Dilution of Phosphate in the Plasma of Tocopherol Deficient Rabbits

It has been observed by MASTER et al.[1] that in tocopherol deficient rabbits the specific activity of the plasma phosphate was decreased at 15, 30 and 60 min after injection of P^{32}; however no attempt to study the kinetics of the dilution of P^{32} phosphate has been made. The kinetics of the dilution of P^{32} phosphate in the plasma of tocopherol deficient rabbits during the first 15 min after the injection of P^{32} will be discussed here.

Materials and methods. Male white rabbits of approximately 500 g were fed the basal tocopherol deficient diet used by CAPUTTO et al.[2]. The control animals were fed the same diet with the addition of 450 mg of α-tocopherol acetate per kilogram of diet. After 3 weeks on the diet, the tocopherol deficient animals stopped growing and developed a fairly rapidly progressing muscular weakness with loss of weight. The animals were used at the time when the difference of weight between the deficient and control animals was not greater than 15% but they presented definite symptoms of muscular weakness.

One millilitre of sterile solution of P^{32} phosphate in saline was injected into the marginal vein of the ear. Samples of a few drops of blood were extracted from the marginal vein of the ear, opposite to that used for the injection of phosphate. The samples were collected in centrifuge tubes cooled at 0 °C, containing 1 ml of saline with 1 mg of heparine. The tubes were centrifuged at 1000 g for 10 min and then the supernatant separated. Duplicate samples of 0.2 ml of the supernatant were dried on planchets and counted. The radioactivity per μmoles of inorganic phosphate *Pi* was calculated with the following formula:

$$\frac{\text{cpm}}{\mu\text{moles } Pi} = \frac{\text{cpm}\left(1 + \frac{w}{1.083}(1\text{-H})\right)}{0.2\left(\frac{w}{1.083}(1\text{-H})\right)\frac{\mu\text{moles } Pi}{\text{ml plasma}}}$$

where W, is the blood weight; H, the hematocrit; 1.083, the value of the blood density, and *Pi* the inorganic phosphate of the plasma. Blood weight was determined by difference between the weights of the centrifuge tube before and after the collection of blood. Hematocrit and inorganic phosphate of the plasma were determined in samples of blood extracted at the end of the experiment. Hematocrit was determined with microhematocrit tubes. For determination of phosphate, a sample of blood was centrifuged for separation of erythrocytes and to the supernatant a concentrate solution of trichloroacetic acid was added to reach a final concentration of 6%. Phosphate was measured in the trichloroacetic extract by the method of FISKE and SUBBAROW[3]. In order to compare rabbits, radioactivity was calculated on the basis of 10^9 count/min injected per kilogram of body weight.

Results and discussion. The determinations of inorganic phosphate of the plasma in the control and deficient animals gave 2.1 ± 0.4 μmoles per millilitre of plasma for both groups of animals.

In Figures 1 and 2 are represented in semilogarithmic plot the values of P^{32} activity per μmoles *Pi* at different times after the injection of P^{32} phosphate for the control and deficient animals respectively. The values of the P^{32} per μmoles *Pi* at each period of time were significantly different ($p < 0.001$) in both groups of animals.

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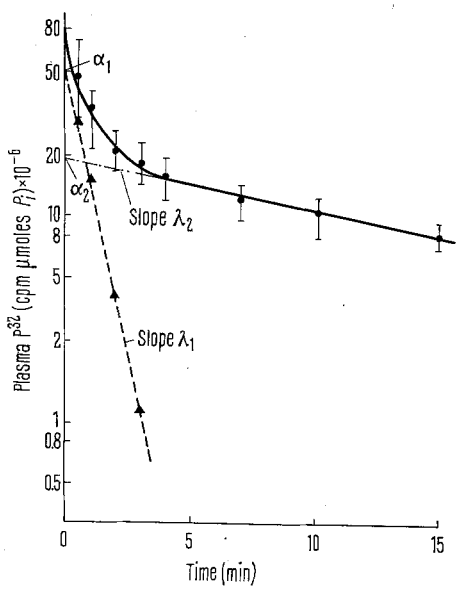

Fig. 1. Semilogarithmic plot of the values of P^{32} activity per μmoles *Pi* at different times after P^{32} phosphate injection to control animals. —●—, experimental data approximated by the equation: $q = \alpha_1 e^{-\lambda_1 t} + \alpha_2 e^{-\lambda_2 t} = 54.5\, e^{-1.26\, t} + 19.0\, e^{-0.058\, t}$ each point that represents the average of determinations in 12 animals fell within the limits indicated by vertical lines. ▲—▲—▲, exponential function $\alpha_1 e^{-\lambda_1 t} = 54.5\, e^{-1.26\, t}$; —.—.—, exponential function $\alpha_2 e^{-\lambda_2 t} = 19.0\, e^{-0.058\, t}$.

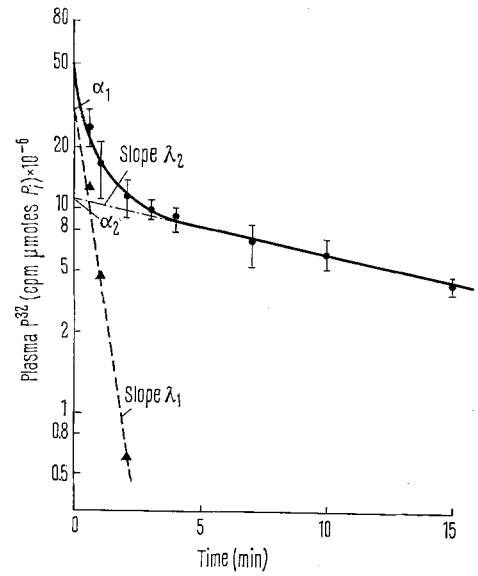

Fig. 2. Semilogarithmic plot of the values of P^{32} activity per μmoles *Pi* at different times after P^{32} phosphate injection to deficient animals. —●—, experimental data approximated by the equation: $q = \alpha_1 e^{-\lambda_1 t} + \alpha_2 e^{-\lambda_2 t} = 31.4\, e^{-1.97\, t} + 11.4\, e^{-0.069\, t}$ each point that represents the average of determinations in 12 animals fell within the limits indicated by vertical lines. ▲—▲—▲—, exponential function $\alpha_1 e^{-\lambda_1 t} = 31.4\, e^{-1.97\, t}$; —.—.—, exponential function $\alpha_2 e^{-\lambda_2 t} = 11.4\, e^{-0.069\, t}$.

