Behaviour of Serum Labile Phosphoric Esters After Amino Acids Administration

A decrease of serum inorganic P follows the intravenous introduction of a casein hydrolysate[1]. It is our purpose to investigate the behaviour of serum labile phosphoric esters after administration of casein hydrolysate, starting from the fact that several papers on labeled amino acids have shown that the latter, when given intravenously, are immediately used up in proteic synthesis processes[2], and that LIPMAN affirms that a transphosphorylation reaction between A.T.P. and amino acids, with formation of acylphosphates, proceeds the construction of pectic bonds[3].

Procedure.–For our purposes we have employed rabbits of 2 kg, fasted for 12 h. We then administrated intravenously 20 ml of a 5% of a casein hydrolysate per kilogram of body weight, according to the method given in a previous paper[1].

Before the injection and 30 and 60 min after it, we drew 8 ml of blood from the marginal vein of the ear, and then determined the inorganic P of serum obtained from the three samples of blood, according to the FISKE and SUBBAROW method[4] and the LOWRY and LOPEZ method[5]. It is known that with the former method one determines, as inorganic P, also the P present in the labile phosphoric esters (phosphocreatine, acetyl-phosphate, ribose-1-phosphate) which are quickly hydrolysed in a molibdate acid solution[6], so that the difference between data obtained by the two methods expresses the P value of labile phosphoric esters[7].

Results.–The results obtained are summarized in Figure 1 (average of six experiments).

A decrease of inorganic P of serum with an increase of P value of labile phosphoric esters follows the administration of casein hydrolysate, since the initial value of 0·69 mg ± 0·14% before casein administration increases to 0·85 mg ± 0·18% 30 min after the administration, and then regains the initial value at the end of the experiment.

Our purpose has been to establish whether there is an analogous behaviour after glucose administration, since in this case also a decrease of serum inorganic P occurs[8].

The experiments were made by the same procedure as in the previous experiments, administering, by i.v. injection, 20 ml per kilogram of body weight of a 10% of a glucose solution to rabbits of 2 kg fasted for 12 h.

The results are collected in Figure 2 (average of six) experiments.

A decrease of serum inorganic P follows the introduction of glucose, without a parallel increase of labile phosphoric esters value, which, in some tests, showed a light decrease.

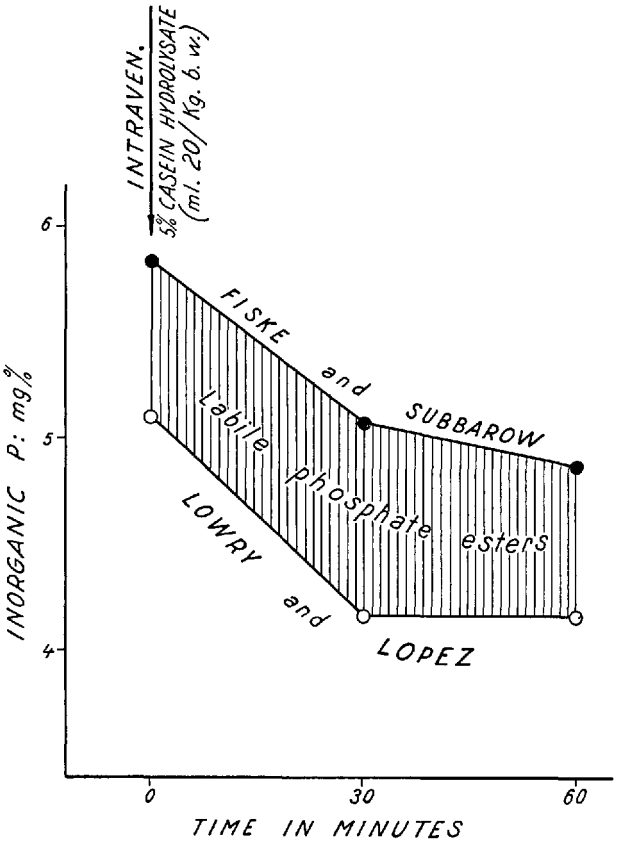

Fig. 1.

