creas into serum: the tempting speculation that the low molecular weight of the enzyme may play a role in its diffusibility does not agree with present data. The mechanisms of entry of the pancreatic enzyme into blood serum are not clearly understood[9], and may vary from one animal species to another. The contribution of pancreatic to serum amylase has already been challenged, in some animal species, on the basis of different experimental approach[10-12].

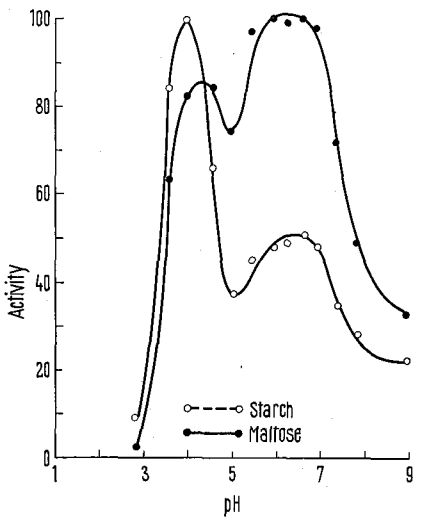


Fig. 2. pH curves, in maltose and starch hydrolysis, of the partially purified enzyme from horse serum.

*Riassunto.* Mediante gel-cromatografia si è confermata la presenza di α-glucosidasi e l'assenza di α-amilasi nel siero di cavallo. La α-amilasi è anche assente nelle ghiandole salivari ma presente nel pancreas dello stesso animale.

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## A Comparative Study of the Intracellular $Ca^{++}$ Movements in White and Red Muscle

In a recent study on the distribution of in vivo injected $^{45}Ca^{++}$ among the subcellular fractions of the heart, the largest part of the radioactivity was recovered in the mitochondria[1]. The movements of $Ca^{++}$ were more active in mitochondria than in the sarcoplasmic reticulum, a result suggesting a role for mitochondria in the $Ca^{++}$ movements associated with the contraction and relaxation of the heart. Results pointing to the same conclusion have recently been reported by FEHMERS[2,3]. Studies of $Ca^{++}$ transport in the isolated sarcoplasmic reticulum have, on the other hand, led to the view that the movements of $Ca^{++}$ linked to the contraction and relaxation of muscle are under the control of the sarcoplasmic reticulum[4-6]. However, most of these studies have been carried out on white muscles, which contain very few mitochondria and a very well developed sarcoplasmic reticulum. The possibility was thus considered that the intracellular movements of $Ca^{++}$ were under the control of different subcellular organelles in white and red muscles. Sarcoplasmic reticulum would be predominant in the former, and mitochondria in the latter: a similar possibility has been suggested by GERGELY et al.[7] a few years ago.

Rabbit masseter (red) and adductor magnus (white) were used. The rabbits were injected i.v. with 60 μC $^{45}Ca^{++}$ (equal to about 6 μg $CaCl_2$) 15 min before the sacrifice. The masseter and adductor magnus muscles were quickly excised, cut into small pieces, soaked in several changes of 0.4 *M* sucrose, at 0 °C, and squeezed between layers of filter paper to minimize the contamination by highly labelled blood plasma. They were freed from the connective tissue, minced with scissors, and homogenized in 0.1 *M* KCl-0.005 *M* histidine-Cl, pH 7.0 (masseter) or in 0.4 *M* sucrose (adductor magnus): a lucite Potter homogenizer was used for the masseter, and a Waring Blendor for the adductor magnus. Total $Ca^{++}$ and $^{45}Ca^{++}$ were determined on aliquots of the homogenates. The myofibrils, the nuclei, and the cell debris were discarded at 600 g for 10 min, and the mitochondria were separated at 12,000 g for 10 min; the sarcoplasmic reticulum was sedimented at 125,000 g for 45 min, after having discarded an intermediate fraction at 34,000 g for 20 min. The purity of the fractions was checked by determining the total cytochrome oxidase activity and the RNA/protein ratio. The yields were as follows: about 2.8 mg and 0.1 mg of mitochondrial proteins/g of masseter and of adductor magnus, respectively. About 1.1 mg and 1.3 mg of sarcoplasmic reticulum protein/g of masseter and of adductor magnus, respectively. $^{45}Ca^{++}$ was counted on aliquots of the suspensions of the various

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$^{45}Ca^{++}$ 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.

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