The values of the slopes of the curves of the exponential functions were:

$\lambda_1 = (1.26 \pm 0.16)$ min^{-1} and $\lambda_2 = (0.058 \pm 0.014)$ min^{-1} for the control and

$\lambda_1 = (1.97 \pm 0.23)$ min^{-1} and $\lambda_2 = (0.069 \pm 0.008)$ min^{-1} for the deficient animals. The value of λ_1 for the deficient animals was significantly higher ($p < 0.001$) than that for the control animals. Assuming that λ_1, is the rate of passage through the capillar barrier, the higher value obtained in the deficient animals could be interpreted as if in these animals the capillar permeability is increased.

The values of the coefficients of the exponential functions were: $\alpha_1 = (54.5 \pm 8) \times 10^6$ cpm/μmoles *Pi* and $\alpha_2 = (19.0 \pm 5) \times 10^6$ cpm/μmoles *Pi* for the control, and $\alpha_1 = (31.4 \pm 4) \times 10^6$ cpm/μmoles *Pi* and $\alpha_2 = (11.4 \pm 2) \times 10^6$ cpm/μmoles *Pi* for the deficient animals. Significant differences ($p < 0.001$) have been found between the corresponding values of α_1 and α_2 for the control and deficient animals. The total space of phosphate, determined by the sum of the coefficients α_1 and α_2 of the exponential functions, gave a value of 73.5×10^6 cpm/μmoles *Pi* for the control and 42.8×10^6 cpm/μmoles *Pi* for the deficient animals.

The present results proved conclusively that the rate of elimination of P^{32} from the plasma was increased in tocopherol deficient rabbits. The difference observed between the control and deficient animals could be due to an increase of the capillar permeability and the extraplasmatic accumulation of phosphate. The increased extraplasmatic accumulation of phosphate could be the consequence of a higher uptake of phosphate by the skeletal muscle or other tissues and an increase of the cellular permeability toward phosphate. An increase of the extracellular space has been found by DIEHL[4] in tocopherol deficient rabbits. Higher values for the ratios of both inorganic and organic phosphate respectively to that of plasma phosphate has been observed in the deficient rabbits[1]. Since the muscle weight represents 70% of the body weight the observed difference could account for the higher dilution rate of P^{32} in the tocopherol deficient animals[5–7].

Résumé. L'étude de la cinétique de la dilution du phosphate dans le plasma des lapins déficients en α-tocophérol a mis en évidence que, chez ces animaux, la vitesse de disparition du phosphate du plasma ainsi que l'espace de phosphate se trouvent augmentés.

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Versuche zur chemischen Gedächtnisübertragung von farbdressierten Goldfischen auf undressierte Tiere

Untersuchungen über das Gedächtnis und zur Gedächtnisübertragung werden nach den grundlegenden Versuchen der letzten Jahre (Kurz- und Langzeitgedächtnis[1], Gedächtnisübertragung[2,3]) an vielen Stellen durchgeführt.

Material und Methodik. Wir haben 10–14 cm lange Goldfische in Gruppen zu je zwei Tieren in straffreier Differenzdressur mit automatischer Aufzeichnung des Verhaltens auf grünes Licht (hier Belohnung durch Futter) gegen spontan bevorzugtes rotes Licht dressiert (Versuchsanordnung und optische Dressur[4]). Nach Ausbildung des bedingten Reflexes werden die Gehirne der dressierten Tiere (= «Spender») herausoperiert, anschliessend in Anlehnung an eine Arbeitstechnik von UNGAR[5] homogenisiert, dialysiert und das gefriergetrocknete niedermolekulare Material in Kaltblutringer gelöst an undressierte Tiere (= «Empfänger») i.p. verabfolgt. Gleichzeitig injizierten wir undressierte Kontrollgruppen (= «Kontrollempfänger») mit niedermolekularen Gehirndialysaten nicht dressierter Tiere (= «Kontrollspender»). Anschliessend testeten wir die Empfängergruppen mehrmals täglich unter gleichen Bedingungen in ihren Reaktionen auf grünes und rotes Licht. Dem Präparator der Extrakte sowie dem Versuchsleiter war nicht bekannt, welche Empfängergruppe das Kontroll- und welche das Spenderdialysat erhalten hatte (Doppelter Blindversuch).

Ergebnisse. 1) Alle Empfängergruppen zeigen vor der Injektion (Figur 1B) eine Spontanbevorzugung für rotes Licht[4].

2. Die Gruppen der Kontrollempfänger verhalten sich in den Testsitzungen nach der Injektion gegenüber den gebotenen Farben genauso wie in den Tests vor der Injektion (Figur 2). Mitunter konnten wir im Verlauf späterer Tests eine leichte Abnahme der Rot-Reaktion beobachten. Der Übergang zu einer Grün-Bevorzugung liess sich jedoch bei den Kontrollgruppen nie feststellen.

3. Bei den Gruppen der «Empfänger» konnten wir 6–12 h nach der Injektion des «Gedächtnisdialysates»

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