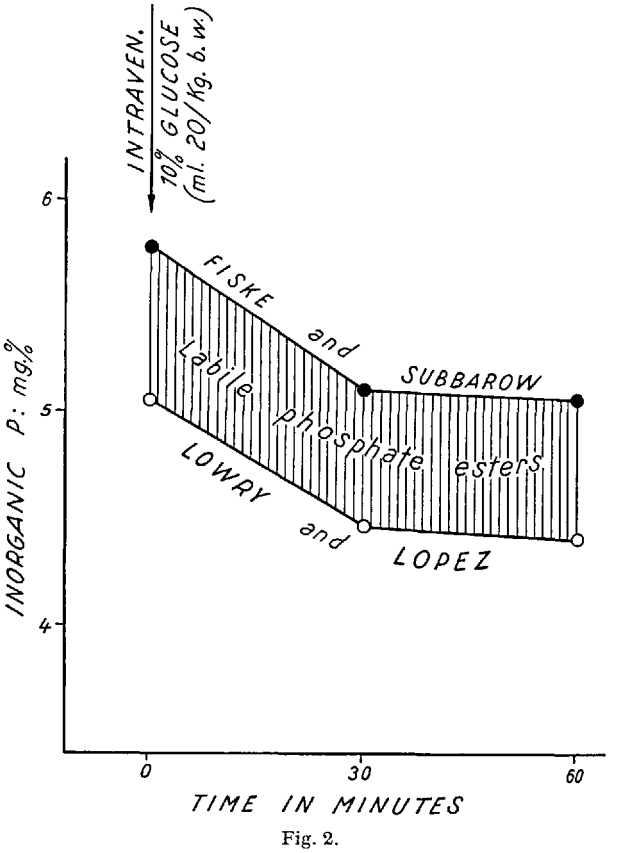

Fig. 2.

[1] F. PASQUINELLI, A. GIUNTA, G. NATALI, and A. D'ALESSANDRO Lo Sperimentale *102*, 134 (1952).

[2] T. WINNICK, F. FRIEDBERG, and D. M. GREENBERG, J. Biol. Chem. *173*, 189 (1948).– D. M. GRENNBERG, and T. WINNCK, J. Biol. Chem. *173*, 199 (1948). – H. BORSOOK, C. L. DEASY, A. J. HAAGEN-SMITH, G. HEIGHLEY, and P. H. LOWRY, J. Biol. Chem. *187*, 839 (1950).

[3] F. LIPMANN, Adv. Enzymol. *1*, 99 (1941).

[4] C. H. FISKE and T. SUBBAROW, J. Biol. Chem. *66*, 375 (1925).

[5] O. H. LOWRY and J. A. LOPEZ, J. Biol. Chem. *162*, 421 (1946).

[6] O. H. LOWRY and J. A. LOPEZ, J. Biol. Chem. *162*, 421 (1946). – F. LIPMANN, Adv. Enzymol. *6*, 231 (1946).

[7] V. R. POTTER, J. Biol. Chem. *169*, 17 (1947).

[8] A. BOLLIGER and F. W. HARTMAN, J. Biol. Chem. *64*, 91 (1925). – H. K. BARRENSCHEN, F. DOLESHALL, and L. POPPER, Biochem. Z. *177*, 50 (1925). – H. POLLACK, R. F. MILLET, H. E. ESSEX, and F. C. MANN, Amer. J. Physiol. *110*, 117 (1934). – A. BODANSKY, J. Biol. Chem. *104*, 473 (1934). – V. BECCARI and G. AURICCHIO, Boll. Soc. Biol. Sper. *23*, 1066 (1947).– F. DE VENANZI, Proc. Soc. exp. Biol. Med. *76*, 770 (1951).

Discussion and conclusion.—The present work has shown that while the decrease of serum inorganic P which occurs after casein hydrolysate administration is accompanied by an increase of serum labile phosphoric esters, this does not occur after glucose administration, although a sharp decrease of inorganic P is shown in this case also.

The behaviour observed after glucose administration eliminates the possibility that the increase of labile phosphoric esters of serum observed after casein hydrolysate administration, is to be attributed to the administration of compounds which by being oxidated by KREBS cycle, supply the necessary energy for labile phosphoric bonds synthesis.

The increase of labile phosphoric esters after casein hydrolysate administration may be explained if it is then for granted, according to LIPMANN, that the amino acids administrated are phosphorylated with formation of acylphosphates.

If this last hypothesis is accepted, we can understand how a part of the acylphosphate formed at tissues level and not utilized in proteosynthesis processes, may avoid the high acylphosphatasis activity of tissues[1], and pass into the blood stream causing a temporary increase of labile phosphoric esters.

This would explain the lack of increase of labile phosphoric esters after glucose administration while inorganic P decrease, observed either after glucose administration or after amino acids administration, might be due, in both cases, to the utilisation of inorganic P for the resynthesis, at tissues level, of the A.T.P. largely involved in forming hexose-6-phosphate, in the first case, and acylphosphates in the second case.

