< 0.05
7	12	328.5 ± 52.4	+15.1	< 0.01	373.2 ± 70.2	+47.0	< 0.01
7	24	275.6 ± 41.9	− 3.6	> 0.05	287.2 ± 45.6	+13.2	> 0.05
7	48	271.9 ± 41.9	− 4.8	> 0.05	238.3 ± 21.0	− 5.7	> 0.05
7	72	249.0 ± 40.0	+ 3.0	> 0.05	241.4 ± 34.4	− 4.9	> 0.05



Arginase activity and mitotic activity during liver regeneration of saline-treated (A) and phenobarbital-treated (B) rats. ——, number of mitoses/1000 nuclei; – – –, arginase activity.

To bring the activity of liver arginase in correlation with the control of DNA synthesis[3–5], we examined arginase activity at the time of active cellular proliferation induced by phenobarbital and partial hepatectomy.

The experiments were carried out on white male rats weighing between 130 and 150 g. 2 groups of animals were submitted to partial hepatectomy. 1 group of animals was injected with phenobarbital-Na by i.p. way in daily doses of 100 mg/kg body weight, for 5 days before operation and the other group with saline solution. The treatment was the same after the operation up to the sacrifice. Partial hepatectomy was performed in both groups under ether anesthesia by HIGGINS' and ANDERSON'S[6] method. The animals were sacrificed at different intervals after the operation. In each group of animals, intact liver arginase activity was compared with corresponding postoperative values. Standard method of VAN SLYKE and ARCHIBALD[7] was applied for determination of arginase activity. The quantity of enzymes which 1 μmole of urea creates for 1 min, under standard conditions, was taken as the unit of arginase activity.

In the course of the regeneration of normal rat's liver, we noted a statistically significant increase in arginase activity during the premitotic phase in relation to the intact untreated liver. The increase of arginase activity 6 h after the operation amounts to 11.7% ($p < 0.01$), while 12 h after the operation it is about 15% ($p < 0.01$). There were no statistically important variations at the other examined intervals (Table). During the regeneration of the liver of phenobarbital treated animals (Table), we have also found an increased arginase activity in the premitotic phase of regeneration which is more pronounced than in the liver of saline-treated animals. The increase of arginase activity 6 h after the operation amounts to 17%, while 12 h after the operation it is about 50%. Both increases in arginase activity, in relation to the intact liver of phenobarbital treated animals, are statistically significant. The variations during other test intervals are not statistically significant.

It is of interest to consider the results of our experiments in relation to data and hypothesis of LIEBERMAN and OVE[4], OTSUKA and TERAYAMA[3] and HOLLEY[5]. The results of these authors indicate that arginase might have an inhibitory role in DNA synthesis. Our results show that the proliferative activation of the liver after partial hepatectomy, of both normal and phenobarbital treated animals is accompanied by an increase in arginase activity during the premitotic phase of regeneration (Figure), when a highly intensive DNA synthesis takes place in the parenchymal liver cells. Accordingly, it is obvious that under our conditions one cannot attribute the role of an inhibitor of DNA synthesis to liver arginase, as the increase of its activity during premitotic phase of liver regeneration coincides with a spectacular increase in DNA synthesis.

Résumé. Au cours de la régénération du foie normal des rats, on a constaté une augmentation – statistiquement significative – de l'activité arginasique dans la phase prémitotique. Chez des animaux traités par le phénobarbital, l'activité arginasique augmente encore davantage dans la phase prémitotique du foie régénéré.

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[3] H. OTSUKA and H. TERAYAMA, Biochim. Biphys. Acta *123*, 274 (1966).
[4] I. LIEBERMAN and P. OVE, Biochim. Biophys. Acta *28*, 153 (1960).
[5] R. W. HOLLEY, Biochim. Biophys. Acta *145*, 525 (1967).
[6] G. W. HIGGINS and R. N. ANDERSON, Am. Med. Ass. Arch. Pathol. *12*, 186 (1931).
[7] D. D. VAN SLYKE and R. M. ARCHIBALD, J. Biol. Chem. *165*, 293 (1946).