F. PASQUINELLI and A. D'ALESSANDRO

Institute of General Pathology, University of Florence, February 18, 1953.

Riassunto

Alla introduzione endovenosa di un idrolisato di caseina, consegue un abbassamento del P inorganico del siero con aumento degli esteri fosforici. Gli autori pensano che tale reperto possa interpretarsi ammettendo che gli aminoacidi introdotti vengano fosforilati con formazione di acilfosfati prima di essere uniti in legami peptidici.

[1] F. LIPMANN, Adv. Enzymol. *6*, 231 (1946). – E. SHAPIRO and E. WERTHEIMER, Nature *156*, 690 (1945).

Studies on Arterio-Venous Anastomoses in the Lungs

It is now generally accepted that arterio-venous anastomoses exist in the lungs. As regards the conditions however in which they may act as haemodynamic shunts the literature is controversial[1]. Further nothing is known as to the percentage amount of blood coming from the pulmonary artery that passes through these anastomoses and thus bypassing the lung capillary circulation. We therefore undertook the following studies to get quantitative data regarding the functioning of these channels in various species of animals.

Methods. In principle the method consisted in injecting at a slow fixed rate a known quantity of radioactive spheres (P^{32})[1] with diameters of 25 to 30 μ into the jugular vein of narcotised animals and sacrificing the animals after 15 to 30 min to determine the radioactivity retained in the lungs. The difference in activity of the spheres injected and those retained in the lungs corresponds to the amount of the spheres that have passed through anastomotic channels. This difference may be considered to be a criterion of the percentage volume of blood from the pulmonary artery passing through these shunts. The studies were conducted on rats, guinea pigs, rabbits and cats.

Table I

Data sheet indicating the injected, residual and the total activity of ten specimens of spheres

Sample No.	Weight of spheres mg	Activity of the spheres cts./min		
		glass *a* (injected)	glass *b* (residual)	*a* + *b* (total)
1	5	7789	2602	10 391
2	5	7100	3361	10 461
3	5	7097	2936	10 033
4	5	7552	2850	10 402
5	5	7650	2855	10 505
6	5	7479	3114	10 593
7	5	7566	2657	10 223
8	5	7614	2684	10 298
9	5	7948	2650	10 598
10	5	7841	2257	10 098
Mean				10 360
Standard Deviation*				∓ 1,9%

* Standard Deviation $= \sqrt{\frac{1}{N\text{-}1} \cdot S^n (x\text{-}\bar{x})^2}$.

Rats, guinea pigs and rabbits were narcotised with 2 g/kg urethane subcutaneously and cats with 40 mg/kg sodium pentothal intraperitoneally. The suspensions of the spheres were prepared freshly each time just before the injection. 5 mg of spheres were put into a flask containing 2 ml of normal saline with 4 mg of Dioctyl-Sodium-Sulfosuccinate (aerosol) for the rats and double the above quantities for the other species. The aerosol was used to prevent adhesion of the spheres and to give a homogenous suspension. The suspensions were put into a syringe and injected for 2 min at a uniform speed. The spheres left over in the syringe and flask were carefully collected and their activity determined. ¼ to ½ h after the injection the animals were bled to death, chest opened and lungs removed. The organs were washed free of blood and the wet weight recorded. They were then emulsified thoroughly and 1 g of the homogenate was wet ashed and taken for determining radioactivity.

Since in our method the percentage flow in the anastomoses is calculated by noting the difference in the activity of the spheres injected with those retained in the lungs, accurate knowledge of the exact amount injected is of primary importance. In order to assess the accuracy and the degree of variation in our technique the following experiments were carried out:

[1] M. CLARA, *Die arterio-venösen Anastamosen* (J. A. Barth, Leipzig, 1939). – H. V. HAYEK, Z. Anat. *112*, 221 (1943). – A. HÜRLIMANN, Arch. int. Pharmacodyn. *80*, 99 (1949). – K. PRINZMETAL, E. M. ORNITZ, Jr., B. SIMKINS, and H. C. BERGMANN, Amer. J. Physiol. *152*, 48 (1948). – H. RAHN, R. C. STROUD, and C. E. TOBIN, Proc. Soc. exp. Biol. Med. *80*, 239 (1952).

[1] H. EMMENNEGGER, A. HÜRLIMANN, and K. BUCHER, Helv. physiol. Acta *9*, 224 (1951).